https://doi.org/10.31351/vol33iss3pp1-16

Biomarkers of Oxidative Stress in Diabetic Microvascular Complications Review Article

Sarah Hashim Mhaibes*,[1] and Shatha H. Ali[1]

[1]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
***Corresponding author**
Received 12/6/2023, Accepted 21/8/2023, Published 15/9/2024

Abstract

Reactive oxygen species (ROS) are produced as a result of biochemical processes that are not in balance with the body's antioxidant defense mechanisms. This metabolic dysfunction is referred to the oxidative stress (OS). Metabolic dysfunction-associated diseases are affected by changes in the redox balance. It is now widely recognized that oxidative stress significantly affects diabetes mellitus (DM), particularly type 2 diabetes. The biochemical changes associated with DM could disturb the oxidative milieu, leading to several microvascular complications in diabetic patients. Thus, DM is a perfect disease to explore the harmful consequences of oxidative stress and how to treat it. Oxidative stress triggered by hyperglycemia is an important contributor to the effects of diabetic microvascular diseases. Uncontrolled hyperglycemia carried by deficiencies in insulin secretion or action produces a number of problems, such as peripheral vascular disorders, nephropathy, neuropathy, retinopathy, increased morbidity and/or mortality, as well as the incidence of DM are rising globally. The development and progression of diabetic problems are strongly correlated with ROS and oxidative stress, according to a wide body of research. Antioxidant supplementation may be a helpful tactic for controlling diabetic complications and enhancing antioxidant capacity. This review aims to explore various markers of oxidative stress and the role of ROS in the pathogenesis and progression of late diabetic microvascular complications.
Keywords: Diabetes Mellitus, Hyperglycemia, Microvascular Diseases, Oxidative Stress, ROS.

المؤشرات الحيوية للإجهاد التأكسدي في مضاعفات الأوعية الدموية الدقيقة لمرض السكري

سارة هاشم محيبس[١] و شذى حسين علي[٢]

[١]العلوم المختبرية السريرية ،كلية الصيدلة ،جامعة بغداد، بغداد، العراق

الخلاصة

يتم إنتاج أنواع الأكسجين التفاعلية نتيجة للعمليات الكيميائية الحيوية التي لا تكون متوازنة مع آليات دفاع الجسم المضادة للأكسدة. يشار إلى هذا الخلل الأيضي بالإجهاد التأكسدي. تتأثر الأمراض المرتبطة بضعف التمثيل الغذائي بالتغيرات في توازن الأكسدة والاختزال. من المعترف به الآن على نطاق واسع أن الإجهاد التأكسدي يؤثر بشكل كبير على مرض السكري وخاصة مرض السكري من النوع الثاني. يمكن أن تؤدي التغيرات الكيميائية الحيوية المرتبطة بداء السكري إلى اضطراب البيئة المؤكسدة ، مما يؤدي إلى العديد من مضاعفات الأوعية الدموية الدقيقة لدى مرضى السكري. وبالتالي ، فإن داء السكري هو مرض مثالي لاستكشاف العواقب الضارة للإجهاد التأكسدي وكيفية علاجه. الإجهاد التأكسدي الناجم عن ارتفاع السكر في الدم هو مساهم مهم في آثار أمراض الأوعية الدموية الدقيقة لمرض السكري. ينتج عن ارتفاع السكر في الدم غير المسيطر عليه الناتج عن نقص في إفراز الأنسولين أو تأثيره عددًا من المشاكل ، مثل اضطرابات الأوعية الدموية الطرفية ، واعتلال الكلية ، واعتلال الأعصاب ، واعتلال الشبكية ، وزيادة معدلات المراضة و / أو الوفيات ، فضلاً عن ارتفاع معدلات الإصابة بداء السكري على مستوى العالم. يرتبط تطور وتفاقم مشاكل مرض السكري ارتباطًا وثيقًا بأنواع الأكسجين التفاعلية والإجهاد التأكسدي ، وفقًا لمجموعة واسعة من الأبحاث. قد تكون مكملات مضادات الأكسدة وسيلة مفيدة للسيطرة على مضاعفات مرض السكري وتعزيز القدرة المضادة للأكسدة. تهدف هذه المراجعة إلى استكشاف العلامات المختلفة للإجهاد التأكسدي ودور أنواع الأكسجين التفاعلية في التسبب في مضاعفات الأوعية الدموية الدقيقة لمرض السكري وتطورها.
الكلمات المفتاحية : داء السكري ، ارتفاع السكر في الدم ، أمراض الأوعية الدموية الدقيقة ، الإجهاد التأكسدي ، انواع الأكسجين التفاعلية

Introduction

High levels of blood glucose are a hallmark of diabetic mellitus (DM), which is metabolic illness. An abnormality in insulin action, secretion, or both could possibly be the origin of this Hyper-glycemia. [(1)]. Diabetes mellitus morbidity and mortality are greatly enhanced by the vascular complications that arise from the disease. Because of this, identifying diabetes complications is extremely important.

While acute problems such hypoglycemia, hyperglycemia with ketoacidosis, or hyperosmolar hyperglycemia can occur unexpectedly and pose significant risks to life, chronic consequences are correlated with the length of DM and glycemic management. Chronic complications are divided into microvascular complications, which affect small blood vessels involving retinopathy, nephropathy, and neuropathy, and macrovascular complications, which affect arteries and encompass ischemic heart disease, peripheral vascular disease, as well as stroke [2].

Both microvascular and macrovascular complications are frequently associated with Type 2 DM [3]. Vascular problems can be identified in the early stages of Type 2 DM or even in the prediabetes stage, which makes it difficult to diagnose because the illness is frequently moderate or asymptomatic [3]. Finding biomarkers that can forecast the beginning, progression, and occurrence of Type 2 Diabetes Mellitus and its accompanying comorbidities can be useful for early recognition and intervention, which can halt the disease's course [4]. The onset of diabetes and its complications have frequently been linked to oxidative stress, which is characterized by an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense systems [5].

Hyperglycemia induces the overproduction of ROS through different metabolic abnormalities. Excessive and sustained ROS production can jeopardize the integrity and physiological functions of macromolecules, which contribute to the pathogenesis of secondary complications in diabetes mellitus [6].

Advanced glycation products (AGEs), oxidative stress, the release of pro-inflammatory cytokines, and cellular death are all consequences of the disruption of signaling pathways caused by hyperglycemia in different organs. It is becoming more and clearer that the main processes underlying the cellular damage associated with diabetic complications in both Type 1 and Type 2 diabetes mellitus are oxidative stress and inflammation [7]. This review focuses on exploring the oxidative stress biomarkers in diabetes mellitus and evaluates the essential role of ROS participation in the development and genesis of advanced microvascular problems.

Diabetic Complications

Several organs, including the heart, kidneys, eyes, nerves, and blood vessels, might suffer damage and reduced performance as a result of persistent hyperglycemia [8]. Microvascular effects (related to small blood vessels) and macrovascular effects (related to bigger blood vessels like arteries and veins) are two ways that diabetes can have negative impacts on the body. Diabetic nephropathy, retinopathy, and neuropathy are examples of microvascular problems, whereas coronary artery disease, peripheral arterial disease, and stroke (heart attack) are examples of macrovascular complications [9].

A reduction in glomerular filtration rate, proximal tubular epithelial cells hypertrophy, and albuminuria are all signs of diabetic nephropathy. This syndrome may be accompanied by mesangial enlargement and an accumulating amount of extracellular matrix proteins (ECM), which can progress to end-stage renal failure [9]. Changes in glomerular filtration lead to abnormal albuminuria, which progresses through various stages, including microalbuminuria and macroalbuminuria. This can ultimately result in end-stage renal disease [10]. In addition, diabetic retinopathy is a leading cause of visual impairment worldwide. It arises due to injury to the light-sensitive tissue and small blood vessels in the retina at the back of the eye [11]. The ongoing degradation of neuronal activity, deterioration of the sensory nerves, and injury to the motor nerves are characteristics of diabetic neuropathy. Endothelial cell destruction, reduced blood supply to the nerves, and neuronal injury all contribute to this and decrease impulse conduction [12].A lack of perspiration, numbness, a warm feeling, and slowed movements are among the common symptoms of diabetic neuropathy [12].

Vascular problems in diabetes can be avoided by maintaining intense treatment at relatively normal glycemic control levels. Several large-scale randomized controlled studies have looked at whether intensive glycemic control minimizes the risk of long-term microvascular complications, including the Diabetes Control and Complications Trial (DCCT), United Kingdom Prospective Diabetes Study (UKPDS), Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE), and Action to Control Cardiovascular Risk in Diabetes (ACCORD) [13-16].

One commonly used marker to assess glycemic management is HbA1c, which is produced through the non-enzymatic glycation of hemoglobin. Maintaining an almost normal level of glycemic monitoring with glycated A1c (HbA1c) levels below 6.5-7.0% will help prevent microvascular complications in diabetes mellitus [13–16]. Hyperglycemic metabolic memory's long-term effects may be lessened with early DM therapy. In order to prevent the microvascular manifestations of DM, it is emphasized that achieving strict glucose management in the early phases of DM is a successful preventative strategy [9].

Furthermore, there is an agreement on the importance of maintaining optimal glycemic control (with HbA1c levels below 6.5%) and suggestions to personalize HbA1c goals based on individual factors [17]. Higher levels of HbA1c have been linked to diabetic complications, making it a well-established standard for assessing glycemic control [18]. Since

mean HbA1c does not give a thorough picture of glycemic fluctuation, long-term glycemic exposure, and the underlying causes of diabetes' adverse consequences and indicators of those problems that take the duration of hyperglycemia into account are being studied [19]. Therefore, due to the drawbacks of using HbA1c, there is a shift towards exploring alternative approaches for forecasting diabetic complications [20].

Oxidative Stress's Sources

During typical physiological processes, reactive oxygen species (ROS) are created and removed in a balanced way (Figure 1). To keep the balance between ROS production and elimination, which prevents oxidative damage, a cell's intracellular compartments must maintain a reducing environment with high amounts of glutathione (GSH). This redox balance is crucial for downstream signaling processes that require an oxidative state to activate secondary messengers. The electron transport chain (ETC) in mitochondria, which takes part in the process of oxidative phosphorylation, is one of the main sources of superoxide, which can be converted to hydrogen peroxide by the catalytic activity of superoxide dismutase (SOD). Mitochondria are a vital component of a cell since they are the main ATP-producing energy producers. The ability of mitochondria to produce energy can gradually decline as a result of age-related somatic mutations in mitochondrial DNA (mtDNA) [21]. It is believed that mtDNA mutations occur 10-100 times more frequently than nuclear genome mutations. Numerous factors contribute to lower replication fidelity, such as the oxidative environment in mitochondria, the lack of protective histones for mtDNA, and the restricted capacity of DNA polymerase for proofreading [22,23].

For physiological activities, low ROS levels produced by oxygen metabolism are beneficial. The ETC generally maintains a voltage gradient across the inner mitochondrial membrane in order to activate ATP-synthase and promote ATP production. Oxygen is crucial because it serves as the process' last electron acceptor and forms water when combined with two protons. The partial reduction of oxygen results in the formation of superoxide (O2), which can then combine with protons to produce further reactive species such as hydrogen peroxide (H2O2) and the hydroxyl radical (OH). A variety of protein complexes (complexes I–IV) that are involved in the oxidative phosphorylation pathway are found in the mitochondrial membrane [24].

While hydrogen ions are driven across the mitochondrial membrane, electron donors including nicotinamide adenine dinucleotide (NADH2) and flavin adenine dinucleotide (FADH2) transfer electrons between these protein complexes [24]. When oxygen and electrons from complexes I and III mix, hydrogen peroxide, hydroxyl radicals, and other reactive oxygen and nitrogen species are created [25]. Free radicals are also produced by the cytochrome P450 system and nitric oxide synthase activities, among other things [26]. When a cell creates an excessive amount of ROS, various biological components, including DNA, proteins, lipids, and metabolites, can oxidize. Protein clumping, altered gene and protein expression patterns, and cellular dysfunction may all be consequences of this oxidative stress. Endogenous ROS can also promote the lipid hydroperoxide synthesis that is necessary for ROS-mediated activities. Free radicals and non-radical oxidants are neutralized by the majority of cells' antioxidant defense system, which combines enzymatic and non-enzymatic elements to avert negative effects [27].

Metal-binding proteins (such as albumin, ferritin, and lactoferrin), retinol (Vitamin A), ascorbic acid (Vitamin C), tocopherol (Vitamin E), coenzyme Q10, and polyamines (such as spermine, spermidine, and putrescine) are a few intracellular non-enzymatic defenses against excessive ROS. The enzymes that respond to ROS in protective mechanisms include superoxide dismutase (SOD), catalase, peroxiredoxins, glutathione peroxidase, and glutathione reductase, to name just a few. The three main SOD isoforms are: cytosolic (SOD-1 or CuZn-SOD), mitochondrial (SOD-2 or Mn-SOD), and extracellular (SOD-3 or EC-CuZn-SOD), each of which is characterized by its cellular location. Despite the retina's high CuZn-SOD activity, the EC-SOD and Mn-SOD activities were modest [28]. The nuclear factor erythroid-related factor-2 (Nrf2) is an essential component of the cellular antioxidant response [29]. When a change in redox equilibrium activates the transcription factor nuclear factor erythroid-related factor-2 (Nrf2), it binds to enhancer areas located in the promoters of antioxidant genes known as antioxidant response elements. Fighting intracellular ROS is aided by the antioxidant proteins that produce it and the transcription that they support [30].

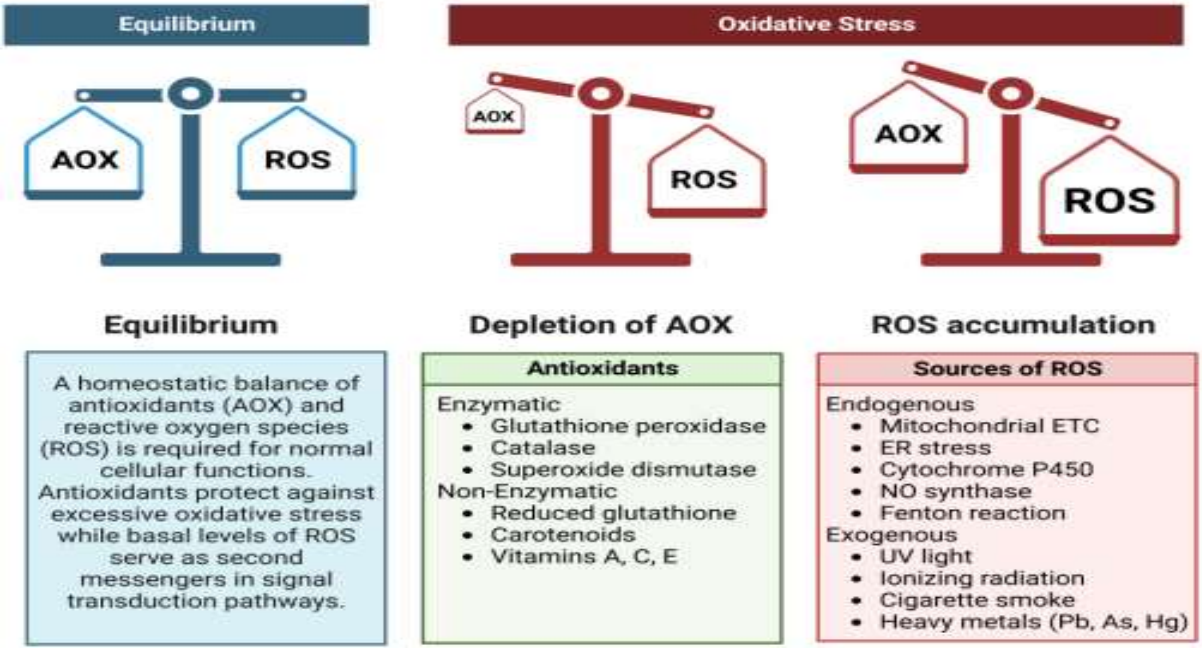

Figure 1. Redox homeostasis [31] Reactive oxygen species {ROS} , Antioxidants {AOX} ,Lead {Pb}, Arsenic {As}, and Mercury {Hg}.

Role of Oxidative Stress in Diabetes

Free radicals are highly reactive chemical entities with a brief half-life and one or more unpaired electrons. They can damage cells by oxidizing molecules and cellular parts [32]. Free radicals are an essential component in the growth and development of life, while often being extremely reactive and unstable. They may be created by ionizing radiation, non-enzymatic oxidation processes of organic molecules, or both external and endogenous sources from the environment and the cell. Free radicals can also be produced via the mitochondrial process of oxidative phosphorylation. Cellular damage may eventually arise from the buildup of free radicals and the oxidative stress that follows [33].

Free radical generation might be triggered by high blood glucose levels. A damaged immune system makes it difficult for the body to properly combat the production of ROS, which causes an imbalance between ROS and cellular defenses and oxidative stress. ROS and antioxidant ratio disturbances can have negative consequences for health. During the respiratory burst process, ROS may also be produced by macrophages and neutrophils, which helps neutralize antigens [34]. The Extracellular-Signal-Regulated Kinase (ERK) and Mitogen-Activated Protein Kinase (MAPK) pathways, which are activated by free radicals, can alter gene expression and result in cell death that is mediated by Superoxide Dismutase (SOD). These radicals' decreased capacity to activate insulin-dependent insulin signaling elements and glucose transport function leads to insulin resistance [35].

The formation of ROS is influenced by oxidized glucose, increased lipid peroxidation, and non-enzymatic protein glycation in diabetes. As a result, the cellular machinery is harmed, oxidative stress is increased, and enzymes are harmed, all of which result in insulin resistance [36]. There is emerging evidence that oxidative stress plays a significant role in the systemic inflammation that underlies the pathogenesis of many macro and microvascular problems. Cells with diabetes mellitus have different lipid compositions, which increases their susceptibility to lipid peroxidation [37].

Additionally, the production of free radicals can activate signaling pathways that boost the expression of antioxidant enzymes [33]. Only a few of the mechanisms by which hyperglycemia harms tissue include the development of Advanced Glycation End Products (AGEs), overexpression of the AGEs receptor and its activating ligands, activation of Protein Kinase-C isoforms, and increased activity of the hexosamine pathway [38]. Free radicals are important in the emergence and development of diabetes problems because of their capacity to harm lipids, proteins, and DNA. Changes in oxidative marker levels increase the susceptibility of tissues to oxidative stress, which leads to the development of diabetes complications. Among the oxidative stress-related consequences of diabetes mellitus are neuropathy, nephropathy, retinopathy,

coronary artery disease, and stroke. In addition, vascular disease, not hyperglycemia, is the main factor contributing to diabetic death [38]. The effects of ROS exposure can change both biomolecules and the molecules of the antioxidant system. These altered substances are also known as "oxidative stress biomarkers." [39]. Numerous investigations have demonstrated that oxidative stress markers are particularly concentrated in high levels in persons with diabetes mellitus [40, 41].

ROS is among several indicators that can be detected, including oxidative stress. Nevertheless, due to the high reactivity and short-lived nature of ROS, it is more advantageous to assess the oxidation products resulting from ROS activity in order to evaluate the extent of oxidative stress. Damage to oxidative nucleic acids, oxidized proteins, and lipid peroxidation may result from these oxidation target products [42]. Following are examples of oxidative stress biomarkers in diabetes.

Lipid peroxidation and their products

Diabetes mellitus frequently results in the disturbance of the lipid profile, which raises the levels of lipid peroxidation. Highly reactive aldehydes produced by this mechanism include malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). In plasma, serum, and other diabetic tissues, MDA, a biomarker of oxidative stress brought on by lipid peroxidation, is routinely tested [43]. Because oxidative damage plays a crucial role in the development of diabetes complications, diabetic individuals have elevated MDA levels, especially in cases of atherosclerosis and brain diseases [44]. In addition, hydroperoxides, conjugated dienes, dicarboxylic acids, and F2-isoprostanes are examples of lipid peroxidation byproducts [45] .

Protein oxidation and their products

The proteins, which are crucial cellular macromolecules, are engaged in a variety of physiological processes, such as cellular transport and signaling. Proteins can undergo ROS-mediated alterations as well, which can have a detrimental effect on both their structure and function. Just a few examples of side chains that can be targeted for protein oxidation include cysteine, methionine, and tyrosine. Carbonyls, which are results of protein oxidation, are reliable signs of oxidative stress [46]. Stable end products are developed after transient radical species are produced as a result of oxidant stimuli such chloramines and nitrogen/carbon radicals. Glycation has been shown to trigger the formation of protein carbonyls such ketoamine derivatives, which generate reactive radicals and sustain a destructive cycle [47]. The accumulation of protein oxidation products caused by protein damage can have negative effects on cellular function. One of the key factors contributing to the accumulation of damaged proteins that are unable to function is the increased glycol- and lipooxidation. [48]. The importance of protein oxidative and glycoxidative damage in individuals with prediabetes and type 2 diabetes was the focus of Gradinaru et al.'s investigation. In order to ascertain how protein status in diabetic patients related to diabetes, it was shown that AGEs, low-density lipoprotein oxidation susceptibility (oxLDL), and products of the nitric oxide metabolic pathway (NOx) were crucial indications [49]. The non-enzymatic interactions between proteins, amino phospholipids, or nucleic acids and reducing sugars or oxidized lipids are known as amino-carbonyl reactions, and they result in the production of AGE [50]. The production of AGEs may have an intracellular impact on a protein's capacity to control gene expression [51]. The development of AGE, oxidative stress, and hyperglycemia have all been linked in several investigations on both humans and animals [51–53]. Advanced oxidation protein products (AOPPs) are regarded as a useful indicator of protein oxidation during oxidative stress because they reflect the overall state of proteins in the cell or tissue [54, 55].

Protein oxidation can have a major impact on a number of physiological processes in diabetic patients. The higher amounts of protein carbonyls and AOPPs in these people demonstrate the relevance of protein conformational changes in the genesis of diabetic nephropathy [56]. Patients with age-related diabetes have a propensity to accumulate AOPPs, also known as pro-inflammatory and pro-oxidative compounds, which may be significantly contributing to the increased incidence of endothelial dysfunction and the ensuing cardiovascular diseases. Dityrosines, which are abundant in AOPPs and enable disulfide bridges, carbonyl groups, and crosslinking. The primary catalyst for the production of hypochlorous acid and chloramines is myeloperoxidase [57]. Numerous studies have demonstrated that adult participants with type 2 diabetes have greater levels of both AOPPs and oxidative stress markers, regardless of whether they have micro- or macrovascular issues [49, 58].

Antioxidants

Antioxidants are essential for maintaining stable free radicals and preventing the onset of oxidative stress, which strengthens our defense system. They can be taken from a variety of dietary sources. Reactive oxygen species removal techniques both enzymatic and non-enzymatic exist in nature. Ascorbic acid, retinol, glutathione, carotenoids, tocopherols, trace minerals like

selenium, copper, zinc, and coenzyme Q10, uric acid, folic acid, riboflavin, and thiamine are all components of the non-enzymatic antioxidant system. While serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the primary antioxidant enzymes [5]. Hydrogen peroxide is transformed into water and oxygen by the enzymes GPx (located in the mitochondria) and lysosomes. GSH reductase, GSH S-transferase, peroxiredoxin, thioredoxin, and thioredoxin reductase are other antioxidant enzymes [6].

Antioxidants are present in human plasma, which can be utilized as a marker to assess oxidative stress. Plasma contains a variety of substances that shield cells and cellular biomolecules from oxidative damage. The total activity of all plasma molecules serves as a proxy for the plasma's antioxidant capacity. A rise in oxidative stress and a decline in antioxidant capacity are two effects of diabetes mellitus that are linked to insulin resistance and DNA damage. The weak antioxidant defense in plasma can lead to a number of problems, including blindness, nerve damage, cardiovascular disease, and nephropathy [59].

Mechanisms Involved in The Pathogenesis of Oxidative Stress in Diabetes Mellitus

All cells in any tissue are affected by diabetes-induced hyperglycemia, but some cell types are more vulnerable to being endangered because they are unable to regulate the amount of glucose taken up by the cell. These delicate cells include the mesangial cells in the kidney glomerulus, the neuronal and Schwann cells in peripheral nerves, and the retinal capillary endothelial cells. Due to their inability to maintain a stable glucose concentration in the cell under hyperglycemic conditions, these cells are consequently disproportionately affected by problems resulting from diabetes-induced tissue damage [60].

1. Polyol pathway flux

The enzyme aldose reductase's typical function is to transform harmful aldehydes into inert alcohol. The catalyst induces a reaction that changes glucose into sorbitol instead of consuming the NADPH cofactor when there is an excessive amount of glucose present in the cell. Sorbitol is consequently changed into fructose [61]. The formation of glutathione, an essential antioxidant for the cell, is decreased as NADPH levels drop. As a result, the cell's internal milieu becomes more conducive to oxidative agents [62].

2. Intracellular production of AGE precursors

These products are connected through three different mechanisms: The most important mechanism is that they alter intracellular proteins first [63], particularly those that control gene transcription. Second, AGE precursors have the ability to disrupt cellular function via disrupting extracellular matrix and intercellular signaling. Thirdly, these AGE precursors may interact with circulating proteins including albumin and AGE receptors to produce inflammatory cytokines and growth factors that damage vascular tissue [64].

3. Protein Kinase-C (PKC) activation

Increased synthesis of diacylglycerol results from PKC isoform activation spurred on by intracellular hyperglycemia. As a result of this molecule's impact on gene expression, less of the essential products for healthy cellular function and more detrimental ones are produced. Reduced generation of endothelial nitric oxide and increased production of the vasoconstrictor endothelin-1 are two examples of this [65].

4. Increased Hexosamine Pathway activity

A considerable amount of glucose enters the glycolysis process when the cell's glucose levels rise. A portion of the generated fructose 6-phosphate makes its way into the signaling pathway during this step, where it is eventually converted into glucosamine 6-phosphate by the enzyme glutamine-fructose 6-phosphate amidotransferase. Following its conversion, this glucosamine becomes uridine diphosphate (UDP) N-acetyl glucosamine, which binds to serine and threonine residues found in transcription factors. In the end, this glucosamine might cause pathogenic alterations in gene expression [66,67].

The common pathway, which is indicated by an excess of superoxide in the mitochondrial electron transport chain, is followed by all of the pathogenic mechanisms brought on by hyperglycemia despite the fact that each one has its own distinctive effects. All cell types that are hyperglycemic create more reactive oxygen species (ROS) [68]. High glucose levels cause the synthesis of mitochondrial superoxide and the activation of Poly ADP-ribose Polymerase (PARP). The polymerase subsequently inhibits glyceraldehyde-3 phosphate dehydrogenase (GAPDH), which causes the onset of all four pathogenic processes [69].

Inhibition of GAPDH results in an increase in the glycolytic intermediates that precede GAPDH, such as 3-phosphate glyceraldehyde. This glyceraldehyde 3-phosphate is the main intracellular precursor of AGE, which starts the AGE pathway. Additionally stimulating PKC, glyceraldehyde 3-phosphate also promotes the conventional PKC

pathway. Inhibition of GAPDH results in the activation of the hexose amine pathway, which is initiated by fructose 6-phosphate and leads to the synthesis of UDP-N-acetylglucosamine (UDP-GlcNAc). This pathway ultimately increases glucose levels, which are then lowered by aldose reductase with concurrent NADPH consumption [64]. DNA strand breaks and the production of ROS, which activates PARP, are brought on by elevated glucose levels. The resulting ADP-ribose binds to GAPDH and other nuclear proteins, inhibiting GAPDH [70].

Adipocytes with insulin resistance, on the other hand, contain larger levels of free fatty acids (FFAs). These FFAs build up inside macrovascular endothelial cells, which triggers mitochondrial oxidation and boosts the production of FADH and NADH. As a result, ROS production increases [71].

The Role of Nrf2 in Oxidative Stress-Induced Diabetes

The oxidative stress is significantly controlled by the nuclear factor erythroid-2-related factor -2 (NRF2 or NFE2L2), which also has anti-inflammatory characteristics. More than 50 genes involved in redox homeostasis and almost 200 genes impacting metabolism and repair are regulated by this factor [72]. Cells are protected by Nrf2 and are helped while responding to stress from the environment. Keap1, a protein connected to the cap-n-collar homology factor generated from erythroid that resembles kelch, controls Nrf2 negatively. Numerous research teams have thoroughly investigated the roles of Nrf2 and Keap1, both of which are essential in cellular redox signaling [73]. While Nrf2 is constitutively expressed in the cytoplasm under normal physiological circumstances, Keap1 simultaneously inhibits this expression. However, free radicals and other cellular stressors cause Nrf2 to translocate into the nucleus, increasing the production of antioxidant proteins. Additionally, Nrf2 has been linked to a number of chronic conditions like diabetic, vascular, metabolic, and neurological disorders that are brought on by oxidative stress and inflammation. As a result, scientists are interested in finding out how Nrf2 and Nrf2 activators might be used to treat and prevent diabetes and its consequences. Due to the Nrf2-Keap1 system's essential function in cellular protection by releasing antioxidant and drug-metabolizing enzyme genes, it has received much research into its crucial role in diabetes. In a diabetic mouse model, Yagishita etal. showed that Nrf2 protects pancreatic -cells from oxidative and nitrosative damage [74]. Under ROS/RNS-induced stress conditions in transgenic animal models, induced NO synthase (iNOS) is overexpressed in pancreatic beta-cells. However, it has been demonstrated that activating Nrf2 in cell lines greatly reduces ROS/RNS levels and guards against -cell damage. Nrf2 is essential for shielding pancreatic -cells from damage caused by arsenate in addition to suppressing ROS/RNS-mediated deterioration [75]. In cells, activation of Nrf2 has been demonstrated to support glucose homeostasis and improve insulin sensitivity [76]. Researchers examine Nrf2's role in diabetes using both computational and experimental models. According to a large body of research [77–80], pterostilbene has been discovered to activate Nrf2 and reduce oxidative stress and pro-inflammatory cytokine toxicity via regulating the Nrf2 signaling network.

Numerous studies have demonstrated the protective effects of a variety of Nrf2 activators, including resveratrol [81], sulforaphane [82], curcumin [83], quercetin [84], and CDDO (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid) [85]. These substances help to restore the function of the -cells while also protecting them against oxidative stress-induced apoptosis and necrosis. The critical involvement of Nrf2 in activating a series of genes, including heme oxygenase-1 (HO-1), superoxide dismutase (SOD), NAD(P)H quinone oxidoreductase (NQO1), and glutathione S-transferase (GST), which play a significant role in antioxidant activity, detoxification, cellular redox homeostasis, glutathione homeostasis, etc. has been well documented [77,78].

Potential Nrf2 activators have demonstrated promise as a therapeutic target for managing diabetic complications. Numerous studies have shown that the overexpression of Nrf2-dependent phase 2 genes protects cells, animals, and people from numerous hazardous substances such ROS, RNS, carcinogens, electrophiles, and radiation [86].

Chronic Complications of Diabetes

Diabetic nephropathy

Diabetic nephropathy (DN) is a common microvascular diabetic complication. Albuminuria, a persistent reduction in glomerular filtration rate (GFR), and elevated blood pressure are symptoms of this disorder [87]. A significant number of individuals with DM experience diabetic kidney disease (DKD), which is a prevalent consequence of DM. Studies have revealed that 20–40% of DM patients develop DKD, and that the interaction between hyperglycemia and hypertension is responsible for 80% chronic renal failure in those individuals [88]. As a result, DKD is very intimately related to cardiovascular disease (CVD). Mesangial expansion and sclerosis, glomerular endothelial cell dysfunction, glycocalyx degradation, thickening of

the basement membrane, effacement of the podocyte foot process, tubular basement membrane thickening, elevated apoptosis, and interstitial fibrosis are pathological changes that contribute to DKD. These modifications may result in ongoing proteinuria and a gradual GFR decline [89]. DKD involves a variety of components, including hereditary factors, oxidative stress, inflammatory response, cytokines, glycolipid metabolic problem, insulin resistance, and hemodynamic changes. It should be noted that the development of DKD is heavily influenced by oxidative stress, which can be brought on by the activation of the polyol pathway, the buildup of AGEs, and a variety of cytokines. These elements cause lesions in the kidney's tiny blood veins, aggravating kidney damage [90].

Numerous free radicals are formed when the body and the environment are exposed to hazardous stimuli. These species cannot be completely scavenged by the antioxidant defense system, which causes physiological and pathological reactions in cells and tissues [90]. Additionally, a number of variables, including hemodynamic changes and disorders of glycolipid metabolism, activate pathways including the polyol and hexosamine pathways, leading to significant ROS generation and an oxidative stress response in kidney. Inflammation, autophagy, and fibrosis are subsequently brought on by the activation of the downstream cellular signaling pathways, hastening the pathological alterations and functional abnormalities that result in DKD [90].

Numerous tissue cells commonly have dormant NF-jB. In response to activators (such as proinflammatory cytokines, Toll-like receptors, p38-MAPK, HO-1, and ROS), NF-jB dimers are released into the nucleus, which regulate target gene expression and result in inflammatory and immunological responses in the body [91]. The development of DKD is linked to NF-jB hyperactivation. For instance, the NF-jB signaling pathway is activated by high glucose stimulation, which also results in the generation of ROS and inflammatory molecules (such as TNF-a and IL-6). Following this, the activated NF-jB encourages the transcription and translation of TGF-b1, adhesion molecules, proinflammatory cytokines, and chemokines. This causes tissue fibrosis and cell death and necrosis, which expedites the development of DKD [92]. Hofmann et al.'s study demonstrated a positive correlation between proteinuria in the general population and the amount of NF-jB expression, which was shown to be significantly greater in DKD patients compared to non-diabetic people. Therefore, regulating the activation of the NF-jB signaling pathway may be the main focus of research into the prevention and treatment of DKD [93].

Diabetic retinopathy

Over 80% of individuals with type 1 or type 2 diabetes have diabetic retinopathy after 15 to 20 years of living with the disease. The length of diabetes and the intensity of hyperglycemia have major roles in the progression of the illness. An individual has a higher risk of developing retinopathy the longer they have diabetes. The condition is asymptomatic in its early stages and has no impact on vision. However, retinal examination reveals progressively more serious retinal damage as the illness worsens, starting with tiny bulges in the retinal blood vessels known as microaneurysms. The development of lipid deposits, cotton-wool patches, hemorrhages, and intraretinal microvascular abnormalities may then occur. These signs may eventually result in neovascularization, in which weak new blood vessels grow and may rupture, obstructing vision. Blindness may arise from neovascularization's ability to lift and detach the retina [94].

Even though diabetic retinopathy is regarded as a microvascular complication of diabetes, nonvascular cells including retinal neurons and glial cells are also impacted. These cells are susceptible to quick apoptosis because of their abnormal metabolism [95].

Numerous metabolic pathways and molecular alterations brought on by hyperglycemia are blamed for the onset of diabetic retinopathy. The production of AGEs, activation of the polyol pathway, activation of PKC, and the hexosamine pathway are the primary mechanisms included in the multifactorial pathophysiology of this illness. These pathways both create and are activated by ROS, which damages the mitochondria and increases capillary cell death [96]. Diabetic retinopathy begins as a result. Additionally, because oxidative stress increases inflammatory cytokines, there is a direct connection between oxidative stress and inflammation., which in turn raise ROS. As a result, an unhealthy loop of oxidative stress develops, speeding up the onset and progression of diabetic retinopathy [97].

Intracellular antioxidants serve to maintain a balance between ROS and antioxidants in the body, which creates ROS naturally during normal metabolic activities [98]. Free radicals can accumulate when there is an imbalance between the two, which can happen when there are metabolic disturbances. This might be brought on by an increase in the formation of free radicals, a decrease in antioxidants, or even both. When free radical levels are out of control, they can damage proteins,

lipids, and nucleic acids, which can cause cellular dysfunction, altered cell signaling, genetic alterations, higher utilization of energy, and inflammation. Free radicals are necessary for crucial redox signaling [(99)]. The retina is particularly vulnerable to diabetes because it produces more free radicals and has a weaker antioxidant defense mechanism, making it more difficult for it to neutralize them [(100)]. Diabetes results in retinal damage from both cytosolic and mitochondrial ROS, and NADPH oxidases (Noxs) catalyze the generation of superoxide anion by oxidizing cytosolic NADPH to NADP [(101)].

The excess reactive oxygen species (ROS) produced by diabetes-related imbalances between free radicals and antioxidants can activate the matrix metalloproteinases MMP-2 and MMP-9, which can damage the mitochondrial membrane. This damage can cause the apoptotic process by allowing cytochrome c to leak into the cytoplasm [(102)]. In the etiology of diabetic retinopathy, cytosolic ROS generation is followed by mitochondrial damage and apoptosis activation. As a result of increased glucose-derived pyruvate oxidation, diabetes increases electron donors entering the electron transport chain, which in turn increases superoxide production [(103)]. Damage to the mitochondria compromises the mechanism of the electron transport chain, and mitochondrial DNA is also affected. MtDNA biogenesis tries to repair mitochondrial damage in the early stages of retinopathy, but as the condition worsens, it is finally overpowered and the injured mitochondria continue to self-replicate the ROS cycle [(104)]. Diabetes negatively affects the retina's efficient enzymatic and non-enzymatic defense systems [(105)]. The antioxidant GSH is decreased, the superoxide scavenger MnSOD is inhibited, and the master transcription factor Nrf2, which regulates the ARE-mediated transcription of antioxidant enzymes, is less active. Therefore, oxidative stress plays a significant role in the progression of diabetic retinopathy [(106)].

Diabetic neuropathy

Diabetic neuropathy is a condition that affects sensory neurons and can result in neurological pain accompanied by central or peripheral symptoms [(107)]. It's important to note that, despite any related neurological abnormalities, about one-third of diabetes patients report experiencing pain. Diabetes severity and levels of HbA1c are key indicators of diabetic neuropathy [(108)].

Vascular endothelial cells, glial cells, and nerve cells in the central as well as peripheral nervous systems are among the cells affected by diabetes because they have a restricted capacity to regulate their glucose consumption. It is unknown if high hyperglycemia triggers axonal degeneration by activating intrinsic programs within axons, or if peripheral axons or the glial cells that accompany them are the primary targets of axonal degeneration. Clinical studies, however, suggest that patients with diabetic neuropathy might specifically target glial cells [(109,110)].

Hyperglycemia affects vascular permeability and blood circulation in the initial phases of diabetes., while defective glucose metabolism lowers intracellular NADPH levels and diminishes the production of myo-inositol, which is necessary for neurons to function normally [(111)]. Therefore, hyperglycemia may directly damage cells and indirectly impair cellular functioning, both of which may contribute to diabetic neuropathy. Peripheral nerve degeneration results from microvascular cell loss occurring concurrently with decreased endothelial and neuronal cell formation [(112)].

In diabetic mice with diabetic neuropathy, free radical accumulation and decreased antioxidant enzyme activity were seen, supporting the opinion that oxidative stress contributes to diabetic neuropathy progression. Antioxidant therapy has been shown to alleviate diabetic neuropathy symptoms in diabetic mice [(113)]. Impairment of mitochondrial glucose oxidation is regarded to be one of the primary causes of ROS production in diabetes. Axons filled with mitochondria have immediate links to the blood supply of the nerve. Due in part to their reliance on local mitochondria for energy, axons are particularly susceptible to ROS-mediated damage in hyperglycemia. This leads to axonal degeneration as a result of the neuron's lacking the capacity to eliminate excessive ROS and inadequate ATP synthesis [(114)].

Targeting Oxidative Stress as A Treatment for Diabetic Mellitus

Numerous research has been conducted to explore the antioxidant effects of various substances, including naturally occurring antioxidants of plant origin, owing to the significance of OS in a range of disorders associated with DM [(115)]. Numerous antioxidants, encompassing glutathione, CoQ10, and α-lipoic acid, have been shown to help restore insulin sensitivity [(116)]. Additionally, it has been discovered that a number of antioxidants, including lutein, zeaxanthin, lycopene, - and -tocopherol, retinol, -cryptoxanthin, ascorbic acid, - and -carotene, might dramatically lessen DM problems [(117-120)]. Due to their potent anti-radical and anti-inflammatory properties as well as their capacity to control the activities of -glucosidase and lipase, lower blood

sugar levels, enhance pancreatic function, and interact synergistically with hypoglycemic medications, phytochemical components of food and medicinal plants are highly effective in treating DM [121]. Anthocyanins and polyphenols are antioxidants perform their antioxidant action by inhibiting the production of prostaglandins, inflammatory cytokines, and transcription factors like NF-B. Because of its anti-inflammatory and antioxidant effects, curcumin is regarded to be suitable for both avoiding and reducing the risk of DM complications [122].

Diabetes has been demonstrated to benefit from several specific antioxidants. For instance, the antioxidant polyphenol butein, which has been demonstrated to protect pancreatic -cells from excessive inflammation and limit the generation of NO in vitro, may be helpful in halting the course of DM1 [123]. Resveratrol affects the expression of genes related to the development of DM2 by enhancing the activity of many -cell genes and insulin in pancreatic -cells [124] . Experimental research has been done on the effects of different antioxidant-active ingredients on the development of DM. For instance, it has been demonstrated that adding coenzyme Q10 and L-arginine together can suppress OS, significantly increase NO content, and increase the availability of NO because pro-atherogenic cholesterol components are less concentrated, which in turn promotes the production of eNOS [125].

Clinical studies have also demonstrated the efficacy of coenzyme Q10 [126], and the synthetic biguanide derivative N-imino(1-piperidinyl) methyl guanidine has been demonstrated to have favorable effects on free radical levels homeostasis, aconitate hydratase activity, and citrate content in the liver and blood serum of DM2 rats [127]. Due to their antioxidant qualities, phytochemicals control the activity of -glucosidase and lipase, lower blood sugar levels, enhance pancreatic function, and work in conjunction with hypoglycemic medications to treat diabetes [125].

Overall, the antioxidant characteristics of vegetables, fruits, and seeds make them useful in reducing diabetes problems. Antioxidants can be used as a part of the treatment process, but there isn't enough data to say that using them alone can have a fully therapeutic effect [128].

Conclusions

Oxidative stress is a possible target for pharmacotherapy since it plays a role in the onset, progression, and complications of T2DM. Oxidative stress markers may be helpful as early warning signs of problems from diabetes. T2DM is intimately linked to metabolic conditions where there is an excess of mitochondrial superoxide, which causes tissue damage. Oxidative stress plays a role in insulin secretion malfunction and resistance, which ultimately results in diabetic complications. Enzymatic antioxidants like SOD, CAT, GR, and GPX and non-enzymatic antioxidants like vitamins E and C, GSH, and GSSH are important actors in scavenging ROS. A possible therapeutic target, the Keap1-Nrf2-ARE pathway is a crucial defensive mechanism against oxidative stress and xenobiotic damage. DM and other conditions linked to oxidative stress may be prevented and treated with the aid of small medicines that target the Nrf2/Keap1 pathways. To be certain that Nrf2-associated pathways play a role in reducing diabetic complications, more research is required.

A promising strategy to stop oxidative damage brought on by hyperglycemia is activating Nrf2 with natural substances. Additionally, it's conceivable that foods that interact with Nrf2 could balance insulin resistance. Antioxidant supplementation may be a helpful tactic for controlling diabetic complications and enhancing antioxidant capacity. Despite the potential advantages of antioxidant medication, more thorough, randomized studies are required to examine the effectiveness and safety of this therapeutic strategy.

Acknowledgment

The authors wish to express their gratitude to the Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad for their valuable contributions to this review article

Conflicts of Interest

No conflict of interest.

Funding

There is no funding for this article

Ethics Statements

This review article does not involve any original research with human subjects or animals. As such, no ethical statements or approvals are required.

Author Contribution

All authors have substantially contributed to the conception, design, and execution of this review article.

References

1. Fakree NK, Ali SH. Effect of COX-2 Inhibitors Selectivity on Lipid Profile in Hyperlipidemic and Normolipidemic Type 2 Diabetics. Iraqi J. Pharm. Sci. 2009; 18:7-13.

2. Brar, P.C.; Tell, S.; Mehta, S.; Franklin, B. Hyperosmolar diabetic ketoacidosis– review of

literature and the shifting paradigm in evaluation and management. Diabetes Metab. Syndr. 2021, 15, 102313.

3. Aikaeli F, Njim T, Gissing S, Moyo F, Alam U, Mfinanga SG, Okebe J, Ramaiya K, Webb EL, Jaffar S, Garrib A. Prevalence of microvascular and macrovascular complications of diabetes in newly diagnosed type 2 diabetes in low-and-middle-income countries: A systematic review and meta-analysis. PLOS global public health. 2022 Jun 15;2(6): e0000599.

4. Ortiz-Martínez M, González-González M, Martagón AJ, Hlavinka V, Willson RC, Rito-Palomares M. Recent developments in biomarkers for diagnosis and screening of type 2 diabetes mellitus. Current Diabetes Reports. 2022 Mar;22(3):95-115.

5. Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, Kumar S, Bhatti GK, Reddy PH. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Radical Biology and Medicine. 2022 May 1;184:114-34.

6. Tan Y, Cheong MS, Cheang WS. Roles of Reactive Oxygen Species in Vascular Complications of Diabetes: Therapeutic Properties of Medicinal Plants and Food. *Oxygen*. 2022; 2(3):246-268.

7. Volpe CM, Villar-Delfino PH, Dos Anjos PM, NogueiraMachado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death Dis 2018; 9:119.

8. Jabbar TL, Kasim AA. Association of Retinol Binding Protein-4 (RBP4) with Glycemia, Dyslipidemia, Hypertension, and Obesity in Type 2 Diabetic Iraqi Patients. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2020 Dec 30;29(2):263-70.

9. Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. Nature reviews nephrology. 2020 Jul;16(7):377-90.

10. Samsu N. Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. BioMed research international. 2021 Jul 8; Volume 2021, Article ID 1497449, 17 pages.

11. Ansari P, Tabasumma N, Snigdha NN, Siam NH, Panduru RV, Azam S, Hannan JM, Abdel-Wahab YH. Diabetic retinopathy: an overview on mechanisms, pathophysiology and pharmacotherapy. Diabetology. 2022 Feb 15;3(1):159-75.

12. Luna R, Manjunatha RT, Bollu B, Jhaveri S, Avanthika C, Reddy N, Saha T, Gandhi F. A comprehensive review of neuronal changes in diabetics. Cureus. 2021 Oct 30;13(10).

13. Diabetes Control and Complications Trial (DCCT). Update. DCCT Research Group. Diabetes Care .1990;13:427–433.

14. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352: 837–853.

15. Study rationale and design of ADVANCE: Action in diabetes and vascular disease–preterax and diamicron MR controlled evaluation. Diabetologia. 2001; 44:1118–1120.

16. Buse, J.B.; Bigger, J.T.; Byington, R.P.; Cooper, L.S.; Cushman, W.C.; Friedewald, W.T.; Genuth, S.; Gerstein, H.C.; Ginsberg, H.N.; Goff, D.C., Jr.; et al. Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial: Design and methods. Am. J. Cardiol. 2007; 99: 21i–33i.

17. Hur, K.Y.; Moon, M.K.; Park, J.S.; Kim, S.K.; Lee, S.H.; Yun, J.S.; Baek, J.H.; Noh, J.; Lee, B.W.; Oh, T.J.; et al. 2021 Clinical Practice Guidelines for Diabetes Mellitus of the Korean Diabetes Association. Diabetes Metab. J. 2021; 45: 461–481.

18. Hoffmann, A.P.; Honigberg, M.C. Glycated Hemoglobin as an Integrator of Cardiovascular Risk in Individuals Without Diabetes: Lessons from Recent Epidemiologic Studies. Curr. Atheroscler. Rep. 2022; 24:435–442.

19. Rigon FA, Ronsoni MF, Vianna AG, Schiavon LD, Hohl A, Sande-Lee SV. Flash glucose monitoring system in special situations. Archives of Endocrinology and Metabolism. 2022 Jun 10; 66:883-94.

20. Yoo, J.H.; Kim, J.H. Time in Range from Continuous Glucose Monitoring: A Novel Metric for Glycemic Control. Diabetes Metab. J. 2020;44: 828–839.

21. Villalpando-Rodriguez GE, Gibson SB. Reactive Oxygen Species (ROS) Regulates Different Types of Cell Death by Acting as a Rheostat. Oxid Med Cell Longev. 2021; 2021:9912436.

22. Cortopassi, G.A.; Shibata, D.; Soong, N.W.; Arnheim, N. A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues. Proc. Natl. Acad. Sci. USA 1992; 89:7370–7374.

23. Song, S.; Pursell, Z.F.; Copeland, W.C.; Longley, M.J.; Kunkel, T.A.; Mathews, C.K. DNA precursor asymmetries in mammalian

tissue mitochondria and possible contribution to mutagenesis through reduced replication fidelity. Proc. Natl. Acad. Sci. USA 2005; 102: 4990–4995.

24. Hernansanz-Agustín P, Enríquez JA. Generation of reactive oxygen species by mitochondria. Antioxidants. 2021 Mar 9;10(3):415.

25. Kampjut, D.; Sazanov, L.A. Structure and mechanism of mitochondrial proton-translocating transhydrogenase. Nature 2019, 573, 291–295.

26. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity. 2017 Oct;2017.

27. Birben, E.; Sahiner, U.M.; Sackesen, C.; Erzurum, S.; Kalayci, O. Oxidative stress and antioxidant defense. World Allergy Organ. J. 2012; 5:9–19.

28. Behndig, A.; Svensson, B.; Marklund, S.L.; Karlsson, K. Superoxide dismutase isoenzymes in the human eye. Investig. Ophthalmol. Vis. Sci. 1998; 39:471–475.

29. Deliyanti, D.; Alrashdi, S.F.; Tan, S.M.; Meyer, C.; Ward, K.W.; de Haan, J.B.; Wilkinson-Berka, J.L. Nrf2 Activation Is a Potential Therapeutic Approach to Attenuate Diabetic Retinopathy. Investig. Ophthalmol. Vis. Sci. 2018; 59:815–825.

30. Tonelli, C.; Chio, I.I.C.; Tuveson, D.A. Transcriptional Regulation by Nrf2. Antioxid. Redox Signal. 2017;29:1727–1745.

31. Shu DY, Chaudhary S, Cho KS, Lennikov A, Miller WP, Thorn DC, Yang M, McKay TB. Role of Oxidative Stress in Ocular Diseases: A Balancing Act. Metabolites. 2023 Jan 27;13(2):187.

32. Paz Aliaga A, Cruz Oviedo AF, Paz Matellini CM. Antioxidants: healthier life projection. Physiological Mini Reviews. 2019;12.

33. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008; 4:89-96.

34. Freitas M, Gomes A, Porto G, Fernandes E. Nickel induces oxidative burst, NFκB activation and interleukin-8 production in human neutrophils. JBIC J Biol Inorg Chem. 2010; 15:1275-83.

35. Tauffenberger, A., Magistretti, P.J. Reactive Oxygen Species: Beyond Their Reactive Behavior. *Neurochem Res* .2021;46, 77–87

36. Singh A, Kukreti R, Saso L, Kukreti S. Mechanistic insight into oxidative stress-triggered signaling pathways and type 2 diabetes. Molecules. 2022 Jan 30;27(3):950.

37. Lugrin J, Rosenblatt-Velin N, Parapanov R, Liaudet L. The role of oxidative stress during inflammatory processes. Biol Chem. 2014; 395:203-30.

38. Wu T, Ding L, Andoh V, Zhang J, Chen L. The Mechanism of Hyperglycemia-Induced Renal Cell Injury in Diabetic Nephropathy Disease: An Update. *Life (Basel)*. 2023;13(2):539.

39. Ho E, Galougahi KK, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. Redox Biol 2013; 1(1): 483-491.

40. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging 2018; 13: 757-772.

41. Bigagli E, Lodovici M. Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications. Oxidative medicine and cellular longevity. 2019 May 12;2019.

42. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clinical chemistry. 2006 Apr 1;52(4):601-23

43. Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation. Antioxidants 2019; 8(3): 1-28.

44. Shabalala SC, Johnson R, Basson AK, Ziqubu K, Hlengwa N, Mthembu SXH, Mabhida SE, Mazibuko-Mbeje SE, Hanser S, Cirilli I, et al. Detrimental Effects of Lipid Peroxidation in Type 2 Diabetes: Exploring the Neutralizing Influence of Antioxidants. *Antioxidants*. 2022; 11(10):2071.

45. Spickett CM, Wiswedel I, Siems W, Zarkovic K, Zarkovic N. Advances in methods for the determination of biologically relevant lipid peroxidation products. Free Radic Res 2010; 44(10): 1172-1202.

46. Milkovic L., Cipak Gasparovic A., Cindric M., Mouthuy P.-A., Zarkovic N. Short overview of ROS as cell function regulators and their implications in therapy concepts. *Cells.* 2019;8:793.

47. R. L. Levine, D. Garland, C. N. Oliver et al., "Determination of carbonyl content in oxidatively modified proteins," Methods in Enzymology.1990; 186 : 464–478.

48. A. Sakul, A. Cumaoglu, E. Aydın, N. Ar, N. Dilsiz, and C. Karasu, "Age-and diabetes-induced regulation of oxidative protein

modification in rat brain and peripheral tissues: consequences of treatment with antioxidant pyridoindole," Experimental Gerontology.2013; 48(5): 476–484.

49. D. Gradinaru, C. Borsa, C. Ionescu, and D. Margina, "Advanced oxidative and glycoxidative protein damage markers in the elderly with type 2 diabetes," Journal of Proteomics.2013; 13: 181– 184.

50. Twarda-Clapa A, Olczak A, Białkowska AM, Koziołkiewicz M. Advanced glycation end-products (AGEs): Formation, chemistry, classification, receptors, and diseases related to AGEs. Cells. 2022 Apr 12;11(8):1312.

51. Senavirathna L, Ma C, Chen R, Pan S. Proteomic Investigation of Glyceraldehyde-Derived Intracellular AGEs and Their Potential Influence on Pancreatic Ductal Cells. *Cells*. 2021; 10(5):1005..

52. Lal MA, Brismar H, Eklöf AC, Aperia A. Role of oxidative stress in advanced glycation end product-induced mesangial cell activation. Kidney International. 2002 Jun 1;61(6):2006-14.

53. 53.M. Kalousova, J. ´ Skrha, and T. Zima, "Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus," Physiological Research.2002; 51(6) : 597–604.

54. K. B. Pandey and S. I. Rizvi, "Resveratrol may protect plasma proteins from oxidation under conditions of oxidative stress in vitro," Journal of the Brazilian Chemical Society.2010; 21(5) : 909–913.

55. V. Witko-Sarsat, M. Friedlander, C. Capeillere-Blandin et al."Advanced oxidation protein products as a novel marker of oxidative stress in uremia," Kidney International.1996 ;49(5): 1304–1313.

56. Kehm R, Baldensperger T, Raupbach J, Höhn A. Protein oxidation - Formation mechanisms, detection and relevance as biomarkers in human diseases. *Redox Biol*. 2021;42:101901.

57. A. Piwowar, "Advanced oxidation protein products. Part I. Mechanism of the formation, characteristics and property," Polski Merkuriusz Lekarski.2010; 28(164): 166–169.

58. A. Piwowar, M. Knapik-Kordecka, and M. Warwas, "AOPP and its relations with selected markers of oxidative/antioxidative system in type 2 diabetes mellitus," Diabetes Research and Clinical Practice.2007;77(2): 188–192.

59. Khan AN, Khan RA, Ahmad M, Mushtaq N. Role of antioxidant in oxidative stress and diabetes mellitus. Journal of pharmacognosy and phytochemistry. 2015;3(6):217-20.

60. Chowdhury S, Ghosh S, Das AK, Sil PC. Ferulic acid protects hyperglycemia-induced kidney damage by regulating oxidative insult, inflammation and autophagy. Front Pharmacol 2019; 10(27): 1-24

61. Behl T, Kaur I, Kotwani A. Implication of oxidative stress in progression of diabetic retinopathy. Surv Ophthalmol 2016; 61(2): 187-196.

62. Kilanczyk E, Saraswat Ohri S, Whittemore SR, Hetman M. Antioxidant protection of NADPH-depleted oligodendrocyte precursor cells is dependent on supply of reduced glutathione. ASN Neuro 2016; 8(4): 1-13.

63. de Paula MLA, Rodrigues Villela AM, Negri MM, Kanaan S, de Carvalho Cardoso Weide L. Role of advanced glycation end products related to the onset of diabetic kidney disease complications. Clin Biomed Res 2017; 37(4): 341-348.

64. Ighodaro OM. Molecular pathways associated with oxidative stress in diabetes mellitus. Biomed Pharmacother 2018; 108: 656-662.

65. 65.Yadav SK, Tripathi R, Tripathi K. Toll-like receptor in diabetic vascular endothelium dysfunction. In: Chawla R. (eds.) RSSDI diabetes update. New Delhi: Jaypee Brothers Medical Publishers; 2018, p. 11-15.

66. Lotfy M, Adeghate J, Kalasz H, Singh J, Adeghate E. Chronic complications of diabetes mellitus: A mini review. Curr Diabetes Rev 2017; 13(1): 3-10.

67. Uhde K, van Tol HTA, Stout TAE, Roelen BAJ. Exposure to elevated glucose concentrations alters the metabolomic profile of bovine blastocysts. PLoS One 2018; 13(6): 1-13.

68. Pesta M, Cedikova M, Dvorak P, Dvorakova J, Kulda V, Srbecka K, et al. Trends in gene expression changes during adipogenesis in human adipose derived mesenchymal stem cells under dichlorodiphenyldichloroethylene exposure. Mol Cell Toxicol 2018; 14(4): 369-379.

69. Li WJ, Xu M, Gu M, Zheng D, Xie M, Guo J, et al. Poly (ADP-ribose) polymerase inhibition restores bladder function by suppressing bladder apoptosis in diabetic rats. Int J Clin Exp Pathol 2017; 10(4): 4451-4460.

70. Czapski GA, Cieślik M, Wencel PL, Wójtowicz S, Strosznajder RP, Strosznajder JB. Inhibition of poly (ADP-ribose) polymerase-1 alters expression of mitochondria-related genes in PC12 cells: Relevance to mitochondrial homeostasis in neurodegenerative disorders. Biochim

Biophys Acta (BBA)- Mol Cell Res 2018; 1865(2): 281-288.

71. Tibaut M. Oxidative stress genes, antioxidants and coronary artery disease in type 2 diabetes mellitus. Cardiovasc Hematol Agents Med Chem (Formerly Curr Med Chem Hematol Agents) 2016; 14(1): 23-38.

72. Hayes, J.D.; Dinkova-Kostova, A.T. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem. Sci. 2014; 39: 199–218.

73. Kensler, T.W.; Wakabayashi, N.; Biswal, S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu. Rev. Pharmacol. Toxicol. 2007;47: 89–116.

74. Yagishita, Y.; Fukutomi, T.; Sugawara, A.; Kawamura, H.; Takahashi, T.; Pi, J.; Uruno, A.; Yamamoto, M. Nrf2 protects pancreatic β-cells from oxidative and nitrosative stress in diabetic model mice. Diabetes 2014;63:605–618.

75. Yang, B.; Fu, J.; Zheng, H.; Xue, P.; Yarborough, K.; Woods, C.G.; Hou, Y.; Zhang, Q.; Andersen, M.E.; Pi, J. Deficiency in the nuclear factor E2-related factor 2 renders pancreatic β-cells vulnerable to arsenic-induced cell damage. Toxicol. Appl. Pharmacol. 2012;264:315–323.

76. Bhakkiyalakshmi, E.; Sireesh, D.; Rajaguru, P.; Paulmurugan, R.; Ramkumar, K.M. The emerging role of redox-sensitive Nrf2—Keap1 pathway in diabetes. Pharmacol. Res. 2015; 91:104–114.

77. Sireesh, D.; Ganesh, M.R.; Dhamodharan, U.; Sakthivadivel, M.; Sivasubramanian, S.; Gunasekaran, P.; Ramkumar, K.M. Role of pterostilbene in attenuating immune mediated devastation of pancreatic β cells via Nrf2 signaling cascade. J. Nutr. Biochem. 2017;44:11–21.

78. Bhakkiyalakshmi, E.; Shalini, D.; Sekar, T.V.; Rajaguru, P.; Paulmurugan, R.; Ramkumar, K.M. Therapeutic potential of pterostilbene against pancreatic β-cell apoptosis mediated through Nrf2. Br. J. Pharmacol. 2014;171: 1747–1757.

79. Bhakkiyalakshmi, E.; Dineshkumar, K.; Karthik, S.; Sireesh, D.; Hopper, W.; Paulmurugan, R.; Ramkumar, K.M. Pterostilbenemediated Nrf2 activation: Mechanistic insights on Keap1:Nrf2 interface. Bioorganic Med. Chem. 2016;24:3378–3386.

80. Ramkumar, K.M.; Sekar, T.V.; Foygel, K.; Elango, B.; Paulmurugan, R. Reporter protein complementation imaging assay to screen and study Nrf2 activators in cells and living animals. Anal. Chem. 2013;85:7542–7549.

81. Wang, B.; Sun, J.; Li, X.; Zhou, Q.; Bai, J.; Shi, Y.; Le, G. Resveratrol prevents suppression of regulatory T-cell production, oxidative stress, and inflammation of mice prone or resistant to high-fat diet-induced obesity. Nutr. Res. 2013;33:971–981.

82. Song, M.Y.; Kim, E.K.; Moon, W.S.; Park, J.W.; Kim, H.J.; So, H.S.; Park, R.; Kwon, K.B.; Park, B.H. Sulforaphane protects against cytokine- and streptozotocin-induced β-cell damage by suppressing the NF kappaB pathway. Toxicol. Appl. Pharmacol. 2009;235:57–67.

83. Rashid, K.; Sil, P.C. Curcumin enhances recovery of pancreatic islets from cellular stress induced inflammation and apoptosis in diabetic rats. Toxicol. Appl. Pharmacol. 2015;282:297–310.

84. Coskun, O.; Kanter, M.; Korkmaz, A.; Oter, S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Pharmacol. Res. 2005;51:117–123.

85. Li, W.; Wu, W.; Song, H.; Wang, F.; Li, H.; Chen, L.; Lai, Y.; Janicki, J.S.; Ward, K.W.; Meyer, C.J.; et al. Targeting Nrf2 by dihydro-CDDO-trifluoroethyl amide enhances autophagic clearance and viability of β-cells in a setting of oxidative stress. FEBS Lett. 2014; 588:2115–2124.

86. Li, W.; Kong, A.N. Molecular mechanisms of Nrf2-mediated antioxidant response. Mol. Carcinog. 2009;48:91–104.

87. Salih BH, Ali SH, Allehibi KI. Serum Aldosterone Levels in Patients With Diabetic Nephropathy in Relation to Vascular Calcification. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2019 Jun 11;28(1):53-63.

88. Filippatos G, Anker SD, Agarwal R, et al. Finerenone and cardiovascular outcomes in patients with chronic kidney disease and type 2 diabetes. Circulation. 2021;143(6):540–552.

89. Lassén E, Daehn IS. Molecular Mechanisms in Early Diabetic Kidney Disease: Glomerular Endothelial Cell Dysfunction. Int J Mol Sci. 2020 Dec 11;21(24):9456

90. Kao MP, Ang DS, Pall AA, Struthers AD. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. Journal of human hypertension. 2010 Jan;24(1):1-8.

91. Wen C, Ying Y, Zhao H, et al. Resistance exercise affects catheter-related thrombosis in

rats through miR-92a-3p, oxidative stress and the MAPK/NF-jB pathway. BMC Cardiovasc Disord. 2021;21(1):440.

92. Zoccali C, Mallamaci F. Nonproteinuric progressive diabetic kidney disease. Curr Opin Nephrol Hypertens. 2019;28(3):227–232.

93. Hofmann MA, Schiekofer S, Isermann B, et al. Peripheral blood mononuclear cells isolated from patients with diabetic nephropathy show increased activation of the oxidative-stress sensitive transcription factor NF-kappaB. Diabetologia. 1999;42(2): 222–232.

94. Viswanath, K.; McGavin, D.D. Diabetic retinopathy: Clinical findings and management. Community Eye Health 2003; 16:21–24.

95. Frank, R.N. Diabetic Retinopathy. N. Engl. J. Med. 2004; 350:48–58.

96. Kowluru, R.A.; Kowluru, A.; Mishra, M.; Kumar, B. Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. Prog. Retin. Eye Res. 2015;48: 40–61.

97. Kowluru, R.A.; Mohammad, G. Epigenetic modifications in diabetes. Metabolism 2021; 126:154920.

98. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Frontiers in physiology. 2020 Jul 2;11:694.

99. Poljsak B, Šuput D, Milisav I. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxidative medicine and cellular longevity. 2013 Oct;2013.

100. Kang Q, Yang C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. Redox Biol. 2020;37:101799.

101. Kowluru RA. Cross Talks between Oxidative Stress, Inflammation and Epigenetics in Diabetic Retinopathy. Cells. 2023;12(2):300

102. Kowluru RA. Diabetic Retinopathy: Mitochondria Caught in a Muddle of Homocysteine. *Journal of Clinical Medicine*. 2020; 9(9):3019.

103. Kowluru, R.A.; Mishra, M. Regulation of matrix metalloproteinase in the pathogenesis of diabetic retinopathy. Prog. Mol. Biol. Transl. Sci. 2017;148:67–85.

104. Murphy, M.P. How mitochondria produce reactive oxygen species. Biochem. J. 2009;417: 1–13.

105. Kowluru, R.A.; Kowluru, A.; Veluthakal, R.; Mohammad, G.; Syed, I.; Santos, J.M.; Mishra, M. TIAM1-RAC1 signalling axismediated activation of NADPH oxidase-2 initiates mitochondrial damage in the development of diabetic retinopathy. Diabetologia 2014;57: 1047–1056.

106. Kowluru, R.A. Diabetic retinopathy, oxidative stress and antioxidants. Curr. Top. Nutraceutical Res. 2005;3:209–218

107. Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, Bril V, Russell JW, Viswanathan V. Diabetic neuropathy. Nature reviews Disease primers. 2019 Jun 13;5(1):41.

108. G. Sloan, P. Shillo, D. Selvarajah et al., "A new look at painful diabetic neuropathy," Diabetes Research and Clinical Practice.2018; 144: 177–191.

109. Liu H, Bian W, Yang D, Yang M, Luo H. Inhibiting the Piezo1 channel protects microglia from acute hyperglycaemia damage through the JNK1 and mTOR signalling pathways. Life Sci. 2021;264: 118667.

110. Vargas-Soria M, García-Alloza M, Corraliza-Gómez M. Effects of diabetes on microglial physiology: a systematic review of in vitro, preclinical and clinical studies. Journal of Neuroinflammation. 2023 Dec;20(1):1-30.

111. Chhetri DR. Myo-Inositol and Its Derivatives: Their Emerging Role in the Treatment of Human Diseases. Front Pharmacol. 2019;10:1172.

112. H. E. Poulsen, E. Specht, K. Broedbaek et al., "RNA modifications by oxidation: a novel disease mechanism?" Free Radical Biology & Medicine.2012; 52(8): 1353–1361.

113. Oyenihi AB, Ayeleso AO, Mukwevho E, Masola B. Antioxidant strategies in the management of diabetic neuropathy. Biomed Res Int. 2015 Oct;2015(515042):515042.

114. Yang C, Zhao X, An X, Zhang Y, Sun W, Zhang Y, Duan Y, Kang X, Sun Y, Jiang L, Lian F. Axonal transport deficits in the pathogenesis of diabetic peripheral neuropathy. Frontiers in Endocrinology. 2023 Mar 28;14:1136796.

115. Rahimi-Madiseh M, Malekpour-Tehrani A, Bahmani M, Rafeian-Kopaei M. The research and development on the antioxidants in prevention of diabetic complications. Asian Pac. J. Trop. Med. 2016;9(9):825-831.

116. Serhiyenko V, Hotsko M, Serhiyenko A, Snitynska O, Serhiyenko L, Segin V. The impact of alpha-lipoic acid on insulin resistance and infammatory parameters in

patients with type 2 diabetes mellitus and cardiac autonomic neuropathy. Am. J. Int. Med. 2020;8(5):197-203.

117. Asbaghi, O., Nazarian, B., Yousefi, M. *et al.* Effect of vitamin E intake on glycemic control and insulin resistance in diabetic patients: an updated systematic review and meta-analysis of randomized controlled trials. *Nutr J*.2023; 22:10

118. Shaun A. Mason, Michelle A. Keske, Glenn D. Wadley; Effects of Vitamin C Supplementation on Glycemic Control and Cardiovascular Risk Factors in People With Type 2 Diabetes: A GRADE-Assessed Systematic Review and Meta-analysis of Randomized Controlled Trials. *Diabetes Care* 1 February 2021; 44 (2): 618–630.

119. Jin Y, Arroo R. The protective effects of flavonoids and carotenoids against diabetic complications—A review of in vivo evidence. Frontiers in Nutrition. 2023 Mar 24;10:1020950.

120. Leh HE, Lee LK. Lycopene: A potent antioxidant for the amelioration of type II diabetes mellitus. Molecules. 2022 Apr 4;27(7):2335.

121. Yedjou CG, Grigsby J, Mbemi A, Nelson D, Mildort B, Latinwo L, Tchounwou PB. The Management of Diabetes Mellitus Using Medicinal Plants and Vitamins. *International Journal of Molecular Sciences*. 2023; 24(10):9085.

122. Pivari F, Mingione A, Brasacchio C, Soldati L. Curcumin and type 2 diabetes mellitus: prevention and treatment. Nutrients. 2019;11(8):1837.

123. Liu J, Li X, Cai R, Ren Z, Zhang A, Deng F, Chen D. Simultaneous study of anti-ferroptosis and antioxidant mechanisms of butein and (S)-butin. Molecules. 2020;25(3):674.

124. Hoseini A, Namazi G, Farrokhian A, Reiner Ž, Aghadavod E, Bahmani F, Asemi Z. The efects of resveratrol on metabolic status in patients with type 2 diabetes mellitus and coronary heart disease. Food Function. 2019;10(9):6042-6051.

125. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative stress: pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. Bulletin of experimental biology and medicine. 2021 May;171(2):179-89.

126. Dzugkoev SG, Kaloeva MB, Dzugkoeva FS. Efect of combination therapy with coenzyme Q10 on functional and metabolic parameters in patients with type 1 diabetes mellitus. Bull. Exp. Biol. Med. 2012;152(3):364-366.

127. Skliarova EI, Popova TN, Shulgin KK. Efects of N-[Imino(1- Piperidinyl)Methyl] Guanidine on the Intensity of Free Radical Processes, Aconitase Activity, and Citrate Level in the Tissues of Rats with Experimental Type 2 Diabetes Mellitus. Bull. Exp. Biol. Med. 2016;161(2):261-265.

128. Sun C, Liu Y, Zhan L, Rayat GR, Xiao J, Jiang H, Li X, Chen K. Anti-diabetic effects of natural antioxidants from fruits. Trends in Food Science & Technology. 2021 Nov 1;117:3-14.