Review

Natural Biologics Accelerate Healing of Diabetic Foot Ulcers by Regulating Oxidative Stress

Jianying Song[1,2,†], Aiai Liu[3,†], Bo Liu[2], Wei Huang[1,4,5,6], Zongzhe Jiang[4,5,6], Xue Bai[1,4,5,6], Lixin Hu[1,2], Silin Zheng[3], Shengming Guo[3,*], Jianming Wu[7,*], Qi Chen[1,2,3,4,5,6,*]

[1]Department of Endocrinology and Metabolism, The Affiliated Hospital of Southwest Medical University, 646000 Luzhou, Sichuan, China
[2]School of Nursing, Southwest Medical University, 646000 Luzhou, Sichuan, China
[3]Department of Nursing, The Affiliated Hospital of Southwest Medical University, 646000 Luzhou, Sichuan, China
[4]Metabolic Vascular Disease Key Laboratory of Sichuan Province, 646000 Luzhou, Sichuan, China
[5]Sichuan Clinical Research Center for Nephropathy, 646000 Luzhou, Sichuan, China
[6]Cardiovascular and Metabolic Diseases Key Laboratory of Luzhou, 646000 Luzhou, Sichuan, China
[7]Department of Pharmacology, School of Pharmacy, Southwest Medical University, 646000 Luzhou, Sichuan, China
*Correspondence: Shengminguo@swmu.edu.cn (Shengming Guo); jianmingwu@swmu.edu.cn (Jianming Wu); qichen@swmu.edu.cn (Qi Chen)
[†]These authors contributed equally.

Abstract

Difficult or even non-healing diabetic foot ulcers (DFU) are a global medical challenge. Although current treatments such as debridement, offloading, and infection control have resulted in partial improvement in DFU, the incidence, amputation, and mortality rates of DFU remain high. Therefore, there is an urgent need to find new or more effective drugs. Numerous studies have shown that oxidative stress plays an important role in the pathophysiology of DFU. The nuclear factor erythroid 2-related factor (Nrf2) signaling pathway and the advanced glycated end products (AGEs)-receptor for advanced glycation endproducts (RAGE), protein kinase C (PKC), polyol and hexosamine biochemical pathways play critical roles in the regulation of oxidative stress in the body. Targeting these pathways to restore redox balance can control and alleviate the occurrence and development of DFU. Natural biologics are a major source of potential drugs for these relevant targets, and their antioxidant potential has been extensively demonstrated. Here, we discussed the pathophysiological mechanism of oxidative stress in DFU, and identified natural biologics targeting these pathways to accelerate DFU healing, in order to provide a new or potential direction for clinical treatment, nursing and related basic research of DFU.

Keywords: diabetic foot ulcers; diabetic wound; oxidative stress; natural biologics; targeted therapy

1. Introduction

The incidence and prevalence of diabetes mellitus (DM) is increasing worldwide. In 2021, 536.6 million people in 20–79-year-olds had diabetes worldwide, which is estimated to rise to 783.2 million in 2045 [1]. DFU are a common complication of DM. It is estimated that 19–34% of diabetic patients will develop DFU [2]. With the increase in patients with diabetes, the number of patients with DFU is increasing. Importantly, DFU not only have a high incidence [3], but also a high rate of disability and mortality [4,5], and once DFU appear, they can be difficult to heal or even become nonhealing. They lead to great suffering both physically and mentally. Current treatments for DFU mainly include debridement, offloading, and infection control [6], but the effect is not ideal. Therefore, more effective treatment strategies need to be explored. Although the exact mechanism of the occurrence and development of DFU is still unclear, there is no doubt that oxidative stress plays a key role in DFU [7].

Oxidative stress is a physiological process, and a reasonable level of oxidative stress can promote wound healing [8,9]. However, in DFU, there is an imbalance between the body's reactive oxygen species (ROS) levels and antioxidant enzymes [10]. The pathological oxidative stress not only damages the stages of inflammation, proliferation, and remodeling, making each stage unable to proceed smoothly and orderly, but also leads to vascular, neuropathy, or local infection, and ultimately worsens DFU [7]. Nrf2 is the central regulator of oxidative stress [11]. Damage to the AGEs-RAGE, PKC, polyol and hexosamine biochemical pathways is the key to the imbalance of redox levels [12]. Therefore, seeking drugs targeting the Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine pathways to promote the body's redox balance may become an important strategy to accelerate the healing of DFU.

Natural biologics have always been a research hotspot in the world due to their advantages of less toxicity and side effects, wide sources, and cost efficiency. Many natural biologics can scavenge oxygen free radicals or increase antioxidant levels by targeting the Nrf2 signaling pathway, AGEs-RAGE, PKC, polyol and hexosamine pathways, and ultimately play a beneficial role in the repair of DFU.

Therefore, the aim of this review was to investigate the role of oxidative stress in the pathophysiology of DFU through a literature review. We then introduced the Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine pathways as pharmacological targets, providing up-to-date information on natural biologics that target these pathways to accelerate DFU healing.

2. Oxidative Stress in DFU

Oxidative stress was defined as: "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage" [13]. When the skin barrier structure is destroyed, oxygen immediately flows in, and the injury-induced O2 influx fuels local ROS production [14]. At low concentrations, ROS act as cellular messengers to accelerat wound healing [8,9]. However, in DFU, abnormalities in the Nrf2 signaling pathway and AGEs-RAGE, PKC, Polyol and Hexosamine biochemical pathways result in excessive oxidative stress on the wound surface, which is one of the main reasons for the difficulty in healing of DFU. The results of clinical specimen experiments showed that there was uncontrolled oxidative stress and decreased antioxidant capacity in DFU, which resulted in redox imbalance [10]. Excessive oxidative stress can damage all stages of DFU repair, such as inflammation, proliferation, and remodeling.

2.1 Excessive Oxidative Stress Causes DFU to Stagnate During the Inflammatory Phase

In patients with DFU, continuous hyperglycemia will lead to excessive oxidative stress. Oxidative stress induced by hyperglycemia can enhance neutrophil gene expression and secretion of S100A8 and S100A9 [15,16]. S100A8/A9 promotes leukocytosis via increased proliferation of Bone Marrow progenitor cells [17]. For example, S100A8/A9 induces macrophages to secrete more proinflammatory mediators, such as IL6 [15]. Moreover, sustained hyperglycemia exposure increases neutrophil metabolism, and ROS production as a byproduct through the mitochondrial electron transport chain also increases [16,18]. ROS in the neutrophils stimulate NETosis, capturing and killing pathogens via neutrophils extracellular traps (NETs) [19], through both the NOX-dependent and NOX-independent pathways [16]. Reasonable formation of NETs is beneficial to the normalization of the immune microenvironment, but excessive formation of NETs worsens the abnormal inflammatory response of wounds, which is also the key pathological manifestation of diabetic wounds (DW) that is difficult to heal [20–22]. In addition, NETs also reduce the responsiveness of neutrophils to lipopolysaccharide, leading to an imbalance of M1/M2 macrophages [23]. Neutrophils and macrophages are the core cells in the inflammatory phase, and their abnormal function or phenotype often leads to a continuous low-efficiency inflammatory reaction on the wound. Natural biologics play an import role in decreasing

inflammatory response and oxidative stress markers [24]. Solving the problem of pathological oxidative stress in the wound and precise regulation of the start and end of inflammation are the key to promote the repair of DFU.

2.2 The Balance of Redox Homeostasis is the Basis for the Smooth Progress of the DFU Proliferation Phase

Excessive ROS production in DFU leads to ROS accumulation in endothelial cells, which mediates endothelial dysfunction [25,26]. Inhibition of oxidative stress can enhance the proliferation of human umbilical vein endothelial cells and human skin fibroblasts, restore angiogenesis, promote granulation tissue formation and healing of epithelialization, overall demonstrating markedly improved healing of DW [27]. In addition, fibroblasts promote keratinocyte proliferation through Jun-dependent expression of PTN and SDF-1 [28], which is beneficial to epithelial reformation [29]. However, in DFU, excessive oxidative stress impaired the proliferation and differentiation of fibroblasts [27]. In DFU, intense oxidative DNA damage, low vascular endothelial growth factor (VEGF) and transforming growth factor β1 (TGF-β1) expression, and increased apoptosis all contribute to delayed healing of DFU [30]. Excessive oxidative stress results in a harsh microenvironment for DFU; and endothelial cells, fibroblasts, etc., cannot proliferate ideally to promote the smooth progress of wound proliferation.

2.3 Imbalance in Redox Homeostasis Causes DFU Remodeling Disorder

In DFU, excessive oxidative stress leads to impaired proliferation of fibroblasts. Fibroblasts play an important role not only in reepithelialization but also in remodeling. Fibroblast derived keratinocyte growth factor-1 (KGF-1), also known as fibroblast growth factor 7, accelerates wound contraction by promoting TGF-β1-induced fibroblast contraction via the TGF-β1/Smad signaling pathway [31]. KGF-1 deficiency can significantly reduce the wound contraction rate during healing [32]. Myofibroblasts are responsible for contracting dermal tissue and they can produce several components of the extracellular matrix (ECM) such as collagens of types I, III, IV, and V to promote tissue remodeling [33]. TGF-β1 is also required for fibroblast differentiation into myofibroblasts [31]. However, the expression levels of TGF-β1 were significantly lower in DFU [34]. Ilya A Demyanenko *et al.* [35] confirmed that the mitochondria-targeted antioxidant 10-(6′plastoquinonyl) decyltriphenylphosphonium can not only reduce the number of neutrophils, promote the M1/M2 macrophages to restore balance, also promote fibroblast migration and increase the number of myofibroblasts, eventually improveing DW healing. Therefore, remodeling is also impaired by excessive oxidative stress.

3. Signaling Pathways in the Regulation of Oxidative Stress in DFU

3.1 Nrf2 Signaling Pathways

Nrf2, a member of the cap 'n' collar subfamily of basic region leucine zipper transcription factors [36], maintains the balance of redox in the body by regulating many genes, including but not limited to GSH, catalase (CAT), heme oxygenase 1 (HO1) and quinone oxidoreductase 1 (NQO1) [37,38]. Research targeting Nrf2 shows great potential and application value in cancer [39], Alzheimer's disease [40], cardiovascular disease [41] and DW [42] or chronic wound healing [43].

In a normal physiological environment, Nrf2 specifically binds to the amino-terminal Neh2 domain to mediate the ubiquitination and degradation of Nrf2, thus maintaining a low intracellular concentration [44]. When the body is in a state of oxidative stress, the accumulation of ROS or electrophiles will weaken the interaction between Nrf2 and Keap1, thus releasing Nrf2 and inhibiting Nrf2 ubiquitination, leading to the enhancement of Nrf2 nuclear translocation and the formation of heterodimerizes with one of the small Maf proteins, and binding with antioxidant response elements to activate the transcription of antioxidant protein genes. Ultimately, it acts as an antioxidant and protects cells from stress factors [45,46]. Therefore, low levels of oxidative stress can be regulated by Nrf2 to promote the recovery of redox homeostasis. However, continuous excessive oxidative stress will lead to the imbalance of ROS, free radicals, etc., and antioxidants in the body, which is one of the pathological mechanisms that mediate the development of DFU. Under hyperglycemia, the activation of Nrf2 is inhibited, and the expression of antioxidant genes including NQO1 and HO1 are significantly down-regulated, which exacerbates the occurrence and development of oxidative stress [37]. The study of Rajan Teena *et al*. [47] using human clinical specimens showed that Nrf2 expression was significantly reduced in type 2 DM subjects and DFU subjects compared with normal glucose tolerant subjects. Peri-ilesional skin tissues from patients with diabetes are often in a more severe state of oxidative stress. Although the compensatory Nrf2 pathway is activated due to high oxidative stress, such compensatory Nrf2 activation cannot restore the redox balance of the body [42]. Activation impairment of Nrf2 not only aggravates oxidative stress, but also causes abnormal inflammation of the wound surface [37]. Moreover, due to severe oxidative damage to the skin tissue of diabetic patients, cell apoptosis often occurs [42]. Silencing Nrf2 by siRNA not only increases cell apoptosis, but also slows down the migration rate of HaCaT cells [42], and the Nrf2 transcription factor is also a novel target of KGF [48]. An Abnormal Nrf2 pathway is not conducive to the smooth progression of wound proliferation and remodeling. There is no doubt that targeted activation of Nrf2 is an effective way to accelerate healing of DFU. The team of Min Li *et al*. [37] and Ying Li *et al*. [49]

confirmed this idea in streptozotocin-induced diabetic rat wound models using dimethyl fumarate, an Nrf2 activator. In addition, Nrf2 also promotes angiogenesis through multiple pathways, such as MALAT1/HIF-1α [50], which is also beneficial for the healing of DFU. It has been reported that hyperbaric oxygen therapy, which is commonly used in clinical practice, can accelerate wound repair by activating Nrf2 and stimulating angiogenesis [51]. In conclusion, the persistent and high level of oxidative stress on the DW is closely related to the refractory DW, and the disorder of redox homeostasis is mainly related to abnormal Nrf2 in the tissue of DFU.

3.2 AGEs-RAGE Pathways

AGEs are a heterogeneous group of modified molecular species [52] that play a central role in oxidative stress. The accumulation of AGEs *in vivo* can be not only produced endogenously but also acquired by exogenously. For endogenous AGEs, the most classic is the Maillard reaction. The Maillard reaction is a complex network of amino acids and reducing sugars [53]. This non-enzymatic reaction can generate intermediate carbonyl precursors of AGEs, and glucose can also generate dicarbonyls through autoxidation, the polyol pathway and lipid peroxidation, which greatly contribute to the formation of AGEs [54]. Worldwide, the age-standardized prevalence of daily smoking was 25.0% for males and 5.4% for females [55]. Cigarette smoke promotes the production and activation of advanced glycation end products [56]. Food is also an important source of AGEs in the body. Approximately 10–30% of AGEs in food can be absorbed into the systemic circulation [54,57]. AGEs/RAGE interactions can activate or strengthen numerous signaling pathways to enhance oxidative stress such as the Ras-mediated extracellular signal-regulated kinase (ERK1/2), stress-activated protein kinase/cJun N-terminal kinase (SAPK/JNK), mitogen-activated protein kinase (MAPK), and Janus kinase signal transducer and activator of transcription (JAK/STAT) pathways [54].

Under normal circumstances, there are only moderate amounts of AGEs in the body. However, for DFU patients, the continuous high glucose environment leads to the continuous accumulation of AGEs in the body [58,59]. Moreover, many diabetic patients are obese, and the obese patients often acquire more exogenous AGEs orally [60]. The binding of AGEs to RAGE promotes the production of ROS and the inflammatory cascade by reinforcing oxidative stress. For example, excessive accumulation of AGEs decreased the migratory and adhesive functions of neutrophils [61], inhibited the influx of early macrophages, and disrupted the phagocytic function of M1 macrophages [62], which is not conducive to the formation of an early proinflammatory microenvironment on the wound surface. At the late stage of wound healing, accumulated AGEs promote overactivation of the NLRP3 inflammasome by gener-

ating ROS [63], which promotes the continued polarization of macrophages toward the M1 type. In addition, AGEs and RAGE binding can affect Ras homolog family member A/Rho kinase signaling to inhibit the anti-inflammatory function of exocytosis [64], putting the wound in a continuous stage of an inefficient inflammatory response. The adverse immune microenvironment prevented the wound from progressing to the stage of proliferation and remodeling from the stage of inflammation. In the proliferation phase, AGEs significantly inhibited HaCaT cells proliferation and migration by down-regulating the expression of miR-146a and upregulating the expression of an anchoring protein 12 (AKAP12) [65]. Moreover, excessive activation of the AGEs-RAGE pathway disrupts collagen I maturation and prevents its deposition in the ECM, which also further impairs DFU remodeling [66,67]. In addition to these adverse consequences, unfortunately for patients with DFU, the accumulation of AGEs in tissues was independently correlated with vascular lesions that may lead to ischemic lesions in diabetic feet, leading to the adverse consequences of amputation [68,69]. The accumulation of AGEs is also a risk factor for the development of diabetic neuropathy [70], which may cause the patient's feet to lose protective pain sensation. Undoubtedly, vascular and neuropathy are an added hazard for an already difficult-to-heal DFU. More troubling is that AGEs induced the release of extracellular DNA (eDNA) by positively affecting sigB transcription and downregulating lrgA expression, thereby promoting the formation of S. aureus biofilms [71], which may aggravate DFU local infection. AGEs not only adversely affect the inflammatory, proliferative, and remodeling phases of DFU repair, but also codamage DFU by inducing or aggravating vasculopathy, neuropathy, and local infection. Targeting AGEs-RAGE pathways could be a good way to treat DFU.

3.3 PKC Pathways

Protein kinase C isozymes (PKCs), serine-threonine protein kinases, are widely distributed in mammalian tissues and play a critical role in many physiological functions such as regulating cell growth and proliferation, senescence, and apoptosis [72,73]. They are grouped into three categories according to their domain composition: conventional PKCs (PKCα, PKCβI, PKCβII, PKCγ), novel PKCs (PKCδ, PKCε, PKCη, PKCθ), and atypical PKCs (PKCζ, PKCλ/ι) [74,75]. Conventional PKCs are activated by diglycerol and Ca^{2+}, novel PKCs are activated by diglycerol, and atypical PKCs are regulated by protein:protein interactions [76]. All PKCs remain in an autoinhibited conformation until they bind to the correct second messengers or protein scaffolds for atypical PKCs [77], thus releasing the pseudosubstrate and activating PKC [76,78]. Activation of PKC regulates substrate phosphorylation and downstream signal transduction.

In the high-glucose environment of DFU, PKC is continuously activated [79]. Excessive activation of PKC ag-

gravates the neutrophil respiratory burst and releases a large number of NETs [80,81]. Sushant Kumar Das *et al*. [82] also confirmed that the expression of PKC βII was significantly upregulated in DFU, which primes neutrophils to undergo NETosis and promotes PKC βII mediated downregulation of the VEGF-dependent Akt/eNOS pathway. The respiratory burst of neutrophils, the occurrence of NETosis and the reduction of VEGF, which worsen inflammation, endothelial cell damage, and ECM degradation, are important reasons for the difficult healing or even nonhealing of DFU. In addition, excessive activation of PKC signaling also damages the vascular endothelium. With the further research, the abnormal expression of PKC in DFU does not remain at the level of animal and cell experiments. Zhichuan Liu *et al*. [10] also verified the significant upregulation of p-PKCβ in DFU at the population level. High glucose levels may impair the vascular endothelium by activating the PKCβ-p66shc signaling pathway [10]. Inhibition of PKC signaling promotes the stabilization of endothelial barrier function by reducing moesin phosphorylation [83]. Furthermore, PKCδ protein and mRNA levels were increased by 7-fold and 3-fold, respectively, in discarded tissues obtained from active DFU compared with levels detected in control (without diabetes) tissues [84]. Increasing PKCδ expression aggravates the difficulty in DFU healing by impairing fibroblasts. Equally worrisome is that the PKC signaling pathway is closely related to peripheral neuropathy, and abnormal PKC can exacerbate peripheral neuropathy in diabetic patients [85]. Numbness and sensory disturbances are common symptoms of peripheral neuropathy that cause protection from painful stimuli. This is an important reason why the injured feet cannot be detected and treated in time, resulting in wound infection, deterioration, and missed optimal treatment time. An abnormal PKC signaling pathway not only intensifies oxidative stress but also impairs the healing of DFU. However, currently, in view of the special complications of diabetic foot, the most studied category is conventional PKCs (PKCβ), and other categories need to be further explored.

3.4 Polyol Pathways

The polyol pathway includes a family of monomeric nicotinamide adenine dinucleotide phosphate (NADPH) dependent aldo-keto reductase enzymes that catalyze the conversion of carbonyl compounds to sugar alcohols [72]. Part of glucose can be converted into sorbitol via aldolase reductase (AR). Then sorbitol is slowly converted to fructose by sorbitol dehydrogenase hindering antioxidant activity [86]. This step uses NAD+ as a cofactor. Substrate-driven reactions mediated by NADPH and NAD+ play a significant role in maintaining the redox balance [87].

However, compared to nondiabetic wounds, higher levels of oxidative stress were present in the tissue of DFU [47]. The increased sorbitol pathway activity is one of the important mechanisms for the development of DFU [88].

AR is the rate-limiting enzyme for this reaction. Under normal conditions, AR has a very low affinity for glucose, and no more than 3% of glucose is processed through this pathway [89]. However, under the condition of continuous hyperglycemia, hexokinase is saturated, and then AR is continuously activated [90], which consumes NADPH and reduces the function of GSH reductase impairing antioxidant activity [86]. The accumulation of sorbitol may cause osmotic damage and lead to cell death. At present, there is no direct evidence that excessive accumulation of sorbitol in DFU tissue can aggravate the death of cells that are beneficial to wound healing such as endothelial cells. Further research is needed. However, the team of Bradley P Mudge [91] confirmed that the level of GSH in DFU was significantly lower than that in nondiabetic wounds. Treatment with local GSH accelerated the recovery of redox levels, which reduced wound tissue biofilm production, increased antibiotic sensitivity and ultimately promoted the healing of DFU [92]. What also bothers us is that the abnormal increase of AR in DFU may make DFU fall into a worse situation [93]. In addition, fructose was abnormally increased in DFU tissue. Excessive fructose is metabolized by fructokinase to produce overly acetyl-CoA, which increases protein acetylation and results in protein dysfunction [94,95]. Fructose can generate AGEs [96], which are detrimental for the repair of DFU. Continuous abnormal oxidative stress in DFU increases the collagen I-to-collagen III ratio in collagen accumulation and lipid atrophy and may eventually lead to amputation in DFU patients [95]. Therefore, an abnormal polyol pathway may hinder the repair of DFU.

3.5 Hexosamine Pathways

The hexosamine pathway (HP) is identical to the first two steps of glycolysis. The glycolytic intermediate fructose-6 phosphate is converted to glucosamine-6 phosphate under the action of glutamine-fructose-6-phosphate aminotransferase, the rate-limiting enzyme of HP [97]. Subsequent enzymatic steps then lead to acetylation and activation using UTP to produce the amino sugar UDP-N-acetylglucosamine (UDP-GlcNAc) [98]. UDP-GlcNAc is an important metabolic compound for the formation of glycosyl chains of proteins and lipids [99]. Moreover, under the action of Oglycosyltransferase (OGT), UDP-GLcNAC can modify the protein o-GlcNAC [100]. The HP is composed of a series of anabolic reactions [98] and plays an important role in the pathophysiology of diabetes complications [101–103].

Under normal blood glucose levels, only 2–5% of fructose 6-phosphate enters the HP [104]. However, in diabetic patients, excess fructose-6-phosphate is shunted into the HP. The increase in HP flux leads to an increase in UDP-GlcNAC levels, which in turn increases the flux through OGT, leading to an increase in O-GlcNAc levels [99,105]. O-GlcNAc modification is a posttranslational modification that can regulate inflammatory response by targeting the NF-κB signaling pathway [106], Treg cells [107] and receptor-interacting serine/threonine-protein kinase 3 [108]. O-GlcNAc overexpression leads to immune dysregulation. Overexpression of UDP-GlcNAc, a precursor for a variety of O-GlcNAc modifications, leads to increased flux through OGT, but OGT inhibits the activation of classically activated macrophages that promote inflammation by catalyzing ribosomal protein S6 kinase β-1 O-Glcnacylation and inhibiting S6 kinase 1 and mTORC1 signaling [109]. However, compared with the DFU-non-Healer group, the proportion of classically activated macrophages promoting inflammation was significantly higher in the DFU-Healer group than in the activated macrophages with anti-inflammatory properties [110]. Andrew P Sawaya *et al.* [111] also confirmed that macrophages were absent in DFU and that the inefficient inflammatory reaction made the wound difficult to heal or even non-heal. Loss of OGT, a key enzyme of protein O-GlcNAc, enhances innate immune activation [108] and normalizes the immune microenvironment. Vascular damage is a key pathological mechanism of DFU. Elevated protein O-GlcNAc modification through the HP may inhibit Akt signaling to impair angiogenesis in endothelial cells [112]. O-GlcNAc overexpression impairs vasodilatory activity [113]. Persistent vascular damage often leads to worsening of DFU, even leading to amputation. Furthermore, excess O-GlcNAc reduces the expression of galectin-7 in keratinocytes, thereby inhibiting keratinocyte migration [114]. Inhibition of O-GlcNAC can accelerate DW healing by alleviating keratinocyte dysfunction [115]. Overexpressed O-GlcNAc level reduces the sensitivity of sphingosine-1-phosphate mediated contraction, thereby inhibiting fibroblast contraction, which is not conducive to wound closure and remodeling [116]. OGT knockdown using shRNA or siRNA can reverse delayed wound closure caused by hyperglycemia [117]. The increase in HP flux in DFU adversely affects the inflammation, proliferation and remodeling phases of wound repair. However, there are few direct studies on HP flux and proliferation of wound repair, and most of them focus on diabetic vascular diseases. The specific mechanism by which increases in HP flux increases adversely affect the smooth progression of wound repair proliferation still needs to be discussed.

4. Natural Biologics against Oxidative Stress in DFU

4.1 Nrf2 Signaling Pathways

As a classical signaling pathway, Nrf2 is the "master regulator" of the antioxidant response [118]. Natural biologics have long been a resource for new drug discovery due to their low toxicity and wide range of sources [119]. In cancer and antibiotic drug development, natural biologics reportedly account for 53% and 59% of all FDA-approved drugs, respectively [120]. Without exception natural biologics are also important sources of Nrf2 agonists [120]. Targeted activation of Nrf2 can drive the balance

of redox homeostasis in the body and ultimately promote DFU repair. We identified some natural biologics targeting Nrf2 to promote DFU healing. Here, we briefly list a few natural biologics and describe how the targeted activation of Nrf2 can promote the repair of DFU. Neferine (Nef), a bisbenzylisoquinoline alkaloid obtained from the seed embryos of Nelumbo nucifera, has various pharmacological effects such as anti-oxidative, anti-inflammatory, anti-arrhythmic and anti-thrombotic. In addition, it can increase the sensitivity of chemotherapy drugs, such as paclitaxel, cisplatin and doxorubicin [121–123]. Juan Li *et al*. [124] showed that Nef can improve insulin sensitivity, which significantly reduced blood glucose levels. Local application of Nef in diabetic rat wounds can activate the Nrf2 signaling pathway, reduce Keap1 expression, increase the expression of downstream factors antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR), and increase the expression of collagen-1, TGF-β and α-SMA in ECM. In addition, Nef can also reduce the production of TNF-α, IL-1β and other inflammatory factors. These results suggested that Nef improved the redox homeostasis of the wound through the targeted activation of Nrf2 and promoted the repair of DW from multiple dimensions of inflammation, remodeling and blood glucose levels. Moreover, targeted activation of Nrf2 to reverse oxidative stress in wounds can not only play a beneficial role in the inflammation and remodeling stages of DW but also improve the proliferation, angiogenesis and neuropathy of DW (important pathological mechanisms or manifestations of the occurrence and development of DW). Resveratrol [125] is a good example of alleviating the effects of oxidative stress on the proliferation and migration of HUVECs through targeted activation of Nrf2, thereby promoting wound proliferation. In addition, procyanidin B2 [126] and rutin [127] promoted DW repair by targeting the Nrf2 pathway to promote angiogenesis and improve neuropathy, respectively. For more examples, please see Table 1 (Ref. [30,62,124–149]). These findings indicate that Nrf2, as a classical and core signaling pathway to maintain redox homeostasis in the body, plays a main role in the development of DFU. Targeted activation of the Nrf2 pathway can increase the expression of downstream factor antioxidant enzymes to exert antioxidant activity, restore the body's redox homeostasis, thereby mediating the smooth progress of various stages of inflammation, proliferation and remodeling in DW repair, promote angiogenesis and improve neuropathy to prevent the deterioration of DFU and ultimately promote the healing of DFU. At present, many natural biologics targeting Nrf2 have been reported, but screening more efficient drugs to promote DFU healing, so as to promote their clinical transformation and ultimately promote the recovery of clinical DFU patients is our ultimate goal. Therefore, further exploration is needed.

4.2 AGEs-RAGE Pathways

AGEs-RAGE pathways is a key factor in maintaining the body's redox balance. Abnormal activation of AGEs-RAGE signaling leads to damage to DFU in all stages. Vascular, neuropathy and local infections also cause patients with DFU to face the threat of amputation or death. Targeting the AGEs-RAGE signaling axis can inhibit abnormal inflammatory responses by regulating the NF-kB/NLRP3 [150], RAGE/RhoA/ROCK signaling pathway [151] or immune cells [64] to normalize the immune microenvironment. Previous studies have reported that topical application of pyridoxamine, a natural vitamin B6 analog, reduced the accumulation of AGEs in the wound tissue of diabetic mice, promoted the influx of macrophages in the early stage of tissue repair, improved the dysfunctional inflammatory response, and accelerated wound healing [62]. Moreover, targeting AGEs-RAGE signaling pathways can also be beneficial in the proliferation and remodeling stages of wound healing such as Centella cordifolia and Shixiang Plaster. Centella cordifolia reversed collagen migratory defects and restored the spreading and attachment of fibroblasts, endothelial cells and keratinocytes over the glycated ECM [133]. Ji Fei *et al*. [134] also used Shixiang Plaster, a traditional Chinese medicine, to promote the reepithelialization of DW by reducing the expression of AGEs and RAGE. In addition, as mentioned above, the accumulation of AGEs *in vivo* is inseparable from exogenous acquisition. Diets with a low AGEs content have effectively improved insulin sensitivity and reduced insulin resistance and cholesterol levels, which are beneficial for DFU repair [152]. There is no doubt that targeting AGEs/RAGE has great potential for the healing of DFU. Natural biologics targeting AGEs/RAGE can also prevent the further deterioration of DFU by regulating angiogenesis or neuropathy. For instance, the extracts of Momordica charantia (MC) induce ERK1/2 phosphorylation and tube formation through the AGEs-RAGE pathway, promoting angiogenesis [135], which may improve diabetic foot microcirculation and prevent diabetic foot ischemic lesions. In addition, salvianolic acid A (SalA), the main efficacious, water-soluble component of miltiorrhiza bunge, also improved peripheral blood perfusion, vasodilation responsiveness and peripheral nerve function by targeting AGEs, which to some extent hindered the development or deterioration of DFU [136]. These research results showed that natural biologics, in addition to their own advantages such as low toxicity and wide sources, were also efficacious for DFU. However, the combination of these natural biologics, which play different roles in different stages of wound healing, may be better for seeking the optimal prescription. Moreover, the current research on the mechanism of natural medicines and diseases mostly focuses on the indirect mechanisms, and the research on the direct mechanisms is not deep enough. This may give us the illusion that natural medicines are less effective. Therefore, an in-depth understanding the exact target of DFU, using

Table 1. Examples of targeted therapy strategies of natural biologics for DFU.

Targeted Pathways	Natural Biologics	Mechanism		Human/Animal/Cell	Wound	Intervention Dose	Administration route	Administration frequency	Findings/ Outcomes	References
Nrf2 pathways	neferine	Activated pathways	Nrf-2	Streptozotocin induced diabetic male rats	Excision wound model (wound diameter is 10 mm)	10% and 20%	Topically applied	Once a day for 14 days	DW healing is accelerated	[124]
	resveratrol	Activated pathways	Nrf-2	HUVECs and Streptozotocininduced diabetic male rats	Excision wound model (wound diameter is 10 mm)	100 nM (HUVECs), 100 mg/mL (diabetic rat)	Co-incubation (HUVECs), Topically applied (diabetic rat)	/ (HUVECs), once a day for 10 days (diabetic rats)	Attenuated oxidative stress-induced impairment of HUVECs proliferation and migration and eventually accelerated DW healing	[125]
	procyanidin B2	Activated pathways	Nrf-2	Endothelial progenitor cell (EPC) and streptozotocin induced diabetic mice	Excision wound model (wound diameter is 8 mm)	2.5 μmol/L (EPC), 10 mg/kg (diabetic mice)	Co-incubation (EPC), Intraperitoneal injection (diabetic mice)	/ (EPC), once a day for 10 days (diabetic mice)	Improved endothelial progenitor cell function and accelerated wound healing, increases angiogenesis in diabetic mice	[126]
	rutin	Activated pathways	Nrf-2	Streptozotocin induced diabetic rats	Excision wound model (wound diameter is 15 mm)	100 mg/kg	Intraperitoneal injection	Once a day for 21 days	Reduceoxidative stress and inflammatory response, promoting DW healing	[127]
	Paeoniflorin	Activated pathways	Nrf-2	Human immortalized keratinocyte (HaCaT) cells and streptozotocin-induced diabetic rats	Excision wound model (wound diameter is 6 mm)	100 μM (HaCaT), 15 and 30 mg/kg (diabetic rats)	co-incubation (HaCaT), intragastric administration (diabetic rats)	/ (HaCaT), once a day for 16 days (diabetic mice)	Increased cell proliferation and migration and accelerated wound healing in diabetic rats	[30]
	Xanthohumol	Activated pathways	Nrf-2	HaCaT, HUVECs and Streptozotocin induced diabetic rats	Excision wound model (wound diameter is 2 mm)	2.5 and 5 μM (HaCaT,HUVECs), 2.5 and 5 mg/kg (diabetic rats)	Co-incubation (HaCaT, HUVECs), Intraperitoneal injection (diabetic mice)	Once a day for 10 days (diabetic rats)	Alleviated oxidative stress-induced cell damage and accelerated DW healing in STZ-induced diabetic rats	[128]
	mangiferin	Increase level	Nrf2	Streptozotocin-nicotinamide-induced type-2 diabetic male rats	/	1% and 2% mangiferin gel	Topically applied	For 21 days	DW healing is accelerated	[129]
	Betulinic acid	Activated pathways	Nrf-2	Human primary aorta smooth muscle cells (HASMCs), HUVECs,Streptozotocin induced diabetic rats	The rat's back was placed in a hot copper column (2 cm in diameter) at 75 °C for 15 s to obtain the burn model	15 μM and 20 μM (HASMC), 2 0μM (HUVECs), 10 mg/kg (diabetic rats)	Intraperitoneal (IP) and Topically applied	Every three days for 4 weeks starting from 1 week before the introduction of burn injury (IP), each day continuously for 3 weeks starting from the second day after the introduction of burn injury (Topically applied)	Diabetic wounds healing is accelerated	[130]
	Luteolin	Inactivation of NF-κB and upregulation of Nrf2		Streptozotocin induced diabetic male rats	Excision wound model (wound diameter is 15 mm)	100 mg/kg	Intraperitoneal injection	Once a day for 14 days	DW healing is accelerated	[131]
	Bee venom	Activated pathways	Nrf-2	Streptozotocin induced diabetic male mice	Excision wound model (wound diameter is 8 mm)	200 μg/kg	Subcutaneously injected	Once a day for 15 days	DW healing is accelerated	[132]

Table 1. Continued.

Targeted Pathways	Natural Biologics	Mechanism	Human/Animal/Cell	Wound	Intervention Dose	Administration route	Administration frequency	Findings/ Outcomes	References
AGEs-RAGE Pathways	pyridoxamine (PM)	Reduced the accumulation of AGEs, promoted the influx of macrophAGEs in the early stage of tissue repair	Human monocytic THP-1 cell (THP-1), Male db/db T2DM mice	Excision wound model (wound diameter is 6 mm)	100 μM (THP-1), 100 μM/20 μL	Co-incubation, Topically applied, then wound was covered with a transparent dressing (3M, 1624W)	Once a day until day 10 post-injury	DW healing is accelerated	[62]
	Centella Cordifolia	Inhibited the expression of AGEs and improved antioxidant capacity	The human vascular endothelial cell line (EAhy926), The mouse embryonic fibroblasts (NIH3t3) cell, The human keratinocytes cell line (Ha-Cat)	/	EAhy926:2 mM; NIH3t3: 500 μg/mL; HaCat: 500 μg/mL	Co-incubation	/	Restored the spreading and attachment of endothelial, fibroblast and keratinocyte cells over the glycated ECM	[133]
	Shixiang Plaster	Inhibited the expression of RAGE and AGEs, Promoted angiogenesis and granulation tissue formation	Streptozotocin induced diabetic Sprague-Dawley rats	Excision wound model (wound diameter is 5 mm)	/ (pastes)	Topical application at a thickness of 2 mm over the wound	Once a day	DW healing is accelerated	[134]
	Momordica Charantia	Blocked RAGE, induced ERK1/2 phosphorylation and tube formation to promote angiogenesis	bovine aortic endothelial cells (BAEC)	/	10, 50 or 75 μg/ mL	Co-incubation for 72 hours	/	promoted angiogenesis	[135]
	Salvianolic acid A (SalA)	Decreased AGEs levels, vascular eNOS expression, and blood glucose, lipid, vWF and malondialdehyde levels	streptozotocin -induced type 2 diabetic rats	/	1 and 3 mg/kg	Per os (p.o) for for 10 weeks	Once a day	Improved diabetic plantar microcirculation and peripheral nerve function	[136]
PKC Pathways	Curcumin	Inhibited PKC-α and PKC-β1 activity	streptozotocin-induced type I diabetic rats	/	100 mg/kg	p.o. for 8 weeks	Once a day	Diabetic nephropathy is attenuated	[137]
	Pueraria tuberosa extract	Downregulated the PKC-α and NF-kB pathway to normaliz the inflammatory microenvironment	streptozotocin-induced diabetic nephropathy rats	/	100 mg/100 g and 50 mg/100 g	p.o for 20 days	/	Inhibited the abnormal inflammatory response in diabetic nephropathy	[138]
	sasa borealis water-extract	Inhibiting the activation of PKC βII and NADPH oxidase	HUVEC	/	1 and 10 μg/ mL	Co-incubation	/	Blocked chronic high glucose-induced endothelial apoptos	[139]
	Verbascoside	Inhibiting PKC/HMGB1/RAGE/NFκB signaling	Smulow-Glickman (S-G) gingival epithelial cell line	/	25, 50 and 100 μM	Co-incubation for 24 hours	/	Mitigated the Suppressed Cell Proliferation and Wound Healing Capacity of Gingival Epithelial Cells under High Glucose Condition	[140]

Table 1. Continued.

Targeted Pathways	Natural Biologics	Mechanism	Human/Animal/Cell	Wound	Intervention Dose	Administration route	Administration frequency	Findings/ Outcomes	References
Polyol Pathways	chlorogenic acid	Enhanced hydroxyproline content, decreased malondialdehyde/nitric oxide levels, elevated reduced-glutathione	streptozotocin-induced diabetic rats	Excision wound model (wound diameter is 15 mm)	50 mg/kg	Intraperitoneal injection	Once a day	DW healing is accelerated	[141]
	AgNPs	Increased GSH peroxidase activity and GSH content	streptozotocin-induced diabetic rats	Incision wound model (straight incisions of 8 cm Length on both sides of the vertebraland closed with interrupted sutures 1 cm apart)	10, 30 μg/kg	Topically applied	Once a day for 21 days	DW healing is accelerated	[142]
	propolis	Increased the GSH and GSH/ GSSG ratio, depleted TNF-α, and increased IL-10 levels	Diabetic patients with foot wounds	/	Propolis (Beepolis®) used was 3% in propylene glycol preparation manufactured	Topically applied (Propolis spray was applied to cover the wound surface in each dressing from week 0 until cicatrization or 8 weeks as a maximum, whichever occurred first.)	/	There was a decrease in the wound area by an average of 4 cm² in the propolis group compared with the control group, which reduced 3 cm²	[143]
	polyphenolic fraction	Inhibited aldose reductase activity as well as their expression in diabetic animals	Diabetic peripheral neuropathy was induced by streptozotocin and maintained	/	100, 200 mg/kg	p.o for 30 days	/	Ameliorated diabetic peripheral neuropathy	[144]
Hexosamine Pathways	APE	Increased hexosamine levels	Streptozotocin-induced type 2 diabetic Sprague-Dawley rats	Excision wound model (wound diameter is 8 mm)	100, 200 and 400 mg/kg (p.o), 5, 10 and 20% APE cream (topically applied)	p.o for 7 days and topically applied for 28 days	p.o (once a day) topically applied (twice a day, approximately 1 mm thick)	DW healing is accelerated and neuropathy is ameliorated	[145]
	ethanolic extract of Melia dubia	Improved total collagen and hexosamine	Female Wistar rats	Excision wound model (wound diameter is 2 cm) and Incision wound model (para-vertebral straight incisions of 6 cm Length and closed with interrupted sutures 1 cm apart)	40 mg/kg b. wt, 200 μL	Topically applied	Once a day until the wounds heal completely	DW healing is accelerated	[146]
	Solanum xanthocarpum	Increased hexosamine levels	streptozotocin-induced diabetic rats	Excision wound model (wound diameter is 10 mm)	10% gel (topically applied) and 200 mg/kg (p.o)	Topically applied and orally	once a day for 14 days	DW healing is accelerated	[147]
	embelin	Increased the level of hexosamine	Streptozotocin-induced diabetic rats	Excision wound model (wound diameter is 2 cm) and Incision Incision wound model (para-vertebral straight incisions of 5 cm Length and closed with interrupted sutures 1 cm apart)	5% embelin-cream(topically applied), 25 and 50 mg/kg (p.o)	Topically applied and p.o	Once a day	Wound contraction was significantly increased and epithelialization was promoted;Diabetic wounds healing is accelerated	[148]
	Withania coagulans	Decreased level of hexosamine	Streptozotocin-induced diabetic rats	Excision wound	10% w/w cream (Topically applied), 500 mg/kg (p.o.)	Topically applied and p.o.	Once a day for 16 days	DW healing is accelerated	[149]

high-throughput screening technology to efficiently and accurately screen natural biologics targeting AGEs-RAGE pathways, and using combination therapy may be an important direction to promote DFU repair.

4.3 PKC Pathways

Abnormal activation of PKC is an important pathological mechanism for the occurrence and development of diabetes and its complications. Ruboxistaurin successfully reversed endothelial progenitor cell dysfunction and prevented the excessive formation of NETs by targeting PKC βII activation [82]. However, the safety of Ruboxistaurin is questionable. A clinical study showed that 87.5% of volunteers experienced one or more treatment-emergent adverse events during the study period such as shoulder pain, Dupuytren contracture and upper respiratory infection [153]. Therefore, the search for safer and more efficient PKC-targeted inhibitors may become a new direction for promoting the repair of DFU. As previously mentioned, natural biologics have become the priority choice for many researchers to study disease treatment strategies due to their rich sources, affordability, low toxicity, and side effects. Although there are few direct studies on natural biologics targeting PKC to promote the healing of DFU, we found through a literature search that there are many natural biologics that can improve diabetes and its complications by regulating PKC. For example, wogonin can prevent hyperglycemia by blocking PKC activation via diacylglycerol [154]. Curcumin [137], Breviscapine [155] and Pueraria tuberosa extract [138] play a beneficial role in diabetic nephropathy by regulating PKC. Sasa borealis water-extract blocks chronic high glucose-induced endothelial apoptosis by the blunting activation of βII and NADPH oxidase promoted [139]. Furthermore, verbascoside downregulated expression of the inflammatory cytokines IL-6 and IL-1β by inhibiting PKC/HMGB1/RAGE/NFκB signaling and ultimately may be beneficial to oral wound healing in diabetic patients [140]. From these findings, we speculate that these PKC-targeted natural biologics for diabetes and its other complications may be potential strategies for DFU therapy because of their same or similar pathogenesis.

4.4 Polyol Pathways

There is no doubt that an abnormal polyol signaling pathway plays a harmful role in DFU repair. Normalizing the polyol signaling pathway may have unexpected benefits for the repair of DFU. As mentioned above, an abnormal polyol signaling pathway will reduce the function of GSH reductase and damage the antioxidant capacity [86]. Ag-NPs, produced by a unicellular spherical cyanobacterium with photo- and hetero-trophic capabilities, also accelerate DW repair by intensifying GSH peroxidase activities and GSH content [142]. In addition to its high antioxidant capacity, GSH can help to normalize the immune microenvironment [156]. Of course, due to the low toxicity and side

effects of natural biologics, they are easy to clinically convert. For example, in a clinical trial, topical application of propolis to DFU also accelerated wound repair by increasing the the GSH and GSH/glutathione disulfide (GSSG) ratio [143]. Moreover, Deniz Bagdas [141] confirmed that systemic antioxidant therapy with chlorogenic acid, a dietary antioxidant, can ultimately promote DW healing by increasing reduced GSH. Dietary care, an important auxiliary strategy to maintain the body's blood glucose and redox balance [157–159], combined with other treatment strategies may result in better outcomes. Because oxidative stress may impair wound healing through neuropathy and lead to loss of protective sensation for patients with chronic DFU, drugs that target polyol pathways in natural biologics to reverse neuropathy are also being sought. Suman Samaddar and Raju Koneri [144] confirmed that the polyphenolic fraction, isolated from Symphyocladia latiuscula, can inhibit aldose reductase activity and expression, thereby improving peripheral neuropathy. Therefore, it can be inferred that targeting the polyol pathway may be an effective auxiliary strategy for promoting DFU healing. However, although there are many natural biologics targeting the polyol signaling pathway [160,161], there are few studies on the application of DFU, and more relevant studies are focused on diabetic retinopathy. For example, total lignans from Fructuse Arctii improve diabetic retinopathy by inhibiting aldose reductase [162]. Moreover, resveratrol (3,4',5-trihydroxy-stilbene) [163] and zinc supplementation [164] can also target the polyol pathway to improve diabetic lens lesions. This may be due to the complex mechanisms such as an abnormal inflammatory response, microvascular damage, neuropathy, and local infection, which cause diabetic feet to be difficult to heal or even nonhealing. There are too many confounding factors to exclude when researchers conduct experiments. It is also possible that DFU, unlike retinopathy, will greatly threaten the patient's life in a short period of time. However, as an increasing number of people suffer from the high incidence and disability rate of DFU, there will surely be an increasing number of studies on improving the natural biologics of diabetic foot via the polyol pathway effects of natural biologics in the future.

4.5 Hexosamine Pathways

As mentioned above, for diabetic patients, the increase in hexosamine flux in the body leads to an increase in the flux through OGT, which in turn increases the level of O-GlcNAc [99,105]. Lowering protein O-GlcNAc expression reverses delayed wound closure caused by hyperglycemia [117]. However, studies on natural biologics targeting O-GlcNAc to promote DFU healing are lacking. Interestingly, it was reported that the hexosamine of DW tissue decreased compared with the normal control group in streptozotocin-induced diabetic rats [146]. Topical application of apple peel extract (APE) significantly increased hydroxyproline and hexosamine levels, thereby enhancing the DW healing
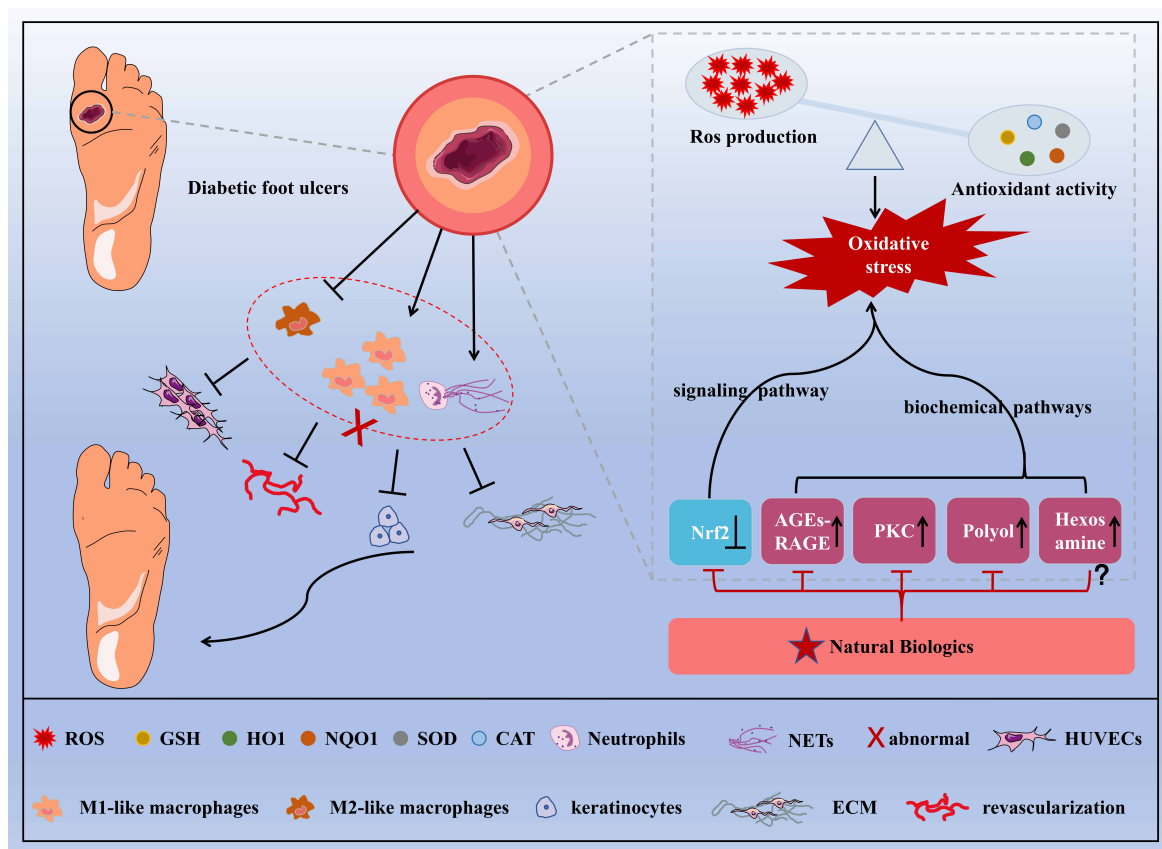
Fig. 1. The potential therapy of natural biologics for DFU. Oxidative damage plays an important role in the pathology of DFU, including leading to arrest DFU healing in the inflammatory stage, which then impairs wound proliferation and remodeling. Nrf2 signaling Pathway and AGEs-RAGE, PKC, Polyol and Hexosamine biochemical pathways are key regulatory targets in DFU. The treatment of natural biologics with antioxidant properties, targeting these signaling pathways and biochemical pathways, can effectively improve the inefficient inflammatory response of DFU, promote the smooth progress of wound proliferation and remodeling, and finally promote the healing of DFU.

process [145]. The application of natural biologics, such as ethanolic extracts of Melia dubia [146] and Solanum xanthocarpum [147] (more examples are shown in the Table 1), during the wound repair in diabetic rats promotes the deposition of hexosamine, which eventually accelerates the wound repair. Theoretically, increased activation of the HP leads to an increase in its downstream product, O-GlcNAc, which impairs wound repair. This is contrary to the results of increased HP flux in diabetic patients. One important reason may be caused by the different model construction and detection methods. Clinical diabetes is often divided into type I and type II. Type I diabetes is mainly caused by immune-mediated destruction of pancreatic beta cells, and patients with type II diabetes face both insulin resistance and relative insulin deficiency. The pathogenesis of type I and type II diabetes is different, and the method of constructing animal models is also different. It is critical to construct and select a full-thickness injury model of diabetic rat skin specifically. Moreover, direct evidence for changes in HP flux in DFU is lacking. There may be other regulatory mechanisms of HP in DFU. Therefore, in order to better

study clinical DFU, it is necessary to collect clinical specimens, use emerging technologies to seek direct evidence, and conduct an indepth study of the specific mechanisms of the onset and difficult healing of DW.

5. Conclusion and Prospects

These studies have shown that continuous exposure to hyperglycemia leads to abnormalities in the Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine biochemical pathways, which results in excessive oxidative stress and an imbalance of redox levels in the body. Excessive oxidative stress on the wound will trigger the release of proinflammatory mediators and trigger an inflammatory cascade, which will cause DFU to be in a stage of persistent low-efficiency inflammatory response state and impair the proliferation and remodeling stages of wound repair. These factors are the bases for the onset, progression, and deterioration of DFU and therapies targeting these pathways have been shown to be effective in promoting the healing of DFU (Fig. 1). This review not only describes the pathology and key mechanisms of oxidative stress in DFU, natural bio-

logics targeting Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine biochemical pathways promoting the healing of DFU are also summarized. Some of these natural biologics have been tested in DW models with promising results. They show the therapeutic potential of antioxidation and anti-inflammatory, promoting proliferation and remodeling, and some natural biologics can also promote wound angiogenesis and improve peripheral neuropathy.

However, targeted natural biologics promoting DFU are more focused on the Nrf2, AGEs-RAGE and HP, while direct studies on the other pathways are currently a shortcoming, but there are many studies on other complications of diabetes (similar to the pathogenesis of DFU). It should also be noted that the specific mechanism of the HP in promoting DW repair is still unclear. Filling in the research gap is bound to happen. Furthermore, due to heterogeneity in animal selection, model construction, blood glucose levels, dosing start, end time, route of administration, frequency of administration, we were unable to identify which natural biologics show better efficacy in the treatment of DFU. The bioavailability, stability and solubility of these natural biologics *in vivo* need further confirmation. In addition, hyperglycemia is closely related to oxidative stress. Hyperglycemia can upregulate markers of chronic inflammation and contribute to increased ROS generation, which ultimately cause pathological oxidative stress. Conversely, pathological oxidative stress can lead to insulin resistance and impaired insulin secretion [165]. Proper treatment of hyperglycemia and inhibition of pathological oxidative stress are crucial for the healing of DFU. On the basis of proper control of hyperglycemia (including dietary care [166]), the application of natural biologics may achieve better curative effect. And in the study of DFU, it is important not only to focus on the effect of natural biologics on DFU healing, but also consider whether they have an effect on blood glucose level. Of course, many synthetic compounds have been produced as antidiabetic agents [167], and it is also an important direction to search for drugs that can promote DFU healing from these antidiabetic agents. Metformin [168] and insulin [169] are good examples. Moreover, seeking high-efficiency drug delivery systems (such as bioactive nanoparticle delivery systems) and combination therapy of multiple pathways (rather than targeting one pathway alone) are better options for promoting the repair of DFU. However, greater thought, investigation, and verification are still required for both drug selection (based on targets such as signaling networks or metabolic pathways or on different stages of wound healing) and the order of administration. However, it is certain that natural biologics have a wide range of sources and excellent cost effectiveness, which can reduce the economic burden of DFU patients to a certain extent. Therefore, it is crucial to devise strategies to study the effects of these natural biologics on patients with DFU, ethically, without compromising patient interests and obtaining informed consent.

Abbreviations

DFU, diabetic foot ulcers; Nrf2, nuclear factor erythroid 2-related factor; AGEs, advanced glycated products; RAGEs, receptor For Advanced Glycation Endproducts; PKC, protein kinase C; DM, diabetes mellitus ; ROS, reactive oxygen species; NETs, neutrophils extracellular traps; DW, diabetic wounds; VEGF, endothelial growth factor; TGF-β1, transforming growth factor β1; KGF-1, keratinocyte growth factor 1; CAT, catalase; HO1, heme oxygenase 1; NQO1, quinone oxidoreductase 1; ERK1/2, extracellular signal-regulated kinase; SAPK/ JNK, stress-activated protein kinase/cJun N-terminal kinase; MAPK, mitogen-activated protein kinase; JAK/STAT, Janus kinase signal transducer and activator of transcription; AKAP12, a anchoring protein 12; eDNA, extracellular DNA; PKCs, protein kinase C isozymes; NADPH, nicotinamide adenine dinucleotide phosphate; AR, aldolase reductase; GSH, glutathione; HP, hexosamine pathway; UDPGlcNAc, UDP-N-acetylglucosamine; OGT, O-Glycosyltransferase; Nef, neferine; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; ECM, extracellular matrix; MC, momordica charantia; SalA, salvianolic acid A; GSSG, glutathione disulfide; APE, apple peel extract; EPC, endothelial progenitor cell; p.o, per os; eNOS, endothelin nictric oxide synthase; S-G, Smulow-Glickman.

Author Contributions

QC, JS and AL conceptualized this study and wrote the manuscript. BL and WH created the figures and tables. ZJ, XB, LH and SZ contributed to the literature search and provided help and advice on grammar. SG, JW and QC reviewed and modified the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Funding

IMR Press

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Research and Clinical Practice. 2022; 183: 109119.

[2] Armstrong DG, Boulton AJM, Bus SA. Diabetic Foot Ulcers and their Recurrence. New England Journal of Medicine. 2017; 376: 2367–2375.

[3] Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and Trends in Diabetes among Adults in the United States, 1988-2012. The Journal of the American Medical Association. 2015; 314: 1021.

[4] Lin CH, Armstrong DG, Liu PH, Lin CW, Huang CH, Huang YY. Survival of Patients Following First Diagnosis of Diabetic Foot Complications: A Nationwide 15-Year Longitudinal Analysis. Frontiers in Endocrinology. 2021; 12: 801324.

[5] Armstrong DG, Swerdlow MA, Armstrong AA, Conte MS, Padula WV, Bus SA. Five year mortality and direct costs of care for people with diabetic foot complications are comparable to cancer. Journal of Foot and Ankle Research. 2020; 13: 16.

[6] Schaper NC, Netten JJ, Apelqvist J, Bus SA, Hinchliffe RJ, Lipsky BA. Practical Guidelines on the prevention and management of diabetic foot disease (IWGDF 2019 update) Diabetes/Metabolism Research and Reviews. 2020; 36: e3266.

[7] Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, et al. The Role of Oxidative Stress and Antioxidants in Diabetic Wound Healing. Oxidative Medicine and Cellular Longevity. 2021; 2021: 1–11.

[8] Gordillo GM, Sen CK. Revisiting the essential role of oxygen in wound healing. the American Journal of Surgery. 2003; 186: 259–263.

[9] Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. International Wound Journal. 2017; 14: 89–96.

[10] Liu Z, Hu L, Zhang T, Xu H, Li H, Yang Z, et al. PKCbeta increases ROS levels leading to vascular endothelial injury in diabetic foot ulcers. American Journal of Translational Research. 2020; 12: 6409–6421.

[11] Uddin MJ, Kim EH, Hannan MA, Ha H. Pharmacotherapy against Oxidative Stress in Chronic Kidney Disease: Promising Small Molecule Natural Products Targeting Nrf2-HO-1 Signaling. Antioxidants (Basel). 2021; 10: 258.

[12] Babel RA, Dandekar MP. A Review on Cellular and Molecular Mechanisms Linked to the Development of Diabetes Complications. Current Diabetes Reviews. 2021; 17: 457–473.

[13] Sies H, Jones D. Oxidative Stress. Encyclopedia of Stress. 2007; 90: 45–48.

[14] Ferreira F, Raghunathan V, Luxardi G, Zhu K, Zhao M. Early redox activities modulate Xenopus tail regeneration. Nature Communications. 2018; 9: 4296.

[15] Kraakman MJ, Lee MKS, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, et al. Neutrophil-derived S100 calcium-binding proteins a8/a9 promote reticulated thrombocytosis and atherogenesis in diabetes. Journal of Clinical Investigation. 2017; 127: 2133–2147.

[16] Johnson J, Jaggers RM, Gopalkrishna S, Dahdah A, Murphy AJ, Hanssen NMJ, et al. Oxidative Stress in Neutrophils: Implications for Diabetic Cardiovascular Complications. Antioxidants Redox Signaling. 2022; 36: 652–666.

[17] Nagareddy P, Murphy A, Stirzaker R, Hu Y, Yu S, Miller R, et al. Hyperglycemia Promotes Myelopoiesis and Impairs the Resolution of Atherosclerosis. Cell Metabolism. 2013; 17: 695–708.

[18] Mikhalchik EV, Lipatova VA, Basyreva LY, Panasenko OM, Gusev SA, Sergienko VI. Hyperglycemia and some Aspects of Leukocyte Activation in vitro. Bulletin of Experimental Biology and Medicine. 2021; 170: 748–751.

[19] Sabbatini M, Magnelli V, Reno F. NETosis in Wound Healing: When Enough Is Enough. Cells. 2021; 10: 494.

[20] Lee YS, Kang SU, Lee M, Kim H, Han C, Won H, et al. GnRH impairs diabetic wound healing through enhanced NETosis. Cellular Molecular Immunology. 2020; 17: 856–864.

[21] Huang W, Jiao J, Liu J, Huang M, Hu Y, Ran W, et al. MFG-E8 accelerates wound healing in diabetes by regulating "NLRP3 inflammasome-neutrophil extracellular traps" axis. Cell Death Discovery. 2020; 6: 84.

[22] Song J, Hu L, Liu B, Jiang N, Huang H, Luo J, et al. The Emerging Role of Immune Cells and Targeted Therapeutic Strategies in Diabetic Wounds Healing. Journal of Inflammation Research. 2022; 15: 4119–4138.

[23] Song C, Li H, Li Y, Dai M, Zhang L, Liu S, et al. NETs promote ALI/ARDS inflammation by regulating alveolar macrophage polarization. Experimental Cell Research. 2019; 382: 111486.

[24] Montazeri RS, Fatahi S, Sohouli MH, Abu-Zaid A, Santos HO, Găman M, et al. The effect of nigella sativa on biomarkers of inflammation and oxidative stress: a systematic review and meta-analysis of randomized controlled trials. Journal of Food Biochemistry. 2021; 45: e13625.

[25] Hou X, Yang S, Yin J. Blocking the REDD1/TXNIP axis ameliorates LPS-induced vascular endothelial cell injury through repressing oxidative stress and apoptosis. American Journal of Physiology-Cell Physiology. 2019; 316: C104–C110.

[26] Shaito A, Aramouni K, Assaf R, Parenti A, Orekhov A, Yazbi AE, et al. Oxidative Stress-Induced Endothelial Dysfunction in Cardiovascular Diseases. Frontiers in Bioscience-Landmark. 2022; 27: 0105.

[27] Xiong Y, Chen L, Liu P, Yu T, Lin C, Yan C, et al. All-in-one: Multifunctional Hydrogel Accelerates Oxidative Diabetic Wound Healing through Timed-Release of Exosome and Fibroblast Growth Factor. Small. 2022; 18: 2104229.

[28] Florin L, Maas-Szabowski N, Werner S, Szabowski A, Angel P. Increased keratinocyte proliferation by JUN-dependent expression of PTN and SDF-1 in fibroblasts. Journal of Cell Science. 2005; 118: 1981–1989.

[29] Hosseini Mansoub N. The role of keratinocyte function on the defected diabetic wound healing. International Journal of Burns and Trauma. 2021; 11: 430–441.

[30] Sun X, Wang X, Zhao Z, Chen J, Li C, Zhao G. Paeoniflorin accelerates foot wound healing in diabetic rats though activating the Nrf2 pathway. Acta Histochemica. 2020; 122: 151649.

[31] Peng Y, Wu S, Tang Q, Li S, Peng C. KGF-1 accelerates wound contraction through the TGF-beta1/Smad signaling pathway in a double-paracrine manner. The Journal of Biological Chemistry. 2019; 294: 8361–8370.

[32] Peng C, Chen B, Kao H, Murphy G, Orgill DP, Guo L. Lack of FGF-7 further Delays Cutaneous Wound Healing in Diabetic Mice. Plastic and Reconstructive Surgery. 2011; 128: 673e–684e.

[33] Hinz B. Formation and Function of the Myofibroblast during

Tissue Repair. Journal of Investigative Dermatology. 2007; 127: 526–537.

[34] Zhang F, Ren Y, Liu P, Ren Y, Wang D. Expression of TGF-beta1 and miRNA-145 in patients with diabetic foot ulcers. Experimental and Therapeutic Medicine. 2016; 11: 2011–2014.

[35] Demyanenko IA, Zakharova VV, Ilyinskaya OP, Vasilieva TV, Fedorov AV, Manskikh VN, *et al*. Mitochondria-Targeted Antioxidant SkQ1 Improves Dermal Wound Healing in Genetically Diabetic Mice. Oxidative Medicine and Cellular Longevity. 2017; 2017: 1–10.

[36] Ma Q. Role of Nrf2 in Oxidative Stress and Toxicity. Annual Review of Pharmacology and Toxicology. 2013; 53: 401–426.

[37] Li M, Yu H, Pan H, Zhou X, Ruan Q, Kong D, *et al*. Nrf2 Suppression Delays Diabetic Wound Healing Through Sustained Oxidative Stress and Inflammation. Frontiers in Pharmacology. 2019; 10: 1099.

[38] Dinkova-Kostova AT, Abramov AY. The emerging role of Nrf2 in mitochondrial function. Free Radical Biology and Medicine. 2015; 88: 179–188.

[39] Sivinski J, Zhang DD, Chapman E. Targeting NRF2 to treat cancer. Seminars in Cancer Biology. 2021; 76: 61–73.

[40] Qu Z, Sun J, Zhang W, Yu J, Zhuang C. Transcription factor NRF2 as a promising therapeutic target for Alzheimer's disease. Free Radical Biology and Medicine. 2020; 159: 87–102.

[41] Chen K, Wang S, Sun QW, Zhang B, Ullah M, Sun Z. Klotho Deficiency Causes Heart Aging via Impairing the Nrf2-GR Pathway. Circulation Research. 2021; 128: 492–507.

[42] Long M, Rojo de la Vega M, Wen Q, Bharara M, Jiang T, Zhang R, *et al*. An Essential Role of NRF2 in Diabetic Wound Healing. Diabetes. 2016; 65: 780–793.

[43] Victor P, Sarada D, Ramkumar KM. Pharmacological activation of Nrf2 promotes wound healing. European Journal of Pharmacology. 2020; 886: 173395.

[44] Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, *et al*. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Development. 1999; 13: 76–86.

[45] Hiebert P, Werner S. Regulation of Wound Healing by the NRF2 Transcription Factor-More Than Cytoprotection. International Journal of Molecular Sciences. 2019; 20: 3856.

[46] Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2-Keap1 signaling in oxidative and reductive stress. Biochimica Et Biophysica Acta (BBA)-Molecular Cell Research. 2018; 1865: 721–733.

[47] Teena R, Dhamodharan U, Jayasuriya R, Ali D, Kesavan R, Ramkumar KM. Analysis of the Exonic Single Nucleotide Polymorphism rs182428269 of the NRF2 Gene in Patients with Diabetic Foot Ulcer. Archives of Medical Research. 2021; 52: 224–232.

[48] Braun S, Hanselmann C, Gassmann MG, auf dem Keller U, Born-Berclaz C, Chan K, *et al*. Nrf2 Transcription Factor, a Novel Target of Keratinocyte Growth Factor Action which Regulates Gene Expression and Inflammation in the Healing Skin Wound. Molecular and Cellular Biology. 2002; 22: 5492–5505.

[49] Li Y, Ma F, Li H, Song Y, Zhang H, Jiang Z, *et al*. Dimethyl fumarate accelerates wound healing under diabetic condition. Journal of Molecular Endocrinology. 2018; 61: 163–172.

[50] Jayasuriya R, Dhamodharan U, Karan AN, Anandharaj A, Rajesh K, Ramkumar KM. Role of Nrf2 in MALAT1/ HIF-1alpha loop on the regulation of angiogenesis in diabetic foot ulcer. Free Radical Biology & Medicine. 2020; 156: 168–175.

[51] Dhamodharan U, Karan A, Sireesh D, Vaishnavi A, Somasundar A, Rajesh K, *et al*. Tissue-specific role of Nrf2 in the treatment of diabetic foot ulcers during hyperbaric oxygen therapy. Free Radical Biology and Medicine. 2019; 138: 53–62.

[52] Del Turco S, Basta G. An update on advanced glycation end-products and atherosclerosis. BioFactors. 2012; 38: 266–274.

[53] Zhang Q, Ames JM, Smith RD, Baynes JW, Metz TO. A Perspective on the Maillard Reaction and the Analysis of Protein Glycation by Mass Spectrometry: Probing the Pathogenesis of Chronic Disease. Journal of Proteome Research. 2009; 8: 754–769.

[54] Khalid M, Petroianu G, Adem A. Advanced Glycation End Products and Diabetes Mellitus: Mechanisms and Perspectives. Biomolecules. 2022; 12: 542.

[55] GBD 2015 Tobacco Collaborators.. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: a systematic analysis from the Global Burden of Disease Study 2015. Lancet. 2017; 389: 1885–1906.

[56] Hirschi-Budge KM, Tsai KYF, Curtis KL, Davis GS, Theurer BK, Kruyer AMM, *et al*. RAGE signaling during tobacco smoke-induced lung inflammation and potential therapeutic utility of SAGEs. BMC Pulmonary Medicine. 2022; 22: 160.

[57] Koschinsky T, He C, Mitsuhashi T, Bucala R, Liu C, Buenting C, *et al*. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. Proceedings of the National Academy of Sciences. 1997; 94: 6474–6479.

[58] Rojubally S, Simoneau A, Monlun M, Foussard N, Blanco L, Domenge F, *et al*. For diabetic type 1 patients, the skin autofluorescence predicts ulcers and amputations. Journal of Diabetes and its Complications. 2021; 35: 107808.

[59] Yuan L, Sun Y, Xu M, Zeng F, Xiong X. MiR-203 Acts as an Inhibitor for Epithelial-Mesenchymal Transition Process in Diabetic Foot Ulcers via Targeting Interleukin-8. Neuroimmunomodulation. 2019; 26: 239–249.

[60] Mahmoudinezhad M, Farhangi MA, Kahroba H, Dehghan P. Personalized diet study of dietary advanced glycation end products (AGEs) and fatty acid desaturase 2 (FADS2) genotypes in obesity. Scientific Reports. 2021; 11: 19725.

[61] Kang Y, Zheng C, Ye J, Song F, Wang X, Liu Y, *et al*. Effects of advanced glycation end products on neutrophil migration and aggregation in diabetic wounds. Aging. 2021; 13: 12143–12159.

[62] Jiang M, Yakupu A, Guan H, Dong J, Liu Y, Song F, *et al*. Pyridoxamine ameliorates methylglyoxal-induced macrophage dysfunction to facilitate tissue repair in diabetic wounds. International Wound Journal. 2022; 19: 52–63.

[63] Wan L, Bai X, Zhou Q, Chen C, Wang H, Liu T, *et al*. The advanced glycation end-products (AGEs)/ROS/NLRP3 inflammasome axis contributes to delayed diabetic corneal wound healing and nerve regeneration. International Journal of Biological Sciences. 2022; 18: 809–825.

[64] Mao QY, He SY, Hu QY, Lu Y, Niu YX, Li XY, *et al*. Advanced Glycation End Products (AGEs) Inhibit Macrophage Efferocytosis of Apoptotic beta Cells through Binding to the Receptor for AGEs. Journal of Immunology. 2022; 208: 1204–1213.

[65] Zhang H, Wen T, Cai Y. Overexpression of miR-146a promotes cell proliferation and migration in a model of diabetic foot ulcers by regulating the AKAP12 axis. Endocrine Journal. 2022; 69: 85–94.

[66] Guillon C, Ferraro S, Clément S, Bouschbacher M, Sigaudo-Roussel D, Bonod C. Glycation by glyoxal leads to profound changes in the behavior of dermal fibroblasts. BMJ Open Diabetes Research Care. 2021; 9: e002091.

[67] Zhu P, Chen C, Wu D, Chen G, Tan R, Ran J. AGEs-induced MMP-9 activation mediated by Notch1 signaling is involved in impaired wound healing in diabetic rats. Diabetes Research and Clinical Practice. 2022; 186: 109831.

[68] Liu C, Xu L, Gao H, Ye J, Huang Y, Wu M, *et al*. The Association between Skin Autofluorescence and Vascular Complications in Chinese Patients with Diabetic Foot Ulcer. the International Journal of Lower Extremity Wounds. 2015; 14: 28–36.

[69] Tanaka K, Tani Y, Asai J, Nemoto F, Kusano Y, Suzuki H, *et al*. Skin autofluorescence is associated with severity of vascular complications in Japanese patients with Type 2 diabetes. Diabetic Medicine. 2012; 29: 492–500.

[70] Papachristou S, Pafili K, Papanas N. Skin AGEs and diabetic neuropathy. BMC Endocrine Disorders. 2021; 21: 28.

[71] Xie X, Liu X, Li Y, Luo L, Yuan W, Chen B, *et al*. Advanced Glycation End Products Enhance Biofilm Formation by Promoting Extracellular DNA Release Through sigB Upregulation in Staphylococcus aureus. Frontiers in Microbiology. 2020; 11: 1479.

[72] Yuan T, Yang T, Chen H, Fu D, Hu Y, Wang J, *et al*. New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. Redox Biology. 2019; 20: 247–260.

[73] Giacco F, Brownlee M. Oxidative Stress and Diabetic Complications. Circulation Research. 2010; 107: 1058–1070.

[74] Newton AC. Protein kinase C: poised to signal. American Journal of Physiology-Endocrinology and Metabolism. 2010; 298: E395–E402.

[75] Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. the FASEB Journal. 1995; 9: 484–496.

[76] Newton AC, Brognard J. Reversing the Paradigm: Protein Kinase C as a Tumor Suppressor. Trends in Pharmacological Sciences. 2017; 38: 438–447.

[77] Tobias IS, Newton AC. Protein Scaffolds Control Localized Protein Kinase Czeta Activity. The Journal of Biological Chemistry. 2016; 291: 13809–13822.

[78] Antal CE, Callender JA, Kornev AP, Taylor SS, Newton AC. Intramolecular C2 Domain-Mediated Autoinhibition of Protein Kinase C betaII. Cell Reports. 2015; 12: 1252–1260.

[79] Du X, Matsumura T, Edelstein D, Rossetti L, Zsengellér Z, Szabó C, *et al*. Inhibition of GAPDH activity by poly (ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. Journal of Clinical Investigation. 2003; 112: 1049–1057.

[80] Nauseef WM, Volpp BD, McCormick S, Leidal KG, Clark RA. Assembly of the neutrophil respiratory burst oxidase. Protein kinase C promotes cytoskeletal and membrane association of cytosolic oxidase components. Journal of Biological Chemistry. 1991; 266: 5911–5917.

[81] Fadini GP, Menegazzo L, Scattolini V, Gintoli M, Albiero M, Avogaro A. A perspective on NETosis in diabetes and cardiometabolic disorders. Nutrition, Metabolism and Cardiovascular Diseases. 2016; 26: 1–8.

[82] Das SK, Yuan YF, Li MQ. Specific PKC betaII inhibitor: one stone two birds in the treatment of diabetic foot ulcers. Bioscience Reports. 2018; 38: BSR20171459.

[83] Liu Y, Chen Z, Liu L, Tang H, Zhu H, Tang S. Role of moesin in the effect of glucagon-like peptide-1 on advanced glycation end products-induced endothelial barrier dysfunction. Cellular Signalling. 2022; 90: 110193.

[84] Khamaisi M, Katagiri S, Keenan H, Park K, Maeda Y, Li Q, *et al*. PKCdelta inhibition normalizes the wound-healing capacity of diabetic human fibroblasts. The Journal of Clinical Investigation. 2016; 126: 837–853.

[85] Abdelkader NF, Ibrahim SM, Moustafa PE, Elbaset MA. Inosine mitigated diabetic peripheral neuropathy via modulating GLO1/AGEs/RAGE/NF-kappaB/Nrf2 and TGF-beta/PKC/TRPV1 signaling pathways. Biomedicine & Pharmacotherapy. 2022; 145: 112395.

[86] Leley SP, Ciulla TA, Bhatwadekar AD. Diabetic Retinopathy in the Aging Population: A Perspective of Pathogenesis and Treatment. Clinical Interventions in Aging. 2021; 16: 1367-1378.

[87] Srikanth KK, Orrick JA. Biochemistry, Polyol or Sorbitol Pathways. 2021. Available at https://www.ncbi.nlm.nih.gov/books/NBK576381/ (Accessed: 13 July 2022).

[88] Obrosova IG. Increased Sorbitol Pathway Activity Generates Oxidative Stress in Tissue Sites for Diabetic Complications. Antioxidants Redox Signaling. 2005; 7: 1543–1552.

[89] Singh M, Kapoor A, Bhatnagar A. Physiological and Pathological Roles of Aldose Reductase. Metabolites. 2021; 11: 655.

[90] Narayanan S. Aldose reductase and its inhibition in the control of diabetic complications. Annals of Clinical and Laboratory Science. 1993; 23: 148–158.

[91] Mudge BP, Harris C, Gilmont RR, Adamson BS, Rees RS. Role of glutathione redox dysfunction in diabetic wounds. Wound Repair and Regeneration. 2002; 10: 52–58.

[92] Dhall S, Do DC, Garcia M, Kim J, Mirebrahim SH, Lyubovitsky J, *et al*. Generating and Reversing Chronic Wounds in Diabetic Mice by Manipulating Wound Redox Parameters. Journal of Diabetes Research. 2014; 2014: 1–18.

[93] Asano S, Himeno T, Hayami T, Motegi M, Inoue R, Nakai-Shimoda H, *et al*. Ranirestat Improved Nerve Conduction Velocities, Sensory Perception, and Intraepidermal Nerve Fiber Density in Rats with Overt Diabetic Polyneuropathy. Journal of Diabetes Research. 2019; 2019: 1–7.

[94] Yan L. Redox imbalance stress in diabetes mellitus: Role of the polyol pathway. Animal Models and Experimental Medicine. 2018; 1: 7–13.

[95] Modaghegh MHS, Saberianpour S, Amoueian S, Shahri JJ, Rahimi H. The effect of redox signaling on extracellular matrix changes in diabetic wounds leading to amputation. Biochemistry and Biophysics Reports. 2021; 26: 101025.

[96] Mou L, Hu P, Cao X, Chen Y, Xu Y, He T, *et al*. Comparison of bovine serum albumin glycation by ribose and fructose *in vitro* and *in vivo*. Biochimica Et Biophysica Acta (BBA)-Molecular Basis of Disease. 2022; 1868: 166283.

[97] Figueroa-Romero C, Sadidi M, Feldman EL. Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. Reviews in Endocrine and Metabolic Disorders. 2008; 9: 301–314.

[98] Denzel MS, Antebi A. Hexosamine pathway and (ER) protein quality control. Current Opinion in Cell Biology. 2015; 33: 14–18.

[99] Ighodaro OM. Molecular pathways associated with oxidative stress in diabetes mellitus. Biomedicine Pharmacotherapy. 2018; 108: 656–662.

[100] Akella NM, Ciraku L, Reginato MJ. Fueling the fire: emerging role of the hexosamine biosynthetic pathway in cancer. BMC Biology. 2019; 17: 52.

[101] McClain DA, Lubas WA, Cooksey RC, Hazel M, Parker GJ, Love DC, *et al*. Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. Proceedings of the National Academy of Sciences. 2002; 99: 10695–10699.

[102] Park K, Saudek CD, Hart GW. Increased expression of beta-N-acetylglucosaminidase in erythrocytes from individuals with pre-diabetes and diabetes. Diabetes. 2010; 59: 1845–1850.

[103] Gurel Z, Sieg KM, Shallow KD, Sorenson CM, Sheibani N. Retinal O-linked N-acetylglucosamine protein modifications: implications for postnatal retinal vascularization and the pathogenesis of diabetic retinopathy. Molecular Vision. 2013; 19: 1047–1059.

[104] Marshall S, Bacote V, Traxinger RR. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. Journal of Biological Chemistry. 1991; 266: 4706–4712.

[105] Padberg I, Peter E, Gonzalez-Maldonado S, Witt H, Mueller M, Weis T, *et al*. A new metabolomic signature in type-2 diabetes mellitus and its pathophysiology. PLoS ONE. 2014; 9: e85082.

[106] Zhang D, Cai Y, Chen M, Gao L, Shen Y, Huang Z. OGT-mediated O-GlcNAcylation promotes NF-kappaB activation and inflammation in acute pancreatitis. Inflammation Research.

2015; 64: 943–952.

[107] Liu B, Salgado OC, Singh S, Hippen KL, Maynard JC, Burlingame AL, *et al*. The lineage stability and suppressive program of regulatory T cells require protein O-GlcNAcylation. Nature Communications. 2019; 10: 354.

[108] Li X, Gong W, Wang H, Li T, Attri KS, Lewis RE, *et al*. O-GlcNAc Transferase Suppresses Inflammation and Necroptosis by Targeting Receptor-Interacting Serine/Threonine-Protein Kinase 3. Immunity. 2019; 50: 576–590.e6.

[109] Yang Y, Li X, Luan HH, Zhang B, Zhang K, Nam JH, *et al*. OGT suppresses S6K1-mediated macrophage inflammation and metabolic disturbance. Proceedings of the National Academy of Sciences. 2020; 117: 16616–16625.

[110] Theocharidis G, Thomas BE, Sarkar D, Mumme HL, Pilcher WJR, Dwivedi B, *et al*. Single cell transcriptomic landscape of diabetic foot ulcers. Nature Communications. 2022; 13: 181.

[111] Sawaya AP, Stone RC, Brooks SR, Pastar I, Jozic I, Hasneen K, *et al*. Deregulated immune cell recruitment orchestrated by FOXM1 impairs human diabetic wound healing. Nature Communications. 2020; 11: 4678.

[112] Luo B, Soesanto Y, McClain DA. Protein Modification by O-Linked GlcNAc Reduces Angiogenesis by Inhibiting Akt Activity in Endothelial Cells. Arteriosclerosis, Thrombosis, and Vascular Biology. 2008; 28: 651–657.

[113] Lima VV, Giachini FRC, Choi H, Carneiro FS, Carneiro ZN, Fortes ZB, *et al*. Impaired Vasodilator Activity in Deoxycorticosterone Acetate-Salt Hypertension is Associated with Increased Protein O-GlcNAcylation. Hypertension. 2009; 53: 166–174.

[114] Huang S, Wu C, Chiu M, Yang H, Chen G, Lan CE. High-glucose environment induced intracellular O-GlcNAc glycosylation and reduced galectin-7 expression in keratinocytes: Implications on impaired diabetic wound healing. Journal of Dermatological Science. 2017; 87: 168–175.

[115] Zhang J, Yang P, Liu D, Gao M, Wang J, Yu T, *et al*. Inhibiting Hyper-O-GlcNAcylation of c-Myc accelerate diabetic wound healing by alleviating keratinocyte dysfunction. Burns Trauma. 2021; 9: tkab031.

[116] Pedowitz NJ, Batt AR, Darabedian N, Pratt MR. MYPT1 O-GlcNAc modification regulates sphingosine-1-phosphate mediated contraction. Nature Chemical Biology. 2021; 17: 169–177.

[117] Runager K, Bektas M, Berkowitz P, Rubenstein DS. Targeting O-Glycosyltransferase (OGT) to Promote Healing of Diabetic Skin Wounds. Journal of Biological Chemistry. 2014; 289: 5462–5466.

[118] Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. Molecular Aspects of Medicine. 2011; 32: 234–246.

[119] Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nature Reviews Drug Discovery. 2015; 14: 111–129.

[120] Zhang DD, Chapman E. The role of natural products in revealing NRF2 function. Natural Product Reports. 2020; 37: 797–826.

[121] Plazas E, Avila M MC, Muñoz DR, Cuca S LE. Natural isoquinoline alkaloids: Pharmacological features and multi-target potential for complex diseases. Pharmacological Research. 2022; 177: 106126.

[122] Bharathi Priya L, Huang CY, Hu RM, Balasubramanian B, Baskaran R. An updated review on pharmacological properties of neferine-A bisbenzylisoquinoline alkaloid from Nelumbo nucifera. J Food Biochem. 2021; 45: e13986.

[123] Marthandam Asokan S, Mariappan R, Muthusamy S, Velmurugan BK. Pharmacological benefits of neferine - a comprehensive review. Life Sciences. 2018; 199: 60–70.

[124] Li J, Chou H, Li L, Li H, Cui Z. Wound healing activity of neferine in experimental diabetic rats through the inhibition of inflammatory cytokines and nrf-2 pathway. Artificial Cells, Nanomedicine, and Biotechnology. 2020; 48: 96–106.

[125] Zhou X, Ruan Q, Ye Z, Chu Z, Xi M, Li M, *et al*. Resveratrol accelerates wound healing by attenuating oxidative stress-induced impairment of cell proliferation and migration. Burns. 2021; 47: 133–139.

[126] Fan J, Liu H, Wang J, Zeng J, Tan Y, Wang Y, *et al*. Procyanidin B2 improves endothelial progenitor cell function and promotes wound healing in diabetic mice via activating Nrf2. Journal of Cellular and Molecular Medicine. 2021; 25: 652–665.

[127] Chen LY, Huang CN, Liao CK, Chang HM, Kuan YH, Tseng TJ, *et al*. Effects of Rutin on Wound Healing in Hyperglycemic Rats. Antioxidants. 2020; 9: 1122.

[128] Lu X, Liu M, Dong H, Miao J, Stagos D, Liu M. Dietary prenylated flavonoid xanthohumol alleviates oxidative damage and accelerates diabetic wound healing via Nrf2 activation. Food and Chemical Toxicology. 2022; 160: 112813.

[129] Lwin OM, Giribabu N, Kilari EK, Salleh N. Topical administration of mangiferin promotes healing of the wound of streptozotocin-nicotinamide-induced type-2 diabetic male rats. Journal of Dermatological Treatment. 2021; 32: 1039–1048.

[130] Xie W, Hu W, Huang Z, Li M, Zhang H, Huang X, *et al*. Betulinic acid accelerates diabetic wound healing by modulating hyperglycemia-induced oxidative stress, inflammation and glucose intolerance. Burns Trauma. 2022; 10: tkac007.

[131] Chen LY, Cheng HL, Kuan YH, Liang TJ, Chao YY, Lin HC. Therapeutic Potential of Luteolin on Impaired Wound Healing in Streptozotocin-Induced Rats. Biomedicines. 2021; 9: 761.

[132] Hozzein WN, Badr G, Badr BM, Allam A, Ghamdi AA, Al-Wadaan MA, *et al*. Bee venom improves diabetic wound healing by protecting functional macrophages from apoptosis and enhancing Nrf2, Ang-1 and Tie-2 signaling. Molecular Immunology. 2018; 103: 322–335.

[133] Alqahtani AS, Li KM, Razmovski-Naumovski V, Kam A, Alam P, Li GQ. Attenuation of methylglyoxal-induced glycation and cellular dysfunction in wound healing by Centella cordifolia. Saudi Journal of Biological Sciences. 2021; 28: 813–824.

[134] Fei J, Ling YM, Zeng MJ, Zhang KW. Shixiang Plaster, a Traditional Chinese Medicine, Promotes Healing in a Rat Model of Diabetic Ulcer Through the receptor for Advanced Glycation End Products (RAGE)/Nuclear Factor kappa B (NF-kappaB) and Vascular Endothelial Growth Factor (VEGF)/Vascular Cell Adhesion Molecule-1 (VCAM-1)/Endothelial Nitric Oxide Synthase (eNOS) Signaling Pathways. Medical Science Monitor. 2019; 25: 9446–9457.

[135] Aljohi A, Matou-Nasri S, Liu D, Al-Khafaji N, Slevin M, Ahmed N. Momordica charantia extracts protect against inhibition of endothelial angiogenesis by advanced glycation end-products *in vitro*. Food Function. 2018; 9: 5728–5739.

[136] Yang X, Sun L, Xu P, Gong L, Qiang G, Zhang L, *et al*. Effects of salvianolic scid a on plantar microcirculation and peripheral nerve function in diabetic rats. European Journal of Pharmacology. 2011; 665: 40–46.

[137] Soetikno V, Watanabe K, Sari FR, Harima M, Thandavarayan RA, Veeraveedu PT, *et al*. Curcumin attenuates diabetic nephropathy by inhibiting PKC-alpha and PKC-beta1 activity in streptozotocin-induced type I diabetic rats. Molecular Nutrition & Food Research. 2011; 55: 1655–1665.

[138] Shukla R, Banerjee S, Tripathi YB. Pueraria tuberosa extract inhibits iNOS and IL-6 through suppression of PKC-alpha and NF-kB pathway in diabetes-induced nephropathy. The Journal of Pharmacy and Pharmacology. 2018; 70: 1102–1112.

[139] Choi YJ, Lim HS, Choi JS, Shin SY, Bae JY, Kang SW, *et al*. Blockade of chronic high glucose-induced endothelial apoptosis by Sasa borealis bamboo extract. Experimental Biology and

Medicine. 2008; 233: 580–591.

[140] Hsieh PF, Yu CC, Chu PM, Hsieh PL. Verbascoside Protects Gingival Cells against High Glucose-Induced Oxidative Stress via PKC/HMGB1/RAGE/NFkappaB Pathway. Antioxidants. 2021; 10: 1445.

[141] Bagdas D, Etoz BC, Gul Z, Ziyanok S, Inan S, Turacozen O, *et al*. *In vivo* systemic chlorogenic acid therapy under diabetic conditions: Wound healing effects and cytotoxicity/genotoxicity profile. Food and Chemical Toxicology. 2015; 81: 54–61.

[142] Younis NS, Mohamed ME, El Semary NA. Green Synthesis of Silver Nanoparticles by the Cyanobacteria Synechocystis sp.: Characterization, Antimicrobial and Diabetic Wound-Healing Actions. Marine Drugs. 2022; 20: 56.

[143] Mujica V, Orrego R, Fuentealba R, Leiva E, Zúñiga-Hernández J. Propolis as an Adjuvant in the Healing of Human Diabetic Foot Wounds Receiving Care in the Diagnostic and Treatment Centre from the Regional Hospital of Talca. Journal of Diabetes Research. 2019; 2019: 1–10.

[144] Samaddar S, Koneri R. Polyphenols of marine red macroalga Symphyocladia latiuscula ameliorate diabetic peripheral neuropathy in experimental animals. Heliyon. 2019; 5: e01781.

[145] Kamdi SP, Raval A, Nakhate KT. Effect of apple peel extract on diabetes-induced peripheral neuropathy and wound injury. Journal of Diabetes Metabolic Disorders. 2021; 20: 119–130.

[146] Thangavel P, Pathak P, Kuttalam I, Lonchin S. Effect of ethanolic extract of Melia dubia leaves on full-thickness cutaneous wounds in Wistar rats. Dermatologic Therapy. 2019; 32: e13077.

[147] Parmar KM, Shende PR, Katare N, Dhobi M, Prasad SK. Wound healing potential of Solanum xanthocarpum in streptozotocin-induced diabetic rats. The Journal of Pharmacy and Pharmacology. 2018; 70: 1389–1400.

[148] Deshmukh PT, Gupta VB. Embelin accelerates cutaneous wound healing in diabetic rats. Journal of Asian Natural Products Research. 2013; 15: 158–165.

[149] Prasad SK, Kumar R, Patel DK, Hemalatha S. Wound healing activity of Withania coagulans in streptozotocin-induced diabetic rats. Pharmaceutical Biology. 2010; 48: 1397–1404.

[150] Yu W, Tao M, Zhao Y, Hu X, Wang M. 4'-Methoxyresveratrol Alleviated AGE-Induced Inflammation via RAGE-Mediated NF-kappaB and NLRP3 Inflammasome Pathway. Molecules. 2018; 23: 1447.

[151] Shu A, Du Q, Chen J, Gao Y, Zhu Y, Lv G, *et al*. Catalpol ameliorates endothelial dysfunction and inflammation in diabetic nephropathy via suppression of RAGE/RhoA/ROCK signaling pathway. Chemico-Biological Interactions. 2021; 348: 109625.

[152] Sohouli MH, Fatahi S, Sharifi-Zahabi E, Santos HO, Tripathi N, Lari A, *et al*. The Impact of Low Advanced Glycation End Products Diet on Metabolic Risk Factors: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Advances in Nutrition. 2021; 12: 766–776.

[153] Casellini CM, Barlow PM, Rice AL, Casey M, Simmons K, Pittenger G, *et al*. A 6-month, randomized, double-masked, placebo-controlled study evaluating the effects of the protein kinase C-beta inhibitor ruboxistaurin on skin microvascular blood flow and other measures of diabetic peripheral neuropathy. Diabetes Care. 2007; 30: 896–902.

[154] Khan S. Wogonin and Alleviation of Hyperglycemia via Inhibition of DAG Mediated PKC Expression. A Brief Insight. Protein and Peptide Letters. 2021; 28: 1365-1371.

[155] Jiang W, Li Z, Zhao W, Chen H, Wu Y, Wang Y, *et al*. Breviscapine attenuatted contrast medium-induced nephropathy via PKC/Akt/MAPK signalling in diabetic mice. American Journal

of Translational Research. 2016; 8: 329–341.

[156] Sinha R, Sinha I, Calcagnotto A, Trushin N, Haley JS, Schell TD, *et al*. Oral supplementation with liposomal glutathione elevates body stores of glutathione and markers of immune function. European Journal of Clinical Nutrition. 2018; 72: 105–111.

[157] Pourrajab B, Fatahi S, Sohouli MH, Găman M, Shidfar F. The effects of probiotic/synbiotic supplementation compared to placebo on biomarkers of oxidative stress in adults: a systematic review and meta-analysis of randomized controlled trials. Critical Reviews in Food Science and Nutrition. 2022; 62: 490–507.

[158] Sohouli MH, Fatahi S, Lari A, Lotfi M, Seifishahpar M, Găman M, *et al*. The effect of paleolithic diet on glucose metabolism and lipid profile among patients with metabolic disorders: a systematic review and meta-analysis of randomized controlled trials. Critical Reviews in Food Science and Nutrition. 2022; 62: 4551–4562.

[159] Xiong P, Niu L, Talaei S, Kord-Varkaneh H, Clark CCT, Găman M, *et al*. The effect of berberine supplementation on obesity indices: a dose– response meta-analysis and systematic review of randomized controlled trials. Complementary Therapies in Clinical Practice. 2020; 39: 101113.

[160] Grewal AS, Thapa K, Kanojia N, Sharma N, Singh S. Natural Compounds as Source of Aldose Reductase (AR) Inhibitors for the Treatment of Diabetic Complications: a Mini Review. Current Drug Metabolism. 2020; 21: 1091–1116.

[161] Aslan HE, Beydemir Ş. Phenolic compounds: the inhibition effect on polyol pathway enzymes. Chemico-Biological Interactions. 2017; 266: 47–55.

[162] Zhang H, Gao Y, Zhang J, Wang K, Jin T, Wang H, *et al*. The effect of total lignans from Fructus Arctii on Streptozotocin-induced diabetic retinopathy in Wistar rats. Journal of Ethnopharmacology. 2020; 255: 112773.

[163] Sedlak L, Wojnar W, Zych M, Wygledowska-Promienska D, Mrukwa-Kominek E, Kaczmarczyk-Sedlak I. Effect of Resveratrol, a Dietary-Derived Polyphenol, on the Oxidative Stress and Polyol Pathway in the Lens of Rats with Streptozotocin-Induced Diabetes. Nutrients. 2018; 10: 1423.

[164] Barman S, Srinivasan K. Zinc Supplementation Ameliorates Diabetic Cataract through Modulation of Crystallin Proteins and Polyol Pathway in Experimental Rats. Biological Trace Element Research. 2019; 187: 212–223.

[165] Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. Journal of Physiology and Pharmacology. 2019; 70: 809–824.

[166] Salehi-Sahlabadi A, Varkaneh HK, Shahdadian F, Ghaedi E, Nouri M, Singh A, *et al*. Effects of Phytosterols supplementation on blood glucose, glycosylated hemoglobin (HbA1c) and insulin levels in humans: a systematic review and meta-analysis of randomized controlled trials. Journal of Diabetes Metabolic Disorders. 2020; 19: 625–632.

[167] Egbuna C, Awuchi CG, Kushwaha G, Rudrapal M, Patrick-Iwuanyanwu KC, Singh O, *et al*. Bioactive Compounds Effective against Type 2 Diabetes Mellitus: a Systematic Review. Current Topics in Medicinal Chemistry. 2021; 21: 1067–1095.

[168] Ochoa-Gonzalez F, Cervantes-Villagrana AR, Fernandez-Ruiz JC, Nava-Ramirez HS, Hernandez-Correa AC, Enciso-Moreno JA, *et al*. Metformin Induces Cell Cycle Arrest, Reduced Proliferation, Wound Healing Impairment *In vivo* and Is Associated to Clinical Outcomes in Diabetic Foot Ulcer Patients. PLoS ONE. 2016; 11: e0150900.

[169] Bhettani MK, Rehman M, Altaf HN, Altaf OS. Effectiveness of Topical Insulin Dressings in Management of Diabetic Foot Ulcers. World Journal of Surgery. 2020; 44: 2028–2033.