

A review article on Therapeutic Potential of Astaxanthin Against Diabetic Progression and Secondary Complications.

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Abstract:

The progression and long-term effects of Diabetes Mellitus (DM) can be largely attributed to oxidative stress (OS). It has been shown that when high blood glucose levels trigger reactive oxygen species (ROS), they reduce the ability of pancreatic beta cells to produce insulin; they impair insulin signaling and sensitivity in responsive tissue; and they negatively affect endothelial cell function in both Type 1 and Type 2 DM. A xanthophyll carotenoid called Astaxanthin (ASX), which is an extremely powerful antioxidant without any harmful side effects, has been identified as a possible way to prevent or manage conditions associated with DM. Although it is currently unclear exactly how ASX works to modify various OS pathways and thus decrease apoptosis, inflammation, and oxidative stress, many studies have demonstrated its effectiveness in reducing symptoms of DM in animals. Many studies using animal models of Type 1 and Type 2 DM have indicated that when administered either by injection or orally, ASX increases insulin secretion and sensitivity, decreases blood glucose levels and provides protective effects against neuropathy, nephropathy, and retinopathy. However, additional research will need to be conducted to identify optimal parameters for use of ASX. Additionally, there is very little research available to date concerning the effectiveness of ASX in treating people with DM. Therefore, the purpose of this review is to summarize current knowledge regarding the biological effects of ASX on ROS-mediated metabolic problems and their associated complications in DM.

Keywords: astaxanthin; diabetes mellitus; oxidative stress; hyperglycemia; diabetes complication; diabetic retinopathy; diabetic neuropathy; diabetic nephropathy; insulin resistance; antioxidant; ROS

1. Introduction:

Diabetes mellitus (DM), which is the most common metabolic disorder, has become a major global health concern with increasing numbers. Currently, there are 463 million people (9.3% of the world population aged 20–79 years) living with diabetes worldwide. Another 1.1 million adolescents and children under 20 years old have type 1 diabetes (T1DM) [1]. It is projected that these figures will reach 578 million by 2030. The financial burden of DM is enormous. The estimated total cost of healthcare spending due to diabetes is about \$760 billion, and it is predicted that the costs will increase to \$825 billion within the next decade. DM is a chronic disease that causes high blood glucose (HG) either

because the body does not produce enough insulin or because the insulin produced does not work properly, or both. High blood glucose (HG), if not managed carefully, can lead to serious and life-threatening complications such as heart disease, eye damage, kidney failure, neuropathy, and poor wound healing [1]. In addition, DM can lead to increased bone fragility and poor bone repair [2,3]. There are three main forms of DM: type 1 (T1DM), type 2 (T2DM), and gestational DM. Type 2 DM is the most prevalent form, making up nearly 90% of all global cases of DM. Chronic low-grade inflammation and oxidative stress (OS) have been shown to be involved in the onset and pathological consequences of DM [4]. Oxidative stress (OS) occurs when there is an imbalance between cellular antioxidants and oxidants caused by the overproduction of free radicals and reactive oxygen species (ROS). Hyperglycemia (HG) increases indicators of chronic inflammation and enhances ROS production, eventually leading to DM-related complications such as vascular damage [5]. Furthermore, high levels of ROS decrease the amount of insulin released and impair insulin signaling and responsiveness in target cells [5]. Therefore, controlling hyperglycemia (HG) and reducing excess ROS is essential for delaying the initiation and progression of DM and preventing further complications associated with DM. Recent research on the biological properties of antioxidants such as carotenoids has demonstrated that they can prevent and/or improve diabetes and its associated complications [6]. Astaxanthin (ASX), also known as 3,3'-dihydroxy- β,β' -carotene-4,4'-dione is a type of xanthophyll carotenoid [7]. ASX provides a wide array of protective actions against the effects of oxidative stress (OS) and inflammation through its ability to quench free radicals [8] and activate the body's natural antioxidant defense systems through gene regulation [9,10]. In comparison with the various other types of carotenoids, ASX has several distinct advantages. Due to its unique chemical structure it can become part of the phospholipid bilayer of cell membranes while not interfering with their function; thus, it protects these membranes from oxidation [11]. In addition, ASX exhibits significantly greater biological activity than alternatives such as α -carotene, β -carotene [12] or α -tocopherol [13,14]. Consequently, astaxanthin has received considerable attention for its potential therapeutic effects as an antioxidant, anti-inflammatory, and antidiabetic agent, as well as its protective effects on the nervous system, kidneys, retina, and heart [6]. Given the significant role that OS and inflammation play in the development and progression of complications from DM, this review seeks to provide insight into the biological effects and mechanisms of action of astaxanthin with respect to the prevention and treatment of pathologies associated with DM.

2. Pathophysiology of Diabetes-Related Metabolic Disorders and Complications Focus on the Hyperglycemia–Oxidative Stress Interactions

Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) are both chronic diseases characterized by hyperglycemia. The primary difference between these two forms of diabetes is that T1DM is an auto-immune disorder in which the immune system destroys the pancreatic beta-cells (islet cells) that produce insulin. In contrast, T2DM is a chronic metabolic disease with multiple metabolic disorders involving lipid and carbohydrate metabolism, including impaired insulin secretion from the pancreatic beta-cells, insulin resistance, impaired peripheral glucose uptake, and increased hepatic glucose synthesis [15]. Both forms of diabetes cause chronic hyperglycaemia (HG), which results in microvascular and macrovascular damage [16]. Studies have shown that oxidative stress (OS) and reactive oxygen species (ROS) contribute to impaired pancreatic beta-cell insulin secretion, insulin

resistance and endothelial dysfunction in both T1DM and T2DM [15-17]. ROS can significantly damage DNA, RNA, lipids and proteins; therefore, ROS may serve as biomarkers for monitoring the progression of the disease [16]. Additionally, ROS play an important role in the interaction among inflammation, OS and metabolic control in diabetes-related pathological conditions [4,18]. ROS consist of molecular oxygen and its byproducts: superoxide anion (O_2^-), nitric oxide (NO), peroxynitrite ($ONOO^-$), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and lipid radicals. Superoxide anion is the first free radical of oxygen, which may be converted into other more reactive forms that disrupt cellular function. Even though ROS are produced at low concentrations during aerobic metabolism to regulate intracellular signal transduction [19], ROS mainly arise in the endoplasmic reticulum (ER), peroxisomes, microsomes and mitochondrial electron transport chain (ETC) [4]. When there is an overproduction of ROS or when endogenous antioxidant defense systems fail to adequately neutralize them, OS occurs. OS damages nucleic acids, membrane lipids and cellular proteins. Endogenous antioxidants include glutathione (GSH) and superoxide dismutase (SOD); however, each part of the cell (mitochondria, nucleus and cell membrane) has distinct roles in maintaining ROS homeostasis [18]. Consequently, excessive ROS generated during HG or fatty acid oxidation lead to cellular dysfunction, inflammation and apoptosis in insulin-sensitive tissues such as liver, kidney, nerve, pancreas and blood vessels [17,18]. For example, in T1DM, ROS generated through autoimmune or metabolic stress within the pancreas can further exacerbate β -cell injury and reduce insulin production [17]. In T2DM, HG-induced ROS generation is a major predictor of disease progression and damage. HG induces glucose auto-oxidation, alters mitochondrial activity and increases ROS levels. The ensuing OS decreases insulin secretion and impairs insulin signaling in affected tissues [20]. Excess ROS also contributes to the development of diabetic vascular complications [18]. Specifically, vascular injury induced by HG involves multiple mechanisms: (1) enhanced glucose influx via polyol pathway activation; (2) increased endogenous formation of advanced glycation end products (AGE); (3) increased AGE receptor expression and ligand activation; (4) activation of protein kinase C (PKC) isoenzymes; and (5) hexosamine pathway over-activity. It is currently recognized that these pathological processes arise from HG-induced overproduction of the superoxide anion radical ($\bullet O_2^-$) in the mitochondrial ETC. Elevated blood sugar leads to increased AGE formation and subsequent OS [21]. While the exact signaling mechanisms remain largely unknown, AGE accumulation is correlated with diabetes and its complications. Through receptors including AGE-R1/OST-48, AGE-R2/80K-H, AGE-R3/galectin-3, LOX-1 and RAGE, AGEs generate ROS and elevate intracellular levels of oxygen derivatives such as NO, O_2^- and H_2O_2 . The interaction between AGE-RAGE activates and elevates mRNA levels of NADPH oxidase (NOX): NOX1, NOX2 and NOX4. It also stimulates the mitochondrial respiratory chain, xanthine oxidase and microsomal enzymes while increasing the release of arachidonic acid metabolites [21].

Furthermore, AGE-induced ROS increase the activity and expression of antioxidant enzymes such as SOD, glutathione peroxidase (GPx) and catalase.

Additionally, AGE-induced ROS modify signaling pathways including p38 MAPK p21RAS, PI3K/Akt, or RAS/MEK/ERK1/2; promote NF- κ B activation and translocation; and ultimately trigger inflammation, apoptosis and cell proliferation through the NLRP-3 inflammasome and the synthesis of cytokines like MCP-1, TNF- α , IL-6 and IL-1 β [21]. Studies have demonstrated elevated circulating

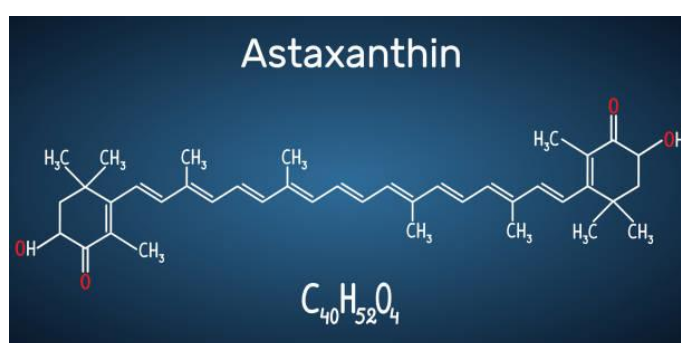
TNF- α and IL-6 in T2DM patients and rodents [22]. These cytokines disrupt peripheral glucose absorption and insulin signaling, leading to lipolysis, insulin resistance and hepatic glucose synthesis.

3. Astaxanthin: Biological Effect on Diabetes Onset, Progression, and Chronic Complications

3.1 General Aspects of Astaxanthin: Structure, Sources, Bioactivity, and Administration Toxicity

3.1.1 Sources and Chemical Structure of Astaxanthin

Astaxanthin (ASX) is a powerful carotenoid that provides antioxidant activity. It is produced either naturally or synthetically. Natural sources of ASX include several species of microbes and aquatic animals such as trout, yeast, krill, salmon, shrimp, microalgae and crayfish along with certain types of vegetation. As the human body cannot produce ASX itself, it must be consumed through diet, specifically seafood [23]. Commercially available ASX is usually produced from chemical synthesis or harvested from *Blakeslea trispora*, *Phaffia rhodozyma*, and *Haematococcus pluvialis*. The main source of natural ASX for human consumption is *Haematococcus pluvialis*. *Haematococcus pluvialis* is a unicellular green microalga that produces ASX under environmental stress conditions such as high light intensity, starvation, salinity, radiation exposure and extremes in temperature [24,25]. The United States Food and Drug Administration (US FDA) approved ASX as a fish feed ingredient in 1987 and as a dietary supplement in 1999 [26]. The European Food Safety Authority (EFSA) has also approved its use as a fish feed ingredient. The maximum allowable daily intake of ASX from *H. pluvialis* by humans is 8 mg per day according to Novel Foods Regulation (EU) 2015/2283 [27]. In the U.S., the FDA considers *H. pluvialis* extract safe for use in dietary supplements at doses ranging from 2 mg to 12 mg per day [28,29]. ASX is chemically defined as 3,3'-dihydroxy- β,β' -carotene-4,4'-dione with a molar mass of 596.84 g/mol and a chemical formula of $C_{40}H_{52}O_4$. ASX is a xanthophyll carotenoid composed of 40 carbon atoms arranged into two oxygenated β -ionone rings connected by a polyene chain [7,30,31].



Structure of astaxanthin

The polyene chain's conjugated double bonds act as a potent antioxidant by neutralizing free radicals and providing electron donors [32]. Additionally, the terminal hydroxyl groups on the rings can form bonds with fatty acids to create diesters or monoesters. Additionally, in some cases, ASX may exist covalently bonded to proteins as exemplified in lobster shells or salmon tissues. These protein/fatty acid interactions provide increased stability to ASX against oxidation when compared to its free form; therefore, the free form is typically synthesized or obtained from yeast [32]. Natural ASX can exist in various geometric isomeric forms, stereoisomeric forms, and esterified and non-esterified forms.

Synthetic forms of ASX may exist in meso (3R, 3S) or chiral ((3R, 3R) or (3S, 3S)) configurations; however, chiral forms are more common. In addition, the double bonds within the polyene chain can exist in either cis or trans configurations. Cis carotenoids are thermodynamically unstable; thus, the trans-isomer is the most common form [11].

3.1.2 Administration Doses and Toxicity Studies

The major obstacle to developing therapies based upon astaxanthin (ASX), including those administered parenterally, is its poor stability in vivo. In order to provide protection for the unstable ASX molecule, improve its stability in vivo, and enable its delivery, investigators have developed various delivery vehicles including lipid-based vehicles, polymeric delivery systems and cyclodextrin inclusion complexes [33, 34]. A polymeric coating or matrix can establish a physical barrier to maintain the biological activity of ASX while increasing its physicochemical stability [23, 35, 36]. Lipid-based delivery systems — such as nanoemulsions, microemulsions or hybrid systems including solid lipid nanoparticles and nanostructured lipid carriers — have also been shown to stabilize ASX [33, 37, 38]. One clinical study demonstrated that incorporation of ASX into a lipid-based delivery system increased the bioavailability of ASX [39]. Nanoemulsions containing both ASX and α -tocopherol were topically applied to skin injury sites in streptozotocin (STZ)-induced diabetic mice. Healing occurred faster in the treated group. Histopathological examination of the injured tissue revealed higher numbers of inflammatory cells, increased fibroblast proliferation and collagen deposition [40]. Cyclodextrins — a family of natural macrocyclic oligosaccharides — are well-suited for encapsulating ASX due to their toroid shape with a hydrophilic outer surface and a hydrophobic inner cavity [41, 42]. Formation of a noncovalent bond between ASX and cyclodextrin improves the aqueous solubility, stability, release characteristics and bioavailability of ASX [43, 44, 45]. Topical application of ASX–cyclodextrin complexes encapsulated within biocompatible poly(vinyl alcohol)-dextran hydrogels inhibited OS in a rat model of ischemia [36].

Furthermore, these systems reduced excess ROS and modified the expression of endogenous antioxidant genes in ischemic tissue, suggesting that localized release of ASX may reduce tissue damage through activation of endogenous antioxidant defenses [42]. Following oral administration of ASX esters, selective hydrolysis occurs during intestinal absorption prior to entering the circulatory system where they are transported to target tissues [46]. Due to the presence of polar termini on the ASX molecule, it is absorbed more efficiently than non-polar carotenoids such as β -carotene or lycopene [24].

However, the low solubility of ASX in gastrointestinal fluid limits its absorption by intestinal epithelial cells and subsequent transport into the lymphatic system as chylomicrons [47, 48].

Although many aspects of the absorption, metabolism and excretion of ASX remain incompletely understood, factors such as sex, age, dietary fat content, food intake and smoking status influence the bioavailability and absorption of ASX in addition to its chemical composition [49]. Studies examining doses of natural ASX ranging from 1 to 4 mg/day and synthetic forms up to 100 mg/day reported a non-linear relationship between dose and plasma concentrations of ASX; esterified forms of ASX exhibited lower bioavailability [49]. There is no evidence from studies on orally administered natural ASX that it poses any health risks [27].

For example, rats administered high daily doses (up to 1240 mg/kg) for 90 days exhibited no signs of toxicity [50]. In humans, daily intake of natural ASX between 8 and 45 mg over a period of 4 to 12 weeks elicited few adverse events [51]. In comparison to placebo arms in randomized clinical trials, ASX exhibits excellent clinical safety at daily doses between 12 and 100 mg [27]. Natural ASX is currently the sole form approved as a human dietary supplement [7].

While preclinical rat studies have employed doses 100-fold to 800-fold greater than those deemed safe for human consumption without causing severe side effects, additional studies are required to determine human-specific dose-response relationships and safety indicators [52]. As of 2010, there have been 34 documented clinical trials involving ASX [53], and 22 have been completed. Most of these trials have evaluated ASX either as a single agent or in combination with other agents to evaluate pharmacokinetic properties and/or safety profiles, or to treat diseases associated with OS including cognitive impairment, skin aging, hyperlipidemia, infertility and stroke. Currently, two ongoing clinical trials (NCT03310359 and NCT03702374) are actively recruiting patients to evaluate an antioxidant regimen composed of ASX plus zinc, copper, vitamin C, vitamin E, zeaxanthin and lutein for treatment of diabetic retinopathy. A previously conducted clinical trial evaluating ASX-based antioxidant therapy for diabetic retinopathy has been completed but results have not yet been published. The increasing number of clinical trials investigating ASX for treatment of diabetic complications reflects the growing interest in this antioxidant.

3.1.3 Bioactivity of Astaxanthin

At cellular and molecular levels, ASX acts against oxidative damage through various mechanisms (Figure 1) including quenching singlet oxygen, scavenging radicals, inhibiting lipid peroxidation, and regulating OS-related gene expression [54]. ASX's antioxidant potential is largely attributed to its interactions with cell membrane lipids [55]. In contrast to other carotenoids, such as lycopene and β -carotene, ASX, thanks to its polar structure, is incorporated in the membrane without disorganizing it, decreases lipid hydroperoxide levels, and does not exhibit harmful pro-oxidative effects [56]; in fact, ASX inhibition of lipid peroxidation is related to its ability to trap ROS within and on both sides of the membrane [57,58]. Growing evidence suggests that ASX improves mitochondrial function by reducing mitochondrial reactive oxygen species (mtROS) production and by increasing ATP production, mitochondrial content, and respiratory chain complex activity [59].

The ability of ASX to scavenge free radicals and its ability to activate the PI3K/Akt, MAPK, and Nrf2/ARE pathways contribute to its ability to control the internal oxidative stress (OS) experienced by diabetic rats with STZ-induced diabetes (12 weeks) [60]. Nrf2 will enter the nucleus after activation of the ERK and PI3K/Akt pathways. Activation of Nrf2/ARE will increase production of Glucathione S-transferase alpha1 (GST- α 1), nicotinamide adenine dinucleotide phosphate (NAD(P)H) quinone dehydrogenase 1 (NQO1) and heme oxygenase-1 (HO-1), initiating an internal antioxidant response [52]. A rat model of experimental subarachnoid hemorrhage has shown that ASX reduces early brain injury through activation of the Nrf2/ARE pathway [61]. ASX additionally suppresses the specificity protein 1 (Sp1)/N-Methyl-d-aspartic acid (NMDA) receptor subunit 1 (NR1) mechanism, which generates intracellular ROS [54,59]. ASX activates ERK and PI3K/Akt as well, and these activations cause a decrease in Bax/Bcl2 ratio. This decreases cytochrome c leakage from mitochondria and thus decreases caspase 3-mediated apoptosis via reduced mitochondrial membrane permeability. When

endothelial cells are under OS, administration of ASX significantly activates PI3K/Akt and decreases expression of Bax genes and endothelial nitric oxide synthase (eNOS) [34].

In addition, ASX decreases caspase-mediated cell death by modifying the p38 MAPK signaling pathway [62]. Finally, ASX regulates the immune response by inhibiting the secretion of pro-inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF- α), intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6) and interleukin-1B (IL-1B) through reactive oxygen species (SHP-1) activation. SHP-1 activation prevents I κ B degradation and NF- κ B expression. Furthermore, ASX directly inhibits I κ B release, preventing NF- κ B from entering the nucleus and enhancing anti-inflammatory effects [10,52,63]. The multiple targets of ASX are related to decreased ER marker expression [64]. For instance, ASX decreases levels of proteins such as X-box binding protein 1 (XBP1), activating transcription factor 6 (ATF6), activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), growth arrest and DNA damage-inducible protein (GADD34) and glucose-regulated protein 78 (GRP78) in human liver hepatocellular carcinoma (HepG2). All of these proteins contribute to apoptosis induced by ER stress.

3.2 Antidiabetic effects of Astaxanthin

Results from clinical studies show that astaxanthin (ASX) can have beneficial effects on the control of symptoms and prevention of diabetes [6]. However, the success of ASX as a treatment will depend on the stage of the disease, and what complications exist. In Figure 2, you will find some of the major findings related to the use of ASX in clinical studies. For example, a study involving participants with type II diabetes found that when they took 8 mg/day of ASX for 8 weeks, they experienced a significant reduction in their plasma glucose and fructosamine levels [65]. Additionally, ASX helps people with type II diabetes process glucose better and has been shown to reduce their blood pressure.

Studies of multiple animal models using ASX to study its antidiabetic effects have produced a range of findings (Figure 2), however, the exact mechanisms responsible for these effects remain largely unknown. The use of db/db mice, which are considered to be an established model of obesity-induced type 2 diabetes mellitus (T2DM), showed a significant reduction in fasting blood sugar after administration of ASX [66-69]. Long-term use of ASX (35 mg/kg for twelve weeks) has been found to be related to the blood-sugar-lowering effect and has been shown to increase serum superoxide dismutase (SOD) activity and decrease malondialdehyde (MDA) levels [70]. The same type of mouse model demonstrated that a one-mg daily dose of ASX for eighteen weeks protected pancreatic β -cells from high glucose-induced damage and enabled them to produce insulin.

In addition to this protective effect, the treatment resulted in a substantial decrease in non-fasting blood glucose levels [69]. Findings reported by Kitahara et al. indicate that ASX protects pancreatic β -cell integrity against cell death caused by endoplasmic reticulum (ER) stress [71]. Exposure of mouse insulinoma (MIN6) cells to palmitate resulted in the release of vascular endothelial growth factor (VEGF) and monocyte chemotactic protein-1 (MCP-1) through the activation of both the PI3K/Akt and JNK pathways. Exposure to ASX attenuated the release of VEGF and MCP-1 through the inhibition of both pathways while also reducing ER stress by up-regulating the chaperone GRP78 and down-regulating CHOP [71]. Animal studies have demonstrated that ASX not only reduces blood glucose

levels, but it also improves glucose absorption and insulin sensitivity. Therefore, ASX effectively counteracts the insulin resistance that characterizes T2DM. According to Arunkumar et al. [72], daily administration of 6 mg/kg of ASX for 45 days markedly improved insulin sensitivity and decreased blood glucose levels and insulin levels in mice fed a high-fat/fructose diet (HFFD). Furthermore, ASX enhances post-receptor insulin signaling through the enhancement of IR- β autophosphorylation, IRS-1 associated PI3-kinase activity, the phospho-Akt/Akt ratio and translocation of GLUT-4 into skeletal muscle tissue [72]. Similarly, Yasuhiro Nishida et al. reported enhanced glucose processing in HFD-fed mice treated with ASX due to enhanced glucose uptake in muscle [73]. Their research indicated that ASX markedly improved glucose tolerance and insulin resistance through activation of AMPK in muscle tissue, a mechanism that does not depend on its antioxidative properties. Additionally, the treatment promoted the development of new mitochondria in muscle tissue [73].

3.2 Astaxanthin: Protective Effects on Diabetes Complications:

Oxidative Stress (OS), has been identified as a common link between the two major biological pathways leading to microvascular and macrovascular complications due to Diabetes Mellitus [87]. Excessive amounts of mitochondrial superoxide are produced in the Heart Muscle, as well as the Endothelial Cells lining vessels of all sizes, when blood glucose levels are too high. In the case of smaller vessels, this excessive production of superoxide results in circulatory injury via multiple metabolic mechanisms; namely accumulation of Advanced Glycation End Products (AGEs), Activation of AGE Receptors, and Activation of the Hexosamine, Polyol, and Protein Kinase C (PKC) Pathways [88]. Inflammatory processes play a critical role in the emergence and progression of diabetic complications [89]. As far as issues affecting the heart and large scale vasculature are concerned, it appears that the damage occurs primarily through Increased Fatty Acid Oxidation, which contributes at least in part to Insulin Resistance via Specific Pathways [88]. Due to its unique chemical composition, ASX is an antioxidant that can travel throughout the body to provide protection for every cell. Through neutralization of free radicals, it is possible that ASX could assist in preventing and managing microvascular diabetic complications through control over Apoptosis, Inflammation, and OS [8].

3.3.1. Astaxanthin: protection against diabetes retinopathy

While the exact mechanism of action for ASX is currently unknown, numerous animal research models have demonstrated that ASX provides protective effects against various diabetes-related conditions including Wound Recovery, Neuropathy, Nephropathy, and Retinopathy. In order to identify the Optimal Dosage for obtaining Beneficial Results in relation to complications, comprehensive human clinical trials need to be conducted to evaluate the Safety and Efficacy of ASX; taking into consideration Tissue Distribution, Metabolism, Variation in Response Among Individuals, Bioavailability, and Potential Toxic Effects. Recent studies show that astaxanthin provides many benefits in treating and preventing different eye diseases because it helps to control metabolism and has both antioxidant and anti-inflammatory effects [90]. Diabetic Retinopathy (DR) is one of the most common causes of microvascular complications of diabetes and is the leading cause of blindness in adults [91]. This condition develops slowly, and its incidence increases with the duration of the diabetes. DR results from complex biological processes involving several metabolic changes that affect many types of retinal cells.

Persistent hyperglycemia increases oxidative stress (OS) in the retina and its capillaries leading to functional damage and microvascular damage [92]. Hyperglycemia promotes the formation of NADPH oxidase (NOX), which damages mitochondrial membranes and increases intracellular levels of reactive oxygen species (ROS). OS in the retina may activate several metabolic pathways - including the polyol pathway, hexosamine pathway, advanced glycation end products (AGEs) pathway, protein kinase C (PKC) pathway, p38 mitogen-activated protein kinase (MAPK) pathway, and autophagy pathway - while altering levels of growth factors such as vascular endothelial growth factor (VEGF) [93]. Moreover, OS may induce activation of the nuclear factor-kappa B (NF- κ B) transcription factor promoting pro-inflammatory cytokines that contribute to the destruction of retinal cells and the progression of DR [94]. In addition to OS, the diabetic condition reduces the body's capacity to detoxify superoxide radicals by decreasing the activity of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase (SOD), catalase GSH, glutathione reductase, and manganese superoxide dismutase (MnSOD), while also inhibiting enzymes involved in histone modification and DNA repair [92]. Many studies demonstrate the protective effect of ASX against DR (Figure 3). ASX reduced the elevation of ROS, AGEs, lipid peroxidation, cell proliferation, and mRNA expression of MMP2 and VEGF induced by high glucose in laboratory cultured human retinal pigment epithelial cells [95]. Oral administration of ASX to rats with STZ-induced diabetes maintained the structure and function of their retinas and therefore protected them from developing DR. ASX decreased markers of OS (such as acrolein, nitrotyrosine and 8-hydroxy-2'-deoxyguanosine), enhanced antioxidant enzymes such as peroxiredoxin and heme oxygenase-1 and suppressed inflammatory factors including fractalkine, monocyte chemoattractant protein-1 and intercellular adhesion molecule-1 likely through inhibition of NF- κ B factor [96]. Recently, research demonstrated that ASX inhibits aldose reductase (AR) activity, a critical enzyme in the polyol pathway associated with diabetic complications as observed in *Psammomys obesus* (a member of the Gerbillidae family) used as models for Type II Diabetes Mellitus (T2DM) [97]. In db/db mice, ASX treatment increased oscillatory potentials (OPs) and decreased retinal tissue markers of OS including MnSOD, 8-OHdG (an indicator of oxidative DNA damage), MDA (an indicator of lipid peroxidation) and superoxide anions [98]. Therefore, it is hypothesized that ASX, acting as an antioxidant, will likely protect against DR by reducing internal OS, which in turn reduces detrimental cellular events such as inflammation, VEGF production and uncontrolled cell proliferation.

3.3.2 Cardiovascular Protective Effect of Astaxanthin in Diabetes

The most common cause of death among patients suffering from type 2 diabetes mellitus (T2DM), according to the National Institute of Diabetes and Digestive and Kidney Diseases, is cardiovascular disease. Cardiovascular disease is a direct result of damage to the blood vessels, resulting primarily from hypertension [111]. High glucose levels in the bloodstream lead to the formation of advanced glycation end products (AGEs), which ultimately contributes to the development of arteriosclerosis [112]. Furthermore, increased production of nitric oxide radicals due to increased activity of the enzyme NADPH oxidase (NOX) results in the formation of thrombi and aggregation of platelets. Under normal physiological circumstances, ROS production is regulated by intracellular antioxidant enzymes present in blood platelets [113]. Due to impaired production of these antioxidant enzymes in patients with diabetes, ASX may reduce thrombosis by stabilizing intracellular redox reactions. Additionally, as shown in previous studies, ASX has been found to inhibit low-density lipoprotein (LDL) oxidation in a

dose-dependent manner and therefore may prevent or slow down the development of arteriosclerosis [114]. Furthermore, by reducing ROS-mediated vasoconstriction, ASX may considerably influence blood viscosity and arterial pressure in hypertensive animal models [115]. Previous studies have demonstrated the cardioprotective effects of ASX and its derivatives when administered either orally or intravenously to various species. In addition to reducing inflammation and OS, both of which are known contributing factors to the development of atherosclerotic cardiovascular diseases [101], ASX may have a number of potential positive effects on cardiac pathology. The potential benefits of ASX on cardiac pathology are summarized in several review articles [49,116,117]. Besides the use of tobacco products, the presence of vascular wall infections and elevated blood pressure, there are multiple additional factors that can influence vascular health.

For example, Zhao and associates evaluated the effects of daily administration of 10 mg/kg ASX for six weeks on the endothelial cell function of STZ-induced diabetic rodent models. The authors found that ASX treatment resulted in decreased concentration levels of oxidized low-density lipoprotein (oxLDL) and a lowered expression level of its receptor, LOX-1, thereby normalizing eNOS levels [118]. Likewise, Chan and colleagues showed that adding astaxanthin to the diet of diabetic rats at concentrations of 0.01% and 0.05% had both anti-coagulation and anti-inflammation effects [85].

Moreover, a clinical trial conducted using human participants with T2DM and receiving daily doses of ASX ranging from 6 to 12 mg demonstrated a significant reduction in inflammatory markers and an improvement in blood coagulation disorders without adversely affecting renal performance [119]. To better understand how ASX works and what its potential benefits may be in relation to diabetes-related complications, additional research will need to be performed.

Anti-inflammatory effects of Asthaxanthin in Diabetes:

Inflammation plays a major role in the development and advancement of Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), and their related pathologies [120]. Cytokines such as TNF-alpha, IL-1beta, and IFN-gamma activate immune cells that cause injury to pancreatic beta-cells. One key feature of T2DM is chronic, low-grade inflammation, in which both metabolic processes and inflammatory responses interact through adipokines and interleukins like IL-1, IL-6, IL-10, and TNF-alpha. Obese individuals often exhibit infiltration of macrophages into adipose tissue. These macrophages serve as an important source of TNF-alpha production and inflammatory action [121]. Numerous studies have demonstrated that ASX possesses anti-inflammatory effects. Treatment with ASX causes a change in macrophage polarization, as indicated by a decrease in pro-inflammatory M1 indicators (MCP-1 and CD11) while increasing anti-inflammatory M2 markers (IL-10) [77,122]. Additionally, Kim et al. [122] found that ASX reduced monocyte recruitment, thereby reducing the localized proliferation of macrophages.

In addition to decreasing the amount of inflammation-related molecules produced in response to HG, including PGE2, iNOS, COX, IL-1beta, MCP-1 and TNF-alpha; ASX also decreases the amounts of pro-inflammatory cytokines (TNF-alpha and IL-6) and activated macrophages (MCP-1) present in the renal tissue and circulating blood of diabetic rats treated with STZ and fed either 0.01% or 0.05% of ASX in their diet [85]. Bhuvaneswari et al. [82] demonstrated that feeding ASX at 2 mg/kg/day to rats

fed a high fructose, high fat diet prevented phosphorylation of I κ B, thus preventing translocation of the NF-kappaB p65 subunit into the nucleus. Studies have shown that the anti-inflammatory effects of ASX in diabetes are mediated through suppression of NF-kappaB activation [123,124]. Thus, by decreasing inflammation, ASX could potentially aid in managing and preventing diseases associated with diabetes.

4. Conclusions and Future Perspectives:

The role of oxidative stress (OS) and inflammation in advancing diabetes mellitus (DM), along with all its related secondary pathology, has been well-documented. Many laboratory-based studies indicate that astaxanthin (ASX) has multiple biological effects on many different types of cells associated with DM, including: pancreatic β -cells, T-cells, macrophages, adipocytes, hepatocytes, endothelial cells, glomerular mesangial cells, and retinal pigment epithelial cells. ASX reduces cellular inflammation and OS through neutralizing reactive oxygen species (ROS) and preventing lipid peroxidation. Although there are many different OS-related pathways regulated by ASX, the exact mechanism(s) of action and cell-specific effects of ASX remain unclear. A large portion of the current literature suggests that ASX may provide a unique therapeutic opportunity for controlling diabetes. The fact that ASX is a non-toxic pharmaceutical product provides many advantages over other drugs used to control diabetes. It also appears to produce positive results in both Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) animal models when administered orally or parenterally. These positive results include: (1) improvements in glucose tolerance and insulin sensitivity, (2) reductions in high blood sugar levels, (3) activation of antioxidant enzymes and reduction of OS, and (4) anti-inflammatory actions. Therefore, ASX may be beneficial in preventing the development and progression of DM, as well as protecting against diabetic complications. However, comparing results of various studies is challenging due to the diversity of DM models employed, variations in source material of ASX, differences in dosage levels, methods of administration, and metrics evaluated. Although several human clinical trials involving ASX exist, additional research concerning the biological pathways related to diabetes is needed. In order to develop effective treatments for diabetic complications, future human trials must identify the most appropriate dosage for treating these complications. Additionally, assessments of safety and efficacy must be performed in conjunction with considerations such as metabolic processing, bioavailability, tissue distribution, individual variation in response to treatment, and potential toxicity.

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