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REVIEW

The Therapeutic Role of Carotenoids in Diabetic Retinopathy: A Systematic Review

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Background: Carotenoids are a large group of natural pigments that occur in many foods, fruits, and vegetables. Several studies have shown a number of biological properties of carotenoids, particularly beneficial impacts on cancer, metabolic, neurodegenerative, and cardiovascular diseases. However, recent evidence has shown that these compounds could prevent, delay, and ameliorate diabetic retinopathy (DR). The aim of current study was to review the therapeutic effects of carotenoids in the treatment of DR and discuss the molecular mechanisms that are behind these pharmacological activities.

Methods: Six online databases (Medline/PubMed, Scopus, Web of Knowledge, Embase, ScienceDirect, and ProQuest) were searched until September 2019. The systematic review was carried out using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.

Results: A total of 25 studies were included after the final retrieval. A relationship was observed between carotenoids and management of DR. Findings also demonstrated that the underlying mechanism of beneficial effects of these compounds was antioxidant, anti-inflammatory, anti-angiogenic, and neuroprotective properties.

Conclusion: Carotenoids potentially delay the initiation and prevent the progression of DR; however, ample preclinical studies are required to confirm their effect, and adequate clinical trials are needed to really understand how well these compounds influence DR among humans.

Keywords: diabetic retinopathy, carotenoids, oxidative stress, inflammation, neuroprotection

Introduction

Diabetic retinopathy (DR) is the main leading causes of morbidity in patients with diabetes and the primary cause of vision loss around the world.[1] The total prevalence of DR is about 34.6% (92.6 million adults) among diabetic patients, of which 28.4 million adults with vision impairment.[2,3] The severity of DR depends on several factors, including the type of diabetes (in type 1 is more severe than type 2), duration of diabetes, glycemic control status, and the presence of some pathological conditions such as hypertension, smoking, and dyslipidemia.[4]

Although the underlying pathophysiology of DR is not precisely known, it is primarily caused by the metabolic impacts of chronic hyperglycemia.[5] Several lines of studies have shown various biochemical mechanisms about how potentially hyperglycemia causes DR, including increased polyol pathway flux, activation of protein kinase C (PKC) pathway, increased hexosamine pathway flux, and accelerated advanced glycation end-product (AGE) formation.[6] These pathogenic mechanisms involved in retinal oxidative stress and inflammation, overexpression of

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growth factors (particularly vascular endothelial growth factor, VEGF), retinal hemodynamic changes, and impairment in neurotrophic factor receptors and their signaling pathway, which damage to retinal vessels, neurons, and glial cells.[7]

The most essential strategies to delay the onset and progression of DR are strict glycemic control, educational program and treatment of high blood pressure and probably dyslipidemia.[7,8] Laser photocoagulation and vitrectomy are now the only approved clinical treatments of DR.[9] The introduction and development of various pharmacotherapies over recent decades have significantly improved the management of vision loss and visual acuity impairment.[10] Advanced intravitreal treatment with anti-angiogenic agents (anti-VEGF therapy) or anti-inflammatory agents (glucocorticoid therapy) is the most commonly used pharmacotherapy for both prevention and treatment of established DR.[11]

Additionally, other pharmacological agents such as aldose reductase inhibitors, PKC inhibitors, hexosamine biosynthesis inhibitors, AGE formation inhibitors, peroxisome proliferator-activated receptor (PPAR)-gamma receptor agonists, angiotensin-converting enzyme (ACE) inhibitors, antioxidants, and anti-inflammatory agents are investigating and developing to manage DR. However, these agents are still on preclinical or clinical trial stages.[12–14] Natural products as potentially valuable and easily available remedies for DR also get the attention of the researchers.

Carotenoids are a large group of organic and lipophilic pigments, which are produced by plants, algae, and several bacteria and fungi.[15] More than 600 ubiquitous carotenoids have been known in nature, but only about 40 carotenoids are present in human diets, including foods, fruits, and vegetables, and fewer carotenoids have been identified in human serum and organs.[16]

The chemical composition of carotenoids contains a central carbon chain with alternating single and double bonds and various groups on this backbone.[17] These compounds are classified into two groups; carotenes (which purely contain carbons and hydrogens) and xanthophylls (which additionally contain oxygen).[18] The most important, naturally occurring carotenoids were summarized in Table 1.

Several lines of studies have conducted on the beneficial effects of carotenoids in the prevention and management of a large number of diseases, including cancer,[19] cardiovascular diseases,[20] diabetes,[21] osteoporosis,[22] eye diseases,[23,24] Alzheimer's disease,[25] and also infectious diseases.[16] Moreover, it has been shown that the serum levels of some carotenoids are inversely associated with the progression of DR in humans.[26–28] Recent studies on the biological impacts of different carotenoids have shown these compounds could prevent, delay, and ameliorate retinopathy in diabetes. A number of observed biological properties of these compounds including quenching of reactive oxygen/nitrogen species (ROS/RNS), scavenging of free radicals, immune enhancement, antimutagenesis activity, augmentation of self-defense systems, and photo-protection could justify the beneficial effect of these compounds on DR. Several studies have been conducted on the potential preventive and therapeutic effects of different carotenoids on DR. In the present review, the therapeutic effects of carotenoids in the treatment of DR screened and the molecular mechanisms that are behind these pharmacological activities completely discussed.

Table 1 The Most Important Naturally Occurring Carotenoids

	Chemical Structure	Carotenoids
Carotenes	Hydrocarbons	α-carotene, β-Carotene, γ-carotene, δ-carotene, Lycopersene, Phytofluene, Hydrolycopene, Torulene, α-Zeacarotene
Xanthophylls	Alcohols	Zeaxanthin, Rhodopin, Alloxanthin, Gazaniaxanthin, Lutein, Loroxanthin, Lycoxanthin, Saproxanthin
	Glycosides	Oscillaxanthin, Phleixanthophyll
	Ethers	Rhodovibrin, Spheroidene
	Epoxides	Diadinoxanthin, Citroxanthin, Luteoxanthin
	Aldehydes	Rhodopinal, Warmingone, Torularhodinaldehyde
	Ketones	Astaxanthin, Canthaxanthin, Capsanthin, Capsorubin
	Esters of alcohols	Fucoxanthin, Physalien, Astacein, Siphonein
	Apocarotenoids	Crocin, Crocetin, Bixin, Citranaxanthin, Sintaxanthin

Method

The Search Strategy of Systematic Reviews

A literature review was carried out using six online databases (Medline/PubMed, Scopus, Web of Knowledge,

Embase, ScienceDirect, and ProQuest) without any time limitation. The following MeSH terms were used for the primary search: (retinopathy or synonyms: retinal neovascularization, retinal vasculopathy, retinal neuropathy, retinal neurodegeneration, retinal histopathology, and eye tissue damage) AND (diabetes or diabetic) AND (the name of carotenoids, mentioned in Table 1). All obtained studies were imported into an EndNote X5 (Thomson Reuters, Carlsbad, USA) library. The duplicate studies were then removed. Reference lists of all sightly studies were manually checked to find additional studies.

Inclusion and Exclusion Criteria

All English-language studies, which contained results of the impacts of carotenoids on DR in preclinical and clinical studies, were included. Reviews, abstracts, posters, letters, comments, and editorial papers were excluded from the review.

Results

Description of Literature Search

The six online databases search yielded 204 citations. After the removal of duplicate papers, 99 unique studies retuned to evaluate in more detail. Duplicates (n=105), irrelevant (n=67), abstracts and reviews (n=13), and non-English language (n=2) studies were excluded. Twenty-five papers were included. No additional papers identified through manual references checking of included studies. The flowchart of the studies process through the review is shown in Figure 1.

Studies Evaluating the Effects of Carotenoids on DR in Humans

Clinical trials and observational studies investigated the effects of carotenoids on diabetic patients with DR were reviewed. The results of these studies are summarized in Table 2.

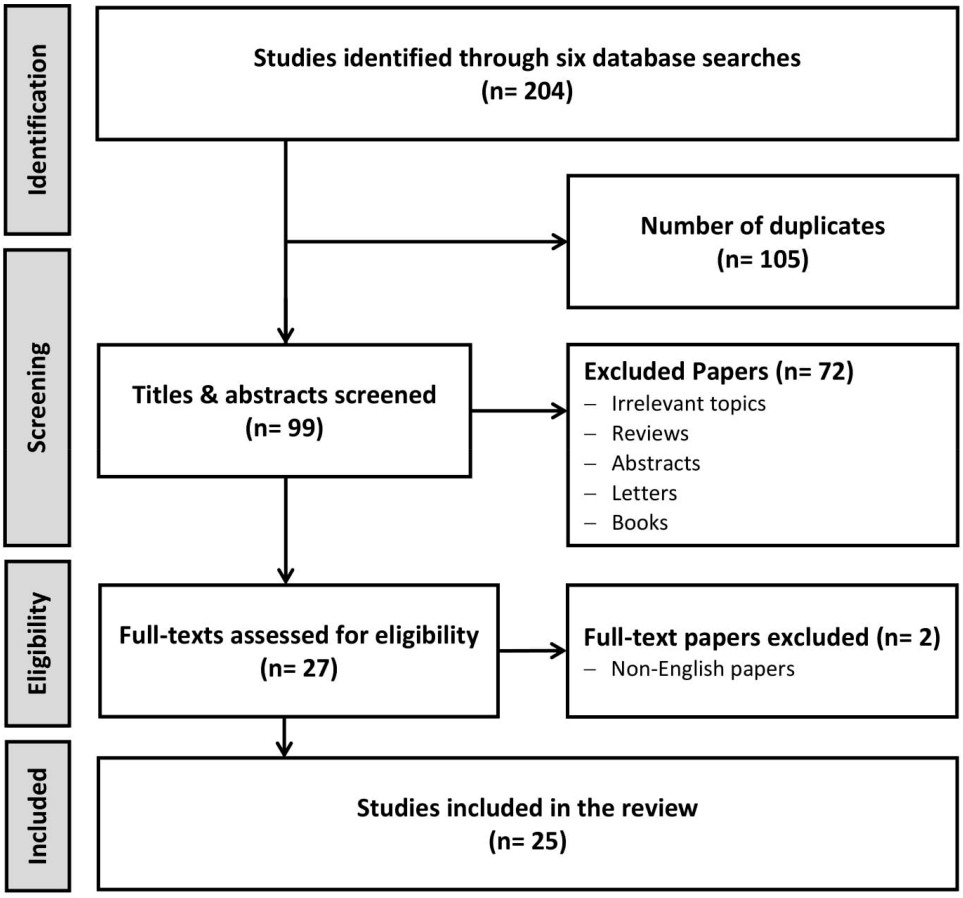

Figure 1 PRISMA flowchart of study selection and retrieval process.
Notes: Adapted from Moher D, Shamseer L, Clarke Met al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* 2015;4:1.[29]

Table 2 Studies Evaluating the Effects of Carotenoids on Diabetic Retinopathy in Humans

	Author, Year	Type of Studies	Number of Subjects		Carotenoids	Effects on Diabetic Retinopathy
			Controls	Cases		
1	Moschos, 2017[30]	Interventional	–	60	Lutein and zeaxanthin-containing supplement	• Improve visual acuity • Improve central foveal thickness • Increase retinal response density
2	Sahli, 2016[31]	Prospective cohort	-	1430	Lutein -rich diet	• No preventive effect on DR
3	Zhang, 2013[32]	Interventional placebo-control	NDR=15	15	Lutein	• No beneficial effects on visual acuity • Increase contrast sensitivity • Slight increase in glare sensitivity
4	Tanaka, 2013[33]	Prospective cohort	-	978	Carotene-rich fruits	• Decease the DR incidence
5	Hu, 2011[34]	Interventional	NDR=30 NS=30	30	Lutein and zeaxanthin-containing supplement	• Improve visual acuity • Increase contrast sensitivity • Reduce macular edema

Abbreviations: NDR, nonproliferative diabetic retinopathy patients; NS, normal subjects.

Moschos et al investigated the effects of carotenoid supplementation on retinal histological changes and macular function of diabetic patients in a two-year prospective cohort study. The results of the present study revealed that lutein (10 mg/day)-, zeaxanthin (2 mg/day)-, and meso-zeaxanthin (10 mg/day)-containing supplement improved retinal histological change and visual function.[30]

Sahli et al revealed that consumption of high lutein and zeaxanthin food was not associated with a decrease in DR incidence in a six-year prospective cohort study.[31]

Zhang et al study the impacts of lutein on diabetic patients with DR in a placebo-controlled clinical trial. They demonstrated intervention with lutein (10 mg/day) for 36 weeks caused a non-significant improvement of visual acuity and glare sensitivity. However, contrast sensitivity increased at special low frequencies.[32]

Tanaka et al reported that consumption of the carotene-containing fruits was able to decline the risk of DR among diabetic patients based on the results of an eight-week prospective cohort study.[33]

Hu et al investigated the effects of carotenoid-containing supplement on DR in a randomized clinical trial. They showed that lutein (6 mg/day) and zeaxanthin (0.5 mg/day) improved visual acuity, increased contrast sensitivity, and decreased macular edema after three months of intervention.[34]

Studies Evaluating the Effects of Carotenoids on Animal Models of DR

In this part, all experimental studies that evaluated the effects of different carotenoids on animal models of DR were reviewed. A summary of the results of these studies is presented in Table 3.

McClinton et al studied the beneficial effects of carotenoid-containing diet consumption on the DR in STZ-induced diabetic rats. The results demonstrated that the retinal function of treated animals aggravated after nine weeks of intervention.[35]

Sharavana et al showed treatment with lutein (0.1 mg/kg) for eight weeks suppressed retinal oxidative stress. It down-regulated the retinal expression of VEGF, VEGF receptor (VEGFR), VEGF co-receptor, and VEGFR transcriptional factor in streptozotocin (STZ)-induced diabetic rats. Additionally, this compound prevented retinal morphological changes, including retinal ganglion cell (RGC) loss and different retinal layers thinning.[36]

Yeh et al indicated that astaxanthin (3 mg/kg) and lutein (0.5 mg/kg) in a treatment period of eight weeks reduced retinal oxidative stress and inflammatory mediators in STZ-induced diabetic rats. Moreover, these carotenoids improved retinal histological and function changes.[37]

Kowluru et al reported the consumption of lutein- and zeaxanthin-containing diet for 11 months decreased

Table 3 Studies Evaluating the Effects of Carotenoids on Retinal Tissue of Diabetic Animal Models

	Author, Year	Animal Models	Carotenoids	Effects on Diabetic Retinopathy
1	McClinton, 2019[35]	STZ-induced diabetic rat	Carotenoid-rich diet	• Aggravate a- and b-wave amplitude in electroretinography • Aggravate a-, OP- and b-wave latency in electroretinography
2	Sharavana, 2017[36]	STZ-induced diabetic rat	Lutein	• Increase GSH levels and SOD activity • Decrease carbonyl and MDA levels • Decrease expression of VEGF and VEGF receptor • Decrease co-receptor and transcription factor of VEGF receptor • Decrease hypoxia-inducible factor • Prevent RGC loss and maintain INL thickness
3	Yeh, 2016[37]	STZ-induced diabetic rats	Astaxanthin Lutein	• Decrease 8-OHdG, nitrotyrosine and acrolein levels • Enhance hemeoxygenase, peroxiredoxin and thioredoxin activity • Decrease NF-kB levels • Decrease MCP-1, fractalkine and ICAM-1 levels • Prevent RGC loss and maintain TR, IPL, INL, and ORL thickness • Restore b-wave amplitude in electroretinography
4	Kowluru, 2014[38]	STZ-induced diabetic rats	Lutein, zeaxanthin-rich diet	• Decrease ROS and increase total antioxidant capacity • Decrease VEGF, NF-kB, IL-1β levels • Reduce mitochondrial DNA damages • Reduce capillary cell apoptosis • Restore a- and b-wave amplitude in electroretinography
5	Yu, 2013[39]	Transgenic diabetic mice	Zeaxanthin, Lutein-rich wolfberry diet	• Enhance expression of TFAM and PCG-1 • Decrease expression of hypoxia-inducible factor, VEGF and HSP60 • Enhance expression of AMPK
6	Tang, 2011[40]	Transgenic diabetic mice	Zeaxanthin, Lutein, Cryptoxanthin-rich wolfberry diet	• Enhance expression of thioredoxin, SOD and FOXO3α • Decrease BiP, PERK, ATF6, caspase-3 and caspase-12 levels • Enhance activation of AMPK • Suppress capillary-like structure formation • Decrease disorganized nuclear distribution in the ONL • Prevent RGC loss and restore RPL and INL thinking
7	Sasaki, 2010[41]	STZ-induced diabetic mice	Lutein-rich diet	• Decrease ROS levels • Increase BDNF and synaptophysin levels • Inhibit activation of ERK signaling pathway • Reduce cell apoptosis and caspase-3 levels • Prevent RGC loss and maintain INL and IPL thickness • Restore OP amplitude in electroretinography
8	Kowluru, 2009[43]	Alloxan-induced diabetic rats	β-carotene-containing supplement	• Decrease NF-kB levels • Decrease expression of iNOS • Decrease lipid peroxides, nitrotyrosine and NO levels
9	Kowluru, 2009[42]	Alloxan-induced diabetic rats	β-carotene-containing supplement	• Decrease lipid peroxides • Inhibit activation of caspase-3

(*Continued*)

Table 3 (Continued).

	Author, Year	Animal Models	Carotenoids	Effects on Diabetic Retinopathy
10	Arnal, 2009[44]	STZ-induced diabetic rats	Lutein	• Decrease MDA and nitrotyrosine levels • Enhance glutathione peroxidase activity and GSH levels • Reduce apoptosis • Prevent RGC loss and maintain INL ONL, and TR thickness • Restore b-wave amplitude and latency in electroretinography
11	Kowluru, 2008[45]	STZ-induced diabetic rats	Carotenoid-rich diet	• Decrease Lipid peroxide, nitrotyrosine, and 8-OHdG level • No beneficial effect on GSH levels • Decrease expression of iNOS • Increase expression of SOD and mitochondrial complex III • Decrease VEGF and ICAM-1 levels
12	Kowluru, 2008[45]	STZ-induced diabetic rats	Carotenoid-rich diet	• Decrease nitrotyrosine and 8-OHdG level • Increase expression of SOD and catalase • Increase expression of iNOS and mitochondrial complex III • Decrease acellular capillaries
13	Muriach, 2006[47]	Alloxan-induced diabetic mice	Lutein	• Decrease MDA and NF-kB levels • Increase GSH levels and glutathione peroxidase activity • Restore b-wave amplitude in electroretinography
14	Dene, 2005[48]	STZ-induced diabetic rats	β-carotene	• No beneficial effect on gamma-glutamyltransferase activity • Increase glutathione reductase activity
15	Kowluru, 2001[46]	STZ-induced diabetic rats	β-carotene-rich diet	• Decrease glutamate levels • Decrease lipid peroxides and NO levels

Abbreviations: GSH, reduced glutathione; MDA, malondialdehyde; VEGF, vascular endothelial growth factor; RGC, retinal ganglion cell; TR, total retina; IPL, inner plexiform layer; INL, inner nuclear layer; ORL, outer retinal layers; RPL, retina photoreceptor layer; OP, oscillatory potential; 8-OHdG, deoxyguanosine; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; NF-kB, nuclear factor kB; ROS, reactive oxygen species; IL-1β, interleukin 1 beta; HSP60, heat shock protein 60; AMPKα, AMP-activated protein kinase alpha; BiP, binding immunoglobulin protein; PERK, protein kinase RNA-like ER kinase; ATF6, activating transcription factor 6; SOD, superoxide dismutase; FOXO3α, forkhead O transcription factor 3 alpha; TNF-α, tumor necrosis factor alpha; BDNF, brain-derived neurotrophic factor; NO, nitric oxide; iNOS, inducible nitric oxide synthase; ERK, extracellular signal-regulated kinases.

expression of retinal oxidative stress, inflammation, and angiogenesis process in STZ-induced diabetic rats. Retinal capillary cells apoptosis, as well as retinal function, were improved in this study.[38]

Yu et al investigated the effects of wolfberry (1% of total calories) in a treatment period of eight weeks on transgenic diabetic mice. Wolfberry attenuated diabetes-induced mitochondrial dysfunction, hypoxia condition, and oxidative stress in retinal tissue. The results of the study demonstrated the active compounds of wolfberry diet, zeaxanthin and lutein, declined expression of VEGF, and retinal neovascularization.[39]

In another study, Tang et al studied DR in transgenic diabetic mice after eight weeks of intervention with wolfberry (1% of total calories). They showed that zeaxanthin, lutein, and also cryptoxanthin as active compounds of wolfberry diet lowered retinal oxidative stress and cell apoptosis. Furthermore, these compounds restored retinal morphological changes in diabetes.[40]

Sasaki et al reported a lutein-rich diet (0.1 w/w) decreased retinal oxidative stress in STZ-induced diabetic mice after four months of intervention. Lutein prevented retinal neurodegeneration and apoptosis through inhibition of ERK overactivation, preservation of synaptophysin, and improvement of retinal brain-derived neurotrophic factor (BDNF) levels. This carotenoid had a protective role in retinal histology and function.[41]

Kowluru et al evaluated the effects of the β-carotene-containing supplement on alloxan-induced diabetic rats in two studies. The results of these studies indicated that an eight-week treatment with β-carotene (45 mg/kg) as one of the active compounds of the supplement ameliorates retinal oxidative stress and inflammation. In this study, retinal cell

apoptosis was significantly decreased in the presence of the supplement.[42,43]

Arnal et al showed lutein (0.5 mg/kg) decreased intracellular oxidative stress in STZ-induced diabetic rats after 12 weeks of treatment. Retinal cell apoptosis also reduced by lutein administration. More importantly, this carotenoid improved the function of diabetic retinal tissue.[44]

Kowluru et al demonstrated that consumption of carotenoid-rich diet (0.02% w/w) for eight weeks decreased retinal oxidative stress and oxidative-induced damages in STZ-induced diabetic rats. Carotenoids inhibited hyperglycemia-induced retinal neovascularization.[45]

In other studies, Kowluru et al evaluated the effects of a carotenoid-rich diet (0.02% w/w) on STZ-induced diabetic rats in different studies. These compounds attenuated the retinal oxidative stress, mitochondrial overproduction of ROS, and oxidative damages of retinal microcapillary.[45] Additionally, β-carotene as an active compound of this diet decreased retinal glutamate and neurotoxicity.[46]

Muriach et al studied the effects of two-week administration of lutein (0.2 mg/kg) on DR in alloxan-induced diabetic mice. The results of the current study revealed that lutein suppressed oxidative stress, ameliorated inflammatory responses, and improved functions of retina.[47]

Dene et al reported that β-carotene (10 mg/kg) had no antioxidant impacts on retinal tissue in STZ-induced diabetic rats after two weeks of treatment.[48]

Studies Evaluating the Effects of Carotenoids on in vitro Models of DR

Experimental studies evaluated the effects of different carotenoids on in vitro models of DR were reviewed. A summary of these studies is presented in Table 4.

Hwang et al investigated the effect of various concentrations of lutein on human adult retinal pigment epithelial cells (ARPE-19) cultured in high concentrations of glucose. Their results demonstrated lutein inhibited hyperglycemia-induced oxidative stress and premature senescence in cells. They also showed these beneficial effects are related to the upregulation of silent information regulator 2 (SIRT1) mRNA and protein levels.[49]

Yang et al reported the result of their study on the retinal microglial cell lines, BV-2 and N9, which cultured in high-glucose and free fatty acid conditions. Crocin prevented the oxidative stress and inflammatory response of hyperglycemic and hyperlipidemic stress. Moreover, they showed these neuroprotective effects of crocin are related to activation of PI3K/Akt signaling pathway.[50]

Baccouche et al studied the effect of astaxanthin on primary retinal cells isolated from Psammomys obesus cultured in high-glucose condition. The results revealed that astaxanthin decreased cell apoptosis, improved mitochondrial function, and enhanced the neurons and glial cells viability.[51]

Umigai et al evaluated the effects of crocetin on human retinal microvascular endothelial cells (HRMEC) and human umbilical vein endothelial cells (HUVEC), which cultured in the presence of VEGF and high concentration of glucose. Although crocetin had no beneficial effect on cell viability, VEGF-induced formation of capillary-like structures in HUVEC culture, and migration of HRMEC suppressed. Furthermore, activation of p38 MAPK and expression of adhesion and tight junction proteins decreased.[52]

Tang et al investigated lutein and zeaxanthin impacts on the ARPE-19, which cultured in high concentrations of glucose. The study demonstrated that both zeaxanthin and lutein increased the activation of AMPK, resulting in attenuation of oxidative stress, and normalization of endoplasmic reticulum stress.[40]

Sun et al investigated the effects of lutein and astaxanthin on ARPE-19 in another study. These carotenoids exhibited suppression of oxidative stress and inhibition of carboxymethyllysine formation, an AGE indicator. Additionally, the deleterious effects of high exogenous AGE on ARPE-19 attenuated in the presence of these compounds.[53]

Kowluru et al found that β-carotene attenuated intracellular oxidative stress and also prevented apoptosis in HRMEC, which cultured in high concentrations of glucose.[42]

Discussion

DR is the leading cause of vision loss around the world with a high socioeconomic burden.[1] Despite the advances in the pharmacotherapy of diabetes, the global prevalence of DR has increased over the last years.[3] Hyperglycemia is the most important known cause of retinopathy.[6] The polyol pathway flux increases under hyperglycemic conditions. Aldose reductase reduces intracellular glucose to sorbitol and aldehydes to inactive alcohols with depleting of NADPH and reduced glutathione (GSH). Subsequently, sorbitol dehydrogenase oxidizes sorbitol to fructose with the consumption of NAD+.[54] Moreover, the intracellular

Table 4 Studies Evaluating the Effects of Carotenoids on in vitro Models of Diabetic Retinopathy

	Author Year	Cell Lines	Carotenoids	Effects on Diabetic Retinopathy
1	Hwang, 2018[49]	ARPE-19	Lutein	• Decrease ROS levels • Decrease SA-b-gal activity • Increase expression and protein level of SIRT1
2	Yang, 2017[50]	BV-2 N9	Crocin	• Decrease intracellular ROS and nitrites levels • Decrease expression of iNOS and COX-2 • Decrease IL-1β and TNF-α levels • Decrease expression of CD11b and Iba-1 • Activate the PI3K/Akt signaling pathway
3	Baccouche, 2017[51]	PRC	Astaxanthin	• Enhance mitochondrial dehydrogenase activity • Reduce cell apoptosis • Increase positive rhodopsin photoreceptors • Increase parvalbumin-positive neurons • Increase NCAM positive cells
4	Umigai, 2012[52]	HRMEC HUVEC	Crocetin	• Inhibit VEGF-induced cell proliferation and migration • Inhibit VEGF-induced p38 MAPK activation • Decrease expression of vascular endothelial cadherin • Suppress tube-like structure formation
5	Tang, 2011[40]	ARPE-19	Zeaxanthin Lutein	• Decrease ROS levels • Enhance FOXO3, SOD, and thioredoxin activity • Activate the AMPK signaling pathway
6	Sun, 2011[53]	ARPE-19	Astaxanthin Lutein	• Decrease ROS, RNS, and MDA levels • Suppress carboxymethyllysine formation • Inhibit AGE-induced cell proliferation • Inhibit AGE-induced overexpression of VEGF and MMP-2
7	Kowluru, 2009[42]	HRMEC	β-carotene	• Decrease lipid peroxide levels • Inhibit caspase-3 activity

Abbreviations: BV-2, microglial cells; N9, microglial cells; PRC, primary retinal cells; HRMEC, human retinal microvascular endothelial cells; HUVEC, human umbilical vein endothelial cells; ARPE-19, human adult retinal pigment epithelial cell; ROS, reactive oxygen species; RNS, reactive nitrogen species; SA-b-gal, senescence-associated b-galactosidase; SIRT1, silent information regulator 2; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; IL-1β, interleukin 1 beta; TNF-α, tumor necrosis factor alpha; PI3K/Akt, phosphoinositide-3-kinase-protein kinase/protein kinase B; NCAM, neural cell adhesion molecule; VEGF, vascular endothelial growth factor; p38 MAPK, p38 mitogen-activated protein kinases; AMPKα, AMP-activated protein kinase alpha; FOXO3α, forkhead O transcription factor 3 alpha; SOD, superoxide dismutase; AGE, advanced glycation end products; MDA, malondialdehyde; MMP-2, matrix metalloproteinase-2.

production of advanced glycation end-products increases. Non-enzymatic modifications of intracellular and matrix proteins alter various cellular functions and cause abnormal interactions between several matrix proteins and integrins. Changes of plasma proteins produce ligands which bind to advanced glycation end-products receptors and causes the generation of intracellular reactive oxygen species, and changes in gene expression.[55] Intracellular hyperglycemia also increases diacylglycerol content and activates protein kinase C, which has numerous pathogenic effects, especially activation of nuclear factor-κB, and NAD(P)H oxidases.[56] Furthermore, the hexosamine pathway activation in hyperglycemic condition results in several alternations in both gene expression and protein function.[57] Oxidative stress as a common link of the four pathogenic mechanisms results in hyperglycemia-induced damages. A body of evidence has demonstrated hyperglycemia-induced oxidative stress is responsible for retinal microvasculopathy as well as early neuropathy in the pathogenesis of DR.[1,58,59]

Carotenoids are a large group of organic and lipophilic pigments produced by plants, algae, and several bacteria and fungi.[15] Several studies have conducted on the beneficial effects of these compounds in the prevention and management of a large number of diseases, particularly diabetes and diabetic complications. It has also been shown that the levels of serum carotenoids are associated with the prediction and severity of DR.[26–28] Although the

therapeutic effects of carotenoids in DR have been evaluated in several different types of studies, the exact effects of these individual compounds on DR are not completely established.

In the present review, all studies evaluating the effects of a number of carotenoids on DR were systematically reviewed. The underlying mechanism of carotenoids on the retinal oxidative stress, inflammation, neovascularization, neurodegeneration, histology, and function in different types of diabetes models and diabetic patients were screened.

Different studies have reported that carotenoids exert retinal protection during oxidative stress, which is the most important underlying mechanism involved in the pathogenesis of DR.[60,61] As previous studies have reported, carotenoids or their active metabolites may act as both free radicals quenching and ROS\RNS scavenging in oxidative milieu.[62] The expression and activity of retinal oxidoreductases, including SOD,[36,40,45] glutathione reductase,[48] and glutathione peroxidase,[44,63] as well as intracellular antioxidant molecules including GSH[36] and thioredoxin,[40] increased in the presence of carotenoids. However, the levels of retinal GSH did not improve after intervention with carotenoids.[45] Moreover, retinal gamma-glutamyl transferase, a crucial enzyme involved in glutathione metabolism, increased.[48] Forkhead box protein O1 (FOXO1), a factor involved in catalase and SOD upregulation, enhanced after exposure with carotenoids.[40]

Carotenoids also have improved mitochondrial dysfunction as an important source of ROS/RNS in DR. These compounds enhanced the expression of mitochondrial dehydrogenase[51] and electron transport complex III[45] in the retinal tissue of diabetic animals. Heat shock protein 60 (HSP60) as a major mitochondrial biomarker of stress ameliorated after treatment with carotenoids.[39] Oxidative stress-induced endoplasmic reticulum (ER) stress has critical effects on retinal cell apoptosis. Both activating transcription factor 6 (ATF6) and protein kinase RNA-like ER kinase (PERK), signaling pathways involved in ER stress, and ER stress sensor protein binding immunoglobulin protein (BiP) ameliorated by carotenoids.[40]

The production of ROS and RNS in retinal tissue[38,41] or cell culture[40,50,53] of diabetes models decreased in the presence of carotenoids. High glucose-induced oxidative stress intracellular products including nitrotyrosine,[37,45] protein carbonyl,[36] acrolein,[37] oxidized deoxyguanosine,[37,45] and lipid peroxides[36,42–47,53] also diminished after treatment with carotenoids. Retinal expression of inducible nitric oxide synthase (iNOS)[43,45,46,50] and overproduction of NO-induced damages modulated by carotenoids.

Some studies reported that carotenoids are able to decrease hyperglycemia-induced AGE. Carotenoid reduced the formation of carboxymethyllysine and prevented neuronal and vascular damages of this AGE.[53]

Retinal inflammation, along with oxidative stress, is an essential part of DR pathogenesis. Treatment with carotenoids augmented the activation of retinal microglia, which have critical participation in the inflammatory responses of retina.[50] The expression of pro-inflammatory mediators in retinal tissue, including different cytokines,[37,38,45,50] chemokines,[37] adhesion molecules for leukocytes,[37] caspases-12,[40] and glutamate decreased in treatment with Catranides. The activation of NFκB[37,38,43,47] and extracellular signal-regulated kinases (ERK) signaling pathways[41] in the activated retinal microglia significantly decreased after consumption of carotenoids.

Additionally, carotenoids enhanced the expression of SOD and attenuated ER stress by upregulating the expression and activation of AMP-activated protein kinase (AMPK).[39,40] Carotenoids also activated the retinal phosphoinositide-3-kinase-protein kinase/protein kinase B (PI3K/Akt) signaling pathway, which plays a crucial role in ameliorating oxidative stress, diminishing the pro-inflammatory response, and reducing neurodegeneration.[50]

Retinal vasculopathy also plays a fundamental role in the process of DR initiation and progression. Carotenoids can significantly improve retinal endothelial dysfunction.[64] It has been shown that retinal capillary in DR reduced in the presence of carotenoids.[45] Blood-retinal barrier breakdown and vascular leakages have recovered after treatment with carotenoids.[52] More importantly, carotenoids had significant beneficial effects on retinal neovascularization, particularly by decrease the expression of VEGF,[36,38,39,45,52] VEGF receptors,[36] and VEGF transcription factors.[36] Increased hypoxia-inducible factor (HIF) due to retinal vasculopathy modulated by carotenoids.[36,39]

Retinal neuronal and vascular cell apoptosis also involved in the pathogenesis of DR. Several studies showed carotenoids decreased apoptosis in retinal tissue and cell of diabetes models.[38,41,44,51] Moreover, it has been shown that these compounds mitigated the activation of caspase-3.[40,42]

Recent studies have shown that retinal neuropathy plays a crucial role, especially in the early stage of DR.[65,66] Carotenoids increased the expression of retinal BDNF and prevented retinal neurodegeneration.[41] Furthermore, it has

been demonstrated that carotenoids diminished RGC loss,[36,37,40,41] thinning of the different retinal layer including total retina (TR),[37] inner plexiform layer (IPL),[37,41] inner nuclear layer (INL),[36,37,40,41] outer retinal layers (ORL),[37] retina photoreceptor layer (RPL)[40] in animal models of diabetes.

Several lines of evidence demonstrated retinal electrophysiological function in preclinical and clinical models of DR has improved after treatment with carotenoids. The amplitude of a-wave,[38] b-wave,[37,38,44,47] and oscillatory potentials[41] as well as the implicit time of b-wave[44] in electroretinography of diabetic animals restored with carotenoids. However, the administration of carotenoids aggravated a-, b-waves amplitude and a-, b-wave, and oscillatory potentials latency in electroretinography.[35]

Although there is not much evidence for the effects of carotenoids on DR in clinical trials, some beneficial effects of these compounds have investigated in diabetic patients. These compounds improved central foveal thickness,[30] retinal response density,[30] visual acuity,[34] contrast sensitivity,[32,34] and macular edema.[34] However, in some studies, carotenoids had no advantageous effects on visual acuity and glare sensitivity,[32] and the absence of these effects might be related to the short period of intervention. It has been shown in an eight-year prospective cohort, consumption of carotenoid-rich diet declines the DR incidence among diabetic patients.[31] On the other hand, the intake of food rich in lutein and zeaxanthin was not associated with a decrease in DR incidence.[31]

Carotenoids showed a higher success rate in the improvement of DR in experimental models compared to diabetic patients. This discrepancy might be related to low doses of carotenoids and short duration of intervention in clinical studies. Moreover, a carotenoids-rich supplement or diet was used in most clinical studies. These types of studies have evaluated the effects of several compounds instead of a specific carotenoid and might be reported confounding results.

It will be advantageous to evaluate the impacts of carotenoids on DR in good quality, placebo-controlled, and large prospective clinical trials in a more prolonged period of time. Furthermore, these trials must also evaluate the safety and tolerability of different carotenoids.

Conclusion

In summary, carotenoids potentially delay the initiation and prevent the progression of DR; however, ample preclinical studies are required to confirm their effect, and adequate clinical trials are needed to prove their effectiveness among humans.

Disclosure

The authors claim that there is no conflict of interest.

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