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1 *Type of the Paper: Review*

2 **Impact of astaxanthin on diabetes pathogenesis and chronic complications**

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14 **Abstract:** Oxidative stress (OS) plays a pivotal role in the diabetes mellitus (DM)
15 onset, progression, and chronic complications. Hyperglycemia-induced reactive
16 oxygen species (ROS) have been shown to reduce insulin secretion from pancreatic
17 β -cells, to impair insulin sensitivity and signaling in insulin-responsive tissues,
18 and to alter endothelial cells function in both type 1 and type 2 DM. As a powerful
19 antioxidant without side-effects, astaxanthin (ASX), a xanthophyll carotenoid, has
20 been suggested to contribute to the prevention and treatment of DM-associated
21 pathologies. ASX reduces inflammation, OS, and apoptosis by regulating different
22 OS pathways though the exact mechanism remains elusive. Based on several
23 studies conducted on type 1 and type 2 DM animal models, orally or parenterally
24 administrated ASX improves insulin resistance and insulin secretion, reduces
25 hyperglycemia, and exerts protective effects against retinopathy, nephropathy,
26 and neuropathy, however more experimental support is needed to define
27 conditions for its use. Moreover, its efficacy in diabetic patients is poorly
28 explored. In the present review, we aimed to identify the up-to-date biological
29 effects and underlying mechanisms of ASX on the ROS-induced DM-associated
30 metabolic disorders and subsequent complications. The development of an
31 in-depth research to better understand the biological mechanisms involved and to
32 identify the most effective ASX dosage and route of administration deems
33 necessary.

34 **Keywords:** astaxanthin; diabetes mellitus; oxidative stress; hyperglycemia;
35 diabetes complication; diabetic retinopathy; diabetic neuropathy; diabetic
36 nephropathy; insulinresistance; antioxidant; ROS

37
38 **Abbreviations:** AGE, advanced glycation end-products; AKT, protein kinase B; ARE,
39 antioxidant response element; ASX, astaxanthin ; COX-2, cyclooxygenase 2; DM, diabetes mellitus;
40 DNA, deoxyribonucleic acid; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase;
41 ERK1/2, extracellular signal-related kinases 1/2; FN, fibronectin; GPx, glutathione peroxidase; GSH,

42 glutathione; GSTA1, glutathione S-transferase alpha1; HG, hyperglycemia; HO-1, heme
43 oxygenase-1; ICAM-1, intercellular adhesion molecule-1; I κ B, inhibitor of nuclear factor kappa B;
44 IL-1, interleukin-1; IL-6, interleukin-6; IR, insulin receptor ; LOX-1, lectin-like oxidized LDL receptor
45 1; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MDA,
46 malondialdehyde; MMP2, matrix metalloproteinase-2; MnSOD, manganese superoxide dismutase;
47 mtROS, mitochondrial reactive oxygen species; NADPH, nicotinamide adenine dinucleotide
48 phosphate; NF- κ B, nuclear factor-kappa B; NOX, NADPH oxidase; NQO1, NAD(P)H
49 dehydrogenase quinone 1; Nrf2, nuclear factor erythroid 2-related factor 2; oxLDL, oxidized low
50 density lipoprotein; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; RAGE, receptor for
51 advanced glycation end products; ROS, reactive oxygen species; SHP-1, Src homology region 2
52 domain-containing phosphatase-1; SOD, superoxide Dismutase; SZT, streptozotocin; T1DM, type 1
53 diabetes mellitus; T2DM, type 2 diabetes mellitus; TGF- β , transforming growth factor beta; TNF- α ,
54 tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

55 1. Introduction

56 Diabetes mellitus (DM), the most common metabolic disease, has become a
57 major health concern with increasing prevalence worldwide. Currently 463 million
58 people (9.3% of adults aged 20–79 years) are living with diabetes around the world.
59 It is to be added a further 1.1 million children and adolescents under the age of 20,
60 who live with type 1 diabetes (T1DM)[1]. This number is projected to increase to
61 578 million by 2030. The financial burden of DM is also staggering. The cost of the
62 total diabetes-related healthcare is estimated to be around \$760 billion and has been
63 projected to reach up to \$825 billion in the next 10 years. DM is a chronic disease
64 that is recognized by hyperglycemia (HG) resulting from impaired insulin
65 secretion, inappropriate insulin action, or both. The insulin deficit leads to high
66 levels of blood glucose (HG), which if not tightly controlled, leads to disabling and
67 life-threatening health complications including cardiovascular diseases,
68 retinopathy, neuropathy, nephropathy, and prolonged/incomplete wound
69 healing[1]. Moreover, DM increases bone fragility and impaired bone healing[2,3].
70 The main categories of DM are type 1 (T1DM), type 2 (T2DM), and gestational DM.
71 From these three types, T2DM is the most common type, accounting for around
72 90% of all diabetes worldwide.

73 The development of DM and its complications are known to be associated with
74 oxidative stress (OS) and low-grade chronic inflammation[4]. OS, an imbalance
75 between cellular oxidant and antioxidant systems, results from the overproduction
76 of free radicals and associated reactive oxygen species (ROS). HG upregulates the
77 markers of chronic inflammation and contributes to the increased ROS generation,
78 which ultimately involves in DM complications including vascular dysfunction[5].
79 Moreover, an increased level of ROS reduces insulin secretion and impairs insulin
80 sensitivity and signaling in insulin-responsive tissues[5]. Proper treatment of HG
81 and inhibition of ROS overproduction is, therefore, crucial for delaying the DM
82 onset and progression, as well as preventing its subsequent complications.

83 Recent advances on biological properties of antioxidants such as carotenoids
84 have suggested that these compounds are able not only to prevent but also to
85 ameliorate diabetes and its subsequent complications[6]. In particular, astaxanthin
86 (ASX) (3,3'-dihydroxy- β , β' -carotene-4,4'-dione), a xanthophyll carotenoid[7], has

87 been reported to exhibit multiple biological activities including modulation of OS
88 and inflammation through free radical quenching[8], and activation of endogenous
89 antioxidant systems via modulation of gene expression[9,10]. It has therefore
90 attracted considerable interest because of its potential pharmacological effects
91 including antidiabetic, anti-inflammatory, and antioxidant activities, as well as its
92 neuro-, nephro-, and retinoprotective, and cardiovascular effects[6].

93 Given the crucial role of OS and inflammation in the pathogenesis and
94 subsequent complications of DM, in this review we aimed to analyze the biological
95 effects and the underlying mechanisms of ASX on the prevention and the treatment
96 of DM-associated pathologies.

97 **2. Pathophysiology of diabetes-related metabolic disorders and complications** 98 **focus on the hyperglycemia - oxidative stress interactions**

99 T1DM and T2DM are complex conditions characterized by elevated blood
100 glucose levels due to impaired insulin production and/or a decrease in insulin
101 sensitivity and function. T1DM is caused by the autoimmune-mediated destruction
102 of pancreatic β -cells and is mainly associated with failure in insulin production.
103 While T2DM is associated with chronic abnormalities of carbohydrate and fat
104 metabolisms in which triggering various pathways induce impaired insulin
105 secretion from the pancreatic β -cells, insulin resistance and decreased glucose
106 utilization in peripheral tissues, and abnormal hepatic glucose production[11]. In
107 both T1DM and T2DM, chronic HG leads to macro- and micro-vascular
108 complications [12]. Today, it is admitted that OS and ROS are involved in the
109 pathogenesis of impaired insulin secretion from the pancreatic β -cells, insulin
110 resistance, and endothelial dysfunction in both T1DM and T2DM[11–13], and that
111 oxidized DNA, RNA, protein and lipid products can be used as possible disease
112 progression markers[12].

113 In the diabetes-related pathologies, ROS play a central role in the interactions
114 involving metabolic control, OS, and inflammation[14][4]. ROS are a family of
115 species including molecular oxygen and its derivatives, superoxide anion (O_2^-),
116 hydroxyl ($HO\cdot$), hydrogen peroxide (H_2O_2), peroxyxynitrite ($ONOO^-$), hypochlorous
117 acid ($HOCl$), nitric oxide (NO), and lipid radicals. Out of these molecules,
118 superoxide has been the initial oxygen free radical, which is then converted to other
119 more reactive species that can damage cell. They are produced in the aerobic
120 metabolism at very low levels and play a crucial role in the cell signaling
121 homeostasis[15]. ROS are mainly produced in the mitochondrial electron transport
122 chain (ETC), endoplasmic reticulum (ER), peroxisomes, and microsomes [4].
123 Overproduction and/or insufficient removal of excessive ROS by endogenous
124 antioxidant systems result in damage to cellular proteins, membrane lipids, and
125 nucleic acids, thereby leading to OS. Besides antioxidant components such as
126 superoxide dismutase (SOD) and glutathione (GSH), different cellular
127 compartments (nucleus, cell membrane, mitochondria) contribute differently to the
128 ROS balance[14].

129 In HG conditions and/or fatty acid oxidation, therefore, ROS overproduction
130 can alter cell function, and induce inflammation and apoptosis in the pancreas,
131 kidney, liver, nerves, and vasculature[13,14].

132 For instance, in T1DM, the generated ROS under metabolic or autoimmune
133 stress in the cells of the pancreatic microenvironment may enhance damage of
134 pancreatic β -cells, thereby reducing insulin secretion[13]. Regarding T2DM, the
135 HG-induced ROS production is considered a major contributor to the damage and
136 disease progression. HG leads to glucose autoxidation, alters mitochondrial
137 dynamics, and enhances ROS production. The induced OS reduces insulin secretion
138 and impairs insulin signaling in target tissues[16]. ROS overproduction is also the
139 underlying contributor to the development of vascular complications of DM [14].
140 HG causes tissue and endothelial cells damage through five primary mechanisms:
141 (1) an increase in the flow of glucose through the activation of the alternative
142 metabolic pathways of glucose, the polyol pathway; (2) an increase in the formation
143 of intracellular advanced glycation end-products (AGEs); (3) an increase in the
144 expression of the AGE receptor and activation of the ligands; (4) activation of
145 isoforms of the protein kinase C (PKC); and (5) hyperactivity of the hexosamine
146 pathway. Today, it has been widely accepted that all of these different pathogenic
147 mechanisms stem from the HG-induced excessive production of superoxide anion
148 radical ($\bullet\text{O}_2^-$) in the mitochondrial ETC.

149 Specifically, high levels of blood glucose increase AGEs formation and subsequent
150 OS[17]. The accumulation of AGEs has been related to diabetes, as well as its
151 associated complications, although the molecular mechanisms and the signaling
152 pathways involved are yet to be clearly defined. AGEs, via their receptors
153 AGE-R1/OST-48, AGE-R2/80K-H, AGE-R3/galectin-3, LOX-1, and RAGE mediate
154 ROS production and increase the intracellular levels of H_2O_2 , O_2^- , and NO. The
155 interaction of AGEs with RAGE specifically increases mRNA levels of NOX1,
156 NOX2, and NOX4, and activates the NADPH oxidases, the mitochondrial
157 respiratory chain, microsomal enzymes, xanthine oxidase, and arachidonic acid[17].
158 Moreover, AGE- induced ROS enhances the expression and activities of antioxidant
159 enzymes such as catalase, glutathione peroxidase (GPx), and SOD. AGE-induced
160 increased ROS formation also modulates the signal transduction pathways such as
161 RAS/MEK/ERK1/2, IP3K/AKT, or p38MAPK p21RAS, enhances NF- κ B
162 translocation and activation, and finally induces inflammation, apoptosis, and
163 proliferation by the NLRP-3 inflammasome and the production of several cytokines
164 such as IL-6, TNF- α , IL-1 β , MCP-1 [18,19]. Many studies have shown increased
165 circulating levels of IL-6 and TNF- α in rodents and T2DM patients[20]. These
166 cytokines impair insulin signaling and peripheral glucose uptake, and contribute to
167 insulin resistance, lipolysis, and hepatic glucose production.

168

169 3. Astaxanthin: biological effect on diabetes onset, progression, and chronic 170 complications

171 3.1. General aspects of astaxanthin: structure, sources, bioactivity, and 172 administration toxicity

173 3.1.1. Sources and chemical structure of astaxanthin

174 ASX is one of the most powerful carotenoids with antioxidant activity which is
175 produced naturally or chemically. Natural sources include various microorganisms
176 and marine animals such as yeast, microalgae, trout, salmon, krill, shrimp, crayfish,
177 complex plants, and some birds. ASX is not synthesized in human body but is
178 ingested in the diet, in particular by seafood[21]. Commercialized ASX is often
179 extracted from *Phaffia rhodozyma*, *Blakeslea trispora*, and *Haematococcus pluvialis*, as
180 well as being produced by chemical synthesis. *H. pluvialis*, the main source of
181 natural ASX for human consumption, is a unicellular green microalga which
182 accumulates ASX under environmental stress conditions such as starvation,
183 irradiation, high salinity, temperature or light[18,19]. ASX was authorized since
184 1987, by the United States Food and Drug Administration (US FDA) as a fish feed
185 additive, then was approved as a dietary supplement in 1999 [22]. ASX has also
186 been registered as a fish feed additive by the European Food Safety Authority
187 (EFSA). According to the Novel Foods Regulation (EU) 2015/2283, ASX from *H.*
188 *pluvialis* has an acceptable daily intake in human up to 8mg[23]. The *H.*
189 *pluvialis*-extracted ASX, included in human dietary supplements, has proved to be
190 safe and accepted by the US FDA at daily doses of 2–12 mg [24,25].

191 ASX (3,3'-dihydroxy- β,β' -carotene-4,4'-dione, molecular formula C₄₀H₅₂O₄, molar
192 mass 596.84 g/mol) is a xanthophyll carotenoid which is composed of 40 carbon
193 atoms organized in two oxygenated β -iononene rings joined by a polyene chain
194 [7,26,27]. The conjugated double bond chain acts as a strong antioxidant by electron
195 donation and by reacting with free radicals[28]. Hydroxyl acid on terminal rings
196 can react with fatty acids and form mono-esters and/or di-ester. In the nature, ASX
197 can also exist as conjugated with proteins (e.g., salmon muscle or lobster
198 exoskeleton). Interacting with fatty acids or proteins stabilizes ASX which in its free
199 form is unstable and particularly susceptible to oxidation; therefore, this form is
200 mainly produced synthetically or from yeast[28]. ASX can be naturally found in
201 stereoisomers, geometric isomers, free and esterified forms. Moreover, synthetic
202 ASX may have different conformations including chiral [(3S, 3'S) or (3R, 3'R)] or
203 meso forms (3R, 3'S), however the chiral stereoisomers are the most prevailing
204 forms. In addition, the polyene chain double bond can be organized as two different
205 conformations: cis or trans. Trans-isomers remain the most widespread form due to
206 the thermodynamic instability of cis carotenoids[29].

207

208

209 3.1.2. Administration doses and toxicity studies

210 Solving ASX stability drawbacks and parenteral administration problems are of
211 crucial importance for the development of ASX-based therapy. In order to
212 encapsulate, deliver, protect, and/or enhance its *in vivo* stability, several vectors
213 including polymeric systems, lipid-based carriers, and inclusion complex using
214 cyclodextrins have been proposed[30,31]. A polymeric matrix or a coating layer
215 around a particular compound provides a physical barrier, preserves its biological
216 activity, and enhances its physicochemical stability[21,32,33]. Lipid-based carriers
217 based on microemulsion, nanoemulsion, or an association of both referred as solid
218 lipid nanoparticles or nanostructured lipid carriers were shown to stabilize ASX
219 [30,34,35]. A clinical trial revealed improvement of ASX bioavailability when
220 incorporated into a lipid-based carrier[36].

221 Nanoemulsions formulated with ASX and α -tocopherol were administered locally
222 to streptozotocin (STZ)-induced diabetic mice wound; a faster wound closure was
223 seen in a while. The tissue composition in the presence of nanoemulsion was
224 characterized by an abundant number of inflammatory cells, fibroblast formation,
225 and collagen syntheses[37]. Cyclodextrins, natural macrocyclic oligosaccharides
226 well known for having toroid-shaped structures with rigid lipophilic cavities, and a
227 hydrophilic outer surface were also proposed as an encapsulation system of choice
228 to solubilize ASX [38,39]. The non-covalent association of the latter with
229 cyclodextrin improves its solubility in water, bioavailability, stability [40], and
230 release[41,42]. The local delivery of cyclodextrin-ASX complexes from
231 biocompatible polyvinyl alcohol-dextran hydrogels was shown to block OS[33]. In
232 addition, in an ischemic rat model, the same systems neutralized excessive ROS and
233 modulated the expression of endogenous antioxidant genes, suggesting that local
234 release of ASX may reduce the damage to tissues by activating the endogenous
235 antioxidant pathways[39].

236 After oral administration, ASX esters are hydrolyzed selectively during absorption
237 with the intestine and transported through circulation to the tissues[43]. Due to the
238 presence of polar ends in its structure, ASX can be absorbed better than other
239 non-polar carotenoids such as lycopene and β -carotene[18]. However, its limited
240 solubility in digestive fluids compromises the uptake by intestinal epithelial cells
241 and its final secretion to lymph as chylomicrons[44,45]. The mechanism involved in
242 the absorption, the metabolic conversion of ASX, and the excretion is not
243 completely elucidated. Besides the chemical structure, the absorption and
244 bioavailability of ASX are influenced by a variety of parameters such as age,
245 gender, smoking, fat, food intake, and so on [46]. Various studies have tested
246 dosages of natural ASX ranging from 1-4 mg/day to 100 mg/day of synthetic ASX
247 and reported a nonlinear dose -response for plasma concentrations, as well as a
248 lower bioavailability of esterified ASX[46].

249 From a safety perspective, studies on oral administration of natural ASX have not
250 documented negative effects [23]. For instance, oral administration of high doses
251 (up to 1240 mg/kg/day) over a long period of time (90 days) in rats did not exhibit
252 any toxic effect[47]. In addition, in humans, natural ASX ranging from 8 to 45
253 mg/day over 4 to 12 weeks showed low adverse effect[48]. ASX has an excellent
254 clinical safety, even when compared to the placebo in randomized studies, at low
255 (up to 12 mg) or high (up to 100 mg) doses of daily administration[23]. Nowadays
256 only natural ASX have been reported as human dietary supplement [7] and
257 although *in vivo* preclinical studies on rats tested at least 100-800 times the actual
258 human safe doses, no critical adverse effect was observed. Safety parameters and
259 specific dose activities in humans should therefore be investigated[49].

260 Since 2010, 34 clinical studies have been reported on the use of ASX
261 (clinicaltrials.gov), from which 22 have been completed until now. ASX has mainly
262 been tested as a dietary supplement alone or in combination with other molecules
263 for safety and pharmacokinetic information or on various pathologies associated to
264 OS (e.g., stroke, infertility, hyperlipidemia, skin aging, cognitive dysfunction).
265 Currently two studies are recruiting to investigate the potential of antioxidant
266 therapy (ASX combined with lutein, zeaxanthin, vitamin C, vitamin E, zinc, and
267 copper as a daily-administered drug) for diabetic retinopathy (unpublished data,
268 clinical trials N° NCT03310359 and N° NCT03702374). The study of antioxidant
269 therapy involving ASX for diabetic retinopathy has been completed; however, the
270 results have not been published yet. Emerging clinical studies using ASX for
271 diabetic complications treatment, reflect the medical interest for this antioxidant.

272 3.1.3. Bioactivity of astaxanthin

273 At cellular and molecular levels, ASX acts against oxidative damage through
274 various mechanisms (Figure 1) including quenching singlet oxygen, scavenging
275 radicals, inhibiting lipid peroxidation, and regulating OS-related gene expression
276 [50]. Growing evidence suggests that ASX improves mitochondrial function by
277 reducing mtROS production, increasing ATP production, mitochondrial content,
278 and respiratory chain complex activity[51].

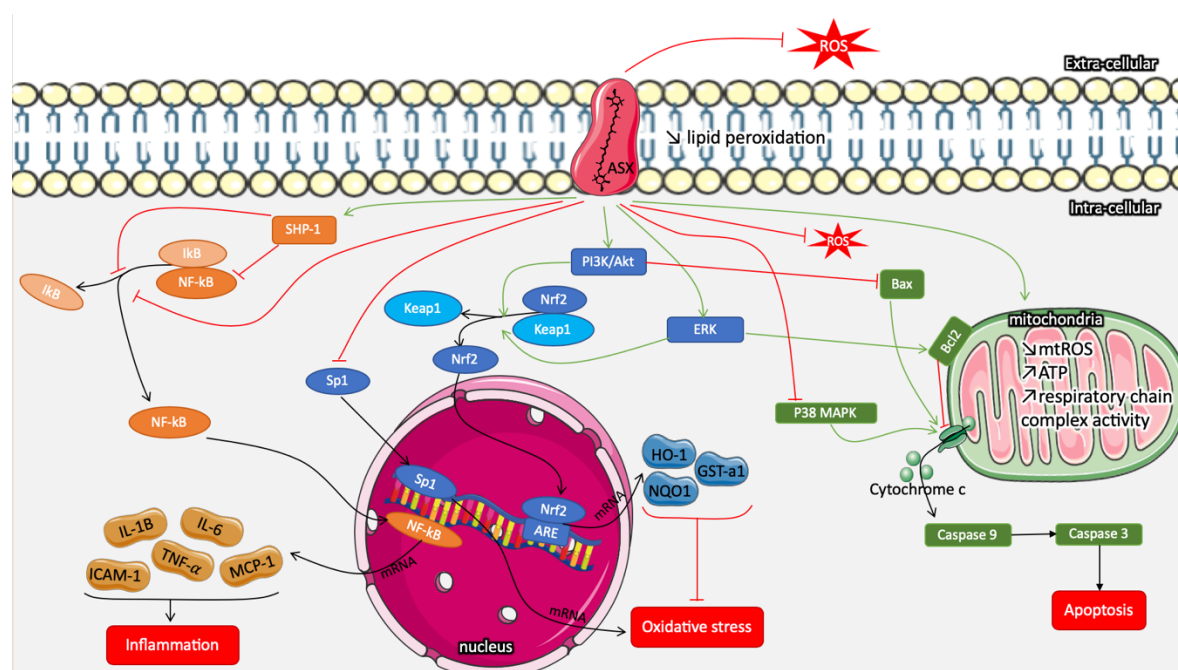


Figure 1. Schematic representation of molecular pathways implied in the protective potential of astaxanthin (ASX). Due to its membrane penetrance, ASX has both intra and extra cellular ROS scavenging actions. Moreover, in the phospholipid membrane, the ASX polyene chain participates in the reduction of lipid peroxidation. Through the regulation of different pathways, ASX reduces inflammation, oxidative stress, and apoptosis. Red arrow indicates an inhibitory action and green arrow shows an enhancement action.

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287 Parallel to natural scavenging, in STZ-induced diabetic rats (12 weeks), ASX
 288 regulates intracellular OS, by mainly activating MAPK, PI3K/Akt, and Nrf2/ARE
 289 signaling pathways[52]. Activation of PI3K/Akt and ERK pathways facilitates the
 290 dissociation and translocation of Nrf2 to the nucleus. Nrf2/ARE increases the
 291 expression of heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1
 292 (NQO1), and Glucathione S-transferase alpha1(GST- α 1), and leads therefore to an
 293 endogenous antioxidant response. ASX was proved to ameliorate the early brain
 294 injury by the activation of Nrf2/ARE signaling pathways in an experimental
 295 subarachnoid hemorrhage rat model[53]. In addition, ASX inhibits specificity
 296 protein 1 (Sp1) / NMDA receptor subunit 1 (NR1) pathway, involved in
 297 intracellular ROS production[50,51].

298 ASX, through PI3K/Akt and ERK activation, also regulates Bax/Bcl2 pathway,
 299 which reduces cytochrome c release from mitochondria and decreases caspase
 300 3-related apoptosis (reduction of mitochondrial permeabilization). In endothelial
 301 cells, under OS, ASX treatment significantly activate PI3K/Akt and reduced the
 302 expression of both eNOS and Bax genes[31]. Moreover, ASX decreases
 303 caspase-associated apoptosis by modulating p38 MAPK pathway[54]. Finally, ASX
 304 modulates inflammatory response by inhibiting the release of pro-inflammatory
 305 cytokines (e.g., IL-1 β , IL-6, ICAM-1, TNF- α , MCP-1) through the activation of
 306 SHP-1, which suppresses NF- κ B expression and I κ B degradation. ASX also has a

307 direct inhibitory effect on I κ B liberation, preventing the nuclear translocation of
 308 NF- κ B and increasing the anti-inflammatory effect [10,49,55].

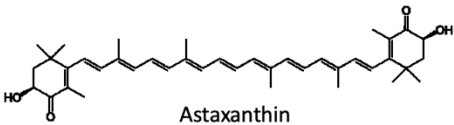
309 3.2. Anti-diabetic effects of astaxanthin

310 ASX has shown some beneficial effects on the prevention and treatment of
 311 diabetes[6]. These effects depend on how advanced the disease is, as well as the
 312 type of complications observed. Table 1 depicts summarized main effects described
 313 in the literature. For example, in clinical studies, oral administration of ASX
 314 (8mg/day for 8 weeks) to patients with T2DM significantly reduced the
 315 fructosamine and plasma glucose concentrations[56]. ASX also improves glucose
 316 metabolism and reduces blood pressure in patients with T2DM.

317

318

319 **Table 1.** Beneficial effects of astaxanthin at different developmental stages of DM and its complications.



Astaxanthin

Early-diabetes	Diabetes	Diabetic complications
<ul style="list-style-type: none"> ↗ Pancreatic βCell protection [60] ↗ Insulin sensitivity [62,68] ↗ Glucose metabolism [62,63,64] ↗ Antioxidant enzymes [69] ↘ Insulin resistance [63, 66, 67] ↘ Blood glucose level [69] 	<ul style="list-style-type: none"> ↗ Antioxidant enzyme [61,69,71,81, 83,91,95] ↗ Adiponectin [66,69] ↘ Hyperglycemia [56-61,66,69] ↘ Lipid Peroxidation [61,65,83,91,95] ↘ ROS/oxidative stress [57,65,71,81,83,91,95] ↘ Inflammation [65,68,71,81,91,95] 	<ul style="list-style-type: none"> ↘ Retinopathy [81,82,83] ↘ Nephropathy [52,57,58,61,91,92] ↘ Neuropathy [93,94,95] ↘ Cardiovascular complication [99,100,101,102,103,104]

320

321 The anti-diabetic effect of ASX has been studied in different animal models with
 322 variable outcomes (Table 1) though the underlying mechanisms have not been
 323 clearly elucidated. Oral administration of ASX significantly reduced fasting blood
 324 glucose in db/db mice – a well-known obese model for T2DM [57–60]. A long-term
 325 administration of ASX (35mg/kg for 12 weeks) showed that hypoglycemic effect is
 326 linked with a lower malondialdehyde (MDA) content and enhanced SOD activity in
 327 serum[61]. In the same animal model, in the presence of ASX (1mg/day during 18
 328 weeks), pancreatic β -cells were protected against glucose toxicity as they preserved
 329 their ability to secrete insulin. The non-fasting blood glucose level also significantly
 330 reduced[60].

331 Besides glucose-lowering effect, animal studies have demonstrated that ASX
 332 improves insulin sensitivity and glucose uptake, thereby lowering insulin
 333 resistance, the hallmark of T2DM. Arunkumar *et al.* [62] reported that ASX (6 mg/kg
 334 per day for 45 days) significantly lowered plasma glucose and insulin levels in
 335 HFFD-fed mice and improved insulin sensitivity. Moreover, ASX potentiates

336 post-receptor insulin signaling events by enhancing the autophosphorylation of
337 insulin receptor- β (IR- β), IRS-1 associated PI3-kinase step, phospho-Akt/Akt ratio,
338 and GLUT-4 translocation in skeletal muscles [62]. Yasuhiro Nishida *et al.* also
339 showed improved glucose metabolism by enhancing glucose incorporation into
340 skeletal muscles in ASX-treated HFD mice [63]. In this study, ASX administration
341 significantly ameliorated insulin resistance and glucose intolerance through
342 regulation of 5' adenosine monophosphate-activated protein kinase (AMPK)
343 activation in the muscle, independent of its antioxidant activity. In addition, it
344 stimulated the muscle mitochondrial biogenesis[63].

345 Furthermore, ASX improves glucose metabolism by increasing glycogen reserves in
346 the liver[64]. It modulates the activity of metabolic enzymes such as hexokinase,
347 pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, and glycogen
348 phosphorylase. It also promotes the IRS-PI3K-Akt pathway of insulin signaling by
349 decreasing serine phosphorylation of IRS proteins. In STZ-induced diabetic rat, a
350 well-known T1DM model, the treatment with ASX (50 mg/kg body weight/day; 18
351 days), significantly decreased the levels of AGEs, ROS, and lipid peroxidation in the
352 liver of diabetic rats. These results suggested that the inhibitory effect of ASX on
353 diabetes-induced hepatic dysfunction could be linked to the blocking of AGEs
354 formation and further anti-inflammatory effect[65].

355 Insulin resistance and compensatory hyperinsulinemia are strongly associated
356 with obesity, dyslipidemia, and hypertension in the metabolic syndrome. The effect
357 of ASX was also evaluated on several parameters of metabolic syndrome[6,66].
358 Hussein *et al.* reported that a 22-week ASX administration (50 mg/kg/day) in SHRcp
359 rats reduced the fasting blood glucose level, improve insulin sensitivity and lipid
360 metabolism parameters including serum adiponectin[66]. Further, it has been
361 shown that ASX administration for 10 weeks prevented and reversed hepatic
362 insulin resistance and ameliorated hepatic steatosis in both genetically (*ob/ob*) and
363 high-fat-diet-induced obese mice[67]. Moreover, these results were associated with
364 enhanced insulin-stimulated phosphorylation of the insulin receptor (IR)- β subunit
365 (p-IR β), and Akt (p-Akt) in the livers of CL+AX mice compared with CL mice[67].
366 ASX may also modulate interactions of PPARgamma with transcriptional
367 intermediary factor 2 (TIF2), steroid receptor coactivator-1 (SRC-1), 3T3-L1 cell
368 differentiation, and lipid accumulation. PPARgamma activation results in changes
369 in adipokine production, and in decreased inflammation of adipose tissue, thereby
370 leading to the improved insulin sensitivity in the liver and skeletal muscle[68]. Ravi
371 Kumar *et al.* described the effect of ASX administration combined with squalene in
372 mice fed with high-fat-high-sucrose diet on glucose/triglyceride levels. Blood
373 glucose levels were decreased while adiponectin levels, and mRNA expression of
374 Sod1 and GPx were increased, underlying a possible benefit of utilizing the two
375 drugs together for the management of obesity/diabetes[69].

376 On the contrary, some other studies reported no significant influences on glucose
377 /insulin metabolism. For instance, Chen *et al.* reported no significant hypoglycemic

378 effect of ASX[70]. Preuss *et al.* examined several parameters of metabolic syndrome
379 in Zucker fatty rats (ZFR); they failed to show an ASX effect on insulin
380 sensitivity[71] at low doses and observed a modulation of glucose-insulin
381 metabolism and nitric oxide at relatively high doses[71]. Differences in dosing
382 and/or animal species used among various studies are plausible explanations of the
383 variation in results and stands for a need to further investigation.

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385 **3.3. Astaxanthin: protective effects on diabetes complications**

386 OS is a common denominator link for the major pathways involved in the
387 pathogenesis of diabetic micro-, as well as macro-vascular complications [72].
388 Hyperglycemia induces the overproduction of mitochondrial superoxide in
389 endothelial cells of both large and small vessels, as well as the myocardium. In
390 microvasculature, the intracellular increased superoxide production causes
391 vascular damage via multiple metabolic pathways including AGEs accumulation,
392 AGEs receptors, protein kinase C (PKC) pathway, polyol pathway, and the
393 hexosamine pathway activation[73]. Inflammation may also play a key role in the
394 development and the progression of diabetes complications[74]. In the diabetic
395 macrovascular complications and in the heart, vascular damage appears to be a
396 consequence of increased oxidation of fatty acids, resulting in part in
397 pathway-specific insulin resistance[73].

398 Owing to its molecular structure, ASX is one of the few antioxidants that can move
399 throughout the body and provide protection to all of our cells. ASX may help in
400 preventing and treating diabetic microvascular complications by modulating OS,
401 inflammation, and apoptosis through free radical quenching[8]. Although the exact
402 mechanism remains elusive, ASX has been reported to exert beneficial effects in
403 preventing DM complications including retinopathy, nephropathy, neuropathy,
404 and wound healing in numerous diabetic models in animal studies. The
405 recommended dose for reaching positive outcomes with diabetic complications
406 should be addressed in adequate human trials that would evaluate the efficacy and
407 safety of ASX considering the bioavailability, individual response, metabolism,
408 tissue delivery, and possible toxicity.

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410 **3.3.1. Astaxanthin: protection against diabetic retinopathy**

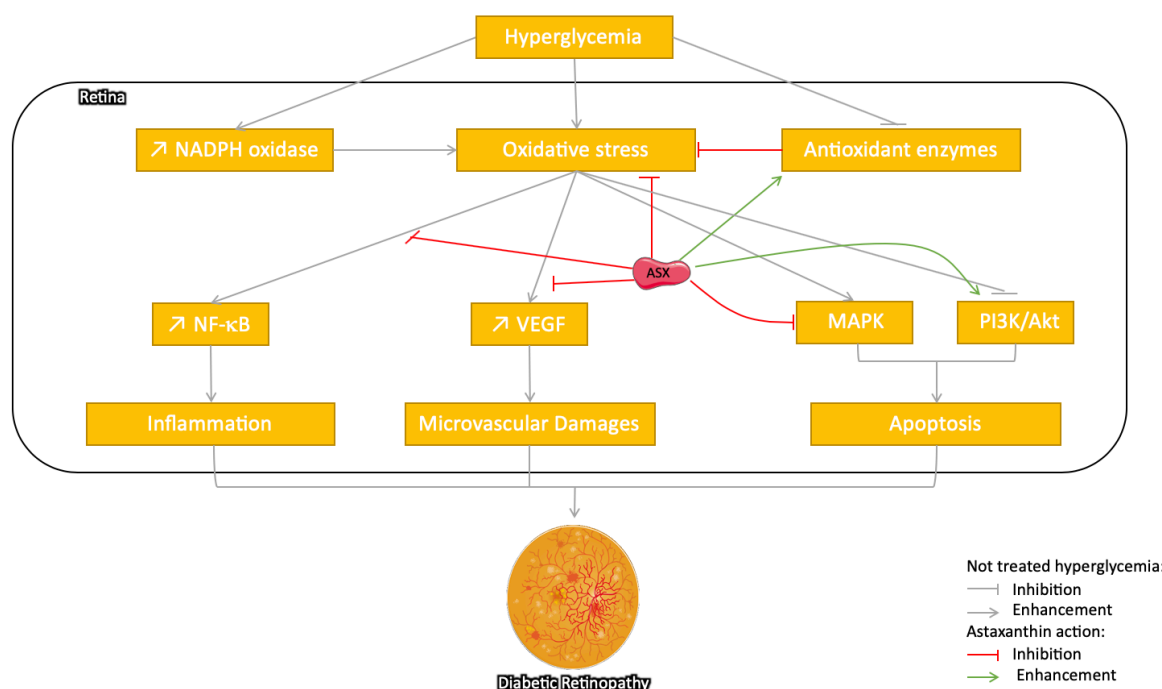
411 Astaxanthin have recently been reviewed to have a beneficial pleiotropic effect
412 in the prevention and treatment of several ocular diseases, due to its
413 anti-inflammatory, antioxidant and metabolism regulatory effect [75]. Diabetic
414 retinopathy (DR) is one of the major microvascular complications of diabetes and
415 the most frequent cause of acquired blindness in adults[76]. It is a slow progressive
416 chronic disease and its prevalence is augmented with the duration of diabetes. The

417 pathophysiology of diabetic retinopathy is complex, as many metabolic changes
418 affect different cell types in the retina. Chronic HG increases OS in the retina and its
419 capillary cells, leading to microvascular damage and retinal dysfunction[77]. HG
420 contributes to NADPH oxidase (NOX) overexpression, leading to cytosolic ROS
421 increase and mitochondrial membrane damage. In retina, OS can activate metabolic
422 pathways including PKC, AGEs, hexosamine pathway, polyol pathway, p38 MAPK
423 pathway, and autophagy pathway, and alter the expression of growth factors such
424 as vascular endothelial growth factor (VEGF)[78]. It can also activate nuclear
425 factor- κ B (NF- κ B) transcription factor, leading to the up-regulation of
426 pro-inflammatory cytokines that contribute to the retinal cell damage and
427 subsequent development of DR[79]. The diabetic environment also compromises
428 the superoxide scavenging system by decreasing the activity of antioxidant
429 enzymes such as SOD, glutathione reductase, glutathione peroxidase, and catalase
430 GSH, MnSOD activity, as well as altering enzymes responsible for DNA and
431 histone modifications[77].

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433 Several studies have reported the protective effect of ASX on DR (Figure 2). In
434 cultured human retinal pigment epithelial cells, ASX reduced the high-glucose
435 exposure-induced increased intracellular level of AGEs, ROS, lipid peroxidation,
436 and the mRNA expression of VEGF and MMP2 and cell proliferation[80]. In STZ
437 rats, oral administration of ASX plays a protective role in the retinal function and
438 architecture and so it prevents DR. ASX reduced levels of OS mediators (e.g., 8-
439 hydroxy-2'-deoxyguanosine, nitrotyrosine, and acrolein), increased levels of
440 antioxidant enzymes (heme oxygenase-1 and peroxiredoxin), and decreased
441 inflammatory mediators (intercellular adhesion molecule-1, monocyte
442 chemoattractant protein-1, and fractalkine) which may be mediated by
443 downregulation of the NF- κ B transcription factor [81].

444 An inhibitory effect of ASX has also been recently reported on the aldose reductase
445 (AR) activity, a key enzyme in the polyol pathway involved in the pathogenesis of
446 diabetic complications including DR, in the gerbils (*Gerbillidae* family, *Psammomys*
447 *obesus* species), animal models for T2DM[82]. In db/db mice, ASX improved
448 oscillatory potentials (OPs) and the levels of OS markers including superoxide
449 anion, MDA (a marker of lipid peroxidation), 8-hydroxy-2-deoxyguanosine
450 (8-OHdG, indicator of oxidative DNA damage), and MnSOD (manganese
451 superoxide dismutase) in the retinal tissue[83]. Thus, it was suggested that ASX as
452 an antioxidant, possibly improved DR by the regulation of intracellular OS which
453 mediates pathological cell functionality (VEGF level, inflammation, excessive cell
454 proliferation).



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Figure 2. Schematic representation of different pathways suggested for the protective effect of astaxanthin on diabetic retinopathy (DR) via its ROS scavenging potential and reduced oxidative stress induced by the enhanced antioxidant and the downstream pathways activated in hyperglycemic states. In parallel, ASX inhibits inflammation through NF- κ B pathway, microvascular damages through VEGF production, and finally apoptosis through the regulation of MAPK and PI3K/Akt pathways. The grey arrow indicates different pathways implied in the development of the pathology, the red arrow indicates the inhibitory/ regulatory effect, while the green ones represent a stimulatory effect.

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3.3.2. Astaxanthin protection against diabetic nephropathy

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Diabetic nephropathy (DN), also called diabetic kidney disease, is a microvascular complication resulting from lesions in the renal glomeruli and tubuli [84]. It is strongly associated with a poor glycemic control [85]. The ROS-related increase of HG induces the recruitment of inflammatory cells [86], the production of inflammatory cytokines [87], and an increased expression of NF- κ B [88]. Renal inflammation has been reported to contribute to nephropathy development. ROS overproduction has been also associated with TGF- β and excessive ECM deposition, leading to the progression of renal fibrosis [89].

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Several animal studies have reported nephroprotective effect of ASX and proposed that it should be explored further as a potential remedy for the prevention and treatment of DN (Figure 3). In DN, the HG-induced oxidative stress is mainly responsible for the disease progression. Chronic exposure to glucose induces an increase in AGEs that activate the transcription factor NF- κ B and the protein kinase C (PKC) system through the cellular RAGEs, leading to the inflammation, and augmentation of synthesis of the ECM constituents [90]. It has been shown that ASX administration for 12 weeks to db/db mice reversed the HG-induced increased urinary albumin and decreased OS markers such as 8-hydroxydeoxyguanosine [57]. In diabetic rats, dietary supplementation with ASX (20 mg/kg daily) has shown

483 direct favorable effects against renal dysfunction with a return to physiological
 484 levels of creatinine and uric acid and a significant reduction in urea level, along
 485 with partial reversal of glomerular hypertrophy [58]. ASX increases the activity of
 486 antioxidant enzymes such as SOD and catalase, and reduces the generated MDA in
 487 the serum. It has been also reported that the ASX accumulated in the mitochondria
 488 of human mesangial cells suppresses the HG- induced ROS production and inhibits
 489 the activation of transcription factors, and production of COX-2, MCP-1, and TGF β 1
 490 [91]. ASX may partially interfere with the pathogenesis of DN by reducing the
 491 accumulation of ECM components as it regulates the overproduction of fibronectin
 492 (FN) and TGF- β 1 and can prevent renal fibrosis[52]. Recently, it has been revealed
 493 that renoprotective effect of ASX on DM partially depends on the signaling of the
 494 redox-regulated transcription factor Nrf2–ARE that exerts major effects in
 495 protecting against oxidative damage[52]. Moreover, in the cultured HG-treated
 496 glomerular mesangial cells and in db/db mice, ASX upregulates the Cx43 protein
 497 level and inhibites c-Src activity. Furthermore, it promotes the nuclear translocation
 498 of Nrf2 and increases the expression level of its downstream protein heme
 499 oxygenase-1 and superoxide dismutase 1 [61]. According to a recent report,
 500 administration of ASX-s-allyl cysteine biconjugate to SZT-treated rats protected the
 501 antioxidant status of kidney and plasma and curbed the progression of secondary
 502 complications of DM[92].

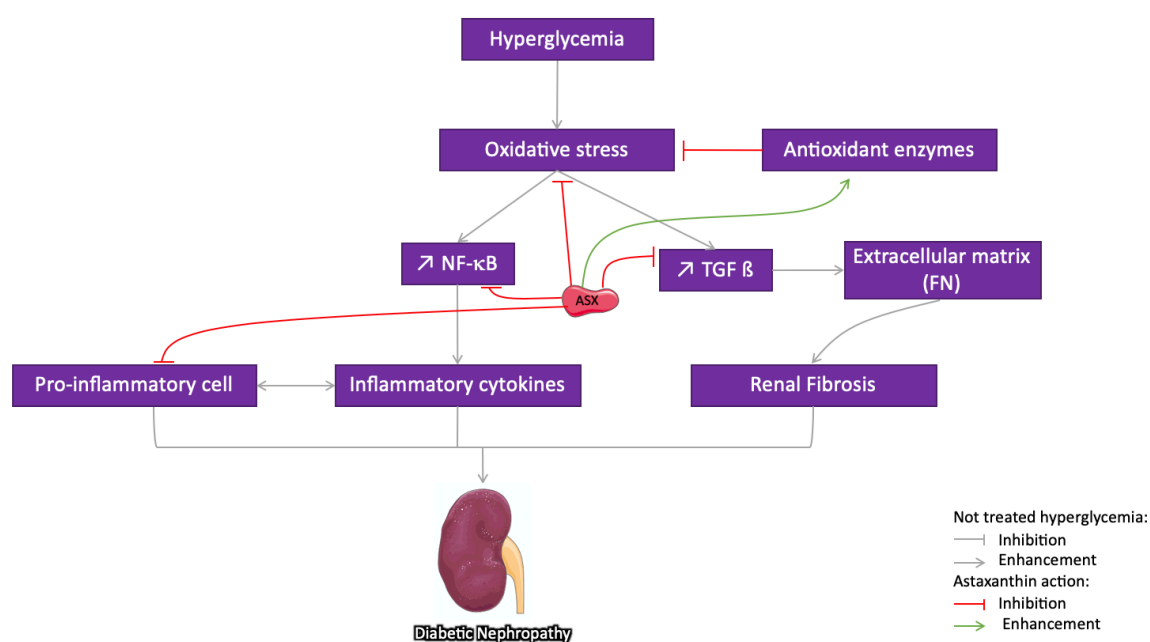


Figure 3. Schematic representation of different pathways suggested for the protective effect of astaxanthin (ASX) on diabetic nephropathy (DN). Through its ROS scavenging and antioxidant potential, ASX reduces oxidative stress and the downstream pathways activated in the development of the disease. In addition, ASX directly inhibits NF- κ B translocation and TGF- β production, as well as reducing inflammation and fibrosis, which are strongly implied in DN. The grey arrow indicates different pathways implied in the DN development, red arrow indicates an inhibitory/ regulatory effect, while the green ones indicate a stimulatory effect.

512 3.3.3. Astaxanthin protection against diabetes-induced neuropathy

513 DM-induced neuropathy and hippocampal-based cognitive deficits are prevalent
 514 diseases with important impact on the life quality of patients. Besides
 515 inflammation, the oxidant/antioxidant imbalance is considered to be the link
 516 between the HG states and neuronal abnormalities[6]. Several animal studies have
 517 therefore considered the neuroprotective effects of ASX on DM (Figure 4), and on
 518 other aging-related diseases, as well[10,49,55]. Specifically, in STZ rats, ASX
 519 administration improves cognitive function[93] and simultaneously reduces
 520 activities of oxidative stress, by increasing the levels of antioxidant enzymes. By
 521 reducing NF- κ B p65 subunit in cerebral cortex and hippocampus, ASX reduces the
 522 levels of inflammatory mediators such as TNF- α , IL-1 β , and IL-6. Furthermore, by
 523 activating PI3K/Akt pathway, it reduces caspase 3 and caspase 9 activities,
 524 protecting brain cells from apoptosis. In another study, ASX ameliorated neuronal
 525 behavior in STZ treated mice. This effect has been related to the decrease of glial
 526 fibrillary acidic protein (GFAP) expression in the brain, the cleaved caspase-3, IL-6,
 527 and IL-1 β down-regulation, and the cystathionine β -synthase (CBS) up-regulation
 528 in the frontal cortex[94]. In a recent study on diabetic mice, ASX increased the level
 529 of SOD and decreased the level of MDA in the hippocampus. It also alleviated
 530 cognitive dysfunction by inhibiting OS and inflammatory responses via Nrf2/ARE
 531 and NF- κ B signaling pathways [95].

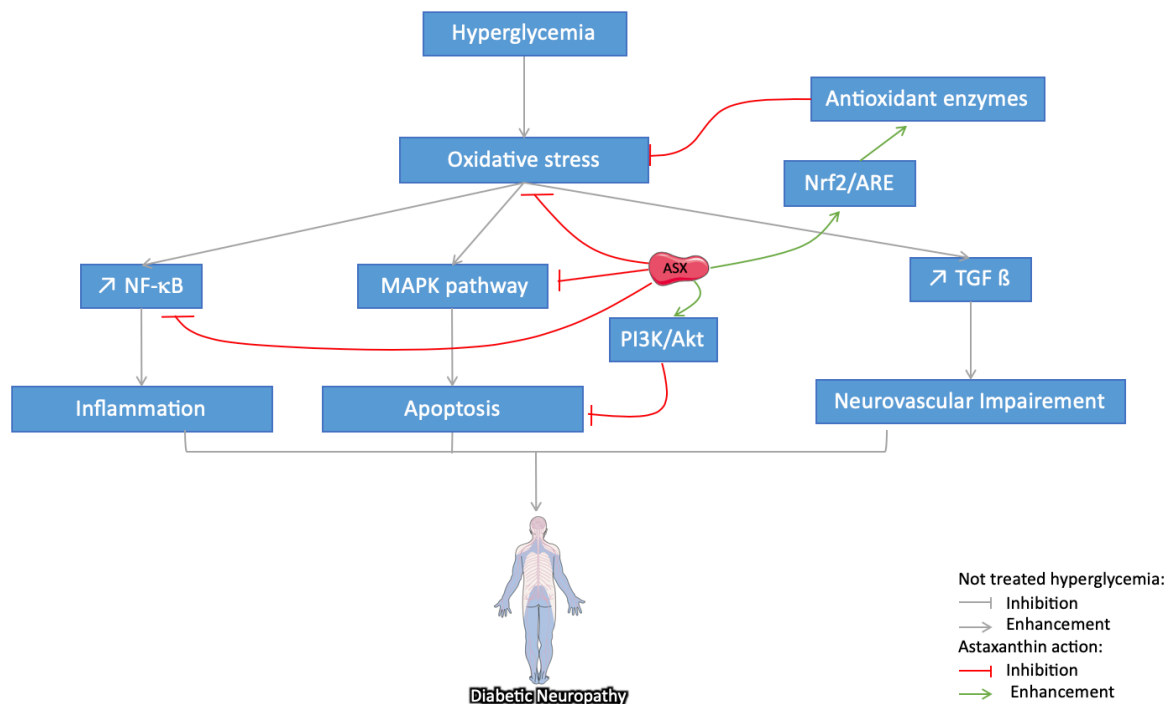
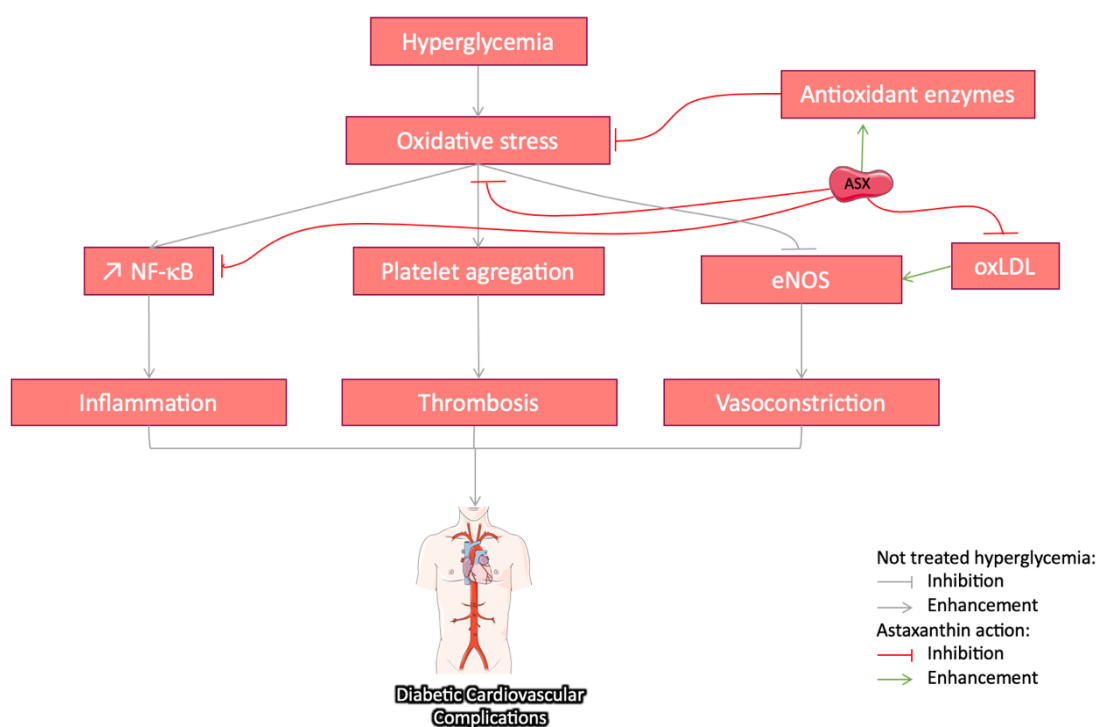


Figure 4. Schematic representation of different pathways suggested for the protective effect of astaxanthin (ASX) on diabetic neuropathy. Through its ROS scavenging potential and the enhancement of antioxidant activities, ASX reduces oxidative stress and the downstream pathways activated in the development of DM-induced neuronal abnormalities. In addition, ASX directly inhibits inflammation through NF- κ B, microvascular damages through VEGF production, and finally apoptosis through the regulation of MAPK and PI3K/Akt pathways. The grey arrow indicates

539 different pathways implied in the development of DM-induced neuronal abnormalities, red arrow
540 indicates an inhibitory/ regulatory effect, while the green ones indicate a stimulatory effect.

541 3.3.4 Cardiovascular protective effect of astaxanthin in diabetes

542 Cardiovascular disease is a major cause of mortality in T2DM patients due to
543 hypertensive vascular damages[96]. HG leads to arteriosclerosis through AGEs
544 increase [97]. OS resulting from NOX enhancement leads to platelet aggregation
545 and thrombosis. It is worth to note that physiologically the antioxidant enzymes in
546 blood platelets regulate ROS overproduction[98]. Considering that antioxidant
547 enzyme production is compromised by diabetic environment, ASX could reduce
548 thrombosis through balancing the redox reactions. By limiting the low density
549 lipoprotein, ASX may also contribute to the prevention of arteriosclerosis in a dose
550 dependent manner [99]. Eventually through the regulation of ROS-induced
551 vasoconstriction, ASX may significantly modulate arterial blood pressure and
552 fluidity in hypertensive animals[100]. The cardiovascular protective effects of ASX
553 and derivatives have been previously reported after prior oral or intravenous
554 administration to different animal species. It also reduces OS and inflammation,
555 and is known to contribute to the pathophysiology of atherosclerotic cardiovascular
556 disease[101]. An overview of the potential effects of ASX 1 (Figure 5) on
557 cardiovascular diseases has been documented in numerous reviews [46;101;102]. It
558 should be noted that DM is one of the contributing events in the cardiovascular
559 physiopathology, besides hypertension, infections of the vessel wall, and smoking.
560 With a focus on the DM context, Zhao *et al.* studied the effect of ASX (10mg/kg daily
561 for 42 consecutive days) on endothelial cells in STZ diabetic rats and reported
562 reduced levels of the oxidized low-density lipoprotein (oxLDL) and the expression
563 of its endothelial-cell receptor LOX-1, leading to the eNOS expression restoration
564 [103]. Chan *et al.* showed that in diabetic rats, dietary intake of astaxanthin (at 0.01
565 and 0.05%) exhibited anti-inflammatory and anti-coagulatory effect [70]. Moreover,
566 a clinical trial in T2DM patients (daily intake up to 12mg ASX) significantly
567 attenuated hemostatic disorders and reduce inflammatory cytokines level, without
568 affecting renal function[104]. Further investigations needed to clarify the
569 mechanisms and the effects of ASX in the DM context.
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Figure 5. Schematic representation of different pathways suggested for the protective effect of astaxanthin (ASX) on diabetic cardiovascular complications in the literature. Though its ROS scavenging potential and the enhancement of antioxidant activities, ASX reduces oxidative stress and the downstream pathways activated in the development of the pathology. In addition, ASX directly inhibits inflammation through NF- κ B pathway. Thrombosis and vasoconstriction reduction are also associated to oxidative stress regulation. Moreover, ASX lowers oxLDL, enhances eNOS, and reduces vasoconstriction. The grey arrow indicates different pathways implied in the development of DM-induced neuronal abnormalities, red arrow indicates an inhibitory/ regulatory effect, while the green ones indicate a stimulatory effect.

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3.3.5. Anti-inflammatory effects of astaxanthin in diabetes

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Inflammation is a determinant factor in the onset and development of T1DM, T2DM, and their complications[104]. Immune cells involved in the pancreatic β -cell destruction are activated through a variety of cytokines including IFN- γ , TNF- α , and IL-1 β . Chronic low-level inflammation is a main feature of T2DM, and TNF- α , IL-1, IL-6, IL-10, and adipokines serve as links between inflammation and metabolism. Obese adipose tissue is characterized by macrophage infiltration which is an important source of inflammation and TNF- α production in this tissue [106].

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The anti-inflammatory effects of ASX have been reported in several studies. ASX supplementation induces macrophage phenotype switch by the decrease of pro-inflammatory M1 markers (CD11, MCP-1) and the increase of anti-inflammatory M2 markers (IL-10)[67,107]. Moreover, Kim *et al.* [107] observed an attenuated monocyte recruitment, thereby reducing *in situ* macrophage proliferation. ASX reduces the HG-induced inflammation-related levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX), MCP-1, prostaglandin E2 (PGE2), IL-1 β , and TNF- α molecules. For instance, in STZ-induced diabetic rats, ASX (0.01% or 0.05% in diet) was shown to reduce the

599 level of activated macrophages (MCP-1) in plasma and kidney and that of the
600 pro-inflammatory cytokines (IL-6, TNF- α) [70]. Bhuvanewari *et al.* demonstrated
601 that in high-fat-high-fructose diet, ASX administration (2 mg/kg/day) inhibited I κ B
602 phosphorylation and therefore the nuclear translocation of NF- κ B p65 subunit[108].
603 Indeed, numerous studies have demonstrated that the anti-inflammatory effect of
604 ASX is mediated via inhibiting the NF- κ B activation in diabetes-induced
605 models[109,110]. In fact, by regulating NF- κ B pathway, ASX reduces the risk of
606 complication in diabetes[81,91,93,95]. Thus, ASX, in targeting inflammation, may
607 improve the prevention and control of the diabetes-related diseases.

608 4. Conclusions and future perspectives

609 OS and inflammation are recognized as determinant factors of the DM
610 development and its associated complications. Several *in vitro* studies have shown a
611 broad range of biological properties in DM-related pathologic cells including
612 pancreatic β -cells, macrophages, T-cells, hepatocytes, adipocytes, endothelial cells,
613 retinal pigment epithelial cells, glomerular mesangial cells among others. ASX is
614 able to reduce intracellular oxidative stress and inflammation by scavenging the
615 ROS and inhibition of lipid peroxidation. It modulates multiple OS pathways but
616 the mechanisms and specificity of action, regarding type of cells, remain elusive.

617

618 The literature reviewed here clearly suggests a promising utilization of ASX in
619 diabetes healthcare. ASX is a safe drug with pleiotropic effects and without crucial
620 adverse effects. The orally/parenterally administrated ASX to T1DM and T2DM
621 animal models has been shown to (i) improve insulin resistance and glucose
622 intolerance, (ii) reduce hyperglycemia, (iii) stimulate activation of antioxidant
623 enzymes and reduce OS, and (iv) have an anti-inflammatory effect. It may have,
624 therefore, a significant preventive role in the onset and progression of DM, as well
625 as a protective role in the development of diabetic complications. However, the
626 heterogeneity of DM models, ASX source, dosage, mode of administration, and
627 outcomes studied make comparisons of the obtained results almost impossible.

628

629 Although a number of clinical trials have already studied ASX effects, research
630 on the biological mechanisms involved in a diabetic context deems necessary. The
631 appropriate dose for reaching positive results with diabetic complications should
632 be addressed in adequate human trials that would evaluate the efficacy and safety
633 considering the bioavailability, individual response, metabolism, tissue delivery,
634 and possible toxicity.

635

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637 VG, DL, and HP: assisted to the review; FA and GPD: did review and editing. All
638 authors have read and agreed to the published version of the manuscript.

639

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