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Review Article

Astaxanthin Structure, Metabolism, and Health Benefits

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Abstract

Astaxanthin (ASTX), a xanthophyll carotenoid, has a unique structure featured by the presence of polar moieties on both end of its polyene chain. This structural property of ASTX confers a great antioxidant activity and allows it to align in the cell membrane for various biological activities. ASTX has been suggested to have health benefits for the prevention of inflammatory diseases, diabetes, cardiovascular disease, nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, which are the major obesity-related health problems in the developed countries. This review discusses the chemical properties of ASTX and its metabolism. It also addresses the current knowledge on the mechanisms for the protective effects of ASTX against oxidative stress, inflammation, insulin resistance as well as the development of the aforementioned metabolic diseases.

ABBREVIATIONS: AP-1: Activator Protein-1; apoE-/-: apolipoprotein E knockout, ASTX: Astaxanthin; CVD: Cardio Vascular Disease; COX-2: Cyclooxygenase; CYP: Cytochrome P450; DEN: Diethylnitrosamine; GSH: Glutathione; *H. pylori*: *Helicobacter pylori;* HDL: High-Density Lipoprotein; 8-OHdG: 8-hydroxy-2-doxyguanosine; iNOS: inducible Nitric Oxide Synthase; IκBα: Inhibitor of NF-κBα; IRS: Insulin Receptor Substrates; IL: Interleukin; Keap1: Kelch-like ECH-associated protein 1; LDL: Low-Density Lipoprotein; LPS: Lipopolysaccharide; MMP: Matrix Metalloproteinase, NO: Nitric Oxide; NAFLD: Non Alcoholic Fatty Liver Disease; NASH: Non Alcoholic Steatohepatitis; NRF2: Nuclear Factor E2 related factor 2; NF-κB: Nuclear Factor kappa B; PPAR: Peroxisome Proliferator-Activated Receptor; PI3K: Phosphatidylinositol 3-kinase; PGE2: Prostaglandin E 2; PTECs: Proximal Tubularpithelial Cells; ROS: Reactive Oxygen Species; SR-BI: Scavenger Receptor class B, type I; SHR: Spontaneously-Hypertensive Rats; SOD: Superoxide Dismutase; TNFα: Tumor necrosis factor α; UV: Ultraviolet; VLDL: Very Low-Density Lipoprotein.

INTRODUCTION

Astaxanthin (ASTX) is a xanthophyll carotenoid abundant in marine animals such as salmon, crab, and crustaceans that live on ASTX-containing planktons and microalgae [1]. It is the main carotenoid found in wild salmons, conferring its unique dark red color [2,3]. *Haematococcus pluvialis* (*H. pluvialis*), a single-celled green alga, is believed to have the highest capacity to accumulate ASTX in nature under environmental stresses such as starvation, high salt, elevated temperature, or irradiation [4,5]. ASTX produced from *H.pluvialis* is a primary natural source of ASTX for human consumption.

Humans and other mammals cannot synthesize ASTX [6]. The natural sources of ASTX are algae, bacteria and fungi [7]. Animals, such as salmon, lobster, shrimp and trout, acquire ASTX by consuming ASTX-containing algae or bacteria, and the accumulation of ASTX in their flesh, skin or exoskeleton gives pinkish or reddish appearances [8]. Therefore, ASTX is also used as a feed ingredient for seafood farming, especially salmon, trout and shrimp, to give their unique reddish color [3]. By consuming ASTX-containing seafood or dietary supplement, either synthetic or extracted from *H. pluvialis*, humans are able to obtain ASTX [8].

ASTX is well known for its strong antioxidant capacity [9], which presumes to largely contribute to its diverse protective properties against inflammation, ulcer, cancer, neurodegeneration, diabetes, and cardiovascular disease (CVD) as well as hepato-protective effects [7]. Therefore, use of ASTX as a dietary supplement for optimal health has been rapidly growing in recent years. In this review, current knowledge of health-promoting properties of ASTX is discussed with focuses on its effects on the prevention/therapy formetabolic diseases such as diabetes, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and CVD.

CHEMICAL PROPERTIES OF ASTX

ASTX (3,3'-dihydroxy-beta,beta-carotene-4,4'-dione) belongs to a xanthophylls carotenoid subclass, which is characterized by

the presence of oxygen molecule in their structures (Figure 1). ASTX structure is similar to β-carotene and other xanthophylls, such as lutein, canthaxanthin and zeaxanthin, in that they share a common semi-symmetric layout with two terminal carbon rings flanking an extended double-bond hydrocarbon chain, also referred to as the polyene chain [3]. However, ASTX is distinctive in its structure from other carotenoids due to the presence of hydroxyl and keto moieties on both ends. The polar-nonpolar-polar structure of ASTX allows it to align in the phospholipid bilayer of cell membrane [10] and to expose both hydrophilic ends to aqueous environment [11]. Free form of ASTX is sensitive to oxidation [9]. Therefore, in nature, ASTX primarily exists as a protein-conjugated form such as in exoskeleton of crustaceans, or a fatty acid-esterified form, i.e., monoester or diester [11]. The predominant form of ASTX in *H. pluvialis* is monoester [12].

Due to the presence of two hydroxyl groups, several stereoisomers of ASTX exist. Depending on the configuration of hydroxyl group on the chiral centers in C-3 and C-3', three ASTX isomers, i.e., two enantiomers (3S, 3'S), (3R, 3'R) and one mesomer (3R, 3'S), can be formed. (3S, 3'S) isomer is the predominant form of natural ASTX. During artificial synthesis of ASTX, the S and R orientation occur equally on each chiral center of ASTX [13]. As (3S, 3'R) isomer is identical to (3R, 3'S) isomer, the mesomer accounts for a half of total ASTX, whereas (3S, 3'S) and (3R, 3'R) enantiomers account for ~25% each in synthetic ASTX [4]. In addition, as ASTX has a polyene chain consisting of multiple double-bonds, geometrical *cis*- or *trans*- isomers of ASTX also exist. *Trans*-ASTX esters predominantly present in nature, whereas *cis*-ASTX esters are thermodynamically less stable but still detectable [14]. ASTX in *H. pluvialis* is composed of 3:1 ratio of *trans*-ASTX to *cis*-ASTX [15]. Studies have shown ASTX isomers may have different bioavailability in humans [16-18]. This aspect is described in detail below.

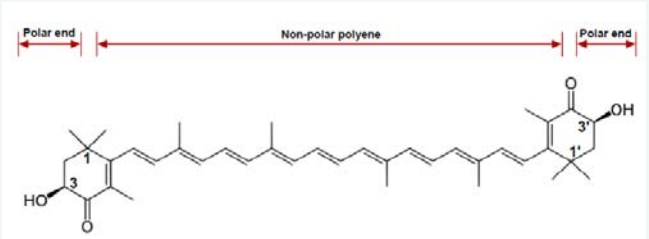

Figure 1 The molecular structure of all-*trans*-ASTXwith polar-nonpolar-polar nature.

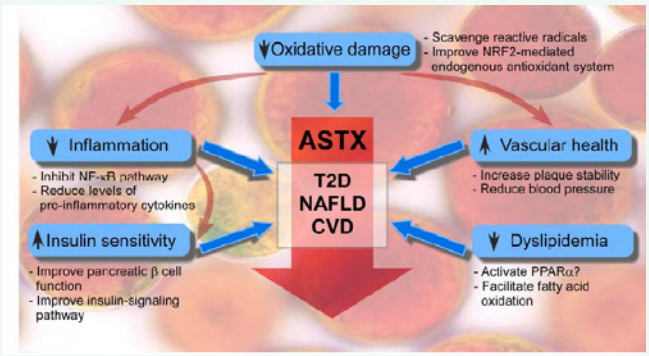

Figure 2 Summary of the effects of ASTX on the prevention of metabolic diseasesand underlying mechanisms. Chronic metabolic disease such as type 2 diabetes, NAFLD and CVD are closely related with oxidative damage, inflammation, insulin resistance, and dyslipidemia. ASTX consumption may provide health benefit effects by preventing oxidative stress via the activation of NRF2-mediated endogenous antioxidant system; inhibiting inflammation by inhibiting NF-κB pathway and subsequent repression of pro-inflammatory cytokine production; increasing insulin sensitivity; inhibiting dyslipidemia;and improving vascular health.

METABOLISM OF ASTX

Digestion, absorption, and transport of ASTX

Due to low solubility in aqueous environment, xanthophyll carotenoids have lower bioavailability than other dietary lipids such as triglyceride [19]. However, due to the presence of polar ends in free ASTX, it can be absorbed better than other non-polar carotenoids, e.g., lycopene and β- carotene [20]. As ASTX is largely present as fatty acid-esters in nature, the ASTX esters need to be hydrolyzed to free ASTX and subsequently incorporated into micelles to get access to intestinal cells for absorption as do dietary lipids. Cholesterol esterase is a likely candidate to hydrolyze ASTX esters [21,22]. The presence of dietary fat is known to affect the degree of ASTX absorption in the small intestine [23]. In humans, incorporation of ASTX into a lipid-based formulation, composing of lipophilic glycerol monooleate, dioleate and an emulsifier polysorbate 80, can enhance ASTX absorption [24]. Furthermore, the absorption of ASTX may be influenced by the type of oil that is consumed with ASTX. ASTX absorption was higher when it was emulsified with olive oil than with corn oil in rat duodenum [25].

The entry of ASTX into enterocytes has been thought to occur primarily by simple diffusion [19]. However, alternatively, facilitated diffusion may also play a role in the absorption of ASTX. Scavenger receptor class B, type I (SR-BI) has been shown to mediate the absorption of β-carotene and xanthophylls, including β-cryptoxanthin, lutein and zeaxanthin, into enterocytes [26,27]. As ASTX shares several structural similarities with these carotenoids, SR-BI may also mediate the intestinal absorption of ASTX. Evidence has suggested that ASTX isomers may be absorbed at a different degree. In humans, after oral administration of a mixture of all-*cis*-ASTX and all-*trans*-ASTX at a ratio of 1:14, the isomers appeared in the plasma at ~1:2 ratio [18]. The observation suggests that all-*cis*-ASTX may be preferentially absorbed or selectively accumulated in the circulation. Additionally, in the subjects who consumed farm-raised salmon for 4 weeks, plasma levels of (3S, 3'S) ASTX isomer accounted for ~80% of total ASTX in the circulation despite the fact that (3R, 3'S) ASTX mesomer was predominantly present in the salmon [28]. Therefore, mechanisms for the selective absorption of ASTX isomers may exist in enterocytes. Future studies are needed to determine whether SR-BI plays a role in facilitating intestinal ASTX absorption and whether each ASTX isomer can be absorbed at a similar extent.

Free, but not esterified, ASTX is detected in all lipoprotein fractions of human plasma, including chylomicron, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), after the consumption of ASTX

esters [17,18]. Therefore, it is likely that enterocytes can package a free form of ASTX into chylomicron for secretion into the lymphatic system. Bioavailability and a half-life of ASTX likely depend on its esterification status.

After one-time oral administrations of 100 mg free ASTX, the maximum plasma levels of ASTX were 1.3 ± 0.1 mg/L with a half-life of 21 ± 11 h in humans [17]. In contract, ingestion of 100 mg of ASTX diesters resulted in plasma ASTX levels of 0.28 ± 0.12 mg/L with extended half-life of 52 ± 40 h [18], implicating the additional hydrolysis step of ASTX esters may slower ASTX absorption rate. Although absorption rate is slower for ASTX esters than free ASTX, whether the amount of absorbed ASTX would differ between two ASTX forms is not clear. Consumption of ASTX capsules with a daily dosage of 1 mg for 4 weeks elevated plasma ASTX levels to ~7.3 ± 5.6 μg/L, whereas the levels reached 11.3 ± 5.8 μg/L as intervention was extended to 12 weeks [29], suggesting plasma ASTX levels can be increased by long-term consumption.

Although there is no information available on ASTX tissue distribution in humans, studies have demonstrated ASTX is present in various tissues. In chickens, the highest ASTX was found in the intestine, followed by adipose, spleen, liver, heart, kidney, skin and muscle [30]. In mice, high levels of ASTX were accumulated in the liver, whereas it was also detectable in the heart and the brain [31]. At the cellular level, the hydrophobic nature of ASTX suggests that it may reside in the lipid droplets [32] or phospholipid bilayer [33], as are the other carotenoids. However, the polar groups in ASTX allow it to expose to hydrophilic environment on the membrane surface, whereas non-polar carotenoids, such as β-carotene and lycopene, tend to be located within the hydrophobic core of phospholipid bilayer membrane or lipid droplets [33]. Theoretically, ASTX may also exist in other intracellular membranes, i.e., mitochondria and endoplasmic reticulum, yet studies on cellular localization of ASTX are very limited. One study has shown that in chicken liver, most ASTX was found in the microsomal fraction with minor proportion being detected in the mitochondria and nucleus [30]. However, the ASTX distribution in different cellular organelles may be different between species. As cellular localization can provide distinctive cellular activities, further study is necessary to determine ASTX distribution in cells.

METABOLITES OF ASTX

ASTX can be metabolized into 3-hydroxy-4-oxo-β-ionone and 3-hydroxy-4-oxo-7,8-dihydro-β-ionone in primary rat hepatocytes [34]. However, enzymes that catalyze synthesis of the metabolites and their potential biological functions have not been elucidated. ASTX has been shown to increase the levels of cytochrome P450 (CYP) enzymes in the hepatocytes [34,35]. However, incubation of ASTX with isolated microsomes containing CYPs did not generate ASTX metabolites and furthermore the induction of CYP activity by ASTX pretreatment did not increase generation of the metabolites in hepatocytes [34]. Therefore, the CYP enzymes are not likely to be responsible for the production of ASTX metabolites. ASTX metabolism occurs through currently unknown mechanisms and future investigation on how ASTX is metabolized and what the functions of its metabolites are needed.

SAFETY OF ASTX

ASTX has been demonstrated safe in multiple animal studies and human trials [4,36]. In a randomized, double-blind and placebo-controlled trial that gave daily supplementation of 6 mg of ASTX from *H. pluvialis* to healthy adults for 8 weeks, there were no significant changes in blood pressure, plasma metabolic panels and blood cell blood count whereas ASTX supplementation slightly increased serum levels of calcium, total proteins and eosinophils within healthy ranges [36]. Moreover, administration of a single dose of 100 mg ASTX in middle-aged male [17], daily dose of 40 mg for 4 weeks in patients with functional dyspepsia [37], or daily dose of 4 mg for 12 months in subjects with macular degeneration [38] did not induce any adverse side-effects. To date, no adverse side-effects of ASTX supplementation have been reported in humans. In 2010, the U.S. Food and Drug Administration acknowledged the "generally recognized as safe (GRAS)" status of ASTX extracted from *H. pluvialis* [39].

HEALTH BENEFITS OF ASTX

Antioxidant properties of ASTX

Oxidative stress has been identified as one of the major underlying causes of aging, CVD, NAFLD, and carcinogenesis [40]. Free radicals and reactive oxygen species (ROS) are highly unstable and react quickly with adjacent molecules to obtain electrons, initiating chain reactions. Excessive accumulation of reactive radicals and ROS can trigger oxidative damages to nucleotides, proteins or lipids, eventually deteriorating cellular activities and causing cell injury and death [41]. Free radicals and ROS are produced in normal metabolic process and quickly neutralized by body's antioxidant defense system, a complex network consisting of endogenous and exogenous antioxidant molecules and enzymes [42]. However, oxidative stresses from smoking, exposure to ultraviolet (UV) light and obesity can increase ROS production while they decrease body's antioxidant defense system, consequently damaging cells and tissues [9,43].

Studies have demonstrated functions of ASTX in scavenging a broad-spectrum of reactive radicals and oxygen species. ASTX showed higher scavenging capacity against peroxyl radicals and hypochlorous acid than that of α-tocopherol, lutein, lycopene, and β-carotene [44,45]. It also exhibited the highest capacity in scavenging hydroxyl radicals comparing to lutein, lycopene, and β -carotene [45]. Moreover, in an in vitro membrane model, ASTX maintained the membrane integrity and effectively repressed lipid peroxide formation, whereas lutein and β -carotene disrupted the membrane structure and increased the levels of lipid hydroperoxides [46]. These results suggest that the antioxidant activity of ASTX is superior to other carotenoids and α-tocopherol. The potent antioxidant capacity of ASTX is at least partially attributed to its unique chemical structure. Carotenoids can capture singlet reactive oxygen and shuttle it along the double-bond polyene chain, thus terminating the chain reaction [47]. In addition, comparing to non-polar carotenoids, such as lycopene and β-carotene, ASTX contains polar ends that can react with phospholipid head groups or water in the aqueous environment, quenching radicals from the surface of or inside the lipid bilayer [48]. It has also been proposed that ASTX locates in

a proximity to vitamin C that is present in aqueous environment [49]. Vitamin C may serve as a sink to accept radicals and restore the electron-transferring capacity of ASTX, allowing ASTX for continuous scavenging activity.

Strong antioxidant effects of ASTX have been documented in cell models exposed to various oxidative stresses. ASTX effectively prevented ultraviolet A (UVA) or UVB radiation-induced photo-oxidation and cytotoxicity in human dermal fibroblast [50] and keratinocyte [51]. Furthermore, ASTX repressed ROS production and increased antioxidant enzyme expressions in retinal cell [52], neuron [53,54], immune cells [55-57], and hepatocytes [58]. As such, due to its potent antioxidant property, ASTX prevents oxidative damages caused by different stimulants and restores normal cellular functions. Due to the antioxidant effects of ASTX in protecting cells in skin, nervous system, immune system and vital organs, it may have preventive activities in the pathogenesis of multiple diseases mediated by oxidative stress in human body.

In addition to the activity of ASTX to scavenge radicals and quench reactive species, studies also suggest that ASTX can enhance nuclear factor E2 related factor 2 (NRF2)-mediated endogenous antioxidant defense system [59]. Activation of NRF2 pathway improves endogenous antioxidant defense by increasing the expression of antioxidant enzymes, such as glutathione peroxidase, glutathione S-transferase and heme oxygenase 1 [60,61]. Moreover, NRF2 enhances antioxidant capacity of glutathione (GSH), a crucial antioxidant molecule maintains cellular redox status, by upregulating the expression of glutathione reductase, an enzyme that restores the reduction capacity of GSH [62]. Under basal conditions, NRF2 is bound to Kelch-like ECH-associated protein 1 (Keap1) in cytosol, inhibiting NRF2 activity and facilitating its ubiquitination for proteosomal degradation [63]. Upon exposure to chemicals or, oxidative or electrophilic stress, NRF2 dissociates from Keap1, translocates into the nucleus, and initiates the transcription of the aforementioned downstream targets [64]. We previously reported that in apolipoprotein E knockout (apoE-/-) mice fed a high fat/high cholesterol, 0.03% ASTX supplementation for 4 weeks significantly elevated the hepatic expression of NRF2 and its downstream antioxidant enzymes with a concomitant decrease in glutathione disulfide, an oxidized form of GSH [59]. The similar protective effect of ASTX was also reported in the liver of Sprague Dawley rats that were treated with cyclophosphamide, an alkylating agent that disturbs antioxidant balance [65]. ASTX treatment for 3 days prior to or for 10 days after cyclophophamide treatment improved NRF2 activation in the rats under oxidative stress. The studies suggested that antioxidant effects of ASTX may be partly mediated through the up-regulation of NRF2 pathway. Future studies are in need to elucidate the underlying mechanism for how ASTX activates NRF2 pathway.

Antioxidant effects of ASTX have been demonstrated in humans. In healthy male subjects, daily consumption of 4 mg ASTX for 3 months lowered the plasma levels of peroxidized lipids, including 12- and 15-hydroxy fatty acids, indicating that ASTX inhibited lipid peroxidation [66]. Supplementation of 5, 20 or 40 mg ASTX per day for 3 weeks decreased plasma levels of lipid peroxidation markers, such as malondialdehyde and isoprostane, comparing to their baseline in healthy smokers, [67]. In this study, ASTX also increased plasma concentrations of superoxide dismutase (SOD) as well as total antioxidant capacity [67]. Overweight and obese subjects who consumed 5 or 20 mg ASTX daily for 3 weeks showed reduction in lipid peroxidation while antioxidant capacity was increased [68]. These studies are supportive of the use of ASTX for the prevention of oxidative stress in humans.

Anti-inflammatory effects of ASTX

Chronic low-grade inflammation triggers the development of metabolic diseases, such as CVD and type 2 diabetes [69]. Studies have shown that ASTX exerts anti-inflammatory properties, at least in part, by inhibiting the activation of nuclear factor kappa B (NF-κB). NF-κB is a transcription factor that directs cell's inflammatory response by regulating the expression of pro-inflammatory genes [70]. In a resting state, NF-κB is located in the cytoplasm bound with inhibitor of NF-κB α (IκBα), which prevents NF-κB translocation into the nucleus. Upon stimulation by pro-inflammatory insults such as lipopolysaccharide (LPS), IκBα kinase phosphorylates IκBα, facilitating its dissociation from NF-κB for degradation by proteasome. Subsequently, NF-κB is free to translocate into the nucleus to increase the expression of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin 6 (IL-6) and IL-1β. [71,72]. ASTX decreased the production of nitric oxide (NO), prostaglandin E 2 (PGE2) and TNFα as well as activity of inducible NO synthase (iNOS) in the LPS-stimulated RAW264.7 macrophages [73,74]. Primary mouse peritoneal macrophages stimulated by LPS showed increases in the production of NO, TNFα and IL-1β, which was ablated by ASTX treatment [74].

Studies suggest that antioxidant property of ASTX is linked to its anti-inflammatory function as well as inhibitory effect on NF-κB pathway in various cell models. ASTX decreased intracellular accumulation of ROS and inhibited the activation of NF-κB with a concomitant decrease in iNOS promoter activity in RAW264.7 macrophages that were stimulated by LPS [74]. In rat alveolar macrophages, ASTX inhibited the production of superoxide anion (O_2-), NO, and TNFα [56]. ASTX decreased the production of pro-inflammatory cytokines, such and TNFα, IL-1β, IL-6, iNOS and cyclooxygenase 2 (COX-2) while it increased NF-κB phosphorylation in THP-1 human macrophages [75]. ASTX also reduced IL-1β, IL-6 and TNFα secretion in hydrogen peroxide-stimulated U937 human macrophages. It also inhibited ROS-induced production of NF-κB which effectively inhibited the production of inflammatory cytokines and restored basal level of SHP-l, a negative regulator of immune cytokine signaling [76]. ASTX inhibited the expression or formation of NO, iNOS and COX-2 and suppressed the protein levels of iNOS and COX-2 in LPS-stimulated murine BV-2 microglial cells, the resident macrophages and immune surveillance cells of the central nerve system [77]. UVB exposure is one of the inflammatory stimuli for the skin. ASTX significantly decreased UVB-induced phosphorylation of NF-κB p65, which could be associated with the significantly suppressed levels of PGE2 or IL-8 secretion via the down-regulation of COX-2 and IL-8 at the gene and/or protein levels in human keratinocytes [51].

Anti-inflammatory effects of ASTX were also observed in vivo. BALB/C mice were treated with ASTX at 40 mg/kg for 12 h prior to intraperitoneal injection of LPS [74]. ASTX pretreatment decreased serum levels of NO, PGE2, TNFα, and IL-1β. Also, in rats with ocular inflammation that was induced by LPS injection into footpad, intravenous injection of ASTX at 10 or 100 mg/kg significantly decreased the number of infiltrating cells into anterior chamber as well as the amount of NO, TNFα and PGE2 in the aqueous humour [78]. The number of activated NF-κB-positive cells in iris-ciliary bodies was also decreased by ASTX.

Helicobacter pylori (*H. pylori*) infection in humans generates a state of gastric inflammation, which can progress to chronic type B gastritis, peptic ulcer disease, and gastric carcinoma [79]. In BALB/cA mice orally inoculated with *H. pylori*, oral treatment with ASTX at a daily dose of 10, 50, or 100 mg/kg for 10 days significantly lowered the number of *H. pylori* in gastric tissue and gastric inflammation score [80]. Also, when patients with functional dyspepsia and positive to *H. pylori* were treated daily with 40 mg of ASTX for 4 weeks, gastric inflammation score was significantly lower than placebo control [81]. In this study, ASTX also markedly up-regulated CD4, a T-helper cell marker, and the result indicated that ASTX may also enhance humoral immune responses.

Inflammation contributes to the pathogenesis of colon cancer [82]. NF-κB is critically involved in the progression of colon tumor progression by transcriptionally regulating invasion-related factors such as matrix metalloproteinases (MMPs), particularly MMP2 and MMP 9, inhibiting apoptosis and promoting proliferations of cancer cells [83-88]. Induction of apoptosis by ASTX in Wistar rats with colon cancer was shown to regulate the expression of NF-κB, COX-2, MMP2 and MMP9, proliferating cell nuclear antigen and extracelluar signal-regulated kinase-2 [89]. ASTX also inhibited inflammation-related mouse colon carcinogenesis and dextran sulfate sodium-induced colitis in male ICR mice. Dietary ASTX significantly inhibited the occurrence of colonic mucosal ulcers, dysplastic crypts, and colonic adenocarcinoma and suppressed expression of NF-κB, COX-2, TNFα, IL-6, and IL-1β, inhibited proliferation, and induced apoptosis in the colonic adenocarcinomas of ICR mice [90].

In addition to NF-κB pathway, several other transcription factors, such as activator protein-1 (AP-1), nuclear factor of activated T-cells, and signal transduction-activated transcription factors, are also known to be involved in inflammation [91-93]. Carotenoids including lycopene, lutein, β-cryptoxanthin, and β-carotene are known to have an anti-inflammatory effect by regulating NF-κB [94-96] and AP-1 [97-102]. Further studies are needed to identify transcription factors that mediate anti-inflammatory effects of ASTX.

Anti-diabetic effects of ASTX

Type 2 Diabetes is a chronic metabolic disease that is characterized by insufficient secretion or action of endogenous insulin and hyperglycemia [103]. Several studies have demonstrated that hyperglycemia-induced oxidative stress promotes insulin resistance and contributes to the pathogenesis of diabetes [104-106]. Consumption of antioxidants that ameliorate oxidative stress can be used as an effective strategy to prevent diabetes and its-associated complications [107]. Anti-diabetic effects of ASTX have been reported in diabetic animal models. When db/db mice, a well-known mouse model of type 2 diabetes, were fed an ASTX supplement at a daily dosage of 1 mg for 12 weeks, non-fasting blood glucose levels were decreased and glucose tolerance was improved [108]. In mice with diabetes induced by alloxan that promotes oxidative stress, postprandial hyperglycemia was suppressed by feeding ASTX at doses of 5 mg/kg and 10 mg/kg daily for 7 days [109]. ASTX supplementation of 20 mg/kg for 30 days reversed the elevated lipid peroxidation and protein carbonyl groups, indicators of oxidative damage to biomolecules, in alloxan-induced diabetic rats [110].

Combination therapy of ASTX with other antioxidants has shown to be effective in improving diabetic conditions. In streptozotocin-induced diabetic rats, supplementation of ASTX (0.1 g/kg) together with α-tocopherol (0.1 g/kg) for 20 weeks ameliorated oxidative injury [111]. After 12 weeks of a diet containing ASTX and flavangenol, a pine bark extract, at doses of 0.1g/kg and 2.0g/kg, respectively, streptozotocin-induced diabetic rats showed decreased levels of lipid peroxides in plasma, liver and kidney, and of plasma triglyceride [112]. In this study, oxidative stress biomarkers, such as lipid peroxidation in liver and kidney and level of urinary 8-hydroxy-2'-doxyguanosine (8-OHdG), were also reduced by the combination therapy.

Oxidative stress induced by hyperglycemia is one of the factors that cause pancreatic β cell dysfunction and disturb insulin signaling [113,114]. ASTX enhanced insulin-stimulated GLUT4 translocation to the plasma membrane and glucose uptake in rat L6 muscle cells whose insulin signaling was interfered by fatty acids [115]. In db/db mice, although there was no significant difference in pancreatic β cell mass between control and ASTX-fed mice, the ability of islet cells to secret insulin were preserved in ASTX-fed group [108]. Consumption of a high fat/high fructose diet supplemented with ASTX at a dose of 6 mg/kg body weight for 60 days ameliorated high fat/high fructose diet-induced hyperinsulinemia and insulin resistance in Swiss albino mice [116]. In addition, mice fed a high fat/high fructose diet containing ASTX at a dose of 2mg/kg body weight for 45 days improved insulin sensitivity by decreasing serine phosphorylation of insulin receptor substrates (IRS), increasing the association of IRS and phosphatidylinositol 3-kinase (PI3K), and increasing Akt phosphorylation in the liver [117]. This study indicates that ASTX promotes hepatic IRS-PI3K-Akt pathway of insulin signaling. Furthermore, when Swiss albino mice were fed a high fat/high fructose diet supplemented with ASTX at 6 mg/kg for 60 days, ASTX improved hyperglycemia and hyperinsulinemia, and decreased plasma levels of TNFα and IL-6 [118]. Improved insulin signaling was also observed in mice fed ASTX by enhancing IRS tyrosin phosphorylation and GLUT4 translocation in skeletal muscle.

Kidney failure is one of the diabetic complications and renal dysfunction in diabetes is primarily due to damages of tubular epithelial cells by apoptosis [119,120]. Hyperglycemia-induced oxidative stress can cause apoptosis in proximal tubular epithelial cells (PTECs), deteriorating kidney functions [121,122]. In PTECs, ASTX inhibited high glucose-induced lipid peroxidation,

production of ROS, iNOS and COX-2, NF-κB nuclear translocation, and pro-apoptotic Bax protein, whereas it increased anti-apoptotic Bcl2 protein levels [123]. The results suggest that ASTX may protect against high glucose-induced oxidative stress, inflammation, and apoptosis in kidney. Diabetic nephropathy is also characterized by the enlargement of glomerular mesangium. Consumption of 0.02% ASTX for 12 weeks prevented the progression of diabetic nephropathy, evidenced by reduced glomerular mesangial area, improved hyperglyceridemia and oxidative stress in db/db mice [124]. Also, ASTX supplementation decreased the expression of genes involved in the mitochondrial oxidative phosphorylation pathway, such as complexes I, III, and IV, in primary glomerular cells from the kidney of db/db mouse fed 0.02% ASTX for 6 weeks [125].

Taken together, studies have supported that ASTX exerts anti-diabetic effects by ameliorating hyperglycemia-induced oxidative stress, which can improving insulin sensitivity by the activation of IRS-PI3K-Akt pathway in several diabetic animal models.

Hepato-protective effects of ASTX against NAFLD

NAFLD, the hepatic manifestation of metabolic syndrome [126], is the most common cause of chronic liver disease in the developed countries [127]. A part of NAFLD patients progress to NASH, which is characterized by hepatocyte damage, necroinflammation, and fibrosis [128,129]. Although the pathogenesis of NASH is controversial, lipotoxicity, oxidative stress, and inflammation have been suggested as key culprits for the progression of benign hepatic steatosis to NASH [130-132]. Because ASTX accumulates in the liver at a high concentration [31] and it has potent antioxidant and anti-inflammatory properties as mentioned earlier, it has a great preventive/therapeutic potential to prevent the development of NASH.

The hepato-protective function of ASTX has been demonstrated in several animal models. Administration of carbon tetrachloride (CCl4), a common chemical inducer of NASH, increases fatty acid synthesis and inhibits lipoprotein secretion in the liver, ultimately leading to excessive lipid accumulation and oxidative stress [133]. Daily oral gavage of 100 mg/kg body weight ASTX for 16 days inhibited lipid peroxidation, and increased the levels of GSH and the activity of SOD in CCl_4-treated rats [134]. In high fat-fed ddY mice, stomach intubation of 30 mg/kg body weight of ASTX per day for 60 days prevented the high fat-induced increase in body weight, and adipose and liver weights, as well as the hepatic triglyceride content without altering energy intake compared to control [135]. The study suggests that ASTX may exert an anti-obese effect by facilitating energy expenditure possibly via increasing thermogenesis and fatty acid oxidation. Lower respiratory quotient in ASTX-administered mice supports this possibility because ASTX increased the utilization of fatty acids, instead of carbohydrate, as energy sources. In addition, daily administration of 6 mg/kg body weight of ASTX for 60 days prevented high fat/high fructose diet-induced obesity and hepatic steatosis in albino mice [136]. This study showed that ASTX improved the liver morphology by reducing lipid droplets and collagen accumulation; and ASTX significantly improved antioxidant status in liver, evidenced by increased GSH level and antioxidant enzymes activities with a concomitant decrease in lipid hydroperoxide levels. Therefore, the studies suggest that ASTX has antioxidant and lipid-lowering activities in the liver, which may act synergistically to prevent the pathogenesis of NAFLD and NASH.

ASTX may be able to prevent the progression of existing NASH conditions. Oval cells, or hepatic progenitor cells, undergo rapid proliferation and differentiate into hepatocytes in response to injury of mature hepatocytes [137]. Excessive differentiation of oval cells, however, may increase the risk of neoplastic transformations and carcinogenesis [137]. Treatment of diethylnitrosamine (DEN), a chemical inducer of hepatocellular carcinogenesis, triggers rapid proliferation and carcinogenesis in oval cells [138]. Expansion of oval cells is a key event for NASH in murine models and humans [139]. When oval cells isolated from partially hepatectomized or DEN-treated rats were cultured with ASTX, the proliferation of oval cells was significantly attenuated by ASTX, suggesting it may have hepato-protective properties against deterioration of existing hepatic injury [140]. Taken together, ASTX has anti-steatotic, antioxidant, and anti-carcinogenic properties in the liver, which can prevent key steps for the development of NAFLD and liver cancer.

Health benefits of ASTX for the prevention of CVD

CVD risk factors include hypercholesterolemia, hypertriglyceridemia, hypertension, and chronic inflammation [141]. Several studies in animal models and humans reported that ASTX can reduce the CVD risk. We also previously reported that apoE-/- mice, a mouse model of atherosclerosis, fed a high fat/high cholesterol diet supplemented with 0.03% ASTX for 4 weeks showed lower plasma total cholesterol and triglyceride than control mice [59]. The hypocholesterolemic effect of ASTX was likely due to increased hepatic expression of LDL receptor, which facilitates LDL uptake to the liver from the circulation, whereas the triglyceride-lowering effect of ASTX was attributed to an increase in the expression of genes involved in fatty acid β-oxidation. In subjects with mild hyperlipidemia, ASTX consumption reduced plasma triglyceride levels but increased HDL cholesterol and adiponectin [142]. The underlying molecular mechanisms for the hypolipidemic effect of ASTX may be mediated through peroxisome proliferator-activated receptors (PPARs). In HepG2 cells, a human hepatoma cell line, ASTX increased the expression of PPARα, yet it downregulated PPARγ, stimulated bile acid synthesis pathway, and inhibited cholesterol biosynthesis [143]. This study also demonstrated that ASTX can function as a PPARα agonist but as a PPARγ antagonist, reducing accumulation in HepG2 cells. The role of ASTX on atherogenesis was also determined. When Watanabe heritable hyperlipidemic rabbits were fed 100 mg/kg of ASTX for 24 weeks, ASTX significantly decreased macrophage infiltration in the atherosclerotic plaques, improved plaque stability, significantly diminished macrophage apoptosis, MMP3 expression [144].

Studies have demonstrated ASTX may improve hypertension. In a spontaneously-hypertensive rat (SHR), oral administration of ASTX for 14 days or 5 weeks at the level of 50 mg/kg reduced blood pressure and delayed stroke incidence [145]. Also, 5 mg/kg of ASTX supplementation reduced plasma levels of nitrite/nitrates, decreased lipid peroxidation, improved vascular elastin, and decreased coronary artery wall thickness in SHR [146,147].

In SHR fed an ASTX-enriched diet at a dose of 200 mg/kg body weight, the systolic blood pressure was lowered and endothelial function was improved concomitantly with a decrease in oxidative stress and an increase in NO bioavailability [148]. Also, vascular oxidative damage, hypertension, and cerebral thrombosis were protected by 3 week-ASTX supplementation at a daily dose of 600 mg/kg body weight in stroke-prone SHR [149]. In Zucker fatty rats, supplementation of ASTX at doses of 5 mg/kg or 25 mg/kg for 75 days also decreased systolic blood pressure [150].

In summary, mounting evidence supports that the preventive effect of ASTX against CVD may be attributed to its hypolipidemic effect by functioning as a PPARα agonist to lower plasma triglyceride levels, improving oxidative damage, and preventing hypertension.

CONCLUSION

Oxidative stress is a major underlying cause for metabolic disorders, such as insulin resistance, CVD and NAFLD. Mounting evidence supports that ASTX has a potent antioxidant effect, which is largely attributed to its unique chemical structure and position in the lipid bilayer of cell membrane. In addition to direct removal of free radicals and ROS, ASTX can also regulate activity of NRF2 and NF-κB to enhance body's endogenous antioxidant defense system and to inhibit pro-inflammatory response, respectively. Although several health-promoting effects of ASTX have been demonstrated (Figure 2), future studies are necessary for better understanding of the functions of ASTX. In particular, it is important to understand mechanisms by which ASTX alters signaling pathways and activities of transcriptional factors. Also, large-scale, well-designed human clinical trials should be conducted to test a therapeutic potential of ASTX to lower the risks of diabetes, NAFLD or CVD.

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