

Safety assessment and pharmaceutical effects of astaxanthin: An overview

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Abbreviations

ASX	astaxanthin
COX	cyclooxygenase
CYP450	cytochrome P450
H ₂ O ₂	hydrogen peroxide
IL	interleukin
iNOS	inducible nitric oxide
ROS	reactive oxygen species
TNF- α	tumor necrosis factor- α

1 Introduction

Carotenoids are tetraterpenoid organic pigments produced by plants, algae, fungi, and several photosynthetic bacteria. These carotenoids exhibit the bright yellow, red, and orange colors in plants, vegetables, and fruits. Among the 750 known naturally occurring carotenoids, only 50–60 are present in the human diet. Of all commonly dietary carotenoids, astaxanthin (ASX) is one of the potent antioxidant xanthophyll carotenoids [1]. In recent years, ASX has gained higher importance as a dietary supplement because of its potent biological activities against diabetes, inflammation, cancer, cardiovascular diseases, and age-related degenerations. ASX, a red-orange carotenoid, is commonly found in microalgae, yeast, and aquatic animals [2]. Several studies revealed that ASX consumption is associated with reduced risk of various metabolic disorders in humans and animals [3–6]. ASX is related to other carotenoids, namely, β -carotene, lutein, and zeaxanthin, in its physiological and metabolic functions. Due to the significance of ASX in human health and diet, there is an increasing concern regarding its safety. Therefore this chapter will emphasize on the biological activities and safety aspects of ASX.

2 Sources of ASX

ASX is distributed mainly in the marine environment like salmon, shrimps, krill, trout, and crayfish and also in some birds [2]. It is primarily synthesized by phytoplankton/microalgae (Table 28.1), a lower chain in the food chain, which in turn accumulate in the zooplankton and subsequently to fish and crustaceans through which ASX enter into the higher organisms (Table 28.2). Apart from the marine sources, ASX is also synthesized in algae, yeast, and plants and through chemical synthesis. The prominent microorganisms for the production of natural ASX includes microalgae, *Haematococcus pluvialis*, and red yeast, *Xanthophylomyces*

Table 28.1: Microalgae, yeast, and bacteria as sources of ASX.

Sources	Total ASX (%) dry weight basis	Primary stereoisomer	References
Microalgae			
<i>Haematococcus pluvialis</i>	3.8	3S, 3'S (esterified)	[9–13]
<i>Chlorococcum</i> sp.	0.2		
<i>Chlorella zofingiensis</i>	0.001		
Yeast			
<i>Xanthophylomyces</i>	0.5	3R, 3'R (free form)	[14]
<i>dendrorhous</i> (JH)	0.5		[15]
<i>Xanthophylomyces</i>			
<i>dendrorhous</i> (VKPM Y2476)			
Bacteria			
<i>Agrobacterium</i>	0.01	3S, 3'S (free form)	
<i>aurantiacum</i>	2.2	3S, 3'S (free form)	[16,17]
<i>Paracoccus carotinifaciens</i>			

Table 28.2: Marine sources of ASX.

Sources	Primary stereoisomer	References
Fishes		
Salmon	3S, 3'S	[18]
• Atlantic (<i>Salmo salar</i>)		[19]
• Pacific (<i>Onchorhynchus</i> species)		
Crustaceans		
• Shrimp (<i>Pandalus borealis</i>)	3R, 3'R (esterified)	[7]
• Crabs		
• Lobster		
• Antarctic krill (<i>Euphausia superba</i>)		

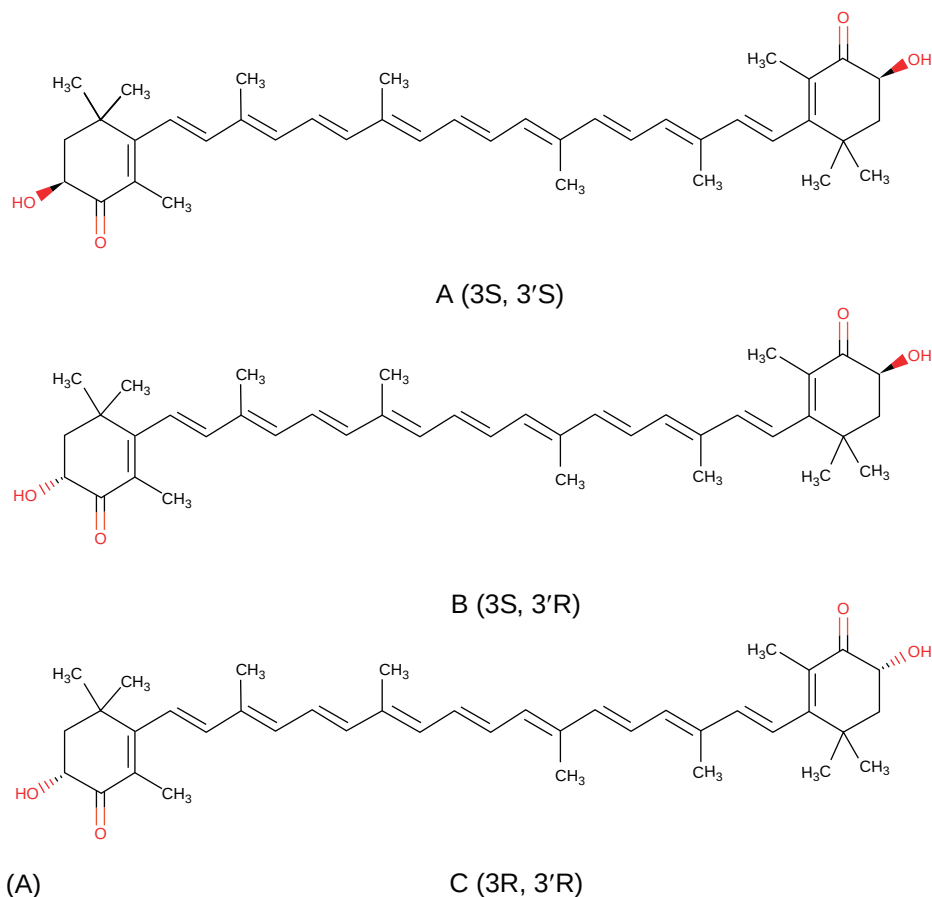
dendrorhous (formerly called as *Phaffia rhodozyma*). In *H. pluvialis*, ASX exists in esterified 3S, 3'S stereoisomer form predominated by monoesters, whereas *X. dendrorhous* contain nonesterified 3R, 3'R form [7]. Depending on the source of synthesis, the ratio in which the stereoisomers occur varies. The primary stereoisomers of ASX present in different sources are given in Table 28.2. However, synthetic ASX encompasses all three different stereoisomers (3S, 3'S, 3R, 3'R, and 3R, 3'R) in the ratio of 1:2:1, respectively [8].

3 Structure of ASX

Generally, carotenoids are categorized into carotenes (nonoxygenated compounds) and xanthophylls (oxygenated compounds) based on the presence of oxygen in their chemical structure. ASX belongs to xanthophyll group. It consists of two β -ionone rings linked by a polyene chain with conjugated double bonds wherein the two β -ionone ring systems contains oxygen in the form of hydroxyl and keto groups. Based on the configuration of the hydroxyl group in β -ionone rings, ASX can exist in three stereo isomeric forms, namely, two chiral (3S, 3'S and 3R, 3'R) and one *meso* (3S, 3'R). Additionally, the configuration of double bonds in the polyene chain also determines the occurrence of ASX either in *trans* and *cis* (E and Z) geometrical isomers (Figs. 28.1A and 28.1B). However, in nature, all-*trans*-ASX (all-*E*-ASX) remains to be a predominant form [8]. It shares the metabolic and physiological functions attributed to carotenoids with other carotenoids like β -carotene, lutein, and zeaxanthin. Besides, ASX has several unique features contributed by its molecular structure. For instance, the existence of hydroxyl ($-\text{OH}$) and keto ($-\text{C}=\text{O}$) in the β -ionone ring gives ASX higher antioxidant activity, the ability to be esterified and more soluble than other carotenoids. In nature, ASX exists either in conjugated forms (with proteins) or esterified with one or two fatty acids (monoester and diester), which is stable compared with its free form due to its susceptibility to oxidation [20].

4 Bioavailability of ASX

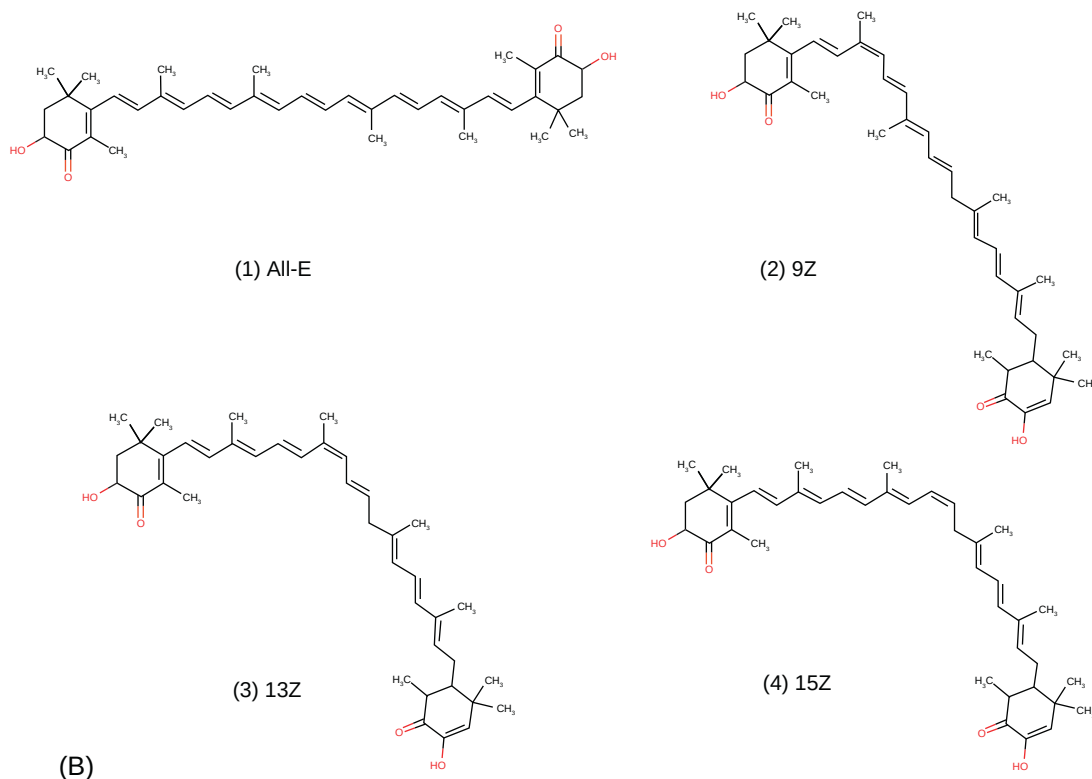
Till now, nearly 750 carotenoids are identified, out of which around 50 carotenoids are present in food. Due to the selectivity in uptake by the digestive tract, only 20 carotenoids are absorbed by the intestine and distributed to organs and tissues. The intestinal absorption of carotenoids is quite similar to the dietary lipids due to their hydrophobic nature [21–23]. As humans cannot synthesize ASX, they need to acquire it from the diet. In general, absorption of carotenoids involves multiple steps like disruption of food matrix, release of carotenoids, accumulation in the lipid droplets within gastric juices, and incorporation into mixed micelles. Later the carotenoids in mixed micelles are absorbed by intestinal mucosal lining by passive diffusion, packed into chylomicrons, and, subsequently, transported to the liver through lymph and blood. In the liver the chylomicrons are digested by lipoprotein lipase and distributed to other tissues along with lipoproteins. For example, the nonpolar carotenoids (β -carotene and lycopene) are

**Fig. 28.1A**

Chemical structure of ASX optical isomers: (A) all-trans-3S, 3'S ASX; (B) all-trans-3S, 3'R ASX; and (C) all-trans-3R, 3'R ASX.

transported by very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) and the polar carotenoids (zeaxanthin, lutein, and ASX) by LDL and high-density lipoproteins. The carotenoids are mainly not only accumulated in the liver and adipose tissues but also detected in the serum, retina, corpus luteum, skin, and testes. Moreover the absorption of ASX is highly dependent on its structure, other dietary (fat content and intake time) and nondietary related factors (age, gender, and smoking) [24]. ASX's bioavailability is also determined by three levels of chemical complexity like (1) degree of esterification, (2) optical stereoisomer form, and (3) geometrical stereoisomer form [25].

Absorption of ASX from different sources and its isomers have been studied in several animal models and in humans [26–29]. From these studies, it is understood that the geometrical and optical isomers are selectively distributed among different tissues. In humans the selective

**Fig. 28.1B**

(1) All-*trans*-ASX, (2) 9-*cis*-3S, 3'-S-ASX, (3) 13-*cis*-3S, 3'-S ASX, and (4) 15-*cis*-3S, 3'-S ASX.

process may involve in the increase of the relative fraction of Z-isomers in plasma compared with all-*E*-isomers before uptake, and its esters are selectively hydrolyzed during absorption [27,30]. The concentration of free ASX is higher in liver compared with the corresponding concentration in plasma indicating the concentrative uptake by the liver. In the liver, its accumulation is more in the microsomal fraction compared with the mitochondrial one [2].

Due to its hydrophobicity, ASX bioavailability is low that restricts its use in well-defined model of human diseases. Several strategies with variable results have been reported to address this limitation. The incorporation of lipid for enhancing carotenoid absorption was effective in mesenteric lymph duct-cannulated rats, where the absorption was influenced by corn oil and olive oil [31]. A similar study conducted by Odeberg et al. [28] in humans showed that ASX oral bioavailability is enhanced by the incorporation of lipid-based formulations. Another strategy to improve solubility is by employing emulsifiers like methyl- β -cyclodextrin, sulfobutyl ether β -cyclodextrin, polyoxyethylene sorbitan monopalmitate (Tween 40), and monooleate form (Tween 80) [32–34]. Other strategies like incorporation into liposomes, microencapsulation, nanoemulsions, and nanoparticle-based delivery have also been suggested

to improve the bioavailability [35–38]. Colloidal delivery system with polysaccharides, proteins, and emulsifiers has been developed to increase the rate of ASX absorption by enhancing its aqueous the solubility [39]. Anarjan et al. [40] reported colloidal system as an effective delivery system by comparing the uptake of colloidal ASX with pure ASX. ASX loaded with DNA/chitosan nanocarriers shown to improve the cellular uptake and antioxidant potential [38].

5 Metabolites of ASX

ASX, being a major carotenoid in marine sources, is commonly found in the human diet. Unlike provitamin-A carotenoids, ASX cannot be converted into Vitamin A in vivo. However, Sangeetha and Baskaran [41] reported that vitamin A-deficient rat could be able to convert ASX in to retinol. Nevertheless, only few reports are available on its metabolism. Studies [42] showed that ASX was converted to zeaxanthin but it was not converted to vitamin A1 or A2 in vitamin A-sufficient trout. In rat hepatocytes, ASX is converted into two metabolites, namely, 3-hydroxy-4-oxo- β -ionone and its reduced form, 3-hydroxy-4-oxo-7,8-dihydro- β -ionone [43]. Kistler et al. [44] reported four metabolites of ASX, the ionones as mentioned previously and their corresponding ionols. Sangeetha and Baskaran [41] reported severities of ASX in rat plasma and liver. ASX is a potent inducer of xenobiotic-metabolizing enzyme cytochrome P450 (CYP450) in rat liver [45,46]. In cultured human hepatocytes, ASX found to be a significant inducer of major CYP450 enzymes, CYP3A4, and CYP2B6, but not the other CYPs [44]. However, the liver microsomes containing CYPs were unable to metabolize ASX indicating that the CYPs induced by ASX pretreatment were not involved in the metabolic process of ASX in hepatocytes [43]. It is evident from the available knowledge that a complete picture of ASX metabolism and its excretion remain unclear. Hence, further investigation is required to understand ASX metabolism.

6 Clinical and safety aspects of ASX

Human cannot synthesize ASX and have to obtain through daily diet. Presently, efforts are made to improve ASX production from natural sources than chemical synthesis. The recommended average daily intake of ASX is 2–4mg. Available studies reveal no significant side effects upon ASX consumption in animals and humans. Europe, Japan, and the United States have approved the ASX-rich microalgae *H. pluvialis* as a dietary supplement for human consumption. Food and Drug Administration (FDA) accepted the ASX intake up to 12 and 24 mg/day for not more than 30 days [47,48]. Initially the European Food Safety Authority (EFSA) recommended daily intake of 2.38mg for a 70kg human and later concluded that 4mg of ASX per day was safe. An acute study in 32 healthy participants with ASX (40mg/day) and a chronic

study (16 and 40mg/day) is recommended as safe [2,49–51]. Intake of ASX (6mg/day) showed no adverse effects on adult humans [5].

Based on the research findings worldwide, ASX has been recognized as safe antioxidant for human health and effective against various health complications. Scientific evidences on ASX are also available for diverse applications [3]. Davinelli et al. [3] published a comprehensive review on the application of ASX with regard to skin health, repair, and disease. In addition to many in vitro and in vivo studies, considerable evidence (clinical studies in humans) suggests that ASX is a wonder antioxidant with health benefits. In clinical studies, ASX has been used as a single component (80%) or in combination with other nutrients (20%). Xue et al. [52] reported that ASX upregulated Nrf2 expression in irradiated cells along with upregulation of the Nrf2-targeted proteins HO-1 and antioxidative enzyme superoxide dismutase 2 (SOD2), catalase (CAT), and glutathione peroxidase 1 (GPX1). Their findings demonstrate that ASX exercises superior antioxidant activities not only via direct radical scavenging but also by triggering the cellular antioxidant defense system through modulation of the Nrf2 pathway.

ASX has been used as natural nutraceutical in human diet [7]. Dry meals of *H. pluvialis* are marketed as a food supplement since 1999 in the United States [53]. Supra therapeutic concentrations of ASX did not show any contrary effects on coagulation, platelet, and fibrinolytic function in humans. These results support the safety of ASX for future clinical studies. Hence, ASX is being recommended along with omega-3 rich foods like fish, walnuts, flaxseed, almonds, and chia as nutraceutical and antioxidant. ASX-rich foods (cream, capsules, and soft gels) are available in the market. Based on the health claim, ASX from natural source considered as a great potential nutraceutical in the global market. ASX esters are certified as “generally regarded as safe” (GRAS) by the US FDA, which allow the use of maximum 0.15mg ASX in baked products, beverages, sports and fruit drinks, cereal products, dairy product analogues, milk products, and ice creams and with processed vegetables (Yuan et al., 2011).

Enhanced absorption of ASX from dietary supplement/food has been achieved when given along with dietary lipids, which helps in passive diffusion into the intestinal cells [54]. The bioavailability and distribution of ASX has been examined in humans by single doses of 40–100mg, and maximum content (0.055–1.3mg/L) in the plasma was recorded between 7 and 21h [9,10,29,54]. The half-life of ASX in the blood is approximately 16h [9,10]. However, the bioavailability and tissue and plasma distribution of ASX is dependent on the individual, type of dietary formulation, and application.

The biological activity of ASX from *H. pluvialis* has been demonstrated clinically like oxidative stress management, cardiovascular health, and skin protection effect and supports antiaging and body recovery from more exercise (Table 28.3).

Table 28.3: Health benefits of dietary ASX in human trials.

Type of dietary supplement	Dose and duration	Age and gender of participant	Targets and mode of action	References
ASX from <i>H. pluvialis</i>	5, 20, 40 mg/day for 3 weeks (<i>n</i> = 13)	39 Smokers and nonsmokers, respectively	↓ Oxidative stress, plasma malondialdehyde, and isoprostane	[55]
ASX	5 and 20 mg/day for 3 weeks	23 Obese adults with BMI >25 kg/m	↑ Superoxide dismutase ↓ Malondialdehyde, isoprostane ↑ SOD	[56]
ASX	2 and 8 mg/day for 8 weeks (<i>n</i> = 14)	Young healthy adult females (average 21.5 years)	↓ DNA damage biomarker, acute phase protein ↑ Lymphoproliferation, natural killer cell cytotoxicity activity, T and B cell and immune response	[57]
ASX	4 mg/day for 90 days	Soccer players	↓ Paraoxonase and oxidative stress	[58]
ASX	4mg/day for 45 and 90 days	40 Male soccer players	↓ Oxidative stress, muscle damage and inflammation ↑ Salivary IgA (sIgA)	[59]
ASX	6 mg/day for 14 days	35 Bilateral cataract patients	↓ H ₂ O ₂ , oxidative stress ↑ SOD scavenging activity	[60]
ASX	6 mg/day for 14 days	16 Males and 19 females aged 71 and 70	↓ VEGF, oxidative stress, H ₂ O ₂ ↑ O ₂ ⁻ scavenging activity	[61]
ASX	12 mg/day for 8 weeks	35 Healthy postmenopausal women	↓ Limb vascular resistance, ↓ blood pressure ↑ Physical symptoms in women with high oxidative stress	[62]
ASX from <i>H. pluvialis</i>	12 mg/day for 12 weeks	96 Healthy middle and elderly aged subjects	Improves cognitive function in healthy aged individuals	[63]
ASX from <i>H. pluvialis</i>	6mg oral supplementation/day for 8 weeks	30 Healthy females 8 weeks and 36 healthy male for 6 weeks	Improves skin wrinkles, age spot size, elasticity, skin texture, moisture content in skin in female Improves feet wrinkle and elasticity and transepidermal water loss in male	[64]

Table 28.3: Health benefits of dietary ASX in human trials—Cont'd

Type of dietary supplement	Dose and duration	Age and gender of participant	Targets and mode of action	References
ASX	2 mg/day combined with collagen hydrolysate 3g/day for 12 weeks	44 Healthy humans	Improves elasticity and barrier integrity in photo aged human facial skin	[65]
ASX and standardized rose hip product	3 mg of rose hip product and 4mg of ASX for 8 weeks	34 Healthy subject (35–65 years of age)	Improves crow's feet wrinkles, skin moisture and elasticity in both the treatments ↑ Cell longevity of erythrocyte cells	[66]
ASX	6 mg/day for 14 days	35 Patients before and after ASX administration	↑ O_2^- scavenging activity	[67]
ASX	12 mg/day for 2 and 4 weeks	20 Healthy volunteers	↑ Total hydroperoxides level ↑ Blood flow velocity	[68]
ASX	1 and 3 mg/day for 4 and 12 weeks	30 Middle and senior Japanese subjects	↑ ASX concentration in plasma and erythrocyte	[69]
ASX	4 mg for 90 days	32 Male elite soccer players	↑ $O_2^\bullet$, sulfhydryl ↓ Total antioxidative status, SOD, serum creatine kinase, aspartate aminotransferase	[70]
ASX	4 mg/day for 28 days	21 Competitive cyclists	Improvement in 20km time trial of cycling	[71]
Algal meal as ASX source	Algal meal equal to 4 mg ASX	40 Healthy paramedic students	Improved fitness of paramedic students supplemented with ASX	[72]
ASX	0, 6, 12, 18 mg/day for 12 weeks	61 Nonobese (without diabetes and hypertension, aged 25–60 years) subjects	↓ Triglyceride ↑ HDL-cholesterol, serum adiponectin	[73]
ASX	Two 4 mg capsule daily	Nonsmoking healthy Finnish men (19–33 years of age)	↓ 12- and 15-hydroxy fatty acids level in plasma and in vivo oxidation of fatty acid	[74]

ASX, astaxanthin; SOD, super oxide dismutase; H_2O_2 , hydrogen peroxide; O_2^- , oxygen radicle.

7 Biological activities of ASX

ASX is an efficient nutraceutical with health benefits like antioxidant, antiinflammatory, antidiabetic, antiangiogenic, anticancer, hepatoprotective, skin protective, and cardiovascular protective. Dietary supplements and functional foods are a decent way to improve the daily consumption of ASX, which otherwise may be consumed in less-than-recommended amounts (2–4 mg). It is a promising strategy to exploit ASX for good health and well-being and offer exciting opportunities for the nutraceutical industry.

7.1 Hepatoprotective effects

ASX exhibited hepatoprotective effect against ethanol-induced liver injury primarily by blocking STAT3 activity [75]. ASX by decreasing ROS production and inflammatory cytokine expression and increasing HIF1 α , HO-1, and Nrf-2 expression showed protective effect in a steatotic liver ischemia reperfusion injury model [76]. A study by Chiu et al. [77] illustrated liposome-encapsulated ASX downregulated nuclear NF- κ B and iNOS and liver protective effect [77]. ASX administration to high fat diet (HFD) fed mice reduced liver proinflammatory cytokines TNF- α and IL-6 and improved liver steatosis and inflammation by activating PPAR α and inhibiting PPAR γ [78]. In a mice model of lipotoxicity-induced insulin resistance and steatohepatitis, ASX decreased hepatic recruitment of T cells and macrophages and an M2-dominant polarization of macrophages/Kupffer cells and thereby attenuated insulin resistance, hepatic inflammation, and fibrosis [79]. Sila et al. [80] demonstrated that ASX prevented early liver injury and exhibited antioxidant effects in diabetic rats. In a CCl₄-induced liver injury model, ASX prevented liver damage by enhancing antioxidant enzyme activities [81]. ASX induced apoptosis, autophagy, and reduced ConA in the liver through JNK/p-JNK signaling [82]. ASX in hepatic IR injured mice suppressed ROS-mediated activation of the MAPK and induced apoptosis and autophagy [83]. Turkez's group in 2012 and 2014 demonstrated protective effect of ASX against TCDD-induced liver injury in rats and rat hepatocytes [84,85]. ASX shown to ameliorate experimental CCL4 and BDL, induced liver fibrosis by decreasing the expression of NF- κ B and TGF- β 1, and downregulate autophagy in hepatic stellate cells [86].

7.2 Skin protection

ASX in combination with ginsenoside Rg2 reduced UVB-induced cytotoxicity and genotoxicity through upregulation of DNA repair in human keratinocytes [87]. Dietary ASX was reported to accumulate in the skin and exert protective effects against features of photoaging induced by UVA radiation in hairless mice [88]. ASX prevented the UVA-induced MMP-1 and SFE/NEP expression in human dermal fibroblasts and thereby showed significant protection against UVA-associated photoaging [89]. Another study performed in human

dermal fibroblasts demonstrated the superior effect of ASX in abrogating the UVA-induced stress compared with the modulatory effects of canthaxanthin, and β -carotene [90]. Lyons and O'Brien [91] showed that ASX in an algal extract reduced the DNA damage induced by UVA irradiation and maintained cellular antioxidant status.

7.3 Cardioprotective effects

ASX treatment to the hypercholesterolemic rabbits showed reduced oxidative stress, thioredoxin reductase activity, and lipid profile [92]. Experimental animals fed with ASX showed improved basal arterial blood flow and exerted cardioprotective effects [93]. A study conducted by Nakao et al. [94] using ASX (0.08%) showed improved mitochondrial membrane potential and contractility index between control and ASX fed mice. Disodium disuccinate ASX (25, 50, and 75 mg/kg body weight) administration for 4 days significantly reduced the size of myocardial infarction and improved the myocardial function [95]. Gross and Lockwood [96] showed disodium disuccinate ASX significantly reduced the myocardial infarct size after occlusion (1 h) and reperfusion (3 h). Disodium disuccinate ASX (50 mg/kg/day) decreased inflammation and thereby improved myocardial function in ischemia-reperfusion rabbit model [97]. Oral pretreatment of disodium disuccinate ASX (125 and 500 mg/kg/day) for 7 days to rats offered cardio protection [98].

Hussein et al. [20] showed oral administration of ASX to spontaneously hypertensive rats for 14 days and in stroke-prone rats for 5 weeks significantly lowered the blood pressure. Another study with spontaneously hypertensive rats revealed ASX restored nitric oxide-induced stress and oxidative parameters [99]. ASX reduced the myeloperoxidase, 8-hydroxy-2'-deoxyguanosine, and 4-hydroxy-2-nonenal-modified protein levels in exercise-induced C57BL/6 mice [100].

7.4 Ocular protection

ASX inhibited ischemia-induced retinal cell death and ROS production in a concentration-dependent manner [101]. ASX showed protection against progression of dry and wet type of age-related macular degeneration [102] and glaucoma [103,104]. ASX offered protection to retinal pigment epithelium against H_2O_2 exposure through Nrf2-mediated upregulation and activated PI3K/AKT pathway in human ARPE-19 cells [105]. Earlier, we have reported the conversion of ASX to retinol and β -carotene in retinol-deficient rats apart from antioxidant potential [41]. Consuming ASX (4 mg/day) for 12 months in macular degeneration patients showed superior retinal action compared with vitamin C and E, zinc, copper, lutein, and zeaxanthin [106]. A study by Naito et al. [107] showed that ASX (4–12 mg/day) ingestion for 28 days significantly enhanced the uncorrected far visual acuity and the accommodation time in healthy volunteers.

7.5 Antidiabetic effects

Diabetes is a metabolic disorder characterized by hyperglycemia due to inadequate insulin secretion or its action. Hyperglycemia-mediated oxidative stress stands to the major factor responsible for the pathogenesis of diabetes [108,109]. Utilization of antioxidants that ameliorate oxidative stress can be an effective strategy to combat diabetes-associated complications [110]. Studies have reported the antidiabetic effect of ASX in vitro and in vivo diabetic models. Kumar et al. [111] stated that ASX and/or squalene reduce the blood glucose and triglycerides levels in obese/diabetic model (male KK-^{Ay} mice) and improved insulin sensitivity. Park et al. [112] found that ASX inhibited diabetic-induced hepatic dysfunction by blocking AGE formation and oxidative stress. ASX improved diabetic retinopathy by attenuating the oxidative stress markers in the retinal tissue of db/db mice [113]. Supplementation of ASX to alloxan-induced rats reverted the deleterious effect of diabetes in neutrophils by reducing the lipid peroxidation and protein carbonyl groups [114]. Bhuvaneswari et al. [115] showed ASX ameliorated hyperinsulinemia and insulin resistance in high fat-/high fructose-fed mice. Further, they have reported that ASX suppressed the serine phosphorylation of insulin receptor substrates and increased Akt phosphorylation in the liver, thereby improving insulin sensitivity [116]. ASX in combination with other carotenoids reported to reduce the effects of diabetes. Supplementation of ASX with tocopherol reduced the oxidative stress in streptozotocin-induced diabetic rats [117]. ASX at a daily dose of 1 mg for 12 weeks mitigated the glucose toxicity and maintained the β -cells function in db/db mouse diabetic model [118].

7.6 Antiobesity effects

Obesity is a chronic disease, which is a significant risk factor for the development of several disease conditions like hyperlipidemia, diabetes, hypertension, and cardiovascular diseases [119–122]. Several studies reported that oxidative stress and inflammation increase under obese conditions, which in turn linked to insulin resistance [123,124]. Thus ASX has drawn attention in the obesity-related investigation due to its potent antioxidant characteristics. In obese adults, ASX supplementation resulted in an increased antioxidant response by suppressing lipid peroxidation [56]. Yang et al. [125] shown that ASX reduced the level of triacylglycerol in plasma, induced hepatic antioxidant gene expression, and mitigated splenocyte inflammatory response in diet-induced obese mice. Kimura et al. [126] also demonstrated the antiobese activity of ASX in Otsuka Long Evans Tokushima Fatty (OLETF) rat model wherein it reduced the visceral adipocyte, decreased free fatty acids, and significantly increased blood high-density lipoprotein level (HDL). In addition, ASX acted as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR- γ) that functions as an antagonist or agonist, thereby ameliorating obesity and insulin resistance [127]. ASX reduced the inflammation in adipose tissue and liver and enhanced the mitochondrial fatty acid

oxidation in skeletal muscle of ob/ob mice [128]. A study conducted by Arunkumar et al. [129] shows that ASX improved insulin resistance, an obesity-related complication by triggering postreceptor insulin signaling in high-calorie diet-fed mice. Also, it reduced the body weight and lipid levels in adipose and skeletal muscles. Briefly, ASX exert antiobese effects and thus prevent its associated complications by reducing oxidative stress, inflammation, and insulin resistance.

7.7 Antiinflammatory activity

ASX exhibited antiinflammatory effect by blocking the nuclear translocation of Rel A and I κ B α degradation [130]. In a skin inflammation model, ASX inhibited the production of inflammatory mediators by blocking NF- κ B activation in human keratinocytes [131]. ASX treatment decreased the UV-induced reactive nitrogen species production (inducible nitric oxide (iNOS)) and inflammatory cytokine expression (cyclooxygenase (COX)-2) and apoptosis in keratinocytes [132]. Recently, Park et al. [133] demonstrated that ASX in a atopic dermatitis animals inhibited the gene expression of proinflammatory cytokines such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) [133].

7.8 Antiangiogenic effects

ASX pretreatment inhibited homocysteine-induced endothelial dysfunction by suppressing the activation of the VEGF-VEGFR2-FAK signaling [134]. For the first time, Kitahara and team demonstrated that ASX inhibited the secretion of MCP-1 and VEGF120 by pancreatic β -cells in hyperlipidemic state [135]. ASX suppressed oxidative stress in the aqueous humor of bilateral cataract surgery patients and prevented age-related macular degeneration and diabetic retinopathy through suppression of neoangiogenesis (Hashimoto et al., 2016). Dietary supplementation of ASX to hamsters prevented tumor development and its progression by inducing apoptosis and impeding neovascularization in oral cancer [136]. A study conducted in choroidal neovascularization-induced mice demonstrated ASX significantly affecting the inflammatory and angiogenic molecules (ICAM-1, MCP-1, IL-6, VEGF, VEGFR-1, and VEGFR-2) [137].

7.9 Anticancer effects

A large number of studies illustrated that ASX, with its antioxidant potential, is beneficial as a therapeutic agent for various diseases including several human cancers. ASX also showed to possess preclinical antitumor efficacy both in vitro and in vivo in several cancer models. For the first time, Montazeri-Najafabady et al. [138] described the antiproliferative effect of ASX on osteosarcoma, where ASX modulated the proliferation and differentiation of osteosarcoma cell line (MG-63) via AhR-dependent pathway. ASX encapsulated in calcium alginate inhibited

human liver cancer cell (HepG2) proliferation by inhibiting aerobic glycolysis and promoting tricarboxylic acid cycle [139]. In *N*-nitrosomethylbenzylamine-induced esophageal cancer model, ASX exerted anticancer effects by inhibiting NF κ B and COX2 [140]. ASX in combination with human serum albumin inhibited the nuclear translocation of NF- κ B and thereby abrogated cell proliferation in human ovarian cancer SKOV3 cells [141]. A study by Chen et al. [142] showed that ASX induced p38 MAPK-mediated XPC downregulation, thereby enhancing the erlotinib-induced cytotoxicity in A549 and H1975 cells. ASX cotreatment suppressed tumor growth and inhibited gemcitabine-resistant pancreatic cancer by targeting hENT1, RRM1, and RRM2 [143]. ASX extracted from shrimp showed synergistic effect when treated in combination with β -carotene and lutein selective killing the MCF-7 cells [144]. In ESCC cells, ASX exerted anticancer effects by inducing apoptosis and G2/M arrest [145].

7.10 Pro- and antiapoptotic effects

In multicellular organisms, programmed cell death or apoptosis is one normal physiological process with distinct morphological characteristics like cell membrane bleeding, nuclear fragmentation, chromosomal DNA fragmentation, and finally cell death [146]. Messina et al. [147] showed that mixture of free and esterified ASX from shrimp by-products inhibited proliferation of HepG2 cells and induced apoptosis via caspase-3 activation. ASX inhibited LS-180 cell proliferation and induced apoptosis by increasing the expression of proapoptotic genes [148]. ASX alleviated TNF- α -induced ECM degradation and chondrocyte apoptosis by inhibiting the NF- κ B signaling [149]. Recently, Wan et al. [150] demonstrated that ASX inhibited inflammatory response and prevented apoptosis in bovine endometrial epithelial cell lines. ASX prevented apoptosis by regulating Nrf2 and NF- κ B pathways in ochratoxin-induced oxidative damage and lung inflammation in experimental mice [151]. ASX showed antiapoptotic effect by downregulating P53 and Bax and upregulating Bcl-2 in NRK-52E cells [152].

A study by Zhu et al. [153] demonstrated the proapoptotic effect of ASX in human hepatic stellate cells mainly by modulating miR-29b levels. In both SCC131 cells and DMBA-induced hamsters, ASX prevented cell proliferation and angiogenesis and induced mitochondria-mediated apoptosis [154]. ASX showed dose-independent reduction in mitochondrial injury and inhibition of apoptosis in the hyperoxia-induced retinopathy C57BL/6Jmouse model [155]. ASX suppressed oxidative stress and prevented coronary microembolization-induced cardiomyocyte apoptosis in Sprague Dawley rats [156]. ASX showed protection against glutamate-induced apoptosis in human neuroblastoma cells by inhibiting glutamate-induced calcium influx and ER stress [157]. In acetaldehyde-treated SH-SY5Y cells, ASX prevented the activation of p38 MAPK and inhibited ERK and Akt-/CREB-mediated cell signals [158]. Zhang and his team showed that ASX prevented pulmonary fibrosis by promoting myofibroblast apoptosis by activating the Drp1-mediated mitochondrial fission pathway [159].

ASX showed protective effects against oxidative stress in severe burn rat model by regulating mitochondrial-related apoptosis [160]. Kavitha et al. [161] demonstrated ASX in a hamster model of oral cancer-inhibited NF- κ B and Wnt/ β -catenin signaling and thereby induced caspase-mediated mitochondrial apoptosis.

8 Conclusion

ASX a naturally occurring ketocarotenoid has shown biological activity against different clinical conditions including the lifestyle diseases. ASX was reported to possess various biomedical significance like hepatoprotective, skin protective, cardioprotective, antiinflammatory, antiangiogenic, antidiabetic, antiobesity, and anticancer. Altogether the broad spectrum of biological activities makes ASX an ideal pharmacological agent. Also, ASX having a superior antioxidant effect has a great scope in the global market of nutraceuticals since there is no such other compound having health claim approved by the FDA or EFSA. Several studies performed with ASX showed its safety without any toxic effect in both in vitro and animal models. Although the molecular mechanisms behind the health-promoting effects of ASX are well documented, still controlled human clinical trials are warranted for determining the required daily dosage of ASX. This review summarizes the recent available evidences regarding ASX safety and potential health beneficial effects in humans.

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Author contributions

PMK, JN, and RJ wrote the book chapter, and VB edited the book chapter and helped review the literature. All the authors approved the final version of the book chapter.

Conflicts of interest

The authors declare that they have no conflict of interest.

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