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Review Article

A Mini Review of Astaxhantin and Oxidative Stress in Aging

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ABSTRACT

There are more than 300 theories attempting to explain old age or senescence, which is affected by genetic and environmental factors. Of all these theories, with respect to free radical theory, oxygen species occurring in the wake of extrinsic and intrinsic factors may trigger senescence and accelerate the aging process. Another reason for free radical generation is decrement of antioxidants and its components intake in diet. Astaxhantin (ASTX) has more antioxidant activity than other carotenoids. Because of this property, astaxhantin may be a substantial antioxidant source in the diet. In this review, we discuss antioxidant effects of astaxhantin on potential mechanisms in senescence.

Keywords: Astaxhantin; Oxidative stress; Senescence

INTRODUCTION

Free radicals, wear and tear, destructive DNA damage, mechanism loss of mitochondria, cellular adaptation and stochastic theories are some of over 300 theories about the aging process [1-4]. With regard to these theories, exogenous factors, such as lifestyle, stress, contribute to aging process, in addition to some metals and naturally occurring free radicals in cells and tissues [1,5].

Lots of agents such as hormones, body proteins and immune system cells also change with age as a result of diversity in gene expression [1]. Diminished physiological function leads defense system up against oxidation and other biochemical variance to fall into a decline and cell survival to become shorter [6].The other reason for oxidative stress increasing with age is decreased intake of exogenous antioxidants from nutrition [7].

Utilization of nutritional supplements against inadequacies due to nutritional diversification seen in the elderly lessens the adverse effects of senescence on immune functions [8]. Antioxidants such as ascorbic acid, phenolic compounds, sterols and carotenoids, which are indigenous to foods, decrease oxidative damage to a minimum level or/and prevent it altogether [9,10].

Various carotenoids, pigments derived from organic hydrocarbons, not only induce antioxidant activity but also have positive effects on glutathione and activation of enzymes such as cyclooxygenase, SOD and caspase-3 and -9 [11-15].There are two subunits of carotenoids in terms of oxygen atom-inclusiveness, carotenes, and xanthophyll's [11]. Astaxhantin (ASTX) is an oxidized carotenoid derivative, including both hydroxy and oxy groups [16].

Additionally, double band and polar-apolar-polar structure help differentiate ASTX from other xanthophyll's and contribute to greater antioxidant activation [17]. Other properties that make ASTX special include indicating vitamin A activity in due course of metabolized in liver, permeant through blood-brain barrier and partaking in grey matter of brain [18,19].

Abdelzaher et al. suggested that ASTX inhibits reactive oxygen species [20]. They stated that incubation of 25 μM ASTX in cell-culture for 24 hours reduces accumulation of free radicals in cells [21]. In another study, individuals aged 20-55 years who consumed 20 mg ASTX for 12 weeks experienced decreased serum malondialdehyde levels, an indicator of oxidation [22]. Astaxhantin administrations in various doses in rats and humans (20 mg/kg/day in rats; 5 mg/day, 20 mg/day, 40 mg/day in humans) provide superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) with activation and enhance total antioxidant capacity [23-25].

Baralic et al. seeking to understand the effect of ASTX on oxidative stress in humans has shown that subjects given 4 mg ASTX for 90 days had increased paraoxonase-1 activity, an

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antioxidant enzyme [26]. In this article, because of the unique properties of ASTX that distinguish it from other carotenoids, and because it is high in antioxidants, we investigated impacts of ASTX on oxidative stress dependent on senescence, and is tackled that it may be protective against oxidative stress.

MATERIALS AND METHODS

Properties of Astaxhantin

Hydroxy (OH-) groups in both two terminal ends of ASTX make polar characteristic available to its pattern while middle part is also nonpolar [27]. Chemical structures of ASTX and its metabolites were shown in Figure 1 [28].

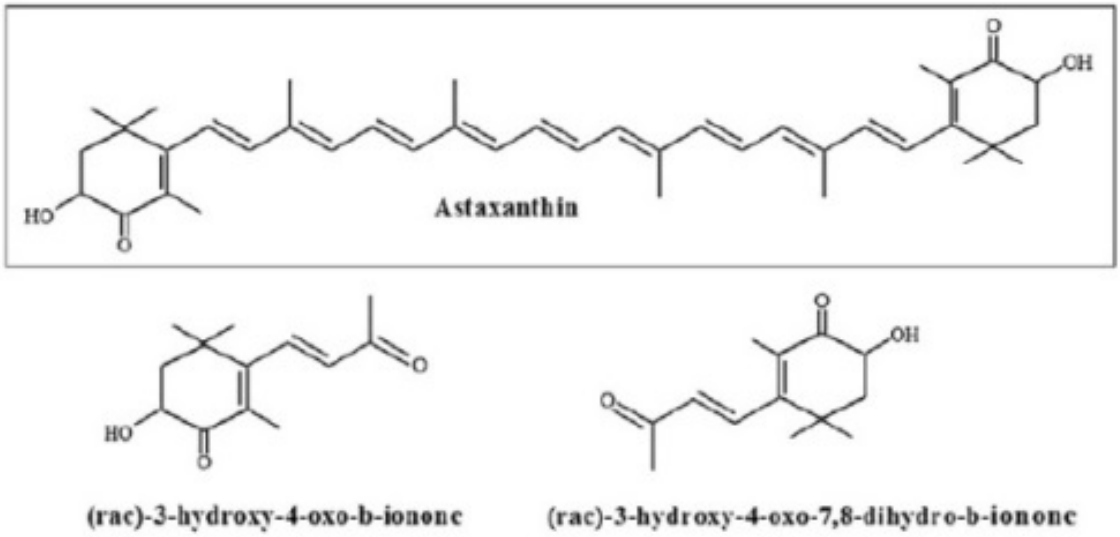

Figure 1: Chemical structures of ASTX and its metabolite.

There are several isomers subject to configuration of OH- groups [17]. Natural ASTX is esterified and artificial ASTX is free form [29]. Astaxhantin stereoisomers are classified by their OH-groups. Astaxhantin isomers are Enantiomer (3S, 3'S), Enantiomer (3R, 3'R), and Mesomere (3R, 3'S) that are identified for C-3 and C-3' carbon atoms in chiral-cores. Of these isomers, enantiomer (3S, 3'S) is the dominant form of ASTX (Figure 2) [30,31].

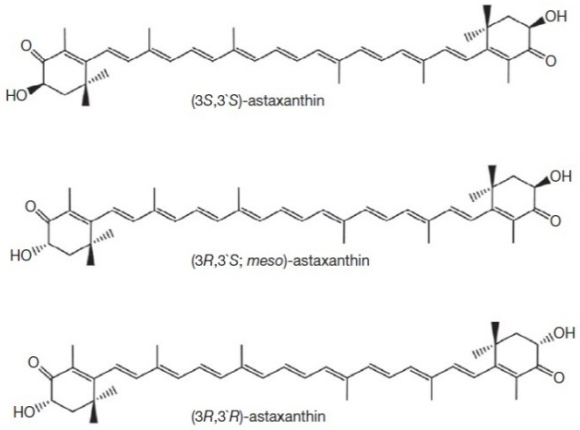

Figure 2: Chemical structures of ASTX isomers.

Table 1: ASTX products being in market.

Once consumed, astaxhantin is first absorbedviapassive diffusion by enterocytes, and is got undergone via facilitated diffusion in the presence of lipids [29]. Its non-esterified form is brought in through chylomicrons to the liver. Then, this form is transported by lipoprotein to other tissues [32]. More than 50% of ASTX taken within 24 hours is metabolized. The main metabolic afterproduct is 3-hydroxy-4-oxo-β-ionone. Afterwards, this last product conjugates to 3-hydroxy-4-oxo-7,8-dihydro-β-ionone (Figure 1) [28,33]. Basic sources of ASTX are phytoplankton and *Haematococcus pluvialis* *H.* (*pluvalis*) in addition to yeast, some mushrooms, bacteria, shellfish, krill, shrimp, salmon, lobster, and some plants [18].

The Food and Drug Administration (FDA) has predicated ASTX grade got *H.pluvalis* in Generally Recognized As Safe (GRAS) since 2010. It is suggested that consumption of up to 40 mg/d in humans doesn't have adverse effects according to this list. Natural ASTX Complex, including extract of *H.pluvalis*, common fatty acids, and other carotenoid esters is recommended at a dose of 2–12 mg/day (mean dose 6 mg/d) by the FDA [34]. There are many products in the market as ASTX supplements (Table 1).

Products	Ingredients	Origin of ASTX	Amounts of ASTX	Advised daily intake	Reference
ZanthoSyn®	Modified food starch, vegetable celulose capsule, microcrystalline, cellulose, vegetable stearate, corn starch, glucose syrup, sodium ascorbate and DI-alpha-tocopherol.	-	12 mg	1 capsule	https://www.gnc.com/gnc-exclusive-brands/539600.html
Solgar®	Sunflower seed oil, gelatin, vegetable glycerin, lutein, canthaxanthin and beta carotene.	*H.pluvialis*	10 mg	1 softgel	https://www.gnc.com/other-antioxidants/216780.html?cgid=other-antioxidants
			5 mg	1 softgel	https://www.gnc.com/other-antioxidants/216781.html?cgid=other-antioxidants
Life Extension®	Phospholipids, sunflower oil, gelatin, glycerin, purified water and extra virgin olive oil.	*H.pluvialis*	4 mg	1-2 softgel	https://www.gnc.com/other-antioxidants/215995.html?cgid=other-antioxidants
AstaPure®	Cold-pressed extra-virgin oil and capsule shell (gelatine, glycerol, beta-carotene, caramel E150a).	*H.pluvialis*	4 mg	1-2 capsule	https://www.amazon.co.uk/Essentials-Astaxanthin-Astapure-Delivering-Capsules/dp/B07BGHKG77
	Extra virgin olive oil and vegetarian softgel (modified food starch, glycerin, carrageenan, purified water). carrageenan, purified water).	*H.pluvialis*	6 mg	1 softgel	https://www.amazon.co.uk/Doctors-Best-Astaxanthin-AstaPure-Softgels/dp/B00KMX35P2 0KMX35P2
AstaReal®	Extra virgin olive oil, gelatin (bovine), glycerin and purified water.	*H.pluvialis*	6 mg	1 softgel	https://www.drvita.com/products/natures-lab-astareal-astaxanthin-6mg-60-softgels
BioastinTM	Safflower oil, gelatin, glycerin, purified water and natural tocopherols.		12 mg	1-3 gel caps	https://www.pureformulas.com/bioastin-natural-astaxanthin-60-capsules-by-nutrex-hawaii.html

Oxidative stress in senescence

Sections of DNA related to aging, progressive faults of protein synthesis, immune attacks in developed organisms against self-antigens, and free radical damage are assumed in the nature of the aging process [35]. Antagonistic pleiotropy theory, fallout accumulation theory, insulin resistance, advanced glycation, wrong destruction theory, autoimmunity, circadian rhythm, and evolutionary theory are some of the propounded theories. Of these theories, free radical theory is involved in both genetic mutations and aggregation of cellular catabolites [36]. Although the aging process and its mechanisms have not been clarified yet, of attractive aging theories, wear and tear, genetic programming telomere, pace of life, mitochondria, endocrine and wrong destraction theory, hypothesis of advanced DNA damage and free radical theory are updated [37].

Harman (1956) has suggested that accrescent free radicals in the wake of molecular oxygen catalyzed in cells cause senescence by their side effects on cells and tissues [5]. Free radicals, forming from exogenic and endogenic resources in cells, have detrimental effects on both the cell nucleus and genomes of mitochondrial DNA [38]. It is asserted that environmental factors, genetic modification, diseases, and naturally-occurring free radicals in aging trigger senescence [39].

There are produced reactive oxidative species in the course of diverse cell reactions. These species are undergone detoxification by enzymes and antioxidant compounds (Figure 3) [40].

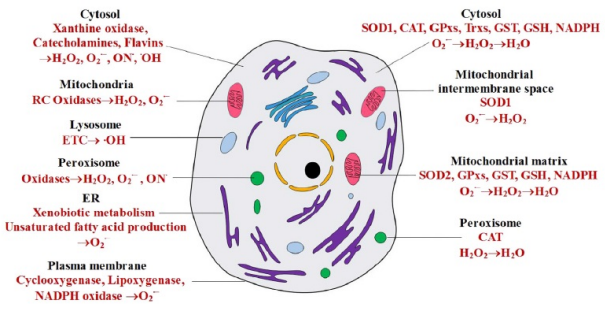

Figure 3: Detoxification of reactive oxidative species by enzymes.

It is discussed that damage signaling pathways increase in company with aging situation, decreasing general physiological capacity and increasing mortality. One of these pathways is DNA damage response (DDR). Inadequency and defectiveness in DDR as well as accumulation of DNA lesion trigger aging and pathological diseases associated with aging. p53 tumor suppressor protein expressed in responsed to DNA damage

modulates cascade I and therefore it is accepted as an important DD [41].

Activity of Nrf2 being another DDR play a repressive role in numerous steps of aging and diseases related with aging. Nrf2 also creates a response by increasing expression of detoxification genes [42].

The longevity-modulating genes found out past two years are preserved by from smallest living to human. Of these genes, Cell aging regulation system (CARS) includes diverse aging effectors modulating longevity, such as lipid unsaturation, mitROS production, mitochondrial DNA repair, autophagy, apoptosis, proteostasis or telomere shortening. Because of these properties, CARS is inclusory such as to previous theories [43].

Mitochondria is origo of intracellular free radical species. Mitochondrial DNA (mtDNA) is exposed to grave oxidative stress. An important oxydant, 8-hydroxydeoxyguanosine (8OHdG), provokes erroneous conjugation and point mutations. Due to the fact that disruptions in mtDNA based on these errors may ocur, it is assumed that mtDNA damage is a reason for aging [44]. Mitochondria theory is descriptive of senescence as increased production of free radicals due to disorders in the mitochondria electron transport chain and premediates that aging is significantly alleviation in enzymes, especially transporter enzymes, with age [45]. One study (2016), seeking out relationship between hippocampal proteins and age, found that proteins in electron transport chains and fusion pathways of synaptic vesicles consistently decrease with age [46]. Unpaired protein-2 (UCP-2), a mitochondrial transporter protein, may have an impact on life span in humans. In reference to this consideration, rats without UCP-2 matureity earlier and have a shorter lifetime [47]. A study (2011) that investigated impacts of senescence on activity of the mitochondria electron transport chain showed that the most reduction is seen in activity of complex-4 while complex-2 does not change [48]. Senescence is associated with oxidative stress and accumulation of mutant mtDNA. Hydrogen peroxide level, releasing of NADPH oxidase-2 (NOX-2), 8OHdG and accumulation of mutant mtDNA increase and caspase-3-dependent apoptosis pathway in a study of Du et al. working on old rats [49]. Dysfunction of Nrf2 in vessels further augments oxidative stress levels occurring with age [50,51], going into diverseness in response of fibroblasts human of old and young to oxidative stress, found that expression of phase-2 enzymes, such as CAT and SOD, is greater in younger individuals than in older [51]. They noted that lifespan of rats without SOD is substantially shorter and these rats age quickly [52].

Based on alterations in mitochondria, diseases with aging, such as Alzheimer's disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD), come in sight. Of these diseases, accumulations of Aβ protein in AD affect Complex-IV and activity of α-ketoglutarate dehydrogenase. This protein also binds to Aβ-binding alcohol dehydrogenase. Of another disease, Lewy bodies accumulate in mitochondria and decrease Complex-I activity in PD. In another disease, ALS, it is a matter of abnormal outturn of mitochondrial ROS depending on SOD 1 mutations. In HD, there is a decrease in Complex-II. All of these mitochondrial disfunctions by diseases are shown in Figure 4 [53].

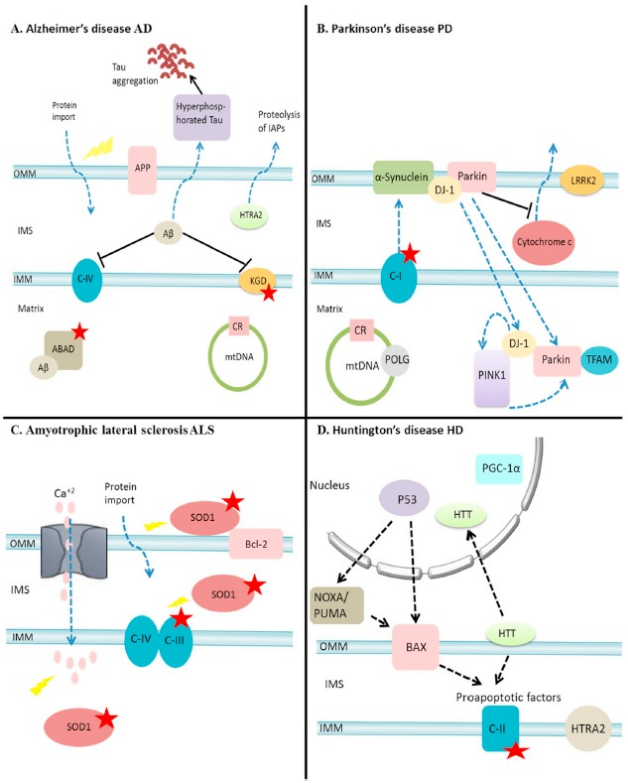

Figure 4: The role of mitochondria in age-related neurodegenerative diseases. (A) In AD (B) In PD (C) In ALS (D) In HD [53].

Effects of Astaxhantin on oxidative stress

β-carotene and vitamin C, being antioxidant, are located in both inside and outside of cell lipid layer of membrane. However, besides participation in the double-layer membrane, the presence of the cell both inside and outside gives ASTX better protection than others (Figure 5) [54].

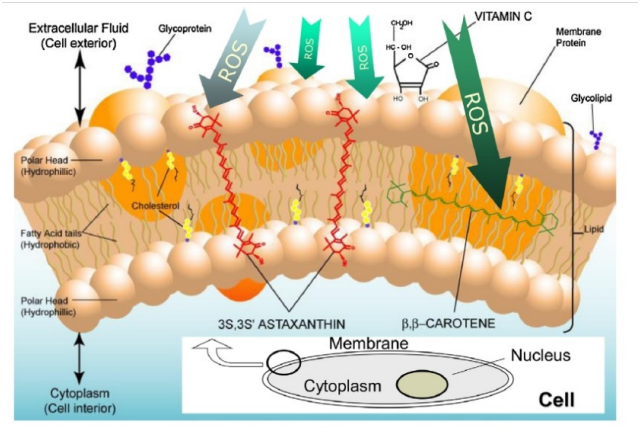

Figure 5: Protection of ASTX against ROS in the double-layer membrane [54].

It is adduced in studies related to astaxhantin that ASTX decreases oxidant stress by inducing antioxidant enzymes, such as Nrf2, PI3K/Akt, SOD and glutathione, and their pathways [55-58].

It is separately researched as mice, human and cell studies.

RESULTS AND DISCUSSION

Mice studies

Effects ASTX on antioxidant enzymes: Al-Amin et al. were studied on mice to measure the levels of anti-oxidative enzymes in the prefrontal cortex (PFC), striatum (ST), hippocampus (HP), and liver (LV) in groups [59]. Treatment of 2 mg/kg ASTX for 28 days induced CAT (in ST and LV) and SOD (ST, HP and LV) while supressed nitric oxide (in PCF, ST, HP and LV), glutathione (in LV) [59]. To investigate effects of ASTX on antioxidant enzymes, 10 mg/kg ASTX were given for 28 days and 100 mg/kg ASTX were given for 7 days to rats and, as a result, SOD activity increased for both dose levels [60,61]. Al-Amin et al. gave 2 mg/kg/day ASTX to old rats and found that activities of CAT and SOD and glutathione levels increased at the end of the study [62].

In a study on adult Wistar male rats for 45 days that Mattei et al. carried on effect of ASTX on oxidative stress due to fish-oil, while only fish-oil (10 mg EPA/kg body weight and 7 mg DHA/kg body weight) decreased activities of SOD, CAT and glutathione reductase, ASTX administration (1 mg/kg body weight) after fish-oil improved these activities [63]. Otsuka et al. investigated effect of ASTX on photoreceptor degeneration induced by fluorescent and noted 8-OHdG level increased in outer core layer. After ASTX administration (100 mg/kg, at 6 h before and at 0, 6, 12, 24, 36, 48, and 72 h after light irradiation) to male albino ddY mice, there was no effect of ASTX on mRNA synthesis of Sod1, metallotionein-II and II [64]. ASTX administration of 100, 250 and 500 mg/kg body weight to ulcerated albino Wistar rats promoted SOD and CAT levels [65].

Al-Amin et al. administrated ASTX (20 mg/kg body weight/day) to Swiss albino male mice for 42 days. Glutathione concentrations of frontal cortex, striatum and cerebellum decreased after administration. In addition to this, malondialdehyde levels diminished in striatum, hypothalamus and hippocampus [66]. Superoxide anion levels in neutrophile of alloxane induced diabetic Wistar male rats fed ASTX (20 mg ASTA/kg of body weight/day, for 30 days) did not change, while there was a diminishment in TBARs levels. At the same time, this administration increased GPx level further [67].

Effects ASTX on reactive oxygen species: Pan et al., who studied adult rats, reported that ASTX given in doses of 5 and 10 mg/kg for a week suppresses reactive oxygen species and activates the defense system of antioxidants [68]. In another study (2016), oxidative stress levels were evaluated, and old rats were given 1.7 mg/day ASTX. They found that the level of plasma oxidative stress was lower while antioxidant levels were higher by the end of 72 weeks [69]. After administration of 25 and 50 mg/kg ASTX in rats, there were decreases in levels of 8OHdG and malondialdehyde [70].

Metabolic disorders such as oxidative stress, happens after high fatty diets and this diet causes neural damage and alterations in neurogenesis and memory. In a study researched effects of antioxidants (ASTX, vitamin C and E; 0,6 g/kg, 0,2 g/kg and 0,2 g/kg, respectively, for 180 days) on potential side-effect of high fatty diet, ASTX administration to male Wistar rats enhanced total antioxidant capacity [71]. In another study sought relevany between ASTX and 8-OHdG level, both 300 and 600 mg/kg/day administration for 3 weeks of ASTX to male SHRSP rats decreased urinary 8-OHdG level [72].

Yeh et al. gave 0.6 mg/kg and 3 mg/kg ASTX to old rats for 8 weeks. They noted that levels of 8OHdG, nitrotyrosine, acrolein and transcription of nuclear factor kappa B (NF-kB) decreased in both groups [73].

Effects ASTX on pathways of oxidative stress: Masoudi et al. asserted that the release of caspase-3 significantly decreased when adult rats were given 10 µL/0.2 mM ASTX [74]. In a rat study, administration of 20 µM/0.1 mM ASTX activated the Nrf2 – ARE pathway [75]. Masoudi et al. to examined the therapeutic potential of AST on adult rats after severe spinal cord injury. 10 µL/0,2 mM ASTX, ASTX reduced expression of Bax and Cleaved-caspase-3 proteins and increased expression of the Bcl-2 protein [74].

Fakhri et al. and Rahman et al. also found that ASTX lessened expression of TNF-α [76,77]. Amyloid plaques appear in pathogenesis of AD and have cytotoxic property. In a study, relation between ASTX and Bexratone was viewed by Fanaee-Danesh et al. [78]. Both two administrations had similar effect. By way of ASTX, Female 3xTg AD mice fed ASTX (80 mg/kg, for 6 days) had reduced Aβ oligomers [78]. All the mice studies mentioned in review are demonstrated in Table 2.

Table 2: Mice studies related with ASTX, their procedures and results.

Study type	Aim of study	Experimental procedure	Results	Reference
Swiss Albino Mice	to measure the levels of anti-oxidative enzymes in the prefrontal cortex (PFC), striatum (ST), hippocampus (HP), and liver (LV) in groups.	Group I: control (0,9% saline) Group II: scopolamine (0,5 mg/kg) Group III: scopolamine (0,5 mg/kg) and ASTX (2 mg/kg) Duration of study: 28 days	In comparison with group II, levels of nitric oxide (in PCF, ST, HP and LV), glutathione (in LV) were significantly lower and higher CAT (in ST and LV) and SOD (ST, HP and LV) in group III.	Al-Amin et al., 2019

Male Wistar Rats	to investigate the potential effects of ASTX to modulate sensory-motor and histopathological dysfunctions, through modifying the NR2B and phospho-p38MAPK (p-p38MAPK) signaling elements as well as TNF-α, in a severe compression model of spinal cord injury (SCI) in rats.	It was separated the rats into 3 groups: Sham, SCI (treated with 5% dimethyl sulfoxide) and SCI (treated with 10 µL of 0,2 mM). Duration of study: 28 days	In comparison to SCI (treated with 5% dimethyl sulfoxide), ASTX treatment significantly decreased expressions of p-p38MAPK on 14-, -21 and -28 day and TNF-α on 14-day.	Fakhri et al., 2018
Female Albino Rats	to search neuroprotective role of ASTX in rats with Alzheimer's disease (AD).	Rats were divided into 5 groups as: Group I: Sham, group II: Aβ(1-42) infused (4 µg/4 µL), group III: 0,5 mg/kg/day and Aβ(1-42) infused (4 µg/4 µL), group IV: 1 mg/kg/day Aβ(1-42) infused (4 µg/4 µL), group V: 1 mg/kg/day	Comparing to group II, both doses of ASTX given with Aβ(1-42) significantly decreased level of TNF-α after 28 days.	Rahman et al., 2019
Adult Rats	to examine whether pretreatment of astaxanthin can protect against ischemic injuries in the adult rats	5 and 10 mg/kg ASTX for seven days	Administration of ASTX supressed ROS	Pan et al., 2017
Female ICR Old Rats	to first determine effects of ASTX on the structural changes by oxidative stress in an aging mouse model	Administration of 1.7 mg/kg ASTX for 72 weeks	ASTX decreased oxidative stress and elevated antioxidant levels.	Kuraji et al., 2016
Male Db/Db Mice	to investigate the effects of astaxanthin on diabetic retinopathy in db/db mice with the reduction of oxidative stress.	Treatment with 25 and 50 mg/kg ASTX	Both of tese doses decreased levels of 8OHdG and malondialdehyde.	Dong et al., 2013
Vascular Cognitive Impairment Mice	to investigate the potential neuroprotective effect of the antioxidant astaxanthin (ATX) in the mice.	10 mg/kg ASTX for 28 days	While the concentration of malondialdehyde was decreased, the levels of glutathione and SOD were increased by ASTX.	Xue et al., 2017b
Sprague-Dawley Rats	to investigate the potential effects of astaxanthin on sepsis and multiple organ dysfunctions.	100 mg/kg ASTX for 7 days	SOD activity was increased by ASTX.	Zhou et al., 2015
Adult Rats	to examined the therapeutic potential of AST on adult rats after severe spinal cord injury.	10 µL/0,2 mM ASTX	ASTX reduced expression of Bax and Cleaved-caspase-3 proteins and increased expression of the Bcl-2 protein.	Masoudi et al., 2017
Old Swiss Albino Mice	to investigate the age-dependent and region-specific antioxidant effects of astaxanthin in mice brain	2 mg/kg/day ASTX	ASTX activated CAT and SOD and increased glutathione levels	Al-Amin et al., 2015
Wistar Rats	to evaluate whether orally administered ASTX protects against oxidative damage	0.6 mg/kg and 3 mg/kg ASTX for 8 weeks	Both doses lowered levels of 8OHdG and NF-kB.	Yeh et al., 2016
Male Sprague-Dawley Rats	to assess the effect of ASTX on the Nrf2-ARE pathway	20 µL/0.1 mM ASTX for 1 hour	It was activated Nrf2-ARE pathway.	Wu et al., 2014b
Swiss Albino Male Mice	to investigate effect of ASTX on oxidative stess incompliance with brain regions	Diets for 42 days (1)CON (control): – 200 ml distilled water was given.	Glutathione concentrations of frontal cortex, striatum and cerebellum decreased after administration. In addition to	Al-Amin et al., 2016

		(2)AlCl3: Aluminum chloride was given at a dose of 50 mg/kg body weight per day (3)AST_AlCl3 – astaxanthin at a dose of 20 mg/kg body weight together with aluminum chloride at a dose 50 mg/kg body weight was given (4)AST – only astaxanthin at a dose of 20 mg/kg body weight was given	this, malondialdehyde levels diminished in striatum, hypothalamus and hippocampus.	
Male Wistar Rats	to search ASTX and antioxidant capacity	Diets for 180 days Antioxidant compounds with amounts 0.2 g/kg of vitamin E, 0.2 g/kg of vitamin C and 0.6 g/kg of ASX HFD consists of 60.9% fat, 18.3% protein, and 20.3% carbohydrate,with a caloric density of approximately 5.24 kcal/g. A standard laboratory rodent chow diet (Lab Diet) was used for the control diet. This control diet has a caloric density of approximately 3.0 kcal/g.	ASTX administration enhanced total antioxidant capacity.	Komaki et al., 2015
Adult Wistar Male Rats	to analyse ASTX and antioxidant enzymes	For 45 days Four experimental groups of 16 animals each were formed: (i) control, fed with 400 µL of 10% Tween-80 aqueous solution (v/v); (ii) ASTA, fed with 1 mg ASTA/kg; (iii) FO (fed for 10 mg EPA/kg and 7 mg DHA/kg); (iv) ASTA/FO, fed with 1 mg ASTA/kg, 10 mg EPA/kg and 7 mg DHA/kg. Each fish oil (FO) capsule of 500 µL contains 9 kcal (38 kJ), 2.0 mg of mixed tocopherols, and 1.0 g of total fat, which 30% are from saturated fats, 20% from monounsaturated fats (mostly palmitoleic and oleic acids), and 50% of polyunsaturated fatty acids (180 mg EPA and 120 mg DHA). AstaREAL A1010 is an astaxanthin-rich natural Haematococcus pluvialis product that contains 5.2–5.8% of total carotenoids, whereas 5.0–5.6% are purely astaxanthin (3.9% as monoesters, 0.9% as diesters, and 0.1% in free form), 0.02% lutein/zeaxanthin, 0.02% adonirubin, 0.02% cantaxanthin, 0.02% β-carotene, and 0.1% others.	While only fish-oil (10 mg EPA/kg body weight and 7 mg DHA/kg body weight) decreased activities of SOD, CAT and glutathione reductase, ASTX administration (1 mg/kg body weight) after fish-oil improved these activities.	Mattei et al., 2011
Male Albino Ddy Mice	to seek effect of ASTX on enzymes	Mice were exposed to 8,000 lux of white fluorescent light for 3 h. Astaxanthin at 100 mg/kg was dissolved in olive oil just before use and was administered orally eight times (at 6 h before and at 0, 6, 12, 24, 36, 48, and 72 h after light irradiation).	After ASTX administration (100 mg/kg, at 6 h before and at 0, 6, 12, 24, 36, 48, and 72 h after light irradiation) to male albino ddY mice, there was no effect of ASTX on mRNA synthesis of Sod1, metallotionein-II and III.	Otsuka et al., 2013
Male Shrsp Rats	to asses ASTX and 8-OHdG	300 or 600 mg astaxanthin/kg every day, for 3 weeks	Both 300 and 600 mg/kg/day administration for 3 weeks of ASTX to male SHRSP rats decreased urinary 8-OHdG level.	Sasaki et al., 2011

Albino Wistar Rats	to research ASTX and antioxidant enzymes such as SOD and CAT levels.	100, 250 ve 500 mg/kg vücut ağırlığı ASTX, for 21 days	ASTX administration of 100, 250 and 500 mg/kg body weight to ulcerated albino Wistar rats promoted SOD and CAT levels.	Kamath et al., 2008
Wistar Male Rats	to asses relation ASTX and oxidative stress parameters	(a)control, fedwith olive oil for 30 days; (b)ASTA, fed with 20 mgASTA/kg BW for 30 days; (c)diabetic, fed initially with olive oil alone for 23 days, then treated with alloxan to induce diabetes, and finally with olive oil for extra 7 days (to complete 30 supplementation days); (d)diabetic+ASTA, fed initially with 20 mg ASTA/kg BW alone for 23 days, then treated with alloxan to induce diabetes and continued with 20 mg ASTA/kg BW for extra 7 days (to complete 30 supplementation days)	Superoxide anion levels in neutrophile of alloxane induced diabetic Wistar male rats fed ASTX (20 mg ASTA/kg of body weight/day, for 30 days) did not change, while there was a diminishment in TBARs levels. At the same time, this administration increased GPx level further.	Marin et al., 2011
Female 3xtg AD Mice	to investigate impact of ASTX on Aβ oligomers	In study I,<1-year-old (32–49 weeks old) female 3xTg AD mice were gavaged for 6 days with vehicle (10% DMSO in corn oil [v/v]; vehicle control group, n=10), Bex (100 mg/kg in DMSO/corn oil; n=9), or Asx (80 mg/kg in DMSO/corn oil; n=8) and compared to non-Tg mice (37–49 weeks; n=5 for vehicle control group; n=6 for Bex; n=7 for Asx). In study II, aged (68–92 weeks old) female 3xTg AD mice were gavaged for 6 days with vehicle (n=8), Bex (100 mg/kg; n=6), or Asx (80 mg/kg; n=8). Body weights were assessed before treatment, at day 4, and before sacrification.	(Results for study II) Both two administrations (ASTX and Bexratone) had similar effect. By way of ASTX, Female 3xTg AD mice fed ASTX (80mg/kg, for 6 days) had reduced Aβ oligomers.	Fanaee-Danesh et al., 2019
Male Sprague-Dawley Rats	to explore whether ATX treatment post SAH could activate the Nrf2-ARE pathway	Subarachnoid hemorrhage (SAH) group (n = 24); SAH + ATX group (n = 24); SAH + vehicle group (n = 24); and control group (n = 24). In the SAH + ATX group, ATX (20 μL of 0.1 mM dissolved in vehicle) was administrated at 30 min after SAH was induced.	Incubation of ASTX activated the Nrf2 – ARE pathway.	Wu et al., 2014b

Cell studies

Effects ASTX on oxidative stress: Yamagishi and Aihara administrated different doses of ASTX (1 nM, 10 nM and 100 nM) to rat retinal ganglion cells and found that the lifespan of cells increased, and the administration of 100 nM ASTX markedly prevented DNA damage and apoptosis [79]. In other study, it was aimed at determination of the effect of ASTX on the interplay of NRF2 and NFκB for its antiinflammatory and antioxidant properties in macrophages. In this study, treatment of macrophages cells with 25 μM ASTX for 24 hours reduced mRNA levels of IL-6, IL-1β and TNF-α [80]. In a culture study, a dose of 1.25-5 μM ASTX increased secretion of HO-1 and Nrf2 and decreased the release of caspase-3, -8 and -9 and lactate dehydrogenase. Antioxidant response components (ARE) also increased in this culture study [81].

Effects ASTX on reactive oxygen species: Another cell culture study that distinct ASTX doses (5 μM, 10 μM and 20 μM) for one hour found that production of intracellular reactive oxygen and cell death due to hydrogen peroxide were diminished [82]. Guerra et al. sought connection between glycolaldehyde and human neutrophils. Combine application of vitamin C (100 mM) and ASTX (2 mM) decreased output of NO and H_2O_2 in cells, unfavourable impacts of glycolaldehyde [83]. Whereas, mitochondrion contributes to energy production by redox, it also causes ROS to occur. Resultant ROS and ASTX were inspected by Wolf et al. [84]. PC12 cells incubated with 200 and 400 nM ASTX for 24 h were protected against cell death due to oxidative stress. However, 800 nM ASTX had a constructive effect on Jurkat cells. HeLa cell cultured with same dose for 6 h also provided a decrease in H_2O_2 levels [84]. In company with ASTX (25, 50, 100, 500 and 1000 nM, for 4 h and 24 h) cultured in human dopaminergic neuroblastoma SH-SY5Y cells,

it was provided a decrease in DHA-OOH levels. ROS production was protected by ASTX in these cells [85]. CDX-085 is a pro-drug, more soluable in water than pure ASTX form. Proinflammatory ONOO- formation was reduced in platelets from C57BL/6 rats fed 0,4% CDX-085 for 2 weeks that were treated with 1 μM ASTX [86].

Fatty acids subject to concentration and type alter leucocyte function. Campoio et al. searched effects of ASTX on excess of oxidative stress based on fatty acids in human peripheral blood lymphocytes. In contrast to adverse effects of fatty acids, ASTX implementation (2 μM of ASTA for 24 h) reduced cellular NO and H_2O_2 production. Beside this, increasing activities in SOD, CAT and GPx by fat was lowered by ASTX [87].

Effects ASTX on enzymes: In a study on cell culture done by Franceschelli et al., horary incubation of 10 μM ASTX also induced antioxidant enzyme activation (CAT and SOD) [88]. ASTX being one of the major xanthophylls indicates an inhibitor impress on CYP isoenzymes. Astaxanthin (from 0,05; 0,5 to 5 μM) implemented on human liver microsome had an weak effect on CYP2C19 and IC50 value was 16,2 μM [52,89].

All the cell studies mentioned in review are demonstrated in Table 3.

Table 3: Cell studies related with ASTX, their procedures and results.

Study type	Aim of study	Experimental procedure	Results	Reference
Rat retinal ganglion cells	To investigate whether ASTX confers a neuroprotective effect against glutamate stress, oxidative stress, and hypoxiainduced apoptotic or necrotic cell death in primary cultures of rat retinal ganglion cells	Treatment with 1 nM, 10 nM, and 100 nM ASTX	DNA damage and apoptosis decreased in 100 nM ASTX treatment.	Yamagishi and Aihara, 2014
HT22 cells	To investigate effects of ASTX on HT22 cells	1,25-5 μM ASTX	Astx increased antioxidant response components, levels of HO-1 and Nrf2 while it decreased caspase-3, -8 and -9 and lactate dehydrogenase.	Wen et al., 2015
U937 cells	To investigate the potential role protective of ASTX	10 μM ASTX for 1 hour	It was induced activations of CAT and SOD.	Franceschelli et al., 2014
ARPE-19 cells	To investigate the protective effect of AST on ARPE-19 cells against oxidative stress	5, 10 and 20 μM ASTX for one hour	It was reduced intracellular ROS and cell death.	Li et al., 2013
Murine RAW 264.7 macrophages	To determine determination of the effect of ASTX on the interplay of NRF2 and nfκb for its antiinflammatory and antioxidant properties in macrophages	25 μM ASTX for 24 hours	Treatment of macrophages cells with ASTX reduced mRNA levels of IL-6, IL-1β and TNF-α.	Farruggia et al., 2018
Human peripheral blood lymphocytes	To investigate effects of ASTX on excess of oxidative stress based on fatty acids in human peripheral blood lymphocytes	The cells were treated with 0.3 mM of the fatty acid mixture (the proportion of fatty acids was as follows: 1.74% lauric (C12:0), 5.2% myristic (C14:0), 31% palmitic (C16:0), 1.1% palmitoleic (C16:1), 41% stearic (C18:0), 4.6% oleic (C18:1), 9.6% linoleic (C18:2), 1.3% linolenic (C18:3), 3.2% arachidonic (C20:4), 0.45% eicosapentaenoic (C20:5), and 1.8% docosahexaenoic (C20:6) acids) added or not of 2 μM of ASTA solubilized in DMSO	ASTX implementation reduced cellular NO and H_2O_2 production. Beside this, increasing activities in SOD, CAT and GPx by fat was lowered by ASTX.	Campoio et al., 2011

		and cultured at 5% CO2 for up to 24 h at 37°C.		
Human Liver Microsome	To investigate the revers-ible inhibitory or time-dependent inhibitory effects of AS on nine CYP isoforms, including CYP1A2, CYP2A6,CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, andcyp3a4/5, in human liver microsomes.	0,05, 0,5 or 5 μM ASTX	Astaxanthin implemented on human liver microsome had an weak effect on CYP2C19 and IC50 value was 16,2 μM.	Zheng et al., 2013
Human Neutrophils	To seek connection between glycolaldehyde and human neutrophils	Glycolaldehyde (1 mM) followed or not by the antioxidants astaxanthin (2 mM) and vitamin C (100 mM) for 24 h at 37°C.	Combine application of vitamin C (100 mM) and ASTX (2 mM) decreased output of NO and H_2O_2 in cells, unfavourable impacts of glycolaldehyde.	Guerra et al., 2012
HeLa human cervical cancer cells, PC12 cells, Jurkat cells	To investigate effects of ASTX on mitochondrial redox	HeLa cells were cultured in DMEM/F12+10% FBS with or without 800 nM AX (control: DMSO) for 6 h, 1 day and 2 days, then exposed to 30 μg/ml antimycin A for 15 min, then 250 nM MitoSOX Red (Invitrogen) and 500 nM Hoechst34580 (Invitrogen) added and incubated for 60 min, and fluorescence recorded using a 20 × (N.A. 0.5) objective. Astaxanthin effect on PC12 cell survival under oxidative stress. Cells were cultured in the presence of 0, 100, 200 and 400 nM AX for 6 h or 24 h. Jurkat cells cultivated in the presence or absence (DMSO) of AX (800 nM) for 6 h, 1 day, and 2 days were treated with antimycin A (30 μg/ml).	PC12 cells incubated with 200 and 400 nM ASTX for 24 h were protected against cell death due to oxidative stress. However, 800 nM ASTX had a constructive effect on Jurkat cells. HeLa cell cultured with same dose for 6 h also provided a decrease in H_2O_2 levels.	Wolf et al., 2010
Human dopaminergic neuroblastoma SH-SY5Y cells	To investigate the effect and the mechanism of astaxanthin on reactive oxygen species (ROS)-mediated apoptosis in dopaminergic SH-SY5Y cells	Human dopaminergic neuroblastoma SH-SY5Y cells were DHAOOH- or 6-OHDA-treated cells. Different concentrations (25, 50, 100, 500, 1000 nM) of astaxanthin were added to the cells for 4 h and 24 h	It was provided a decrease in DHA-OOH levels. ROS production was protected by ASTX in these cells.	Liu et al., 2009
Platelets from C57BL/6 rats	To search effects of pro-drug (CDX-085) on rat platelets	C57BL/6 rats fed 0,4% CDX-085 for 2 weeks that were treated with 1 μM ASTX.	Proinflammatory ONOO- formation was reduced in platelets from C57BL/6 rats fed 0,4% CDX-085 for 2 weeks that were treated with 1 μM ASTX.	Khan et al., 2010

CONCLUSION

Reactive oxygen species emergent through senescence further expedite the aging process. Several studies that investigated effects of ASTX on oxidative stress suggested that ASTX inhibits oxygen species by impacting enzymes and their pathways. In addition to this, ASTX enhances activation of the antioxidant defence system, which decreases with age. Most of studies on rats and limited availability of human studies cause to be restricted proposal usage of ASTX as diet supplement. Besides this situation, indicated classification diary amounts of ASTX by age may put some teeth into safe intake of ASTX.

Effect and important keystone is also confidential doses of ASTX for both rats and human. It should be paid regard to determine these doses for especially human from the viewpoint of gender, age, health or existence of other diseases, used drugs, genotype and their biochemical values.

Senescence is an inexpugnable sooth. Despite this, reasons leading to it may be intercepted and aging may be postponed by antioxidants, nutritional behaviors and studies. Some cell types used in searches, such as microsome, may not represent real physiology. Of other cruces, procedurs of ASTX and researches pertain to perfect physiology should be done more.

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