Asian Journal of Pharmacognosy

Review

Carotenoids and macular pigments

Sherena PA[a,*], Annamala PT[b], Mukkadan JK[c]

[a] *Department of Biochemistry, Little Flower Medical Research Center,Angamaly, Kerala.*

[b] *Professor & Head, Department of Biochemistry, Jubilee Mission Medical College & RI, Angamaly, Kerala.*

[c] *Research Director, Little Flower Medical Research Center, Angamaly, Kerala.*

* Sherena P A: sherenapa@gmail.com

Abstract

Carotenoids are important factors in human health. The essential role of beta-carotene and others as the main dietary source of vitamin A and the protective effects of carotenoids against serious disorders such as cancer, heart disease and degenerative eye disease have been recognized .Stimulated intensive research have been carried out into the role of carotenoids as antioxidants and as regulators of the immune response system. The macular pigment of the eye is composed primarily of three xanthophyll pigments, namely (R,R)-lutein, (R,R)-zeaxanthin and (R,S)-zeaxanthin in the order 36, 18 and 18% of the total carotenoid content of the retina along with the remaining 20% consisting of minor carotenoids like oxo-lutein, epi-lutein and ε.ε-carotene- 3,3'-dione. In this review the dietary sources, naming of carotenoids and its isomers were described. Moreover, antoxidant potential of carotenoids, the macular pigments and its transport, bioavailability and the role in eye health were addressed. Evidences suggested that lutein consumption is inversely related to age-related macular degeneration (AMD) and

cataract. This was supported by the finding that the diet rich in lutein along with its isomer zeaxanthin which are deposited in the lens and the macula lutea would lower the risk of AMD.

Keywords: carotenoids, cis-trans isomers, bioavailability, macular pigment, eye health, lutein, zeaxanthin, retinitis pigmentosa, cataract.

Carotenoids represent as one of the most widely distributed and structurally diverse classes of natural pigments, with important functions in photosynthesis, nutrition, and protection against photo oxidative damage. Carotenoids are a class of more than 600 naturally occurring pigments synthesized by plants, algae, and photosynthetic bacteria. These richly coloured molecules are the sources of the yellow, orange, and red colours of many plants (Britton et al., 2008). Carotenoids derive their name from the work of Wachenroder who isolated β-carotene from carrots in 1831 giving the extracted crystals the name 'carotene'. Berzelius (1837) extracted yellow polar colour from autumn leaves and gave the name 'xanthophyll's'. Later Richard Willstatter (1907) established the empirical formula of carotenoids (C40) and Tswett (1911) separated many pigments using advance chromatographic techniques, which he collectively called as 'carotenoids'. The first effort at chemical identification of the lutein and zeaxanthin in the human eye was reported by Wald (1949). These carotenoids form the 'macular pigments' in the central region of the retina referred as macula lutea and fovea centralis. Bone et al. (1985) had published that the macular pigment is a mixture of lutein and zeaxanthin. Fruits and vegetables provide most of the carotenoids in the human diet. Alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, lycopene, and zeaxanthin are the most common dietary carotenoids. There are about 20 carotenoids in the human diet. Their presence in leafy vegetables is masked by the green of chlorophyll red and purple of anthocyanin, but in other foodstuffs carotenoids are more evident, contributing to their red, yellow and orange colours. Carrots are the major source of β-carotene, although spinach, broccoli and watercress also contain substantial amounts. The predominant dietary source of lycopene is tomatoes. Good dietary sources of lutein include peas, sprouts, greens, broccoli, spinach and peppers. Mangoes, apricots and oranges are sources of cryptoxanthin and also contain some amounts β-carotene (Khachik et al., 1986; Briethuapt & Bamedi, 2001). Structurally, the carotenoids possess end-groups that are either acyclic or a ring of five or six carbons at one or both ends of the molecule (Goodwin, 1980). Carotenes are named according to the end group(s) that they contain in their structures. The conjugated double bonds present in the structures of carotenoids give them the possibility to exist as several "*cis-trans"* geometrical isomers (Zechmeister, 1962). The cis and *trans* to indicate the orientation of substituent groups in a molecule relative to a double bond. Generally, *E* and *Z* are the designations used to describe this geometrical isomerism. In nature, most carotenoids are found in the *all-E* (*trans*) configuration. Studies reported that other than β-carotene *cis-trans* isomerization was observed in all carotenoids. Isomerization from *trans* to *cis* often occurs during chemical reactions. Heat and light being the prominent factors that affects the isomerisation of the carotenoids. Geometrical isomerization leads to a change of shape in the carotenoid molecule. The relative stability of an all-*trans* (*E*) carotenoid is greater than that of *cis* isomers, because of the better electron delocalization and the more planar nature of the chromophore configuration. The Zechmeister's study showed that, in an all-*trans* carotenoid solution, the carotenoid undergoes isomerization continually, first to mono *cis*, next to di *cis*- and during that process the structure star bending until a poly *cis* solution is formed. The thermo stability of the carotenoid solution first decreases during the isomerization, and during the process it gets to a point where the molecule is completely bent, because of its poly *cis* configuration. The c*is* carotenoids are photo chemically sensitive in the presence of iodine. Studies had shown that the all-*trans* isomer has the highest melting point of all isomers. By comparison the *cis* conformers melt at lower temperatures. The spectral characteristics of all-*trans* carotenoids and mixtures of *cis-trans* carotenoids were analysed by Zechmeister and Polgar (Zechmeister & Polgar, 1994). There are carotenoids that have been identified in nature only as *Z* isomers. Bixin was the first *cis*-polyene to be recognized in nature, isolated from annatto seeds (*Bixa orellana*). Pro-lycopene (7Z, 9Z, 7'Z, 9'Z-tetra*cis*-lycopene) was first detected to exist in the tangerine tomato (in 1940) and had an orange colour instead of the all-*E*-lycopene that is

found in most tomatoes Isomerization produces a significant difference in molecular structures that can affect the bioavailability of carotenoids. The *Z* isomers have a greater solubility in mixed micelles and are less likely to aggregate or crystallize; as a result they are more easily become incorporated into bile acid micelles and also increasing the efficiency of their transport to tissues. In fruits and vegetables β-carotene is found in the all-*E* form and only small amounts of the *Z* isomers are detected. An *in vitro* model of digestion showed that the incorporation of *Z* isomers of β-carotene into micelles were 2-3 times more efficient than the *E* form (Khachik et al., 1992). Lutein and zeaxanthin have been found in their *cis* isomers form as (*13 Z*) - and (*13'Z*) - lutein isomers; (*9Z)* - and (*9'Z*) - lutein; and *(13-Z)* - zeaxanthin in processed fruits, vegetables and pasta food products. Carotenoids are pigments which play a major role in the protection of plants against photo oxidative processes. They are efficient antioxidants scavenging singlet molecular oxygen and peroxyl radicals. In Humans, carotenoids are part of the antioxidant defence system. They interact synergistically with other antioxidants, mixtures of carotenoids which are more effective than single compounds (Niki et al., 1995). According to their structure most carotenoids exhibit absorption maxima at around 450 nm. Filtering of blue light has been proposed as a mechanism protecting the macula lutea, against photo oxidative damage. There is increasing evidence from human studies that carotenoids protect the skin against photo oxidative damage. The preventive effects have been associated with their antioxidant activity, protecting cells and tissues from oxidative damage. Lutein and zeaxanthin are powerful antioxidants with ability to quench peroxy-radicals-thus protecting against tumor formation and exerting an anti-mutagenic effect. Oxygenated carotenoids, including lutein, which have little or no pro-vitamin A activity, have been recognized to have antioxidant activities (Krinsky N.I, 1993). Singlet Oxygen generated in biological systems and is capable of damaging proteins, lipids and DNA: Lutein and zeaxanthin absorb visible light and play a role in singlet–singlet energy transfer and the quenching of singlet oxygen (Krinsky & Rock, 1999; Di Mascio et al 1990) . This ability may contribute to the protection of light-exposed tissue, skin and eyes, from light-induced damage (Sies H & Stahl W,2003).The ability of the lipid-soluble carotenoids to quench singlet molecular oxygen may explain some anticancer properties of the carotenoids, independent of their provitamin A activity (Di Mascio et al ,.1991). The effectiveness of carotenoids as antioxidants is also dependent upon their interaction with other co antioxidants, especially vitamins E and C. Lutein was found to scavenge superoxide radicals, hydroxyl radicals and inhibited in vitro lipid peroxidation. The oral administration of lutein in mice for 1 month significantly increased the activity of catalase, superoxide dismutase, glutathione reductase and glutathione in blood and liver while the activity of glutathione peroxidase and glutathione-S-transferase were found to be increased in the liver tissue (Edakkadath R Sindhu et al., 2010). Among the various defense strategies, carotenoids are most likely involved in the scavenging of two of the reactive oxygen species, singlet molecular oxygen, and peroxyl radicals. Further, they are effective deactivators of electronically excited sensitizer molecules which are involved in the generation of radicals and singlet oxygen (Young & Lowe, 2001). The macular pigment of the eye is composed primarily of three xanthophylls pigments, namely (R,R)-lutein, (R,R)-zeaxanthin and (R,S)-zeaxanthin in the order 36, 18 and 18% of the total carotenoid content of the retina along with the remaining 20% consisting of minor carotenoids like oxo-lutein, epi-lutein and ε, ε-carotene - 3,3'-dione (Landrum et al.,1999). Lutein and zeaxanthin are two yellow carotenoids that give the macular area of the retina yellow colour. The reason they are known as macular pigment carotenoids is that they are selectively accumulated in in the macula and its centre, fovea, and lutein distributed in other parts of retina and lens. The concentration of the macular pigment in the central retina approaches 1 mM, more than 1000 times that in human serum and liver (Landrum & Bone, 2001).The difference between these two molecules that compose the macular pigment is the location of one double bond in one of the end-groups and also the configuration of the hydroxyl group in that particular ring. Bone and Landrum showed that the major components of the macular pigment were lutein [(3R,3'R,6'R)- β,ε-Carotene-3,3'-diol], zeaxanthin [(3R,3'R)- β,β-Carotene-3,3'-diol] and meso-zeaxanthin [(3R,3'S)-β,β-Carotene-3,3'-diol]. Neither humans nor animals can synthesize carotenoids, so they must obtain them exclusively from their diet. Green leafy vegetables, fruits, and egg yolk are the primary sources of lutein and zeaxanthin available in nature. Khachik et al. have reported that the macular pigment consist of oxidation products of both lutein and zeaxanthin, such as 3'-epilutein and 3-hydroxy-ß,ε -caroten-3'-one, as well as geometric isomers of the major pigments . He also reported the presence of cis-isomers in the retina, since the macula is exposed to bright light, which is known to isomerize carotenoids. The presence of oxidative metabolites suggests that the

pigments are susceptible to oxidation in the tissue, or that an active metabolic process takes place (Khachick et al., 1997). The percent of lutein, zeaxanthin, and meso-zeaxanthin in the retina is approximately 36%, 18%, and 18% respectively of the total carotenoids (Landrum & Bone, 2001). These carotenoids are concentrated in the fovea, which is the depression located in the retina coinciding with the position of maximum visual acuity.Lutein and zeaxanthin can be characterized by their UV-visible spectra. The spectra may seem very similar but the presence of two β-ionone rings at the ends of the zeaxanthin structure extend the conjugation of the polyene chain and lower the energy separation between the ground and excited state, shifting its spectrum slightly to the red. Lutein absorbs at a slightly shorter wavelength than zeaxanthin (Alves-Rodrigues & Shao, 2004). On the basis of the spectra it can be stated that both carotenoids can filter blue light effectively. The retina is susceptible to oxidative stress because of the high demands for oxygen and its constant exposure to light. A principal function of lutein and zeaxanthin as macular pigments appears to be the protection of the retina against photo-induced damage by acting as antioxidants in addition to their role as a filter for blue light. In 1945, George Wald (1906–1997) observed that the macular pigment in humans had the same absorption spectrum as crystalline leaf xanthophyll. The pigment when extracted from human macula yielded a yellow hydroxy-carotenoid that Wald believed was lutein or leaf xanthophyll itself (Wald, 1945).While a number of studies had identified significant levels of Lutein and Zeaxanthin in different parts of the eye such as the photoreceptor rod outer segments of the peripheral retina (Sommerberg etal., 1999), specific binding sites of the retinal microtubules (Crabtree et al., 2001), in the iris, ciliary body, retinal pigment epithelium and choroid at high concentrations (Bernstein et al., 2001). The most significant presence of Lutein and Zeaxanthin is in the macular region of the retina (Bone et al., 1985) .Almost 36% of the total carotenoid content of the eye is composed of Lutein, while Zeaxanthin occurs in two isomeric forms- 3R, 3′R-zeaxanthin and *meso*-zeaxanthin-each of which represents app. 18% of the total carotenoid (Bone et al., 1988). Lutein esters have also been reported to be present in small quantities in the human skin and serum (Granado et al. 1998). The absorption of lutein and zeaxanthin occurs in the intestinal absorptive cells of the intestinal mucosa. They enter the hepatic portal circulation in the form of chylomicrons (Zaripheh & Erdman, 2002). In the liver, low and high density lipoproteins (LDL, HDL) are synthesized and transport lutein and zeaxanthin to other tissues. It has been demonstrated that HDL is the primary carrier of lutein and zeaxanthin and that LDL is principally responsible for the transportation of carotenes (Shannon Carpentier & Miyoung Suh, 2009). Dietary lutein may be metabolized to produce meso-zeaxanthin within the retina (Bone et al., 1993). A clear pathway of how these carotenoids are transported to the retina is still not well-defined (Li et al., 2010). The general pathway for absorption and metabolism of carotenoids in animal models , after the food matrix is digested, carotenoids will be combined with lipids and bile salts to form micelles. The micelles will move to the intestinal brush border membrane, and carotenoids will be captured and transported into the enterocyte to be metabolized and secreted into the intestinal lumen (cavity where the nutrients are absorbed) where they will be incorporated to chylomicrons and secreted into the lymph. Chylomicrons take the carotenoids to the liver where they can be processed by the liver and stored there or resecreted in VLDL (very low-low density-lipoprotein) and then to the bloodstream in the form of LDL and HDL (Lee et al, 1999). The bioavailability of a carotenoid is considered as the fraction of ingested carotenoids available at the site of action (serum) used for physiological functions or storage. The study aims to estimate the absolute systemic availability of the active substance from the dosage form. Both lutein ester and lutein (free) occur naturally in foods. Lutein ester and lutein are chemically distinct compounds with different physicochemical properties. Lutein esters are typically di ester forms with two fatty acid groups present in the sites of hydroxyl groups of lutein. These fatty acids have to be cleaved off by certain enzymes in the body to obtain lutein in free form. This is confirmed by the fact that only free lutein is observed despite administering lutein ester containing food or supplement.The bioavailability involves the steps such as release of the carotenoid from the carotenoid containing food matrix, incorporation into mixed micelles which consist of bile acids, free fatty acids, mono glycerides and phospholipids. The amount of carotenoid into micelles depends on the polarity of the carotenoid and micellar fatty acid composition and saturation. This requires the presence of dietary fat in the small intestine which stimulates the gall bladder to release bile acids (emulsifiers). Carotenoids are absorbed by intestinal mucosa of the small intestine (mainly duodenum) via passive diffusion (Parker, 1996; Yeum & Russel, 2002). The next step was incorporation into chylomicrons secreted into lymph by delivering to the blood stream through the action of lipoprotein lipase. Lutein

and zeaxanthin are evenly distributed between highdensity lipoprotein (HDL) and low density lipoprotein (LDL) in fasting blood (Wang et al., 2001). Chylomicron levels of xanthophyll's increase early and attaining peak at approximately 2 h after ingestion while peak blood concentrations at about 16 h post-ingestion (Yao et al.,2000) . The blood xanthophyll's level may vary considerably across individuals and population. Lutein and zeaxanthin are major carotenoids in the blood and account for 53 % of the total blood carotenoids in Asians compared with 23 % Americans (Simpson & Urudha-Rojas, 2007). This may be attributed to the consumption pattern of xanthophyll carotenoids in these sample populations. Vision occurs when light reflected from the object passes through the lens and falls on the photoreceptive cells of the retina wherein the captured light is converted into electrical impulses/signals (colour, brightness, etc.) which travel along the optic nerve to the brain. Finally, the brain converts the signals into images that make up human eye sight. In the process of vision perception, macula comprising a yellow pigment, located in the central of the retina, directly behind the lens, is responsible for sharp vision needed to read and see objects clearly. The xanthophyll's comprise of lutein, zeaxanthin and meso-zeaxanthin in high concentrations in the macula and its centre, fovea, and lutein distributed in other parts of retina and lens. Meso-zeaxanthin, not derived from normal diet, is produced from lutein either photo chemically or enzymatically, with in the retinal tissue. Lutein and zeaxanthin are essential dietary constituents and lack of these pigments in the diet may lead to low vision, retinitis pigmentosa, cataract, and premature loss of eye sight leading to macular degeneration (Landrum & Bone, 2001). Retinitis pigmentosa (RP) is the name for a group of eye diseases. RP causes the thin layer of tissue in the back of the eye, which is called the retina, to deteriorate. RP diseases are genetic and are passed down from one or both parents. RP damages the cells in the retina that sense light. These cells are known as rods and cones. The rods are associated with side vision and night vision. Clear central vision and colour vision are associated with the cones. RP mutates the genes of the rod cells and they slowly stop working. As the rod cells stop working, peripheral vision is slowly lost until you can only see a small tunnel of vision straight ahead. RP can cause serious vision loss. Studies strongly associate high dietary intake of Lutein with the possibility of slowing down degenerative eye diseases like retinitis pigmentosa (Dagnelie et al., 2000). Cataract is a cloudiness or opacity in the normally transparent crystalline lens of the eye. The cloudiness can cause a decrease in vision and may lead finally to blindness. The human lens like the retina accumulates the xanthophyll's, lutein and zeaxanthin extensively (Spector A et al. 1981). The lens is composed of approximately 35 % protein and 65 % water. The opacity is by precipitation of oxidative damaged proteins in lens by effect of ultraviolet light and smoke. Higher levels of hydrogen peroxide have been found in cataract affected lenses compared to normal healthy lenses, indicating oxidative stress (Tavani et al., 1996). Diet plays an important role in cataract risk. The focusing lens of the eye receives nutrients indirectly via the aqueous fluid rather than blood stream. In order to maintain lens transparency and clarity, the protective antioxidant levels in the aqueous fluid of the eye must be higher than in the blood plasma (Hankinson et al. 1992). In a prospective study, it was inferred that consumption of lutein-rich vegetables like spinach on long term was inversely related to cataract extraction (Mares-Perlman et al., 1995). The later prospective studies also supported the earlier observation that intake of lutein and zeaxanthin had inverse association with cataract extraction (Chasan-Taber et al., 1999; Lyle et al., 1999; Jacques et al., 2011). A relationship was proposed between the usual nutritive intake and subsequently diagnosed age related nuclear opacities. A long term study showed evidence that antioxidants nutrient such as lutein and zeaxanthin play a significant role in the prevention of the oxidation of lens proteins and the formation of cataract (Karippi et al. 2012). Age-Related Macular Degeneration is a disease that affects the central visual acuity. It is associated with age and is the leading cause of blindness in people over 65 years old (Beatty et al., 1999). Age-Related Macular Degeneration is associated with abnormalities in the retina, degeneration of the retinyl pigment epithelium (RPE), and ultimately loss of the photoreceptors. AMD is a degradation of the central portion of the retina including the macula. AMD is classified into dry AMD (atrophic) wherein there is a white / yellow deposit of fatty protein "Drusen" below the retina causing breakdown, thinning the tissues of the macula and leading to gradual vision loss. The other is wet AMD (neo-vascular / exudative) wherein abnormal blood vessels under the retina begin to grow towards the macula, resulting in rapid loss of central vision(90% vision loss).The main risk factors for AMD are age and sunlight exposure and in addition smoke and nutritional status (Cai et al.,2000). Advanced AMD often leads to irreversible blindness and there is no cure for this (Fine et al., 2000). AMD is a nutrition disease. Based on epidemiological data, from studies of diets rich in these

carotenoids reduce the risk of AMD. Therefore, diet is the only source for macular pigments in the eye and could reduce the risk of developing AMD. Macular pigment optical density is a measurement of the attenuation of blue light by macular pigment and an indication of lutein and zeaxanthin concentrations in the macula. Macular Pigment Density which is directly proportional to the selective accumulation of lutein and zeaxanthin in human and primate retinas (Khachick et al., 1997). In an observational study, a comparison of macular pigment optical density, measured by Resonance Raman spectroscopy showed higher macular pigment densities relative to those not consuming the supplement (Bernstein et al. 2002). Further, AMD subjects who did not consume regularly lutein or zeaxanthin supplements had 32% lower macular carotenoid concentrations than age-matched controls (Richer et al., 2004). In the publications, it had been pointed out that if a deficiency in MPOD is accurately diagnosed by effective intervention, the MPOD can be raised (Richer et al., 2007). Low levels of macular pigments appear to be a risk factor for AMD (Landrum et al., 1997). Since then several studies had provided a growing body of evidence specific to the beneficial role of lutein and zeaxanthin intake in MPOD and their positive effect on eye health and AMD risk reduction. Studies suggest that supplementation of lutein and zeaxanthin may be associated with a decreased risk of AMD. One study found that individuals who ate the largest amounts of food rich in these carotenoids had a 43% lower risk of developing the disease (Seddon J M et al.,1994). In a study of different sources of xanthophylls, three groups of subjects were selected and fed with low vegetable diet, a high vegetable diet (spinach broccoli) and a carotenoid supplement . The case of serum derived from the carotenoid supplement , the lutein level was very much higher (0.64 micromol/L) demonstrating the superior bioavailability of supplement though the spinach had the same equivalent dose of lutein (Van het Hof et al, 1999). The bioavailability of lutein from lutein-fortified fermented milk was studied using in vivo and in vitro approaches. The study showed that fermented milk is a good carrier of lutein esters and also food based approach helps to improve lutein status among subjects (Granado-Lorencio et al., 2010). A study conducted on the comparative bioavailability of lutein esters with that of crystalline lutein in humans providing high doses of the supplements showed the bioavailability of the two is the same (Herbst & Bowen, 1997) . Another study demonstrated that patients taking a lutein oral supplement had significantly increased macular pigment density (Bone R A et al., 1997). Carotenoids as phytonutrients and nature's most wide spread pigments have received substantial attention. Carotenoid structures and their isomeric forms are important factor for their physiochemical properties, bioavailability and biological activities. Around 600 natural carotenoids were reported to be present and most of these are having antioxidant properties. From the studies of the carotenoids it is evident that their antioxidant activity varied depending on the *in vitro* systems used. From the published data there is no mention about the recommended dosage levels, but the data revealed that we should get the sufficient supply of carotenoids by eating fruits and vegetables. In addition there is considerable interest in the health promoting properties of carotenoid. Epidemiological studies demonstrated the protective association of dietary intake of vegetables and fruits against chronic diseases. There are studies support that the supplementation of lutein and zeaxanthin decrease the risk of AMD. The carotenoids of natural origin are considered as an important class of nutraceuticals.

Declaration of conflict of interest

Conflict of Interest declared none

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