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REVIEW ARTICLE

Carotenoids: biochemistry, pharmacology and treatment

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Carotenoids and retinoids have several similar biological activities such as antioxidant properties, the inhibition of malignant tumour growth and the induction of apoptosis. Supplementation with carotenoids can affect cell growth and modulate gene expression and immune responses. Epidemiological studies have shown a correlation between a high carotenoid intake in the diet with a reduced risk of breast, cervical, ovarian, colorectal cancers, and cardiovascular and eye diseases. Cancer chemoprevention by dietary carotenoids involves several mechanisms, including effects on gap junctional intercellular communication, growth factor signalling, cell cycle progression, differentiation-related proteins, retinoid-like receptors, antioxidant response element, nuclear receptors, AP-1 transcriptional complex, the Wnt/β-catenin pathway and inflammatory cytokines. Moreover, carotenoids can stimulate the proliferation of B- and T-lymphocytes, the activity of macrophages and cytotoxic T-cells, effector T-cell function and the production of cytokines. Recently, the beneficial effects of carotenoid-rich vegetables and fruits in health and in decreasing the risk of certain diseases has been attributed to the major carotenoids, β-carotene, lycopene, lutein, zeaxanthin, crocin (/crocetin) and curcumin, due to their antioxidant effects. It is thought that carotenoids act in a time- and dose-dependent manner. In this review, we briefly describe the biological and immunological activities of the main carotenoids used for the treatment of various diseases and their possible mechanisms of action.

LINKED ARTICLES

This article is part of a themed section on Principles of Pharmacological Research of Nutraceuticals. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.11/issuetoc

Abbreviations

ALT, alanine transaminase; AST, aspartate transaminase; ASTA, astaxanthin; Aβ, amyloid-β; CAM, cell adhesion molecules; H_2O_2, hydrogen peroxide; iNOS, inducible NOS; MCP-1, monocyte chemoattractant protein-1; MHC II, major histocompatibility complex class II; MMP, mitochondrial membrane potential; PBMCs, peripheral blood mononuclear cells; PP, Peyer's patch; PTP, protein tyrosine phosphatase; RA, retinoic acid; TLR, toll-like receptor; TG, triglyceride

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Tables of Links

TARGETS	
Other protein targets[a]	**Catalytic receptors**[c]
Bcl-2	IFN-α receptor
Bcl-xL	IGF1R
IL-1β	TLR4
TNF-α	**Enzymes**[d]
Nuclear hormone receptors[b]	AMPK
RARα	CDK2
	COX-2
	iNOS
	MMP9

LIGANDS	
cAMP	LDL (LRP4)
IL-1α	LPS
IL-2	MCP-1 (CCL2)
IL-4	NADPH
IL-6	PGE_2
IL-12	TGFβ1

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d]Alexander *et al.*, 2015a,b,c,d).

Introduction

Carotenoids are colourful liposoluble pigments. They are found in plants, fungi, bacteria and algae and are present in many foods, for example, fruit, vegetables and fish (El-Agamey *et al.*, 2004; Tapiero *et al.*, 2004). There are more than 600 carotenoids with natural structural variants which are divided into carotenes, xanthophylls and lycopene (Jomova and Valko, 2013; Rutz *et al.*, 2016). Only ~40 carotenoids are present in a typical human diet and about 20 carotenoids have been identified in human blood and tissues. These carotenoids in the diet and human body include β-carotene, α-carotene, lycopene, lutein and cryptoxanthin (Rao and Rao, 2007). Carotenoids belong to the tetraterpenes family (C_{40}-based isoprenoid), responsible for the yellow, orange or red colour of fruits, leaves and flowers (Kaulmann and Bohn, 2014; Tapiero *et al.*, 2004). For example, (a) the green vegetables contain high amounts of both hydrocarbon carotenes and xanthophylls; (b) lycopene is a lipophilic red pigment present in ripe tomatoes; (c) the orange colour of carrots is caused by β-carotene; (d) capsanthine is responsible for the brilliant red pigment of peppers; and (e) the pink/red coloration of crustaceans is due to astaxanthin (ASTA) (Astorg, 1997). Most of the carotenoids are composed of a central carbon chain of alternating single and double bonds and carry various cyclic or acyclic end groups (Stahl and Sies, 2005; Figure 1). Epidemiological studies indicated that the use of diets rich in carotenoids is related to a lower incidence of cancer, cardiovascular diseases (CVDs), osteoporosis, diabetes, age-related macular degeneration (AMD), cataract and also infectious diseases such as HIV infections (Rao and Rao, 2007; Pechinskii and Kuregyan, 2014; Saini *et al.*, 2015). HIV patients usually have low plasma concentrations of carotenoids, because all carotenoids (e.g. lutein, cryptoxanthin, lycopene, β-carotene and α-carotene) are significantly destroyed in these patients and this is directly related to an increased risk of death. Both $CD4^+$ and $CD8^+$ lymphocytes can be increased significantly in HIV patients by the administration of 60 mg·day^{-1} of β-carotene and the symptoms of the disease decrease over 24–36 months (Pechinskii and Kuregyan, 2014). In general, a number of biological actions of carotenoids have been demonstrated including antioxidant activity, immune enhancement, regression of malignant lesions and inhibition of mutagenesis (Rao and Rao, 2007). In this review, we briefly describe the biological activities of the main carotenoids used for the treatment of various diseases. Figure 2 shows the major functions and mechanisms of action of carotenoids.

Chemistry of carotenoids

The main carotenoids include lycopene, β-carotene, ASTA, lutein and zeaxanthin (Tapiero *et al.*, 2004). Carotenoids are synthesized by the linkage of two C_{20} geranylgeranyldiphosphate molecules. All carotenoids contain a polyisoprenoid structure, a long conjugated chain of double bond and a near bilateral symmetry around the central double bond (Saini *et al.*, 2015). Carotenoids can be divided into provitamin A (e.g. β-carotene, α-carotene and β-cryptoxanthin) and non-provitamin A compounds (Stahl and Sies, 2005). On the other hand, carotenoids can be classified based on their functional groups as follows: (a) xanthophylls (e.g. lutein, zeaxanthin) containing oxygen as functional group, and (b) carotenes (e.g. α-carotene, β-carotene and lycopene) containing only a parent hydrocarbon chain without any functional group (Saini *et al.*, 2015). Other compounds such as apocarotenoids (e.g. retinoids, vitamin A, β-ionone and α-ionone aromatic volatile compounds) are also derived from carotenoids by oxidative cleavage using carotenoid cleavage dioxygenases (Saini *et al.*, 2015). Generally, carotenoids are hydrophobic molecules with very low solubility in water that act in hydrophobic areas of the cell. Polar functional groups attached to the polyene chain can change the polarity of carotenoids, which may influence their localization within biological membranes and their interactions with different molecules (Jomova and Valko, 2013). Figure 1 shows the structures of the main carotenoids.

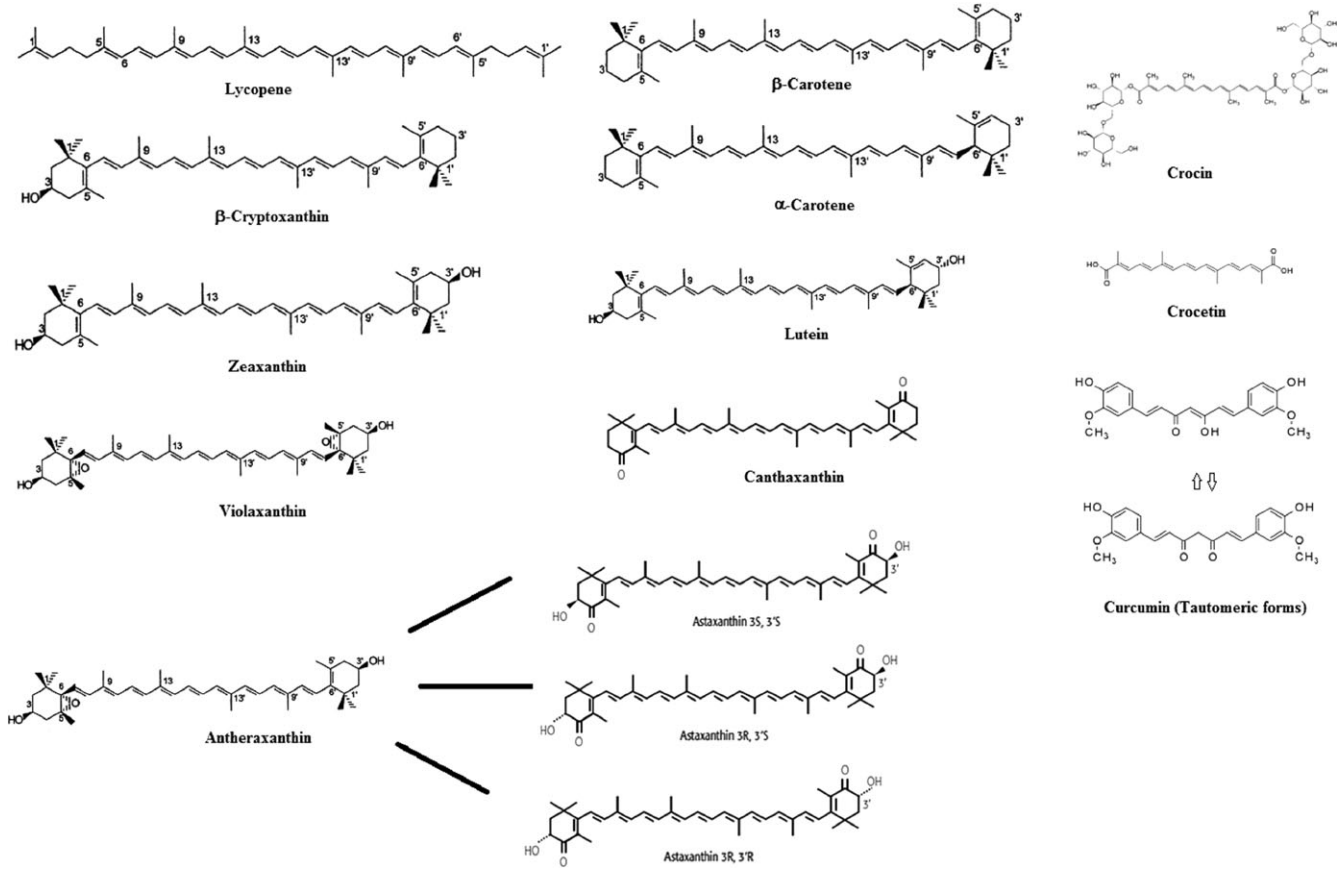

Figure 1

The structures of the major carotenoids.

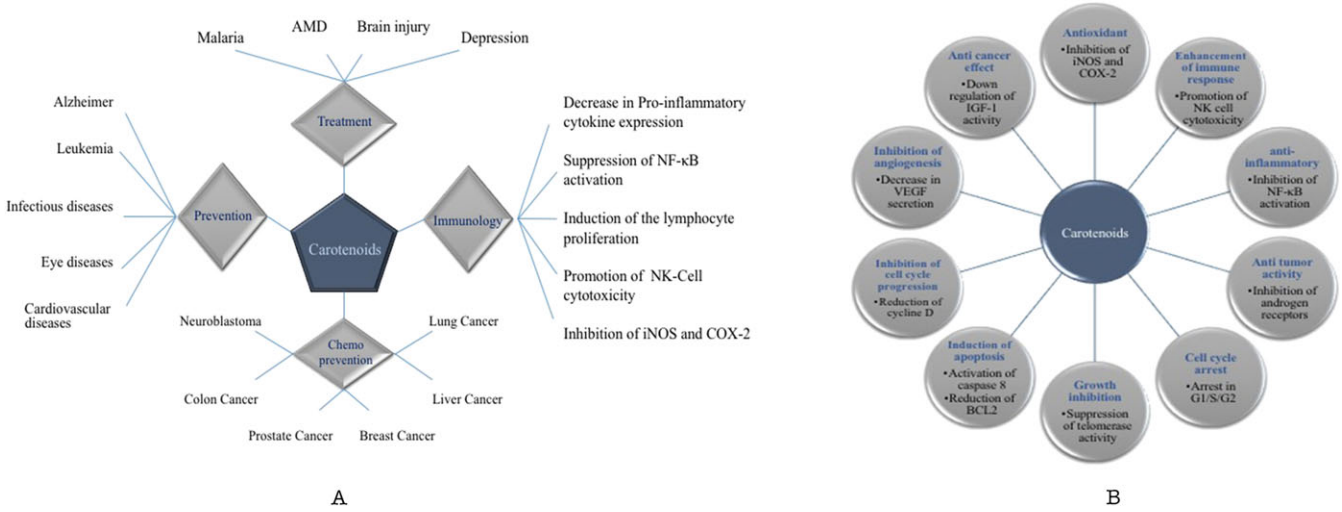

Figure 2

Biological activities of the main carotenoids used in the treatment of various diseases: (A) general effects; (B) their molecular mechanisms.

Antioxidant activities

Reactive oxygen and nitrogen species are produced during pathological processes and aerobic metabolism, and are involved in the pathobiochemistry of degenerative diseases (Stahl and Sies, 2005). The beneficial effects of carotenoids are mainly derived from their antioxidant properties as a main scavenger of the ROS such as single molecular oxygen (O_2) and peroxyl radicals (Stahl and Sies, 2005;

Rao and Rao, 2007). Carotenoids can scavenge radicals in three steps such as electron transfer (oxidation, reduction: $CAR + ROO \rightarrow CAR^{+} + ROO^{-}$), hydrogen abstraction ($CAR + ROO \rightarrow CAR + ROOH$) and addition ($CAR + ROO \rightarrow ROOCAR$) (El-Agamey *et al.*, 2004). The presence of conjugated double bonds enables these compounds to accept electrons from reactive species, and then neutralize free radicals (Rutz *et al.*, 2016). A combination of two lipophilic antioxidants (e.g. vitamins E, C and β-carotene) leads to synergistic effects as a result of scavenging reactive nitrogen species and inhibition of lipid peroxidation, which is significantly higher than that of a single effect (Stahl and Sies, 2005). β-carotene functions as an effective chain-breaking antioxidant. Studies have shown that β-carotene can suppress the up-regulation of haem oxygenase 1 gene expression in human dermal fibroblasts (FEK4) exposed to UV-A, indicating a dose-dependent pro-oxidant effect (El-Agamey *et al.*, 2004; Rao and Rao, 2007). In addition, zeaxanthin can effectively scavenge both water- and lipid-soluble peroxyl radicals, but β-carotene is less effective at preventing lipid peroxidation (El-Agamey *et al.*, 2004). Lycopene is also known to be potent at decreasing the ROS generated by smoke and to modulate redox sensitive cell targets including protein kinases, protein tyrosine phosphatases (PTP), MAP kinase (MAPKs) and transcription factors (Kaulmann and Bohn, 2014).

Carotenoids and anti-cancer properties

Carotenoids have been found to have beneficial effects on the treatment of various cancers. Table 1 shows biochemical activities of main carotenoids against different diseases.

Carotenes (lycopene, β-carotene and β-cryptoxanthin). The carotenoids possessing pro-vitamin A activity are α-carotene, β-carotene, γ-carotene and β-cryptoxanthin. The low bioavailability of β-carotene in natural sources is due to the resistance of carotene-protein complexes and the plant cell walls to digestion and degradation and subsequently its poor release (Donhowe and Kong, 2014). Thermal processing has been shown to enhance β-carotene bioavailability and absorption as compared with mechanical processing (van het Hof *et al.*, 2000; Donhowe and Kong, 2014). Studies have shown that higher circulating levels of these carotenoids in women may reduce the risk of breast cancer (Eliassen *et al.*, 2012). The reports indicate that treatment of human breast cell lines (e.g. MCF-7 cells) with lycopene and β-carotene, for 48 and 96 h, potently inhibits cell proliferation, arrests the cell cycle in different phases and increases apoptosis (Gloria *et al.*, 2014). The findings obtained after treating human chronic monocytic leukaemia (U937) and myeloid leukaemia with β-carotene indicated that β-carotene acts as an antioxidant at lower concentrations (up to 20 μM for 24 h), but shows pro-oxidant properties at higher concentrations. Also, β-carotene has been shown to arrest HL-60 leukaemia cells in the G1 phase (~39.4%) and significantly reduce their viability at a concentration of 20 μM. In fact, the number of apoptotic bodies is enhanced with increasing concentrations of β-carotene (Upadhyaya *et al.*, 2007; Niranjana *et al.*, 2015). Among the different human adenocarcinoma colon cancer cells, COLO 320 HSR, WiDr and LS174 cells are the most susceptible to β-carotene treatment respectively. Treatment with β-carotene significantly decreases the percentage of BCL-2 and Bcl-xL positive cells and induces cell cycle arrest in the G2/M phase by reducing the expression of cyclin A (a key regulator of the G2/M phase progression) in a dose-dependent manner (Niranjana *et al.*, 2015; Palozza *et al.*, 2002). Other studies have shown that supplementation with β-carotene increases the level of apoptotic p53 and decreases anti-apoptotic BCL-2 in a human gastric cancer cell line (AGS cells) after 24 h treatment (Jang *et al.*, 2009; Niranjana *et al.*, 2015). Also, β-carotene reduces the expression of hypoxia-inducible factor 1α, a well-known tumour metastasis regulator, and attenuates the migratory and invasive ability of malignant neuroblastoma cells (i.e. anti-metastasis effect) (Niranjana *et al.*, 2015). Higher serum concentrations of α-carotene and β-carotene as well as some vitamins (e.g. vitamins E and C) are associated with a lower risk of cervical cancer in Chinese women (Guo *et al.*, 2015). In the same way, a significant correlation was observed between high concentrations of serum β-carotene and the risk of prostate cancer (PCa) (Karppi *et al.*, 2012). In addition, natural β-carotene derived from *Dunaliella salina* generated higher rates of cell mortality on MDA-MB-231 breast cancer cells as compared with synthetic β-carotene (Olmos *et al.*, 2015). The findings indicated that treatment with α-carotene significantly suppresses metastasis of human hepatocarcinoma cells (SK-Hep-1), by prevention of invasion, migration and adhesion in a dose-dependent manner (~2.5 μM). The anti-apoptotic effects of α-carotene were stronger than those of β-carotene at the same concentration (Niranjana *et al.*, 2015; Chen *et al.*, 2013a,b). Lycopene, a potent single oxygen quenching agent present in tomatoes, shows different biological effects such as cardioprotective, antioxidant, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities (Bhuvaneswari and Nagini, 2005). A 24 h incubation of cells with lycopene showed that this carotenoid is more efficient than α- and β-carotene in preventing the growth of human endometrial, lung and mammary cancer cells, through inhibition of the insulin-like growth factor (IGF)-induced cell proliferation. IGFs are potent autocrine mitogens for endometrial and breast cancer cells (Levy *et al.*, 1995). Supplementation with lycopene may diminish the growth of PCa by up-regulating Cx43 gap junction protein, decreasing the IGF-1 level and/or increasing IGF binding at protein-3 level (Kucuk *et al.*, 2001). The data showed that the cis form of lycopene found in tomato products was more bioavailable than the trans form found in fresh tomatoes (Boileau *et al.*, 1999). Furthermore, the reduced risk of PCa was slightly stronger for high intakes of cooked tomato products than for high intakes of raw tomatoes (Giovannucci *et al.*, 1995). Lycopene and genistein are known to be potent antioxidants and their combination provides maximum protection against 7, 12-dimethylbenz [α] anthracene (DMBA)-induced mammary carcinogenesis (Sahin *et al.*, 2011). The results showed a late G1-phase cell cycle arrest followed by an increase in the G1 phase cell number (Chalabi *et al.*, 2004). Indeed, lycopene suppressed cell cycle progression through a reduction in the cyclin D level and retention of p27 in cyclinE-cdk2, leading to inhibition of G1 CDK activities (Nahum *et al.*, 2001). On the other hand, lycopene significantly enhanced BRCA1, D11-BRCA1,

Table 1

Biological effects of the main carotenoids against different diseases

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
β-carotene (derived from carrots, apricots, mangoes, red pepper, kale, spinach, broccoli)	Natural	Leukaemia	U937, HL-60 cell lines (*in vitro*)	20 μM	Cell cycle arrest in the G1 phase; induction of apoptosis, Antioxidant properties	Upadhyaya *et al.*, 2007
	Natural	Colon cancer	COLO 320 HSR, WiDr, LS174 cell lines (*in vitro*)	25 μM	Cell cycle arrest in the G2/M phase; reduction of the percentage of Bcl-2 and Bcl-xl positive cells	Palozza *et al.*, 2002
	Natural	Gastric cancer	AGS cell line (*in vitro*)	100 $\mu mol \cdot L^{-1}$	Induction of apoptosis	Jang *et al.*, 2009
	Natural	Adenocarcinoma	WiDr cell line (*in vitro*)	50 μM	Induction of apoptosis by its pro-oxidant properties	Palozza *et al.*, 2001
	Natural	Neuroblastoma	BE(2)C cell line, male BALB/c v/v mice (*in vitro*/ *in vivo*)	20 μM	As a chemotherapeutic agent regulating the invasion and metastasis of neuroblastoma via hypoxia inducible factor- 1 α (HIF-1α)	Kim *et al.*, 2014
	Natural	Hepatocarcinoma, Prostate cancer	SK-Hep1, B16F10, PC-3 cell lines (*in vitro*)	20 μM	Inhibition of proliferation. Decrease in VEGF secretion	Chen *et al.*, 2012
	Natural	Cervical cancer	Chinese women (clinical)	Vitamin diet (8.22% for α-carotene, 6.81% for β-carotene)	Antioxidant compounds (e.g., α-carotene, β-carotene, and vitamins E and C) are beneficial in reducing the risk of cervical cancer	Guo *et al.*, 2015
	Natural	Prostate cancer	Finnish men (clinical)		High concentration of serum β-carotene was associated with low risk of prostate cancer	Karppi *et al.*, 2012
	Natural	HCV infection	HCV patient (clinical)		Decrease in serum retinol, β-carotene, and retinol binding protein 4 (RBP4) was associated with early stages of HCV infection	Kataria *et al.*, 2016

(continues)

Table 1 (Continued)

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
	Natural	Age-related macular degeneration (AMD)	Patients (clinical)	Food intake	Low intake of n-3 fatty acid, α-tocopherol, zinc, vitamin D, vitamin C, β-carotene and lutein was associated with neovascular AMD; but no change for retinol or cryptoxanthin	Aoki *et al.*, 2016
B-carotene, canthaxanthin, phytoene	Natural	Skin tumours	Female SKH·h^{-1} mice (*in vivo*)	3.3 mg (β-carotene) 100 mg (canthaxanthin) 168 mg·kg^{-1}·week^{-1} (phytoene)	β-carotene decreased the number of skin tumours	Mathews-Roth, 1982
α-carotene (AC) and β-carotene (BC) [AC derived from carrots, bananas, pumpkins, peppers, avocados, apricots]	Natural	Hepatocarcinoma	SK-Hep-1 cell line (*in vitro*)	2.5 μM	Inhibition of cell invasion. Anti-tumour effects of AC were stronger than those of BC at the same concentration	Chen *et al.*, 2013a,b
β-carotene	Both forms [natural (derived from *Dunaliellasalina*) and synthetic]	Breast cancer	HaCat, MDA-MB-231 Cells (*in vitro*)	10 μg·mL^{-1}	Induction of apoptosis using both natural and synthetic sources. Natural β-carotene generated considerable higher rates of cell mortality as compared to synthetic form	Olmos *et al.*, 2015
Crocin (s), crocetin	Natural	Liver damage	Male Kunming mice (*in vivo*)	400 mg·kg^{-1}	Low levels of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP)	Chen *et al.*, 2016
Crocin	Natural	Breast cancer	MCF-7 cells (*in vitro*)	50 μg·mL^{-1} (IC_{50}: 60 μg·mL^{-1})	Induction of apoptosis; activation of caspase-8	Lu *et al.*, 2015
Crocin, crocetin	Natural (derived from *Crocus sativus*)	Breast cancer	MCF-7 and MDA-MB-231 cells (*in vitro*)	>200 μM (IC_{50} for MCF-7: 350 μg·mL^{-1}; IC_{50} for MDA-MB-231: 500 μg·mL^{-1})	Inhibition of the proliferation	Chryssanthi *et al.*, 2007

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Table 1 (Continued)

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
Crocin	Natural	Depression	Adult male Wistar Albino rats (*in vivo*)	25 and 50 $mg \cdot kg^{-1}$	Anti-depressant like action by increasing CREB, BDNF and VGF levels in hippocampus	Vahdati Hassani *et al.*, 2014
	Natural	Depression	Adult outpatients (clinical)	30 $mg \cdot day^{-1}$ capsule of saffron	No side effects, treatment of mild to moderate depression (Anti-depressant)	Noorbala *et al.*, 2005
Crocetin	Natural	Brain injury	Adult Sprague–Dawley (SD) rats (*in vivo*)	50 $mg \cdot kg^{-1}$ (once daily)	Decrease in BCL-2 protein expression; inhibition of apoptosis; treatment of traumatic brain injury	Bie *et al.*, 2011
	Natural	Gastric cancer	AGS, HFSF-PI3 cell lines, Male Wistar albino rats (*in vitro*/ *in vivo*)	200 $\mu mol \cdot L^{-1}$	Induction of apoptosis. Reduction of the Bcl-2/ Bax mRNA ratio	Bathaie *et al.*, 2013
	Natural	Malignant cells	A-549 (lung adenocarcinoma), VA-13 (SV-40 transformed fetal lung fibroblast), HeLa cell lines (*in vitro*)	Up to 200 $\mu g \cdot mL^{-1}$ (Non-toxic); IC_{50} (for DNA: 35 or 42 $\mu g \cdot mL^{-1}$, for RNA: 57 or 65 $\mu g \cdot mL^{-1}$, for protein: 45 or 55 $\mu g \cdot mL^{-1}$)	A dose dependent inhibitory effect on DNA/ RNA synthesis in isolated nuclei and suppressed the activity of purified RNA polymerase II; Hela cells were less sensitive to the inhibition of intracellular protein synthesis than the A-549 and VA-13 cells	Abdullaev, 1994
	Natural	Lung cancer	Male Swiss albino mice (*in vivo*)	50 $mg \cdot kg^{-1}$ body weight (Non-toxic)	Inhibition of cell proliferation	Magesh *et al.*, 2009
	Natural	Colorectal cancer	HCT-116, HT-29, SW-480, NSCLC Cell lines (*in vitro*)	3 $mg \cdot mL^{-1}$	Anti-proliferative effects	Aung *et al.*, 2007
	Natural	Cervical cancer caused by HPV infections	TC-1 cell line, Female C57BL/6 mice (*in vitro*/ *in vivo*)	IC_{50} for crocin (2 mM)	Induction of a sub-G1 peak. Apoptosis; anti-tumour effect; prevention of cell growth; chemotherapeutic agent	Khavari *et al.*, 2014

(continues)

Table 1 (Continued)

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
Curcumin	Natural	Breast cancer	MDA-MB-435 cell line, Female athymic nude mice (*in vitro*/ *in vivo*)	50 $\mu mol \cdot L^{-1}$	Enhanced apoptosis; decreased breast cancer metastasis to the lung; suppression of NF-ı B	Aggarwal *et al.*, 2005
	Natural	Alzheimer	APPSw Tg^{+} and Tg^{-} mice (*in vivo*)	160 ppm	Reduction of oxidized proteins and interleukin-1β pro-inflammatory cytokine	Lim *et al.*, 2001
	Natural	Head and neck cancer	CCL23, CAL27, UM-SCC1, UM-SCC14A cell lines. Female athymic nude mice (*in vitro*/ *in vivo*)	50 $\mu mol \cdot L^{-1}$ (toxic at 400 $\mu mol \cdot L^{-1}$)	Growth inhibition	LoTempio *et al.*, 2005
Curcumin	Natural	Bladder cancer	KU-7, 253JB-V cell lines; athymic nude mice (*in vitro*/ *in vivo*)	10 $\mu mol \cdot L^{-1}$ for cell line (toxic at 25 $\mu mol \cdot L^{-1}$); 50 $mg \cdot kg^{-1} \cdot day^{-1}$ for mice	Inhibition of tumour growth	Chadalapaka *et al.*, 2008
	Natural	Skin tumour	Male Swiss ablino mice (*in vivo*)	1% in regimen	Inhibition of the tumour number	Limtrakul *et al.*, 1997
	Natural	*Helicobacter pylori*	C57BL/6 mice (*in vivo*)	50 $\mu g \cdot mL^{-1}$	Growth inhibitor for Indian *H. pylori* strains; healing the overall damage caused by *H. pylori*	De *et al.*, 2009
	Natural	Liver and small intestine cancer	knockout mice Nrf2 ($^{-/-}$) (*in vivo*)	1000 $mg \cdot kg^{-1}$	Chemoprevention	Shen *et al.*, 2006
	Natural	Ovarian cancer	CaOV3 cells (*in vitro*)	50 μM	Curcumin induced AMPK activation	Pan *et al.*, 2008
	Natural	Pancreatic cancer	Patients (clinical)	8 g	Loss of subcutaneous fat and muscle as compared to untreated subjects	Parsons *et al.*, 2016
	Natural	Prostate cancer	LNCaP, PC-3 cell lines (*in vitro*)	40 μM for LNCaP, 30 μM for PC3	Therapeutic effect. Down-regulation of transactivation and expression of androgen receptor (AR) and AR-related cofactors including activator protein-1 (AP-1), NF-ı B, and CREB (cAMP response element-binding protein)-binding protein (CBP)	Nakamura *et al.*, 2002

(continues)

Table 1 (Continued)

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
	Natural	Leukaemia	HL60, Bel7402, SGC7901 cell lines; female BALB/c athymic (ν+/ν+) mice (*in vitro*/*in vivo*)	1 μM (IC_{50} for HL60: 3.11 μM, for Bel7402: 3.8 μM; for SGC7901: 8 μM)	Growth inhibition. Suppression of telomerase activity in the cancer cells, and regulation of telomere length	Cui *et al.*, 2006
Curcumin	Natural	Malaria	Male Swiss mice (*in vivo*)	100 $mg \cdot kg^{-1}$	Malaria therapy. Reduction of blood parasitaemia by 80–90%, and significant enhancement of their survival	Reddy *et al.*, 2005
	Natural	Melanoma	MMAN, MMRU, RPEP, PMWK, Sk-mel-2, Sk-mel-5, Sk-mel-28, MEWO cell lines (*in vitro*)	100 μM	Induction of apoptosis	Bush *et al.*, 2001
Curcuma biscuits	Natural	Cardiovascular disease	Healthy men (clinical)	Curcuma biscuits (daily up to 2 months)	Reduction of total cholesterol and LDL-cholesterol, Prevention of cardiovascular disease	Madaric *et al.*, 2013
Thymoquinon, curcumin	Natural	Influenza	Turkey poults (*in vivo*)	2.5 $g \cdot kg^{-1}$	Synergistic anti-influenza activity, High antibody titer against H9N2 AIV	Umar *et al.*, 2016
Curcumin, tetrahydrocurcumin (THC)	Natural	Colon cancer	Male B6C3F1 mice (*in vivo*)	0.2% (THC) and 0.5% (curcumin) in diet	Chemopreventive agents THC is more active than the curcumin	Kim *et al.*, 1998a,b
Hydrazinocurcumin	Synthetic	Endothelial liver cell	BAECs, HT29, NIH3T3 cells (*in vitro*)	Toxicity: 0.52 μM	New candidate for anti-angiogenic agent	Sup Shim *et al.*, 2002
Hydrazinobenzoyl curcumin (HBC)	Synthetic	Lung cancer	A549 cells (*in vitro*)	Toxicity: 80 μM	Inhibition of the A549 cell proliferation via inducing autophagy	Zhou *et al.*, 2014
Curcumin analogues	Synthetic	Tumour (human fibrosarcoma cells)	HT-1080, BAECs, HUVECs, JB6P+ cell lines (*in vitro*)	5 μM (Non-toxic)	Inhibition of endothelial cell migration, Inhibitory effect of AP-1 transcription and anti-angiogenic activity	Hahm *et al.*, 2004
Lutein/ zeaxanthin (derived from kale, spinach, broccoli, peas, cress, parsley, lettuce, maize, egg yolk)	Natural	Age-related macular degeneration (AMD)	AMD patient (clinical)	Food intake	Strongly associated with a reduced risk for AMD	Seddon *et al.*, 1994

(continues)

Table 1 (Continued)

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
Lutein/ zeaxanthin	Natural	Age-related macular degeneration	Women (clinical)	Food intake	Protection against intermediate AMD in healthy women younger than 75 years	Moeller *et al.*, 2006
	Natural	Age-related cataract	AMD patient (clinical)	Daily lutein/zeaxanthin (10 mg/2 mg)	No statistically significant overall effect on rates of cataract surgery or vision loss	Chew *et al.*, 2013
	Natural	Diabetic retinopathy	Type 2 diabetes pateints (clinical)	Lutein/ zeaxanthin (6 mg/ 0.5 mg)	Potent preventive and also therapeutic effects	Moshetova *et al.*, 2015
	Natural	Epidermal hyperproliferation and acute inflammation	Female SKH-1 mice (*in vivo*)	0.4% lutein plus 0.04% zeaxanthin enriched diet	Reduction of acute inflammatory responses and inhibition of UV-induced rebound hyper proliferation	Gonzalez *et al.*, 2003
	Natural	Skin cancer	Adult patients (clinical)	Dietary foods intake	More than 50% reduction in the risk of squamous cell carcinoma (SCC)	Heinen *et al.*, 2007
	Natural	Atherosclerosis	Female apoE-null mice (*in vivo*)	0.2% (w w^{-1})	Increased dietary intake of lutein is protective against the development of early atherosclerosis	Dwyer *et al.*, 2001
	Natural	Atherosclerosis	Male guinea pigs (*in vivo*)	0.1 g 100 g^{-1} of diet	Prevention of early atherosclerosis development by reducing cholesterol accumulation	Kim *et al.*, 2011
	Semi synthetic	Antioxidant activity	Male Swiss albino mice (*in vivo*)	100 mg	Significant antioxidant activity	Firdous *et al.*, 2010
Astaxanthin	Natural	Colon cancer	Male Crj:CD-1 (ICR) mice (*in vivo*)	200 ppm (No toxicity)	Suppression of colon carcinogenesis by effects on NF-ı B signalling pathway	Yasui *et al.*, 2011
Lycopene [Derived from Tomato, Red watermelon, Pink grapefruit, Papaya, Guava, Rose hip canned]	Natural	Prostate cancer	Men (clinical)	Dietary intake	Decrease in the risk of prostate cancer	Giovannucci *et al.*, 1995

(continues)

Table 1 (Continued)

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
Lycopene	Natural	Prostate cancer	Phase II randomized clinical trial before prostatectomy (clinical)	15 mg (twice daily) (No adverse effects)	Reduction of IGF-1 level; enhancement of IGFBP-3 level; decrease in tumour growth	Kucuk *et al.*, 2001
	Natural	Prostate cancer	DU145, PC-3, LNCaP cell lines; Male BALB/c nude mice (*in vitro*/*in vivo*)	26.6 μmol·L^{-1}, 40.3 μmol·L^{-1}, 168.5 μmol·L^{-1}; In mice:100 and 300 mg·kg^{-1}	Inhibition of tumour growth	Tang *et al.*, 2005
Lycopene	Natural	Breast cancer	Female Wistar rats (*in vivo*)	20 mg·kg^{-1}	Inhibition of tumour growth and expression of apoptosis associated proteins	Sahin *et al.*, 2011
	Natural	Breast cancer	MCF-10a, MCF-7, HBL-100, MDA-MB-231 Cell lines (*in vitro*)	10 μM	Cell cycle arrest in G1/S phase, Increase in expression of BRCA1 and BRCA2 oncosuppressor genes	Chalabi *et al.*, 2004
	Natural	Breast cancer; endometrial cancer	MCF-7,T-47D; ECC-1 cell lines (*in vitro*)	10 μM	Inhibition of cell cycle progression via reduction of the cyclin D level and retention of p27 in the cyclin E-cdk2 complexes	Nahum *et al.*, 2001
	Natural	Breast cancer	MCF-7, MDA-MB-231 cell lines (*in vitro*)	Toxicity: 2.4 μM for MCF-7; 3.0 μM for MDA-MB-231	Enhancement of quinacrine activity synergistically and inhibition of Wnt-TCF signalling through APC	Preet *et al.*, 2013
	Natural	Oral cavity and pharynx cancer	Smokers and non-smokers (clinical)	Dietary intake	Low concentration of plasma lycopene is associated with increased mortality	Mayne *et al.*, 2004
	Natural	Digestive tract cancer	Human (clinical)	Raw tomato intake	Protection against digestive-tract cancers	Franceschi *et al.*, 1994
	Natural	Lung cancer	Male adult ferrets (*in vivo*)	1.1 and 4.3 mg·kg^{-1} body weight·day^{-1}	Protective effects against lung cancer by promotion of apoptosis and inhibition of cell proliferation	Liu *et al.*, 2003
	Natural	Lung cancer	Male B6C3F1 mice (*in vivo*)	50 ppm	Inhibition of lung neoplasian development	Sup Shim *et al.*, 2002

(continues)

Table 1 (Continued)

Carotenoids	Source	Disease	*In vitro*/*in vivo* study	[a]Dose of interest	Biochemical/clinical effects	Ref.
	Natural	HPV infection	Women (clinical)	Dairy foods	Significant inverse association between HPV persistence and plasma cis-lycopene concentrations (56% tumour reduction)	Sedjo *et al.*, 2002
	Natural	Atherosclerosis	Men (clinical)	Dietary intake	Low concentration of serum lycopene is associated with a high carotid atherosclerosis	Rissanen *et al.*, 2003
	Natural	Cardiovascular diseases (CVD)	CVD and healthy pateints (clinical)	7 mg	Improvement of endothelial (vascular) function in CVD patients	Gajendragadkar *et al.*, 2014
Lycopene	Synthetic crystalline	Embryo-fetal toxicity/ teratogenicity	Rats; rabbits (*in vivo*)	For rat: 3000 $mg \cdot kg^{-1} \cdot day^{-1}$; For rabbit: 2000 $mg \cdot kg^{-1} \cdot day^{-1}$	Developmental toxicity	Christian *et al.*, 2003
	Synthetic and natural	–	Healthy participants (clinical)	15 $mg \cdot day^{-1}$	Synthetic and natural lycopene are equivalent sources of lycopene (identical bioavailability)	Hoppe *et al.*, 2003
Lycopene, α- and β-carotene	Natural	Breast cancer; lung cancer	MCF-7; NCI-H226 cell lines (*in vitro*)	1–2 μM	Lycopene inhibited the IGF-induced cell proliferation; lycopene is a more potent inhibitor than α- and β-carotene	Levy *et al.*, 1995
Lycopene and β-carotene	Natural	Breast cancer	MCF-7, MDA-MB-231, MDA-MB-235 cell lines (*in vitro*)	10 μM	Inhibition of cell proliferation; cell cycle arrest in different phases, Induction of apoptosis	Gloria *et al.*, 2014

[a]Dose of interest: the dose at which the sample was tested was non-toxic.

BRCA2 and D12-BRCA2 RNA expression in three breast tumour cells (e.g. MCF-7, HBL-100 and MDA-MB-231 cell lines) (Chalabi *et al.*, 2004). It also decreased serum-induced phosphorylation of the retinoblastoma protein and related pocket proteins (Nahum *et al.*, 2001). Several reports have suggested that topoisomerase inhibitors and lycopene synergistically suppress Wnt-TCF signalling in breast cancer cells without affecting the normal cells (Preet *et al.*, 2013). Generally, the mechanisms involved in the inhibitory effects of lycopene on tumour growth or carcinogenesis include an up-regulation of detoxification systems, ROS scavenging, interference with cell proliferation, inhibition of cell cycle progression, induction of gap-junctional communication and modulation of signal transduction pathways. The anti-inflammatory activity of lycopene is also considered to be an important mechanism involved in its suppressive effect on the promotion and progression of carcinogenesis. Moreover, lycopene can inhibit cell invasion, angiogenesis and metastasis. These activities were shown at physiological concentration in humans. Although the preclinical data strongly suggested lycopene has antitumour activity, several epidemiological studies indicate that its use for the prevention of cancers is controversial. However, because of its multiple tumour-inhibitory activities, lycopene still remains a promising carotenoid for the prevention and treatment of human cancers (Bhuvaneswari and Nagini, 2005; Ono *et al.*, 2015). Furthermore, a significant inverse association has been observed between HPV persistence and plasma *cis*-lycopene concentrations; lycopene induced a ~56% reduction in viral load (Sedjo *et al.*, 2002).

Crocin and crocetin. Saffron contains terpenes, terpene alcohol and their esters (Srivastava *et al.*, 2010). The value of saffron is due to the presence of three main secondary metabolites: crocin, responsible for colour; picrocrocin, responsible for taste; and safranal, responsible for odour (Bolhasani *et al.*, 2005; Bathaie *et al.*, 2007; Alizadeh and Bolhassani, 2015). The stability of its carotenoids depends on storage conditions including light, temperature and humidity (Gutheil *et al.*, 2012). Their hepatoprotective efficacy is mediated through the induction of an antioxidant pathway (Chen *et al.*, 2016). Saffron extract significantly decreases the viability of hepatocellular carcinoma cells (HepG2) in a time- and dose-dependent manner. It can reduce the expression of TNF receptor 1 protein, cell proliferation and oxidative stress, as well as increase the active form of caspase-3, induce apoptosis and down-regulate inflammatory markers, such as COX-2, inducible NOS (iNOS) and NF-κB-p65 *in vivo* (Amin *et al.*, 2011). *Crocus sativus* extract also shows potent anti-proliferative effects on human colorectal cancer cells such as HCT-116, SW-480 and HT-29 (Aung *et al.*, 2007). Crocin significantly inhibits the proliferation of MCF-7 cells and induces apoptosis via mitochondrial signalling pathways such as the activation of caspase-8, up-regulation of Bax, the disruption of mitochondrial membrane potential (MMP) and the release of cytochrome c (Lu *et al.*, 2015). Crocin has also been shown to have efficacy as a treatment of mild to moderate depression by increasing cAMP response element binding protein, brain-derived neurotrophic factor and VEGF levels in the hippocampus (Noorbala *et al.*, 2005; Vahdati Hassani *et al.*, 2014). Furthermore, crocin inhibited amyloid-β (Aβ) fibrillogenesis in male Wistar rats at lower concentrations than dimethylcrocetin (a synthetic carotenoid of saffron), indicating the effective role of the sugars in structure (Khalili, 2010). Our data confirmed that the cytotoxic activity of saffron extract and its ingredients (e.g. crocin) is higher against a malignant TC-1 cell line than non-malignant cells (COS-7) and this is mediated through the induction of apoptosis (Alizadeh and Bolhassani, 2015). In addition, therapeutic DNA vaccination accompanied by oral administration of crocin showed that 100% of mice treated with crocin were tumour-free as compared with those receiving the DNA vaccine alone (~66.7% compared to DNA vaccine + crocin ~33.3%), suggesting the high efficacy of crocin as a chemotherapeutic agent (Khavari *et al.*, 2014). Crocetin was also shown to be an effective treatment for traumatic brain injury; it inhibited apoptosis at the early stages of the injury and enhanced vessel angiogenesis at the sub-acute stage of cerebral trauma (Bie *et al.*, 2011). Crocetin has also been shown to protect neurons from the deleterious effects of 6-hydroxydopamine and was helpful in the prevention of Parkinsonism (Ahmad *et al.*, 2005). Crocetin treatment significantly reduced the Bcl-2/Bax mRNA ratio and increased caspase activity in AGS cell lines. Crocetin can act as a chemopreventive agent against benzo (α) pyrene-induced lung carcinogenesis by protecting the glycoprotein levels in serum and tissues (Magesh *et al.*, 2009). Crocetin has been shown to significantly inhibit the proliferation of human pancreatic adenocarcinoma cell lines (e.g. MIAPaCa-2, BxPC3, Capan-1 and ASPC-1) by suppressing EGF receptor activity and by increasing the Bax/Bcl-2 ratio (Dhar *et al.*, 2009). In addition, the administration of geniposide, crocin and crocetin significantly reduced serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase levels in carbon tetrachloride (CCl4)-treated mice. The decreased levels of GSH and the activities of antioxidant enzymes [SOD and catalase (CAT)] were enhanced by these carotenoids (Chen *et al.*, 2016). Furthermore, saffron extract containing carotenoids has been shown to have anti-convulsant and anti-alzheimer properties in animal and human trials. Administration of saffron extract and its ingredients augmented dopamine and glutamate levels in the brain in a concentration-dependent manner. Moreover, these compounds can interact with the opioid system to decrease withdrawal syndrome (Khazdair *et al.*, 2015).

Xanthophyll (lutein, zeaxanthin and astaxanthin). Xanthophylls are different from other carotenoids because of their oxygenated substituents, that is free hydroxyl groups at each end of the molecule that allow their orientation within cell membranes and lipoproteins (Roberts *et al.*, 2009). Zeaxanthin and lutein act as antioxidants protecting photoreceptor cells from the potential damage caused by free radicals (Krinsky *et al.*, 2003). A high dietary intake of lutein and zeaxanthin has been associated with a reduced incidence of skin cancer (Heinen *et al.*, 2007). Lutein, by lowering very-low-density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) levels, reducing inflammation and oxidative stress in the artery wall and also decreasing IL-10

concentrations, acts as a potent protective factor against the progression of atherosclerosis in animals and humans (Dwyer *et al.*, 2001; Kim *et al.*, 2011). ASTA is unique as it has more hydroxyl groups than other xanthophylls. ASTA contains two terminal rings linked by a polyene chain with both lipophilic and hydrophilic properties (Ambati *et al.*, 2014). ASTA cannot be converted to vitamin A. Humans are not able to synthesize ASTA and need to take it from food. Due to the high lipophilicity of ASTA, it can cross the blood–brain barrier and reach the brain and eye structures (Schweigert *et al.*, 1998). ASTA is a potent antioxidant. Low doses of ASTA have been shown to inhibit colon carcinogenesis in mice by modulating proliferation, G0/G1 phase arrest through down regulation of cyclin D and increasing the expression of p21, p53 and p27. ASTA markedly decreased the incidence of colon adenocarcinoma in mice *in vivo* and increased their survival rate by suppressing the expression of PCNA (Pashkow *et al.*, 2008; Yasui *et al.*, 2011; Niranjana *et al.*, 2015). *In vitro* studies showed that natural ASTA from *Haematococcus pluvialis* microalgae can be more than 50 times stronger than synthetic ASTA in single oxygen quenching and nearly 20 times more effective in eliminating free radicals (antioxidant activity) (Capelli *et al.*, 2013).

Curcumin. Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa*, has a wide range of pharmacological activities (Anand *et al.*, 2007). In a human breast cancer xenograft model, dietary administration of curcumin significantly reduced the incidence of breast cancer metastasis to the lung; this was mediated through the inhibition of the anti-apoptotic transcription factor NF-κB and NF-κB-regulated gene products in the tumour tissue (Aggarwal *et al.*, 2005). This growth suppression was also observed in head and neck cancer (HNC) cell lines (LoTempio *et al.*, 2005; Wilken *et al.*, 2011), human bladder cancer cells (e.g. 253JB-V and KU7) (Chadalapaka *et al.*, 2008), melanoma (Bush *et al.*, 2001) and skin tumours (Limtrakul *et al.*, 1997). Curcumin has been shown to induce apoptosis in melanoma cells via a Fas receptor/caspase-8 pathway independent of p53 (Bush *et al.*, 2001). Both low and high doses of curcumin reduced the levels of IL-1β in TG^{-} mice and also significantly decreased the levels of oxidized proteins, insoluble and soluble amyloid and plaque burden in the brains of APPSw transgenic mice (Lim *et al.*, 2001; Ringman *et al.*, 2005). Curcumin was shown to have therapeutic potential against *Helicobacter pylori* infection irrespective of the disease status. It was highly effective in eradicating *H. pylori* from infected C57BL/6 mice as well as in repairing *H. pylori*-induced gastric damage (De *et al.*, 2009). The new curcumin derivative, tetrahydrocurcumin, was shown to be more active than curcumin in preventing the development of aberrant crypt foci and cell proliferation (Kim *et al.*, 1998a, b; Chauhan, 2002). Furthermore, thymoquinone and curcumin have been shown to have a synergistic inhibitory effect on the influenza virus induced by increasing the efficiency of the immune response and this effect plays a significant role in diminishing the pathogenic effects of H9N2 in turkeys (Umar *et al.*, 2016). Curcumin was found to induce the activation of AMPK in human ovarian cancer cells (CaOV3 cells) in a time- and concentration-dependent manner (~25–50 μM of curcumin; time: 15–120 min), as curcumin activated p38 phosphorylation that is an important downstream signal of AMPK, and elicited CaOV3 cell death (Pan *et al.*, 2008). In addition, the treatment of patients with advanced pancreatic cancer with curcumin showed significantly greater loss of subcutaneous fat and muscle than untreated controls (Parsons *et al.*, 2016). Curcumin has been shown to suppress telomerase expression and activity in three cancer cells (e.g. HL60, Bel7402 and SGC7901 cell lines) by inducing apoptosis, which indicates it has an anti-proliferating effect (Cui *et al.*, 2006). There are data showing that a novel synthetic analogue of curcumin, hydrazinocurcumin, also exhibits anti-angiogenic activity but is less potent (Sup Shim *et al.*, 2002). In contrast, low concentrations of the synthetic analogue of curcumin, hydrazinobenzoylcurcumin, when applied for short time, can inhibit the proliferation of human lung adenocarcinoma A549 cells by inducing autophagy and has potential as a therapeutic anti-cancer agent. This compound is different from other curcumin derivatives, which mostly stimulate cell apoptosis (Zhou *et al.*, 2014). Also, other synthetic derivatives of curcumin, including compounds 4 [1, 7-bis-(3,4-dimethoxyphenyl)-1,6-heptadiene- 3,5-dione] and 8 [1, 7-bis-(4-propargyl-3-methoxyphenyl)-1,6- heptadiene-3,5-dione], have been found to have potent activity against *Leishmania amazonensis* (Gomes *et al.*, 2002). Curcumin and its analogues have been demonstrated to have an inhibitory effect on AP-1 transcription (responsible for cell proliferation and differentiation) and anti-angiogenic activity on the developmental neovascularization of chick embryo. In fact, curcumin analogues can decrease the migration of bovine aortic endothelial cells (BAEC) and HUVEC invasion (Hahm *et al.*, 2004).

Immunopharmacological role of carotenoids

The effects of different carotenoids on immune responses, such as lymphocyte proliferation, cytokine release, phagocytic and microbicidal capacities, natural killer cell cytotoxic activity and inflammation, has been studied *in vitro* and *in vivo*. In this section, we describe the various immunopharmacological roles of individual carotenoids. Table 2 shows the immunological properties of the major carotenoids used to treat different diseases.

Astaxanthin (ASTA). ASTA (3, 3-dihydroxy-β, β-carotene-4, 4-dione) significantly improve the phagocytic and microbicidal capacity of neutrophils and enhances the intracellular calcium concentration (Ca^{+2}) and NO production. These activities were associated with a decrease in the levels of superoxide anion, hydrogen peroxide (H_2O_2), IL-6 and TNF-α cytokines. Indeed, oxidative damage in proteins and lipids is significantly reduced after ASTA treatment. Also, treatment with 5 μM ASTA has been shown to increase both the phagocytic (~30%) and fungicide (~28%) capacities of neutrophils (Macedo *et al.*, 2010). In another study, ASTA treatment was found to decrease the secretion of pro-inflammatory cytokines in stimulated U937 cells. These data indicate that ASTA suppresses the H_2O_2 mediated activation of NF-κB and the secretion of cytokines

Table 2

Immunological properties of major carotenoids against different diseases

Carotenoids	*In vitro/in vivo*	Stimulus	Dose	Immunological effects	Ref.
Astaxanthin	Human	Phytohaemmaglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM)	2 and 8 mg	↓ C-reactive protein (CRP) at 2 mg; ↑Nkc activity and lymph proliferation at 8 mg; ↑IFN-γ and IL-6 at 8 mg; No change in IL-2 and TNF-α	Park *et al.*, 2010
	Dog	Concanavalin A	20 mg	↑ lymph proliferation and NKc cytotoxicity; ↑ IgG, IgM; ↑ Beta cell population; no changes in the populations of $CD4^+$, $CD8^+$ and MHC class II	Chew *et al.*, 2011
	Cat	Con A, PHA, PWM	10 mg	↑ NKc cytotoxicity;↑cd5,cd4 population; ↓Beta cell population; no alteration in CD8 and MHCII; ↑IgG, IgM	Park *et al.*, 2011
	Human	————	4 mg	↑ IgA secretion; no change in leukocyte count; reduction of pro-oxidant-antioxidant balance (PABC)	Baralic *et al.*, 2015
	Neutrophil from human peripheral blood	Lipopolysaccharide (LPS)	5 μM	↑ phagocytic (30%) and fungicide (28%) effects against *Candida albicans*; ↓ TNF-α and IL-6; ↑NO production	Macedo *et al.*, 2010
	U-937 cell line	H_2O_2	10 μM	↓TNF-α, NF-ı B, IL-6 and IL-1β	Speranza *et al.*, 2012
Astaxanthin stereoisomers	Mouse [K562 cell (target for NKc); lymphocytes and peritoneal macrophages]	———–	20 $\mu mol \cdot L^{-1}$	↑Lymph proliferation; ↑phagocytic activity; ↑NKc cytotoxicity; (3S, 3'S)-trans-astaxanthin was better than others	Sun *et al.*, 2016
	Spleen and thymus from C57B/6 mice	PHA, ConA	10^{-7}-10^{-9}M	↑ Ab production; ↑Thy-1+ and Thy-1^- cell populations, No change in IL-2	Jyonouchi *et al.*, 1991
	Human PBMC (peripheral blood mononuclear cells)	PWM (for IgA); trinitrophenol-modified keyhole limpet haemocyanin (TNP-KLH); tetanus toxoid (for IgG)	$10^{-8} mol \cdot L^{-1}$	↑IgM; ↑IgG; ↑IgA	Jyonouchi *et al.*, 1995
	Peritoneal adherent cells of BALB/c mice	*E.coli* lipopolysaccharide (for Ab)	2×10^{-7}- 2×10^{-8}M	↑Thymocyte proliferation; ↑Ab production, ↑ TNF-α and IL-1α	Okai and Higashi-Okai, 1996

(continues)

Table 2 (Continued)

Carotenoids	*In vitro/in vivo*	Stimulus	Dose	Immunological effects	Ref.
Crocin	BV2 mouse microglial cells	LPS	20 μM	↓NO release in the cells stimulated with IFN-γ and amyloid β (Aβ), ↓TNF-α, NF-ᵢB, and IL-1β	Nam *et al.*, 2010
Curcumin	Mice	E.G7/OT1 mouse lymphoma	70 $mg \cdot kg^{-1} \cdot day^{-1}$	↑CD8 cytotoxicity , ↓ TGF-β	Chang *et al.*, 2012
	Mice	Mitogenic anti-CD3/ 28 monoclonal antibodies (mAb) or antigenic stimulation by ovalbumin (OVA)	1% of diet	↓ NF-ᵢB activation; ↓ CD4 proliferation; ↓IL-2 production (29.4%) in antigenic stimulation	Kim *et al.*, 2009
	Rat	Keyhole limpet haemocyanin (KLH) antigen	40 $mg \cdot kg^{-1}$	↑IgG production	South *et al.*, 1997
Curcumin + Cyclosporin-A	Rat	PHA; ConA	40 $mg \cdot kg^{-1} \cdot day^{-1}$	↑proliferation of lymph cells; No change in IFN-γ and IL-2	Varalakshmi *et al.*, 2008
Curcumin	Human gastric epithelial cells (AGS)	*H. pylori*	40 μM for IL-8 80 μM for NF-ᵢB	↓IKK activity, Suppression of IL-8; no change in ERK1/2 and p38	Foryst-Ludwig *et al.*, 2004
Neutral unilamellar liposomes of curcumin	Mice	Sheep red blood cells (SRBC)	200 $\mu mol \cdot Kg^{-1}$	↑Antibody titres; ↑phagocytic activity of macrophages; inhibition of delayed type hypersensitivity (DTH) reaction by about 39.75%	Antony *et al.*, 1999
Curcumin	Primary human CD4+ T cells	anti-CD2/ CD3/CD28 antibody-coated beads	2 $\mu g \cdot mL^{-1}$	↓ T cell expansion; Down-regulation of CD69 at early phase; up-regulation of CCR7 and L-selectin at late phase, ↓IL-10, IL-13, IL-2, TNF-α, and IFN-γ	Kim *et al.*, 2013
	RAW264.7 macrophages from mice	Lipopolysaccharide (LPS)	5 $\mu g \cdot mL^{-1}$	Down-regulation of NF-ᵢB binding to the p40-ᵢB sequence; ↓kB binding activity; inhibition of IL-12 secretion from macrophage	Kang *et al.*, 1999a,b
	Splenic macrophages from mice	LPS; head-killed *Listeria monocytogenes* (HKL)	5 $\mu g \cdot mL^{-1}$	Inhibition of IL-12, ↑IL-4; No change in IL-10; ↓ IFN-γ	Kang *et al.*, 1999a,b
Curcumin	DC from mice	LPS	25 μM	Suppression of CD80,86, MHCII but not MHCI, ↓ TNF-α, NF-ᵢB, IL-6, and IL-1β cytokines	Kim *et al.*, 2005
	Human astrocyte cell line (U373-MG)	LPS	5 μM	↓ IL-6, ↓MMP-9 enzyme activity, and MCP-1 mRNA expression	Seyedzadeh *et al.*, 2014
	CD14+ monocytes, isolated from human peripheral blood	LPS; polycytidylic acid (polyI:C)	30 μM	↓DC-induced CD4 proliferation; ↓dextran uptake by non-stimulated cells; No significant increase in CD83,86;	Shirley *et al.*, 2008

(continues)

Table 2 (Continued)

Carotenoids	*In vitro/in vivo*	Stimulus	Dose	Immunological effects	Ref.
				prevention of DC migration towards CCL19 and CCL21; ↓chemokines fractalkine (CX3CL1) and interferon producing factor (IP-10), ↓ IL-6	
	Human PBMCs; RAW253	PHA (for IL-2); IFN-γ (for NKc); LPS	0.01 and 0.05 $\mu g \cdot mL^{-1}$	Inhibition of PHA-induced T-cell proliferation; ↑NK cell cytotoxicity; no significant decrease in TNF-α, ↓IL-2 and LPS-induced NF-ı B; ↓NO product from macrophages	Yadav *et al.*, 2005
Curcumin	RAW264.7 cells; Ba/F3 cells	LPS	20 μM	↓ NF-ı B, ↓Cox-2 expression; inhibited dimerization of TLR4 in Ba/F3cells	Youn *et al.*, 2006
Curcumin and lutein	Chicks	LPS	200 $mg \cdot kg^{-1}$	Curcumin led to: ↑ beta cell proliferation (%5.6) and T cell proliferation (%30.4) as compared to lutein;	Rajput *et al.*, 2013
Lutein	Human (atherosclerosis patients)	————————	20 $mg \cdot day^{-1}$	↓monocyte chemoattractant protein-1MCP-1), ↓IL-6	Xu *et al.*, 2013
Silk lutein	Mice	LPS; ConA	20 $mg \cdot kg^{-1}$	↑NKc activity; ↑Ab production; ↑CD3, CD4, lymph proliferation; ↑IFN-γ and IL-2	Promphet *et al.*, 2014
FloraGloTM crystalline lutein	Cat	PHA,ConA,PWM	10 $mg \cdot day^{-1}$	↑percentage of CD4,CD21 and IgG; no effect on CD8,MHCII; no change in IL-2	Kim *et al.*, 2000a,b
FloraGloTM crystalline lutein	Dog	PHA,CnoA,PWM	5-20 mg	↑CD4 (at 5 mg); ↑CD8,CD5,MHCII (at 20 mg), ↑IgG	Kim *et al.*, 2000a,b
Lutein	Chickens	*Salmonella* LPS	50 $mg \cdot kg^{-1}$	↓IL12, IL-1β (in liver) ↑TLR-4 mRNA (in spleen)	Moraes *et al.*, 2016
Lutein	Rat Muller cells (rMC-1)	CoCl2	20 μM	↓ COX-2, No change in TNF-α, ↓NF-kB and IL-1β	Li *et al.*, 2012
	RAW264.7 and HaCaT	LPS, INF-γ, TNF-α (for COX-2)	30 μM	↓COX-2 mRNA; suppressed p38, JNK activation; ↓IL-6	Oh *et al.*, 2013
	SW-1353 human	IL-1β	0.1 $\mu mol \cdot L^{-1}$ 1 $\mu mol \cdot L^{-1}$	↑IL-4; IL-10, IL-6 and TNF-α not affected; ↑IFN-γ (1 $\mu mol\ L^{-1}$) and IL-2 (0.1 $\mu mol\ L^{-1}$); ↓NF-kB (0.1 $\mu mol\ L^{-1}$)	Di Filippo *et al.*, 2012
Xanthophylls: lutein and zeaxanthin	Male finch	PHA	7 $\mu g \cdot mL^{-1}$	21% difference in wing-web-swelling between carotenoid-supplemented and control; Lutein and Zeaxanthin are different in cell-mediated immune response by only 3%	McGraw and Ardia, 2004
Meso-zeaxanthin (MZ)	Balb/c mice	LPS	25 $\mu g \cdot mL^{-1}$	↓iNOS and COX-2 in macrophages; ↓TNF-α, IL-1β and IL-6	Firdous *et al.*, 2015
40% of lutein and 60% of zeaxanthin	Hens/ chicks	LPS	40 $mg \cdot kg^{-1}$	↓mRNA of IFN-γ, IL-6, IL-1β; ↓LITAF; ↑IL-10; IL-4 not affected	Gao *et al.*, 2012

(continues)

Table 2 (Continued)

Carotenoids	*In vitro/in vivo*	Stimulus	Dose	Immunological effects	Ref.
β-carotene	Human	————————	60 mg·day^{-1}	↑T cell CD4; ↑percentage of NK; ↑percentage of cells with markers for activation IL-2 and transferrin	Watson *et al.*, 1991
	Dog	ConA; PWM, PHA	50 mg·day^{-1}	↑IgG, not IgM; ↑CD4; CD8,CD21,MHC II not altered; No change in IL-2	Chew *et al.*, 2000
	Fish	*A. hydrophila* infection	100 mg·kg^{-1}	↑phagocytic activity	Anbazahan *et al.*, 2014
Lutein, β-carotene, astaxanthin	Spleen cells from mice (*in vitro*), *In vivo*	T-dependent (TD) Antigen	10–8 mol/1	Lutein (↑Ab *in vitro*); all 3 carotenoids (↑Ab *in vivo*); astaxanthin (↑IgM more than others)	Jyonouchi *et al.*, 1994
β-carotene	Cow	PHA, ConA, PWM	300 mg for phagocyte; 600 mg for proliferation	↑ Phagocyte effect of neutrophils; ↑lymph proliferation	Michal *et al.*, 1994
β-carotene	Healthy women	PHA	30 mg·day^{-1}	No effect on T lymphocyte proliferative response	Gossage *et al.*, 2000
	Jejunum and ileum of mice after weaning	————————	50 mg·kg^{-1}	↑IgA concentrations in the jejunum; ↑IgA antibody-secreting cells	Nishida *et al.*, 2014
	Aged humans	K562 (target for NKc)	45 mg·day^{-1}	↑34% NKc cytotoxicity; ↑31% total T cells	Wood *et al.*, 2000
	Mouse splenocytes; human peripheral blood lymphocytes	————————	2.5, 5 μg·mL^{-1}	For human:↑ tumour cell lysis For murine Nkc: negative effect on NK cells; ↓lysis of YAC-1 lymphoma cells	Ashfaq *et al.*, 2000
	RAW264 macrophage	LPS; INF-γ	10 μM	↓IL-12p40, IL-6 and IL-1β	Katsuura *et al.*, 2009
β-carotene	Peyer's patch (PP) cells were isolated from mice (*ex vivo*)	ConA	5 mg·kg^{-1}·day^{-1}	Weakly decreased the percentage of T cells; ↑IL-2	Yamaguchi *et al.*, 2010
	Spleen and thymus from C57B/6 mice	PHA, ConA	10^{-8} M	Ab and IL-2 production didn't increase	Jyonouchi *et al.*, 1991
	PBMC	PWM (IgA); TNP-KLH; tetanus toxoid (IgG)	10^{-8} M	No increase in IgM and IgG; ↑IgA	Jyonouchi *et al.*, 1995
	Spleen,thymocytes from BALB/c mice	ConA; LPS (for Ab)	2×10^{-8} to 2×10^{-7} M	↑Thymocytes proliferation; ↑Ab production at 2×10^{-7} M; ↑TNF-α and IL-1α	Okai and Higashi-Okai, 1996
β-carotene and Lycopene	Mice	————————	300 mg·kg^{-1}	β-carotene: ↑the percentages and total cell amounts of CD3+,CD4+,CD8+ Lycopene: ↑ the numbers of beta cells and T-helper cells (CD4+ total cell numbers), ↑ IgG	Garcia *et al.*, 2003
	PBMC from human	ConA	Tomato juice (330 mL·day^{-1}): 40 mg lycopene and 1.5 mg β-carotene	↑IL-2 and IL-4 cytokines	Watzl *et al.*, 1999

(continues)

Table 2 (Continued)

Carotenoids	*In vitro/in vivo*	Stimulus	Dose	Immunological effects	Ref.
β-cryptoxanthin	Rat	Myxomatosis vaccine	5, 10 mg·kg^{-1}	↑ CD4; No change in CD8; ↑IgM (all doses at 21 day); ↑IgG (10 mg at 14, 21 day; 5 mg at 21 day); ↑IL-4 (5 mg at 21 day; 10 mg at 14, 21 day); No change in IFN-γ	Ghodratizadeh *et al.*, 2014
	RAW264	LPS; IFN-γ	10 μM	mild suppression of IL-12p40; ↓IL-1β and IL-6	Katsuura *et al.*, 2009
	SW-1353 human	IL-1β	1 μmol·L^{-1}	↓IL-10; IL-4 not affected; inhibition of IL-1α; ↓IFN-γ, NF-ı B and IL-2	Di Filippo *et al.*, 2012
Violaxanthin (isolated from *C. ellipsoidea)*	Murine macrophage RAW 264.7	LPS	60 μM (below 100 μM)	↓NO; ↓PGE_2;↓INOS-COX-2 (mRNA); ↓binding to p65 DNA sequences (↓NF-ı B)	Soontornchaiboon *et al.*, 2012
Lycopene and lutein	Human	–	500 mg full weight; (15 mg of carotenoid in corn oil)	Lycopene: ↑HLA-DR, no change in other MHCII molecules. Lutein: no change	Hughes *et al.*, 2000
Carotenoid extract from *Dunaliella salina* algae (α-carotene, β-carotene, lutein and zeaxanthin)	Murine macrophage RAW264.7	LPS	5, 10, 25 μM	Inhibition of NO and PGE_2; at 5 and 10 μM, the algae extract presented a significantly higher inhibitory activity for NO and IL-1β than all-trans-β-carotene; ↓IL-1β , IL-6, TNF-α and NF-kB	Yang *et al.*, 2013
Lycopene	Mice	Ovalbumin (OVA)	4 mg 200·μL^{-1} of water	↓ eosinophils; ↓IL-4, IL-5; IL-13, and IFN-γ not altered	Hazlewood *et al.*, 2011
Lycopene	Mice	The left anterior descending coronary artery (LAD) for post-myocardial infarction (MI) model in mice	10 mg·kg^{-1}·day^{-1}	↓TGF-β1; ↓ caspase 3,8,9 (all in mRNA); ↓TNF-α, NF-kB and IL-1β	He *et al.*, 2015
	HUVECs	LPS	20 μM	↓CD14,TLR4, TNF-α and NF-ı B	Bae and Bae, 2011
	PBMC	K562 cell	5 μM	↑ NKc cytotoxicity and IFN-γ	Li *et al.*, 2014
	PBMC	LPS; PHA	4 μM	↓IL-10, IL-2 and IFN-γ; IL-6, IL-1ra not affected; ↑TNF-α and IL-1β	Bessler *et al.*, 2008
	Adipose tissue from mice; 3 T3-L1 cells; human preadipocytes	TNF-α	2 μM	↓MCP1in adipose tissue from mice and 3 T3-L1; ↓IL-1β, IL-6 and NF-ı B (↓ phosphorylated IKKα/β in 3 T3-L1)	Gouranton *et al.*, 2011
	Human THP-1 macrophage	Cigarette smoke extract (CSE)	2 μM	↓NF-ı B; ↓IL-8; ↓ROS production; ↓NOX-4 expression	Simone *et al.*, 2011
Lycopene	Pancreatic acinar cells from rat	Cerulein, a cholecystokinin (CCK) analogue	5 μmol·l^{-1}	↓ROS; IL-6, NF-kB activation	Kang *et al.*, 2011
	RAW 264.7 macrophage	LPS	0.5–2 μM	↓JNK phosphorylation; no effect on p38 and ERK1/2 phosphorylation; ↓ TNF-α (at	Marcotorchino *et al.*, 2012

(continues)

Table 2 (Continued)

Carotenoids	*In vitro*/*in vivo*	Stimulus	Dose	Immunological effects	Ref.
				1 μM), IL-1β (at 2 μM), NF-kB (at 2 μM), and IL-6 (at 0.5 μM)	
	PBMCs	Concanavalin A, anti-CD3 (for T-cell activation)	1.18–2.93 $\mu g \cdot mL^{-1}$	↓T lymph proliferation; ↓CD69 in T-cell subsets (at 1.18 $\mu g \cdot mL^{-1}$); ↓IL-2	Mills *et al.*, 2012
	RAW264.7	Gliadin in association with IFN-γ	20 μM	↓iNOS and NF-kB; ↓signal transducer and activator of transcription-1α (STAT-1α); ↓COX-2; ↓interferon regulatory factor-1(IRF-1)	De Stefano *et al.*, 2007
	PBMC	LPS; PMA (for INF-γ, IL-2)	0.25 μM	↓IL-10; ↓IL-1ra; no change in IL-1β; ↓IL-2, TNF-α and IFN-γ	Bergman *et al.*, 2010
	RAW 264.7	LPS	10 μM	↓NO; ↓iNOS (at both protein and mRNA levels); COX-2 not affected	Rafi *et al.*, 2007

by modulating the expression of SHP-1. SHP-1 is a PTP acting as a negative regulator of the cytokine signalling pathway (Speranza *et al.*, 2012). The researchers also showed that all three stereoisomers of ASTA, (3S, 3'S)-trans-ASTA, (3R, 3'R)-trans-ASTA and meso-trans-ASTA, at a concentration of 20 $\mu mol \cdot L^{-1}$ significantly increased lymphocyte proliferation, the phagocytic capacity of peritoneal exudates cells and cytotoxic activity of natural killer (NK) cells. Moreover, the 3S, 3'S enantiomer demonstrated higher immunoregulatory activity than the two other enantiomers *in vitro* (Weihong *et al.*, 2016). ASTA, a carotenoid without vitamin A activity, enhanced the production of human immunoglobulin (IgM, IgA and IgG) by peripheral blood mononuclear cells (PBMCs) in response to T-cell-dependent stimuli (polyclonal stimulants) (Jyonouchi *et al.*, 1995). Dietary ASTA has been found to reduce a biomarker of DNA damage and acute phase protein (plasma C-reactive protein) and enhance immune responses (e.g. NK cell cytotoxic activity and total T and beta cell subpopulations) in young healthy women (Park *et al.*, 2010). In addition, dietary ASTA enhanced the levels of IgG, IgM and beta cell population as well as reducing plasma concentrations of C reactive protein and DNA damage in female Beagle dogs (Chew *et al.*, 2011). ASTA was shown to have similar effects in cats, acting as a potent antioxidant and modulating the immune response (Park *et al.*, 2011). ASTA supplementation also improved the immunological dysfunction, suppression of NK cell activity, induced in mice by stress (Kurihara *et al.*, 2002). Furthermore, this carotenoid can increase the salivary IgA response and attenuate muscle damage in young soccer players, thus preventing inflammation induced by severe physical training or conditions of increased oxidative stress (Baralic *et al.*, 2015).

Crocin and crocetin. Crocin and crocetin carotenoids found in saffron have a variety of pharmacological effects. Crocin derived from stigma of *Crocus sativus* has been confirmed as a powerful antioxidant, stronger than α-tocopherol (Bathaie *et al.*, 2014; Bolhassani *et al.*, 2014; Bolhassani, 2015). Indeed, the neuroprotective effects of crocin and crocetin are mainly associated with their antioxidant properties (Ochiai *et al.*, 2007). Crocin increases intracellular glutathione levels and consequently prevents cell death in hypoxic PC12 cells, a cell culture model for brain ischaemia (Ochiai *et al.*, 2007). Crocetin also inhibits mRNA expression of TNF-α, IL-1β and iNOS in the liver and increased overall survival in a haemorrhagic shock model (Yang *et al.*, 2006). Crocin and crocetin effectively inhibit LPS-induced NO release from cultured BV2 mouse brain microglial cells as well as reducing NF-κB activation, the levels of TNF-α and IL-1β cytokines and intracellular ROS. Crocin and crocetin have been shown to mediate neuroprotection by decreasing the production of different neurotoxic molecules from activated microglia induced by IFN-γ and Aβ (Nam *et al.*, 2010).

Curcumin. Curcumin is a safe and potent immunomodulator of the immune system. Curcumin can induce apoptosis specifically in tumour cells but not in primary cultures or non-transformed cells under similar conditions (e.g. incubation with 25 μM curcumin for 24 h). Curcumin

also modulates adaptive immunity by enhancing the proliferation of T-cells (Varalakshmi *et al.*, 2008). These observations were confirmed by a significant increase in macrophage phagocytic activity and the immuno-stimulatory activity in curcumin-treated Balb/c animals (Antony *et al.*, 1999). Curcumin (diferuloylmethane) can inhibit the cellular mitogenic response of epithelial cells induced by *H. pylori* infection. Curcumin prevented IκBα degradation, the activity of IkB kinases a and b (IKKa and b) and NF-κB DNA-binding in a culture of the AGS infected by *H. pylori*. Indeed, a low dose of curcumin (~40 μM) was found to block *H. pylori*-induced NF-κB activation and IL-8 synthesis (Foryst-Ludwig *et al.*, 2004). In another study, curcumin directly decreased the T-cell-dependent inflammatory stress *in vitro* by modulating the activation of $CD4^+$ T-cells at various levels, such as (a) inhibition of CD2/CD3/CD28-initiated $CD4^+$ T cell proliferation; (b) enhancement of CD69, CCR7, L-selectin and TGFβ1 expression; and (c) suppression of IL-12 production in a dose-dependent manner (Kim *et al.*, 2013). In fact, curcumin-induced inhibition of IL-12 production in mouse macrophages stimulated with LPS could mediate several of the biological effects of curcumin, for example, its anti-inflammatory effects in chronic inflammatory diseases. Curcumin may directly modulate the NF-κB-DNA interaction by forming a complex with NF-κB that is unable to bind kB sites (Kang *et al.*, 1999a,b). A similar report also confirmed that pretreatment with curcumin significantly inhibits IL-12 production by mouse macrophages stimulated with either LPS or head-killed *Listeria monocytogenes*; this could have potential as a therapeutic effect of curcumin on Th1-mediated immune diseases. Curcumin probably inhibits IL-12 production in macrophages by down-regulating the activity of NF-κB on the IL-12 p40 gene, known as the highly inducible component of IL-12 (Kang *et al.*, 1999a,b). The production of Th1 cytokines such as IL-2 and IFN-γ was suppressed in macrophages or splenic T lymphocytes pretreated with curcumin (Gao *et al.*, 2004). Dietary curcumin and limonin have been found to suppress $CD4^+$ T-cell proliferation and IL-2 production, and NF-κB p65 nuclear translocation in activated $CD4^+$ T-cells in DO11.10 transgenic mice (Kim *et al.*, 2009). Furthermore, dendritic cells (DCs) treated with curcumin are highly effective at antigen (Ag) capture through a mannose receptor-mediated endocytosis, and this effect of curcumin is dose-dependent manner. Curcumin inhibits LPS-induced MAPK activation and the translocation of NF-κBp65. Moreover, it significantly inhibited CD80, CD86 and major histocompatibility complex (MHC) class II expression, but not MHC class I expression in DCs. Curcumin suppressed the maturation of bone marrow-derived murine DC at concentration of 25 μM (Kim *et al.*, 2005) and other studies have shown that curcumin prevents DC migration and chemokine secretion. Curcumin suppressed migration towards CCL19 and CCL21 in a chemotaxis assay and also reduced the levels of chemokines fractalkine (CX3CL1) and interferon producing factor (IP-10). The function of both fractalkine and IP-10 is to attract inflammatory cells to sites of inflammation (Shirley *et al.*, 2008). Activated astrocytes have been shown to play a dual functional role in CNS inflammatory disorders such as multiple sclerosis. Using a LPS-induced inflammatory model *in vitro*, curcumin decreased the function of an astrocyte cell line (U373-MG) by inhibiting the release of IL-6 and also the activity of the enzyme MMP-9. Indeed, this anti-inflammatory of curcumin on neuroinflammation resulted in CNS repair (Seyedzadeh *et al.*, 2014). Curcumin also inhibited the dimerization of toll-like receptor (TLR) 4, the degradation of IRAK-1 and NF-κB activation induced by LPS in a dose-dependent manner (~20 μM). Curcumin inhibited both MyD88- and TRIF-dependent pathways in LPS-induced TLR4 signalling (Youn *et al.*, 2006). The immunomodulatory properties of curcumin were evaluated in several studies. Generally, curcumin inhibited phytohaemagglutinin-induced T-cell proliferation, IL-2 production, NO generation, LPS-induced NF-κB and augmented NK-cell cytotoxicity. These data suggest curcumin could have a therapeutic effect in Th1-mediated autoimmune disorders, as it has been shown to have potent immunosuppressive properties *in vitro*. Curcumin is an effective scavenger of ROS such as hydroxyl radicals and superoxide anions (Yadav *et al.*, 2005), and it can affect both endoplasmic reticulum (ER) stress and mitochondria functional pathways. Indeed, curcumin-stimulated apoptosis in activated T-cells was shown to be due to intense ER stress (Zheng *et al.*, 2013). Curcumin may improve the therapeutic efficiency of adoptive T-cell therapy (*i.e.* the *ex vivo* expansion and subsequent transfusion of tumour-specific T lymphocytes) to eradicate tumours. Curcumin, when combined with adoptive therapy in male tumour-bearing C57BL/6 mice, was shown to enhance the cytotoxicity of Ag-specific $CD8^+$ T-cells against the tumours by modifying the tumour micro-environment during treatment. T-cell activity was enhanced by combined treatment due to the blockade of various immunosuppressors (e.g. regulatory T-cells, indoleamine 2, 3-dioxygenase, TGF-β) (Chang *et al.*, 2012). Numerous studies have demonstrated that curcumin modulates the proliferation and activation of T-cells in a dose-dependent manner. Indeed, low-dose curcumin augments the proliferation of splenic lymphocytes, whereas high-dose curcumin reduces this effect in mouse model. Curcumin can successfully restore populations of $CD4^+$ and $CD8^+$ cells in the tumour micro-environment and inhibit the depletion of central and/or effector memory T-cells. Curcumin significantly decreases the levels of IL10 and TGF-β and Treg cell population; pretreatment of $CD4^+CD25^+$ Treg cells with curcumin diminished their immunosuppressive activity (Bose *et al.*, 2015). Tumour-derived exosomal proteins are known to suppress IL-2-induced NK-cell activity in breast carcinoma and Zhang *et al.* showed that curcumin increased the proteasomal degradation of these proteins, partially restoring the NK-cell activity against the tumour. Therefore, curcumin is able to target the immune escape strategies that are crucial for the immune responses (Zhang *et al.*, 2007). Furthermore, oral administration of curcumin (50 $mg \cdot kg^{-1}$) suppressed the mast cell-dependent IgE response in Ag-induced local passive cuataneous anaphylaxis (Srivastava *et al.*, 2011).

Lutein (Lu) and β-cryptoxanthin (βCr). Lutein (~30 μM) significantly decreases several skin inflammatory responses,

such as the increased expression of IL-6 from LPS-treated macrophages, up-regulation of COX-2 from IFN-?/TNF-?-treated aneuploid immortal keratinocyte cells (HaCaT cells) and the enhancement of MMP-9 levels (a marker of acute inflammation) in UV-irradiated keratinocytes (Oh *et al.*, 2013). Lutein acts as a strong antioxidant; it reduces oxidative stress induced by benzo(a)pyrene (Vijayapadma *et al.*, 2014), a hypercholestrolaemic diet (Kim *et al.*, 2012), H_2O_2 (Gao *et al.*, 2011) and D-galactose (Mai *et al.*, 2010). Lutein supplements (~20 mg·day^{-1}) resulted in a significant decrease in serum IL-6 and monocyte chemoattractant protein-1 (MCP-1), triglyceride (TG) and LDL in early atherosclerosis for 3 months (Xu *et al.*, 2013). Cocoons from yellow silkworms have been found to be an abundant source of dietary lutein and this silk lutein extract can increase both innate and adaptive immune functions. The silk extract enhanced IL-2 and IFN-γ production, the populations of $CD3^+$ and $CD4^+$ $CD3^+$ cells, antibody production and NK-cell activity in female BALB/c (Promphet *et al.*, 2014). Domestic cats fed 10 mg lutein on weeks 8 and 12 showed a high plasma IgG level and increased percentages of CD4 and CD21 lymphocytes, supporting the immunomodulatory action of lutein (Kim *et al.*, 2000a,b). Dietary lutein also induced both cell-mediated and humoral immune responses (IgG) and enhanced the percentages of cells expressing CD5, CD4, CD8 and MHC II molecules in domestic canines (Kim *et al.*, 2000a,b). Lutein-supplemented birds showed superior efficiency of pigmentation as compared with curcumin-supplemented birds; however, both groups of birds had reduced lipid oxidation in the liver (Rajput *et al.*, 2013). Lutein protected the retina from ischaemic/hypoxic damage by its anti-oxidative, anti-inflammatory and anti-apoptotic activities (Li *et al.*, 2012). Dietary lutein decreased mammary tumour growth and the expression of the anti-apoptotic Bcl-2 gene as well as increasing the mRNA expression of the pro-apoptotic genes (e.g. p53 and Bax) and the Bax: Bcl-2 ratio in tumours (Chew and Park, 2004). Lutein and β-cryptoxanthin may down-regulate factors involved in the inflammation associated with rheumatoid arthritis and osteoarthritis. βCr suppressed the generation of IFN-γ, IL-1α and IL-2 cytokines while Lu increased their levels. Lu increased the levels of IL-4 and IL-10 while βCr decreased their concentrations. NF-κB p50 production was suppressed by both Lu and βCr (Di Filippo *et al.*, 2012). Some of the immunity-related properties of β-cryptoxanthin and lutein were studied on the murine macrophage cell line (RAW264). β-cryptoxanthin (~10 μM) significantly decreased IL-1β mRNA levels, but it did not reduce IL-6 and IL-12 p40 mRNA levels. Lutein did not significantly inhibit the transcription of these three cytokines (Katsuura *et al.*, 2009). β-cryptoxanthin has been shown to increase the blood $CD4^+$ lymphocytes count and serum IgG, IgM and IgA levels (i.e. humoral immunity) in mammals (Ghodratizadeh *et al.*, 2014). Nishi *et al.* reported βCr administration causes an increase in IL-6 levels but does not affect IL-4 and IFN-γ levels, indicating the significant enhancement of immunoglobulin levels and $CD4^+$ cells count (Nishi *et al.*, 2012). Oxygenated carotenoids like βCr can be esterified with various fatty acid chains and the esterified form of this xanthophyll has a higher bioavailability than its free form (Fu *et al.*, 2010). The esterified βCr with a fatty acid chain could be useful to further the immune stimulatory effect of βCr (Ghodratizadeh *et al.*, 2014).

Lycopene. Lycopene suppresses LPS-induced pro-inflammatory responses by different methods: (a) enhancement of vascular barrier integrity, (b) inhibition of barrier permeability and expression of cell adhesion molecules (CAM) and (c) prevention of leukocyte adhesion and transendothelial migration. Indeed, the anti-inflammatory properties of lycopene are mediated by the down-regulation of NF-κB expression and TNF-α production (Bae and Bae, 2011). The expression of TNF-α-induced intercellular adhesion molecule-1 (ICAM-1) in HUVEC was inhibited by lycopene, whereas the expression of COX-2 and platelet-endothelial cell (EC) adhesion molecule was not influenced in HUVECs. Lycopene attenuated the TNF-α-induced IκB phosphorylation, NF-κB expression and NF-κB p65 translocation from cytosol to nucleus, suggesting its anti-inflammatory effect could be used for the prevention of CVDs. Moreover, IFN-γ-induced ICAM-1 expression was not affected by lycopene, indicating that lycopene firstly influences the TNF-α-induced signalling pathway (Hung *et al.*, 2008). Pretreatment of human THP-1 macrophages with lycopene resulted in a significant inhibition of cigarette smoke extract-induced IL-8 expression at both the RNA and protein levels; the molecular mechanism involved in this effect is mediated through NF-κB inactivation. NF-κB inactivation has been associated with an inhibition of redox signalling and activation of PPAR signalling (Simone *et al.*, 2011). Lycopene has a positive effect on NK- cell viability and cytotoxicity at concentration of 5 μM. Its anti-apoptosis effect on NK-cells is associated with a down-regulation of the expression of caspase 3 and 9 genes. In addition, lycopene did not affect the expression of functional receptors on NK cells including NKG2A, NKG2D, NKp30 and NKp44. Treatment with lycopene increased the expression of IFN-γ at both the gene and protein levels after 7 days (Li *et al.*, 2014). Lycopene has been shown to enhance the production of IL-1β and TNF-α in a dose-dependent manner and decrease IL-2, IL-10 and IFN-γ secretion in human PBMCs, whereas the levels of IL-6 and the IL-1 receptor antagonist were not affected. The increased generation of the pro-inflammatory cytokines (IL-1β and TNF-α), as well as the decreased secretion of the anti-inflammatory cytokine (IL-10), indicate that lycopene can enhance inflammatory responses (Bessler *et al.*, 2008). However, lycopene has been shown to reduce the expression of pro-inflammatory cytokines and chemokines (e.g. IL-6, IL-1β and MCP-1) at both the mRNA and protein levels in a variety of adipose tissues and adipocyte models (Gouranton *et al.*, 2011). A high concentration of lycopene (10 μmol·L^{-1}) showed a strong anti-angiogenic effect that may be associated with the up-regulation of IL-12 (~163%) and IFN-γ (~531%) as observed in HUVEC (Huang *et al.*, 2013). Lycopene may be useful for the prevention or treatment of acute pancreatitis by suppressing the activation of NF-κB and the expression of inflammatory cytokines (IL-6), mediated by a decrease in the intracellular levels of ROS in pancreatic acinar cells (Kang *et al.*, 2011). The anti-inflammatory effects of lycopene on RAW 264.7

macrophages were associated with a decrease in LPS-stimulated migration (Marcotorchino *et al.*, 2012). The studies showed that pre-incubation of mouse peritoneal macrophages with lycopene (~1 μM), lutein (~1 μM) and β-carotene (~2 μM) before the addition of LPS caused a synergistic inhibition of NO, PGE_2 and superoxide production, derived from the down-regulation of iNOS, COX-2 and NADPH oxidase at both the mRNA and protein expression levels and synergistic inhibition of TNF-α secretion (Hadad and Levy, 2012). Indeed, lycopene treatment (~10 μM) decreased LPS-induced iNOS protein and NO production (~40%) in RAW 264.7 in a dose-dependent fashion (Rafi *et al.*, 2007). However, supplementation with lycopene has also been shown to reduce allergic inflammation systemically, especially in the lungs, by diminishing the Th2 cytokine responses. Lycopene prevented the infiltration of inflammatory leukocytes (e.g. neutrophils, eosinophils, lymphocytes and macrophages) into bronchoalveolar lavage fluid and this was associated with the suppression of the Th2 transcription factor GATA-3, the cytokine IL-4 and the activity of eosinophil peroxidase and MMP-9 (Lee *et al.*, 2008; Hazlewood *et al.*, 2011). Whereas lycopene has been shown to attenuate inflammation and apoptosis (e.g. reduction of caspase-3, -8 and -9 expression) in post-myocardial infarction remodeling, in a mouse model, by inhibiting the NF-κB signalling pathway (e.g. NF-κB p65 phosphorylation) (He *et al.*, 2015). Lycopene has been found to inhibit LPS-mediated release of high mobility group1 (HMGB1) and HMGB1-mediated pro-inflammatory signalling responses in both primary HUVEC and animals through down-regulation of cell surface expression of CAMs, as well as HMGB1 receptors, TLR-2 and -4, and receptors for advanced glycation end products, and to initiate pro-inflammatory responses in ECs (Lee *et al.*, 2012). Xanthophylls (containing 60% of zeaxanthin and 40% of lutein) have been shown to regulate anti-inflammatory and pro-inflammatory cytokine expression in various tissues of both chicks and hens. Dietary xanthophyll reduces the expression of pro-inflammatory cytokines (e.g. IFN-γ, IL-6, IL-1β and LITAF) in the liver, duodenum and jejunum of hens and increases anti-inflammatory cytokine expression (e.g. IL-4 and IL-10) in the liver, jejunum and ileum (Gao *et al.*, 2012). Furthermore, lycopene decreases peroxynitrite or oxidative stress-induced DNA damage in Chinese hamster lung fibroblasts (Muzandu *et al.*, 2006; Kim, 2011).

Violaxanthin. Violaxanthin of *Chlorella ellipsoidea*, a non-synthetic natural product, has been proposed to be a safe and efficient anti-inflammatory agent and can also function as an adjuvant. The anti-inflammatory activity of violaxanthin may be based on inhibition of LPS-mediated NF-κB p65 subunit translocation into the nucleus and subsequently inactivation of the NF-κB pathways. Violaxanthin (~60 μM) effectively inhibited the production NO in LPS-treated RAW 264.7 macrophages, as well as the expression of iNOS and COX-2, in a concentration-dependent fashion (Soontornchaiboon *et al.*, 2012).

β-carotene. Treatment of human NK-cells with β-carotene at doses ranging from 2 to 200 $ng{\cdot}mL^{-1}$ induces a decrease in the tumorolytic effect *in vitro*; but treatment with β-carotene at doses ranging from 0.1 to 10 $\mu g{\cdot}mL^{-1}$ induced a significant increase in tumorolytic function. However, other experiments have shown that α-tocopherol increases the tumorolytic effect of mouse splenocytes, while β-carotene suppresses this effect. Indeed, β-carotene inhibits the positive effects of α-tocopherol in mice, but the mechanism of this effect is unclear (Ashfaq *et al.*, 2000). The accumulation of β-carotene in murine macrophage cells (RAW264) was effectively related to cellular lipid peroxidation in a dose- and time-dependent manner, indicating the pro-oxidative activity of β-carotene, and the synthesis of glutathione as an intracellular antioxidant. In addition, supplementation with β-carotene suppressed the transcription of IL-1β, IL-6 and IL-12 p40 cytokines (Katsuura *et al.*, 2009). The number of lymphoid cells with surface markers for NK-cells, IL-2 and transferrin receptors (/or the number of Th and T-inducer lymphocytes) has been shown to be increased in PBMCs from individuals supplemented with oral β-carotene (Watson *et al.*, 1991). Also Seifter *et al.* reported that β-carotene stimulates the growth of the thymus gland and increases the number of thymic small lymphocytes (Seifter *et al.*, 1981). Oral administration of three carotenoids, β-carotene, β-cryptoxanthin and lycopene resulted in modulated Th cytokine production in cultured Peyer's patch (PP) cells stimulated with the mitogen concanavalin A. In fact, oral administration of β-carotene at 1 or 5 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ significantly increased IL-2 production in PP cells after 72 h, while the level of IL-4 was not changed. Also, oral administration of β-carotene with capsaicin (both at 5 $mg{\cdot}kg^{-1}$) considerably improved the levels of IFN-γ and IL-5, three times higher than those induced by capsaicin alone (Yamaguchi *et al.*, 2010). The immunomodulating activities of β-carotene and its associated carotenoids, such as canthaxanthin and ASTA, were studied on the proliferation and functions of murine immunocompetent cells *in vitro* cell cultures. The results showed that canthaxanthin, β-carotene and ASTA induce potent, but different, stimulatory effects on the proliferation of thymocytes and spleen cells from BALB/c mice. All three carotenoids significantly increased the release of IL-1 and TNF-α from murine peritoneal adherent cells at certain concentrations. However, the cytokine-inducing activities were higher for ASTA than those for canthaxanthin and β-carotene respectively (Okai and Higashi-Okai, 1996). For the first time, Yang *et al.* showed that the carotenoid extract of *Dunaliella salina* composed of lutein, zeaxanthin α-carotene and β-carotene inhibits the production of NO, PGE_2 and pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) as well as the expression of iNOS and COX-2 in LPS-activated RAW264.7 cells. Indeed, the extract exhibited anti-inflammatory activities through the inhibition of NF-κB activation and JNK phosphorylation (Yang *et al.*, 2013). Meso-Zeaxanthin is also known as a xanthophyll carotenoid with profound antioxidant activity and has an anti-inflammatory effect in the BALB/c mice model similar to the zeaxanthin supplement (Firdous *et al.*, 2015). Other studies have indicated that lutein and β-carotene enhance *in vivo* antibody production against T-dependent Ags, not T-independent Ags in old B6 mice, although at a lower level

than that induced by ASTA. The *in vivo* or *in vitro* generation of antibody against TD Ags was notably lower in old than in young mice (Jyonouchi *et al.*, 1994). Furthermore, supplementation with β-carotene effectively increased the level of mucosal IgA (both the number and concentration of IgA antibody-secreting cells) in the jejunum and ileum of weanling mice. These effects were mainly due to the mRNA expression of retinoid X receptorα, retinoic acid receptors [(RAR)α and (RAR)γ], and retinoic acid (RA)-mediated immune response (Nishida *et al.*, 2014). Selenium enhances the immune function (NK-cell cytotoxicity) and phenotypic expression of T-cell subsets, whereas β-carotene affects only the immune function. Hence, supplemental selenium and β-carotene could affect the immune function in aged subjects by enhancing the total percentage of T-cells and increasing NK-cell activity (Wood *et al.*, 2000). Nevertheless, β-carotene could be considered as a potential anti-inflammatory agent for DNA-virus infections and, especially, for the human herpes simplex virus, due to its ability to inhibit cytokine expression in *Suid herpesvirus*-induced inflammation via NF-κB inactivation (Lin *et al.*, 2012; Guedes *et al.*, 2014).

Epidemiological studies on carotenoids intake and randomized clinical trials

As carotenoids have been shown to act as antioxidants, the effects of their intake on the risk of developing certain cancer types (e.g. HNC, PCa and colon cancer) and other diseases (e.g. Parkinson's disease) have been extensively studied. Table 3 shows the relationship between intake of carotenoids and prevention of diseases.

Carotenoids and cancer. As is well-known, carotenoid concentrations in blood are biomarkers of fruit and vegetable intake. However, among the six dietary carotenoids, only the intake of β-carotene has been significantly related to a reduced risk of breast cancer (Aune *et al.*, 2012). In contrast, Panic *et al.* showed that there is no significant correlation between the intake of specific carotenoids from dietary sources or combined carotenoids and the risk of colorectal cancer (Panic *et al.*, 2016).In contrast, long-term use of individual β-carotene, retinol and lutein supplements did not show significant preventive effect for lung cancer, especially among smokers (Satia *et al.*, 2009). Also in people with a previous non-melanoma skin cancer, treatment with β-carotene did not reduce the occurrence of novel skin cancers over a 5 year period of treatment with β-carotene (Greenberg *et al.*, 1990). Furthermore, Wang *et al* indicated that dietary β-carotene intake and also its blood levels are not associated with reduced risk of PCa. In contrast, both dietary intake and blood levels of α-carotene and lycopene could decrease the risk of PCa, but not the risk of advanced PCa. In general, α-carotene and lycopene, but not β-carotene, were inversely associated with the risk of PCa (Wang *et al.*, 2015). Moreover, it was suggested that tomatoes can play a modest role in suppressing PCa (Chen *et al.*, 2012). Meta-analysis of four studies in men randomized to receive lycopene did not show any significant decrease in the incidence of benign prostatic hyperplasia (BPH) or PCa. However, two other studies indicated a decrease in prostate-specific antigen levels in men diagnosed with PCa, who had received lycopene. Thus, it is not possible to support the use of lycopene for the prevention or treatment of BPH or PCa, due to the limited number of randomized controlled trials and various qualities of these studies (Ilic and Missob, 2012). Some studies showed that there is a reduced incidence of PCa with higher lycopene intake. Chen *et al.* demonstrated that higher lycopene consumption was linearly accompanied by a reduced risk of PCa with a threshold between 9 and 21 $mg{\cdot}day^{-1}$. In addition, higher levels of circulating lycopene (between 22 and 850 $mg{\cdot}L^{-1}$) significantly decreased the risk of PCa (Chen *et al.*, 2015). Several other findings have supported the hypothesis that lycopene not only enhances the antioxidant responses in prostate cells but is also able to inhibit proliferation, induce apoptosis and decrease the metastatic capacity of PCa cells. However, there is no clear clinical evidence suggesting the use of lycopene in the prevention and/or treatment of PCa. Thus, further studies are required to determine its mechanisms of action in reducing the risk of PCa (Holzapfel *et al.*, 2013). Recently, epidemiological studies were performed to investigate the correlation of the intake of specific carotenoids from dietary sources, with the risk of HNC. The risk reduction of oral cavity cancer and laryngeal cancer associated with β-carotene intake was 46 and 57% respectively. Lycopene and β-cryptoxanthin also decreased the risk of laryngeal cancer. The combination of lycopene, α-carotene and β-cryptoxanthin was associated with at least a 26% reduction in the rate of oral and pharyngeal cancer. These studies showed that dietary carotenoids especially as single nutrients have a protective role against HNC (Leoncini *et al.*, 2015).

Carotenoids and cardiovascular disease. Several clinical trials have shown that carotenoids can reduce the risk of developing. The carotenoids could potentially decrease the risk of CVDs via several mechanisms such as: (a) lowering blood pressure, (b) reducing pro-inflammatory cytokines, (c) decreasing markers of inflammation (e.g. C-reactive protein) and (d) improving insulin sensitivity in liver, muscle and adipose tissues. Furthermore, the expression of specific genes involved in cell metabolism could be modulated by carotenoids (Gammone *et al.*, 2015). There are several major reports about the clinical roles of carotenoids. For example, β-carotene has been shown to have health-enhancing properties including reducing the risk of developing certain diseases. However, it may stimulate disease through the activity of its derivatives that form in the presence of external agents, for example cigarette smoke or ROS generation in the body. An average of 4 years of carotenoids' supplementation showed that the combination of β-carotene and vitamin A had no effect on the risk of death from lung cancer or CVD, and may even have an adverse effect on their incidence (Omenn *et al.*, 1996). The dose-dependent cardioprotective effects and also harmful properties of β-carotene were studied in a rat model. The content of myocardial haem oxygenase 1 (HO1) was significantly elevated at a high dose of β-carotene ($150\ mg{\cdot}kg^{-1}{\cdot}day^{-1}$). It seems that Fe^{2+} generated as a metabolite of HO1 activity may determine the functions of

Table 3

The relationship between carotenoids intake and prevention of diseases

Carotenoid	Disease	Clinical effect	Reference
Dietary carotenoids (combined)	Colorectal cancer	No significant effect	Panic *et al.*, 2016
β-carotene	Prostate cancer	No significant effect	Wang *et al.*, 2015
α-carotene, lycopene	Prostate cancer	Decrease in the risk of prostate cancer, but not at an advanced stage	Wang *et al.*, 2015
Tomatoes rich in carotenoids	Prostate cancer	Modest effect in the prevention of prostate cancer	Chen *et al.*, 2013a,b
Lycopene	Benign prostatic hyperplasia (BPH) or prostate cancer	No significant effect	Ilic and Missob, 2012
Lycopene	Cardiovascular disease	Improvement of endothelial function	Gajendragadkar *et al.*, 2014
Higher doses of lycopene	Prostate cancer	Reduced risk of prostate cancer	Chen *et al.*, 2015
β-carotene	Oral cavity cancer and laryngeal cancer	46 and 57% reduction in the risk of cancers respectively	Leoncini *et al.*, 2015
Lycopene and β-cryptoxanthin	Laryngeal cancer	Reduction in the risk of cancer	Leoncini *et al.*, 2015
Combination of lycopene, α-carotene and β-cryptoxanthin	Oral and pharyngeal cancers	26% reduction in the rate of cancers	Leoncini *et al.*, 2015
Micronutrients (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin and canthaxanthin)	Parkinson's disease	No significant effect	Takeda *et al.*, 2014
Vitamin A or β-carotene supplementation	Maternal or infant mortality in pregnancy	No significant effect on birthweight indicators, preterm birth, stillbirth, miscarriage or fetal loss	Thorne-Lyman and Fawzi, 2012
Vitamin A or β-carotene supplementation	Pregnancy in HIV-positive women	Protective against low birthweight, no significant effects on preterm delivery or small-for-gestational age. Possible harmful effects through increased HIV transmission	Thorne-Lyman and Fawzi, 2012
β-carotene	Non-melanoma skin cancer	No significant effect	Greenberg *et al.*, 1990
β-carotene	Breast cancer	Significant decrease in breast cancer risk	Aune *et al.*, 2012
β-carotene and vitamin A	Cardiovascular disease	No effect and can even stimulate disease	Omenn *et al.*, 1996
Curcumin	Major depressive disorders	Anti-depressant effect	Al-Karawi *et al.*, 2016
Curcumin	Skin diseases	Therapeutic benefits for skin health	Vaughn *et al.*, 2016
Curcumin	Cardiovascular complications	Prevention of ventricular arrhythmias	Wongcharoen and Phrommintikul, 2009
Astaxanthin	Cardiac diseases	Therapeutic role in the management of myocardial injury	Ciccone *et al.*, 2013

β-carotene as an antioxidant or pro-oxidant agent. The high level of this enzyme in response to ischaemia/reperfusion-induced oxidative stress may be a reason for the loss of cardioprotection at the high-dose of β-carotene (Csepanyi *et al.*, 2015). In another study it was found that there was a significant correlation between the consumption of foods rich in β-carotene and a reduced risk of coronary heart disease as compared with β-carotene alone, because foods rich in β-carotene usually possess other antioxidant vitamins and micronutrients effective in preventing diseases (van Poppel, 1996; Kritchevsky, 1999; Tavani and La Vecchia, 1999; Voutilainen *et al.*, 2006). Some studies have suggested that lycopene may also prevent CVD in humans, likely due to significant antioxidant activity *in vitro*.

However, lycopene levels are not lower in smokers than in non-smokers, indicating that its possible preventive activity is not only due to antioxidant properties. In contrast, lycopene may inhibit cholesterol synthesis and enhance LDL degradation. The reports showed that risk of myocardial infarction was decreased in people with higher concentrations of lycopene in adipose tissue (Arab and Steck, 2000). Also lycopene supplementation was found to improve endothelial function in patients suffering from CVD on optimal secondary prevention (Gajendragadkar *et al.*, 2014). It is well-known that lycopene has a low rate of bioavailability. It is incorporated into chylomicrons and other apo-B containing lipoproteins in the blood circulation. The recent studies showed that the accumulation of ROS and NO in oxidative stress may represent a major cause of lycopene depletion in CVD, type 2 diabetes mellitus and ageing; low levels of serum lycopene have been associated with CVD. However, there is a poor relationship between dietary and serum lycopene levels, which is due to the low bioavailability of lycopene from dietary sources (Petyaev, 2016). However, lycopene may improve blood flow, decrease inflammatory responses and induce protective effects against the progression of CVD (Muller *et al.*, 2016). Several epidemiological studies have supported a dose–response relationship between lycopene and CVD, suggesting the role for lycopene in the prevention of CVD (Mordente *et al.*, 2011; Böhm, 2012). Epidemiological reports indicate an inverse relationship between xanthophylls intake and both cataract and AMD suggesting they have a protective role in the eye. Several observational studies also showed that xanthophylls (lutein and zeaxanthin) may reduce the risk of cancer types especially breast and lung cancers, as well as suppressing heart disease and stroke (Ribaya-Mercado and Blumberg, 2004). In contrast, dietary supplementation of long-chain ω-3 polyunsaturated fatty acids or macular xanthophylls as well as daily intake of minerals and vitamins did not decrease the risk of CVD in elderly subjects with AMD (Bonds *et al.*, 2014). Whereas the antioxidant effects of curcumin were shown to attenuate adriamycin-induced cardiotoxicity and also inhibit diabetic cardiovascular complications. The anti-thrombotic, anti-proliferative and anti-inflammatory effects of curcumin as well as decreasing serum cholesterol levels could protect individuals against atherosclerosis. The p300-histone acetyltransferase inhibitory effects of curcumin improve the development of cardiac hypertrophy and heart failure in animal models. In addition, the inflammatory effects of curcumin as well as its role in correcting the Ca^{2+} homeostasis may play a role in the prevention of ventricular arrhythmias (Wongcharoen and Phrommintikul, 2009). Among the carotenoids, ASTA exerts beneficial effects on heart health by different strategies such as: (a) decrease in inflammation associated with atherosclerosis, (b) modification of blood levels of LDL-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) and (c) reduction of apoptosis and macrophage infiltration in vascular lesions resulting in plaque stability by increasing adiponectin. Indeed, ASTA showed therapeutic effects in the management of myocardial injury, oxidized LDL-C, rethrombosis after thrombolysis and other cardiac diseases (e.g. atrial fibrillation). Furthermore, ASTA could regulate macrophage atherogenesis-related functions by suppressing the up-regulation of scavenger receptors, activation of matrix metalloproteinases and expression of pro-inflammatory cytokines (Ciccone *et al.*, 2013).

Other diseases. Takeda *et al.* showed that there is no significant association between micronutrients (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin and canthaxanthin) and the risk of Parkinson's disease (Takeda *et al.*, 2014). Other trials have indicated that vitamin A (VA) or β-carotene supplementation during pregnancy did not have any significant effect on birthweight indicators, preterm birth, stillbirth, miscarriage or fetal loss. Among HIV-positive women, supplementation was protective against low birthweight (<2.5 kg), without significant effects on preterm delivery or small-for-gestational age. VA supplementation could improve haemoglobin levels and decrease anaemia risk ($<110\ g{\cdot}L^{-1}$) during pregnancy. Although, maternal supplementation with VA or β-carotene may show benefits during pregnancy on maternal or infant mortality, it may also trigger harmful effects such as increasing HIV transmission in some individuals (Thorne-Lyman and Fawzi, 2012).

Al-Karawi *et al*. showed that curcumin has anti-depressant effects in patients with major depressive disorders (MDDs). The administration of curcumin induced a significant reduction in the symptoms of depression. Indeed, it had the highest effect in middle-aged patients at higher doses and for a longer duration of administration. Moreover, the administration of novel formulation of curcumin (BCM-95) had no significant effect on depression as compared with the conventional curcumin-piperine formula (Al-Karawi *et al.*, 2016). Turmeric/curcumin products and supplements, both oral and topical, can also provide therapeutic benefits for skin health and improve certain skin diseases. Thus, the development of effective curcumin delivery methods with high bioavailability and solubility would be useful for treatment of skin problems (Vaughn *et al.*, 2016).

Pharmacokinetics, tolerable dose and safety of carotenoids

Carotenoids are absorbed in the body similar to lipids and transported through the lymphatic system into the liver. The absorption of carotenoids depends on dietary ingredients. For instance, a high cholesterol diet increases the absorption of carotenoids, whereas a low-fat diet reduces their absorption (Ambati *et al.*, 2014). All-trans-carotenoids have a better bioavailability than the 9-cis-forms. Elimination of carotenoids takes 5–7 and 2–3 days for β-carotene and lycopene respectively. The bioconversion of β-carotene to retinal is dose-dependent and ranges between 27 and 2% for a 6 and 126 mg dose respectively (Schwedhelm *et al.*, 2003). Pharmacokinetic parameters for trans- and cis-lycopene isomers showed that the formulation was well tolerated with minimal side effects (Gustin *et al.*, 2004). Although, high tissue concentrations of carotenoids showed pro-oxidant activity under certain conditions, there are no known adverse effects at intake levels for dietary or formulated lycopene in healthy population. Therefore, synthetic lycopene, tomato

lycopene extracts and crystallized lycopene extract are recognized as safe for use as an ingredient in a variety of foods (Trumbo, 2005). Lycopene supplementation in men with biochemically relapsed PCa is safe and well tolerated. The plasma levels of lycopene were similar for a wide dose range (15 to 90 mg·day^{-1}) and had plateaued by 3 months (Clark *et al.*, 2006). A physiological pharmacokinetic model was developed to describe the disposition of lycopene, delivered as a tomato beverage formulation in five doses (10, 30, 60, 90 or 120 mg), for a phase I study in healthy male subjects. The absorption rate at the 10 mg dose was significantly higher than at the higher doses; but the amount of lycopene absorbed was not statistically different between doses, suggesting a possible saturation of absorptive mechanisms. Independent of dose, 80% of the subjects absorbed less than 6 mg of lycopene. This may be useful for clinical trials with pharmacological doses of lycopene in cancer prevention, if absorption saturation occurs in the population (Diwadkar-Navsariwala *et al.*, 2003). In clinical trials, the β-carotene supplements were given in the range of 15–50 mg·day^{-1} with good safety in intake level. β-carotene has also been successfully used to treat inherited photosensitivity diseases at a dose of 180 mg·day^{-1} or more, without any adverse effects except for hypercarotenaemia. Toxicity studies in animals showed that β-carotene is not mutagenic, carcinogenic, embryotoxic or teratogenic and did not cause hypervitaminosis A (Bendich, 1988). In several clinical studies, a high incidence of lung cancer was observed after administration of β-carotene in high doses to smokers. As known, carotenoid breakdown products (CBP) including reactive aldehydes, and epoxides are formed during oxidative stress. The lung might be a critical organ in CBP formation. Indeed, the inhibition of mitochondrial respiration in state 3 by CBP was associated with a low content of protein sulfhydryl groups reducing glutathione levels and redox state, and also an elevated accumulation of malondialdehyde. The findings showed possible side effects of β-carotene supplementation in intense oxidative stress induced by CBP indicating a class of lipid oxidation products and unwanted pro-oxidative reactions (Siems *et al.*, 2005).

Curcumin has been shown to protect against hepatic conditions, chronic arsenic exposure and alcohol intoxication. In clinical trials, curcumin has been used either alone or in combination with other agents. Curcumin's pleiotropic activities were derived from its ability to modulate different signalling molecules such as pro-inflammatory cytokines, apoptotic proteins, NF-κB, COX-2, 5-LOX, STAT3, C-reactive protein, PGE_2, prostate-specific Ag, adhesion molecules, phosphorylase kinase, transforming growth factor-β, TG, Endothelin 1 (ET-1), creatinine, HO-1, AST and ALT in human (Gupta *et al.*, 2013). Phase I clinical trials showed that curcumin was safe even at high doses (12 g·day^{-1}) over 3 months but indicated poor bioavailability. Major reasons for the low levels of curcumin in plasma and tissue may be due to poor absorption, rapid metabolism or systemic elimination. Different approaches have been used to improve the bioavailability of curcumin including: (a) the use of adjuvant like piperine interfering with glucuronidation, (b) the use of liposomal curcumin or curcumin nanoparticles, (c) the use of curcumin/phospholipid complex and (d) the use of structural analogues of curcumin (e.g. EF-24). The EF-24 showed a rapid absorption with a high plasma half-life. Despite the lower bioavailability of curcumin, its therapeutic efficiency has been proven in different human diseases including CVDs, diabetes, cancer, arthritis, Crohn's disease and neurological diseases (Anand *et al.*, 2007; Gupta *et al.*, 2013). Crocin could be an effective treatment of patients with MDD without side effects (Talaei *et al.*, 2015). Furthermore, crocin tablets (20 mg) were evaluated for short-term safety and tolerability in healthy volunteers. No major adverse effects were reported during the clinical evaluation (Mohamadpour *et al.*, 2013).

Among several natural carotenoids, ASTA was considered one of the best carotenoids for protection of cells, lipids and membrane lipoproteins against oxidative damage. Its intake includes the following sequential steps: (a) the mixture of ASTA with bile acid after ingestion and generation of micelles in the intestinum tenue; (b) partial absorption of the micelles with ASTA by intestinal mucosal cells; (c) incorporation of ASTA into chylomicra by intestinal mucosal cells; (d) digestion of chylomicra with ASTA by lipoprotein lipase after releasing into the lymph within the systemic circulation; and (e) deletion of chylomicron remnants rapidly by the liver and other tissues. Indeed, ASTA is associated with lipoproteins and is transported into the tissues (Ambati *et al.*, 2014). ASTA was safe, with no side effects when it was used with food. It accumulated in rat tissues, but no toxic effects were observed. After oral administration of ASTA, the levels of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase were significantly increased in animals. However, excessive consumption of ASTA led to yellow to reddish pigmentation of the skin in animal models. On the other hand, the use of 50 mg·kg^{-1} ASTA decreased blood pressure in stroke prone rats and also in hypertensive rats for 5 weeks and 14 days respectively. ASTA also significantly protected animals against naproxen-induced gastric, antral ulcer and suppressed lipid peroxidation levels in gastric mucosa. The higher therapeutic concentrations of ASTA had no adverse effects on platelet, coagulation and fibrinolytic function. The researchers recommended the administration of ASTA (~2–4 mg·day^{-1}) with ω-3 rich seed oils (e.g. walnuts and almonds). In these clinical studies, no side effects were found with higher doses of ASTA (~6 mg·day^{-1}) in adult human subjects (Ambati *et al.*, 2014).

Future directions

Different carotenoids have shown various biochemical and immunological functions. For example, astaxanthin and canthaxanthin carotenoids are known as a neuroprotective agents for neurodegenerative disorders (e.g. Parkinson) due to their anti-oxidative and anti-inflammatory activities, which can enhance the activity of glutathione peroxidase and catalase and reduce the production of IL-1, IL-6 and TNF-α cytokines. Violaxanthin is also an anti-inflammatory agent for therapeutic purposes, due to its ability to inhibit LPS-mediated NF-κB pathways. Lutein can also scavenge ROS generated during the inflammatory process and inhibit TNF-α induction in cultured ECs. In addition, β-carotene blocks nuclear translocation of the NF-κB p65 subunit that is correlated with its inhibitory effect on phosphorylation and degradation of the NF-κB inhibitor. Generally, all carotenoids show antioxidant activities such as quenching free radicals, reducing damage from reactive oxidant species and inhibiting lipid peroxidation. Furthermore, carotenoids can

play important roles in immuno-regulation and immuno-stimulation in vertebrates. However, there are several major issues for the use of dietary carotenoids that should be considered to prevent or treat various disorders including: (a) the efficiency of individual carotenoids may depend on concentrations of other carotenoids. Indeed, carotenoids may interact synergistically and supplementation with a single carotenoid may be ineffective; (b) these compounds are highly sensitive to oxidation, chemical or enzymatic pathways, and consequently, they can be converted to other products, the effects of which are not fully known; (c) different genetic susceptibility can lead to variations in the response to treatment with carotenoids among individuals with similar dietary intakes; and (d) the efficiency of carotenoids is determined in a time- and dose-dependent manner. Finally, the safety, metabolism and molecular biological properties of carotenoids should be elucidated through further studies before they are used to prevent carcinogenesis.

Author contributions

A.M., M.B. and S.S. contributed to the drafting and writing the manuscript. A.B. contributed to the drafting, writing, conception and final approval of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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