

Review

Effects of Flavonoids from Food and Dietary Supplements on Glial and Glioblastoma Multiforme Cells

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Academic Editor: Derek J. McPhee

Received: 31 July 2015 / Accepted: 14 October 2015 / Published: 23 October 2015

Abstract: Quercetin, catechins and proanthocyanidins are flavonoids that are prominently featured in foodstuffs and dietary supplements, and may possess anti-carcinogenic activity. Glioblastoma multiforme is the most dangerous form of glioma, a malignancy of the brain connective tissue. This review assesses molecular structures of these flavonoids, their importance as components of diet and dietary supplements, their bioavailability and ability to cross the blood-brain barrier, their reported beneficial health effects, and their effects on non-malignant glial as well as glioblastoma tumor cells. The reviewed flavonoids appear to protect glial cells via reduction of oxidative stress, while some also attenuate glutamate-induced excitotoxicity and reduce neuroinflammation. Most of the reviewed flavonoids inhibit proliferation of glioblastoma cells and induce their death. Moreover, some of them inhibit pro-oncogene signaling pathways and intensify the effect of conventional anti-cancer therapies. However, most of these anti-glioblastoma effects have only been observed *in vitro* or in animal models. Due to limited ability of the reviewed flavonoids to access the brain, their normal dietary intake is likely insufficient to produce significant anti-cancer effects in this organ, and supplementation is needed.

Keywords: food; dietary supplements; flavonoids; quercetin; catechins; proanthocyanidins; bioavailability; blood-brain barrier; glial cells; glioblastoma multiforme

1. Introduction

Gliomas are malignant tumors of glia, the brain connective tissue. They are ranged from the least severe grade, I, to the most severe grade, IV. Low-grade gliomas (I and II) consist of well-differentiated, slowly proliferating cells while high-grade tumor cells (III and IV) are characterized by their invasiveness and lack of differentiation [1]. Glioblastoma multiforme (GBM) is a type of grade IV glioma notorious for its aggressive clinical manifestation and fatal outcome. Median survival time of GBM patients after diagnosis is only 15 months despite surgical treatment combined with aggressive chemotherapy [2]. In almost all cases, resections fail to remove tumor cells in total, and the cells located in perivascular niches on tumor edges are most likely to be left behind [3]. Many of these cells possess stem-cell like properties such as partial pluripotency, non-differentiated state and self-renewal ability, thus they represent a focal point of new tumor growth [3,4]. Compared with a standard therapy (resection and adjuvant radiotherapy), adjuvant chemotherapy with an alkylating agent temozolomide is unable to extend life for more than two and a half months on average because it is not specifically directed at these stem-like tumor cells [5,6]. GBM tumors readily invade adjacent healthy tissue and metastasize to distant brain regions, but their dissemination outside the brain is usually prevented by the blood-brain barrier (BBB) [7]. Nevertheless, their dissemination within the brain leads to a fatal outcome since it induces brain herniation that disables vital brain centers [8].

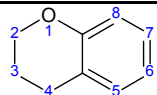
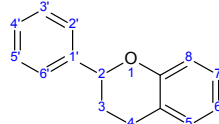
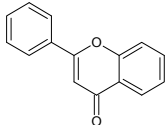
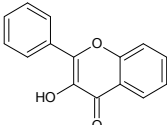
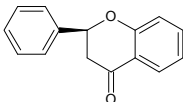
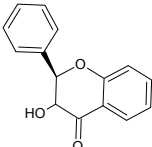
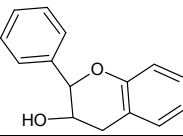
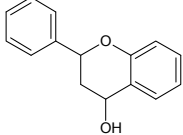
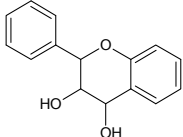
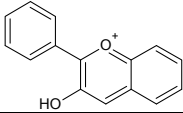
Exposure of the brain to oxidative stress is one of the most important risk factors for GBM [9]. It can be mitigated by intake of antioxidants such as flavonoids, which are a class of polyphenolic compounds synthesized naturally by plants [10]. In developed countries, flavonoids can be found both in normal diet and dietary supplements [11]. The aim of this paper is to review the effects of flavonoids on normal glial and malignant GBM cells, with a focus on flavonoids that frequently appear in food and dietary supplements: quercetin, proanthocyanidins, and catechin derivatives (*i.e.*, catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate). These compounds have been chosen to be reviewed in detail because they are present in common foodstuffs and readily available as supplements.

In the first part of the review, a general molecular structure of flavonoids will be described, followed by their classification into types and assessment of their bioavailability and ability to cross the BBB. In the second part, quercetin, catechins and proanthocyanidins will be reviewed by discussing their prevalence in diet and dietary supplements, reported beneficial effects on health, and effects on glial and GBM cells.

2. Flavonoids

Flavonoids are colored polyphenolic compounds that appear naturally in plants as secondary metabolites. Their main roles in plant physiology are protection against ultraviolet radiation and attraction of pollinating insects [12]. Their molecular structure is characterized by a chromane heterocyclic ring with a phenyl substituent at C-2. Flavonoids are divided into several classes and groups based on substituents at C-3 and C-4, and presence of a double bond between C-2 and C-3 (Table 1). Naturally occurring plant flavonoids often form dimers and oligomers, as well as esters with gallic acid (gallates) or ethers with carbon hydrates (glycosides) [13].

Table 1. Classification and structural formulae of flavonoids.

		Chromane Ring				
		General Structural Formula of Flavonoids (2-Phenylchromane)				
Class	Group	C2-C3 Double Bond	3-OH	4-keto	Structural Formula	Example
Anthoxanthins	Flavones	Yes	No	Yes		Apigenin, luteolin.
	Flavonols	Yes	Yes	Yes		Quercetin, kaempferol
Flavanones	/	No	No	Yes		Naringenin
Flavanonols	/	No	Yes	Yes		Taxifolin
Flavans	Flavan-3-ols	No	Yes	No		Catechin and its derivatives.
	Flavan-4-ols	No	No (4-OH instead)	No		Apiforol
	Flavan-3,4-diols	No	Yes (also 4-OH)	No		Leucocyanidin
	Proanthocyanidins	No	Depending on monomers.	No	Figure 3	Dimers and oligomers of flavanols
Anthocyanidins	/	No (aromatic ring)	Yes	No		Cyanidin

The main absorption site of dietary flavonoids is the small intestine where they mainly appear as glycosides, which are bulky and polar molecules with low permeability coefficients [14]. However, their glycoside moieties may bind to the sodium-glucose transport protein 1 (SGLT 1), which is a type of glucose transporter [15]. Flavonoid glycosides are thus transported in enterocytes where they undergo cleavage of glycoside bond and conjugation with glucuronate, methyl or sulfate groups. Conjugated and unconjugated flavonoids are absorbed from enterocytes into portal circulation. Unconjugated molecules are extensively metabolized during their first pass through the liver, so that the bulk of plasma flavonoids

are conjugates [16]. In enterocytes, flavonoids are also substrates for efflux transporters that transport them back to intestinal lumen [17], further limiting their bioavailability.

Flavonoids can cross the BBB with transcellular diffusion, carrier-mediated transcellular transport or paracellular diffusion through tight junctions between the endothelial cells of the BBB [18]. For both types of diffusion, molecular size is a limiting factor favoring smaller flavonoid molecules such as unconjugated monomers. Passive transcellular diffusion is additionally limited to small molecules with sufficient lipophilicity [19]. Conjugates and oligomers can enter the brain mainly with the help of transporters such as organic anion-transporting polypeptides (OATPs) [20]. However, carrier-mediated transport across the BBB is bidirectional because flavonoids are substrates for P-glycoprotein (P-gp) [21], an efflux transporter that carries substrate molecules from the brain interstitial fluid (ISF) to endothelium [22]. Since all brain regions are not equally perfused with ISF, flavonoids tend to concentrate in more perfused regions [21]. To exert their effects, ISF flavonoids have to enter neurons or glial cells, thus their presence in ISF is not necessarily in correlation with their effects [18]. ISF has a high turnover rate and is continuously secreted across the BBB [20], carrying contained flavonoids with it.

The main systemic action of flavonoids is reduction of oxidative stress. It is mediated by five mechanisms—direct scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS), prevention of Ca^{2+} influx despite increased ROS/RNS levels, increase in the levels of endogenous ROS/RNS scavengers such as glutathione (GSH) [23], alteration of mitochondrial function [24], and decrease in the expression of enzymes involved in ROS/RNS generation such as nitric oxide synthase (NOS) [25]. Reduction of oxidative stress results in neuroprotective and anticarcinogenic activity [24,26]. Antioxidative action of flavonoids supplements that of endogenous ROS scavengers, e.g., ascorbic acid, tocopherols, GSH, thioredoxin and glutaredoxin. ROS are also neutralized by enzymes—catalase, peroxidases and superoxide dismutases (SOD)—which convert them into less reactive compounds. In addition to flavonoids, there are other plant-derived ROS scavengers such as lignin precursors and tannins [27,28].

Other reported beneficial effects of flavonoids include reduction of inflammation due to suppressed expression of cyclooxygenase 2 (COX-2) [25], modulation of efflux proteins such as multidrug resistance-associated proteins (MRPs) [29], inhibition of phase I metabolic enzymes and induction of phase II enzymes [26], inhibition of DNA topoisomerases [30], induction of apoptosis [31], and inhibition of protein kinases involved in proliferative signal transduction [26,32]. Effects of flavonoids on signaling pathways are summarized in Table 2.

Studies in rodents have reported lack of toxicity of flavonoids even in cases of overdose [33,34]. However, flavonoids have a few adverse effects. In high concentrations, flavonoids may promote oxidation even though they otherwise act as antioxidants [35]. If consumed excessively during pregnancy, they may disrupt fetal development, mainly due to their inhibition of DNA topoisomerases, which are highly expressed in fetus [36]. A flavonol myricetin and its 3-galactoside have pronounced cytotoxic effects caused by their inhibition of topoisomerase I [30], while quercetin-mediated inhibition of fetal topoisomerase II results in increased risk of infant leukemia [36]. A flavonon naringenin is highly teratogenic and often lethal for fetus in animal models. Surviving fetuses suffer from developmental retardation and defects of neural tube closure [37]. Other adverse effects of flavonoids include liver damage and contact dermatitis [26]. Flavonoids may also interact with other herbal compounds, as well as with prescribed drugs, altering their bioavailability and therapeutic effectiveness [38].

Table 2. Signaling effects of flavonoids.

		Signaling Pathways (Red = Pathway Inhibition, Green = Pathway Activation)											
Group	Flavonoid	Ras/MAPK	EGF/PI3K/Akt	NF-κB	Wnt/β-catenin	TNFα/NADPH-oxidase	JAK/STAT	Notch	ER	AHR	Nrf2	Ig-E	IRF-1
Flavonols	Quercetin												
	Kaempferol												
	3'-HF												
Flavan-3-ols	Catechin/CG												
	Epicatechin												
	Epicatechin metabolites												
	ECG												
	EGCG												
PACs	Dimeric procyanidins												
	Hexameric procyanidins												
Flavones	Apigenin												
	Luteolin												
	Tangeretin												

References: [39–47]; Abbreviations—Signaling pathways: MAPK = mitogen-activated protein kinase, EGF = epidermal growth factor, PI3K = phosphatidylinositide-3-kinase, Wnt = wingless-related integration site, NF-κB = nuclear factor kappa B, TNFα = tumor necrosis factor alpha, NADPH = nicotinamide adenine dinucleotide phosphate, JAK = Janus kinase, STAT = signal transducer and activator of transcription, ER = estrogen receptor, AHR = aryl hydrocarbon receptor, Nrf2 = nuclear factor 2, Ig-E = immunoglobulin E, IRF-1 = interferon regulatory factor 1; Flavonoids/groups: 3'-HF = 3'-hydroxyflavone, CG = catechin gallate, ECG = epicatechin gallate, EGCG = epigallocatechin gallate, PACs = proanthocyanidins.

3. Quercetin

Quercetin is a member of the class of flavonols (Figure 1), which is characterized by a phenyl substituent at C-2, hydroxyl group at C-3, keto group at C-4 and double bond between C-2 and C-3 [48].

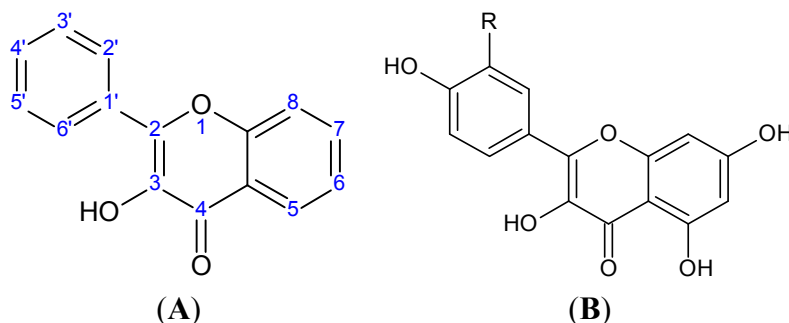


Figure 1. (A) General structural formula of flavonols; (B) structural formula of quercetin (R = OH) and kaempferol (R = H).

Quercetin derivatives are the most abundant flavonoids in Western diet [49] and pure quercetin is also a common ingredient of dietary supplements [50]. Quercetin appears in food mostly in the form of glycoside derivatives such as rutin and quercitrin. Quercetin-containing fruits and vegetables commonly appearing in Western diet are apples, lemons, lettuce, red grapes, cabbage, tomatoes, onions, parsley, pears, plums, cherries, strawberries, blueberries and cranberries, while the richest sources of quercetin are capers, elderberries and lovage leaves [51,52]. Quercetin is also present in black and green tea, red wine, cocoa and olive oil [51]. To achieve quercetin plasma concentration necessary for beneficiary health effects, regular intake of quercetin-rich food is not sufficient [53], thus dietary supplements are needed [54]. Bioavailability of pure quercetin from these supplements is limited by its low solubility in intestinal juice [55], which inhibits its release from a dosage form [56]. On the other hand, larger and more hydrophilic quercetin glycosides are more soluble in intestinal juice but their absorption into enterocytes depends on their carbohydrate moiety. According to Hollman *et al.* [57], after oral administration, the shares of absorbed rutin (quercetin conjugated with the disaccharide rutinose), quercetin glucosides from onions, and quercetin aglycone equal 17%, 52% and 24%, respectively. The difference in absorption of glycosides likely occurs due to SGLT transporters, which facilitate the absorption of glucosides. While its lipophilicity facilitates its diffusion across the BBB, quercetin is also a substrate for P-gp efflux carriers in the BBB [21], which remove it from the brain and lower its net permeability.

Among flavonoids, quercetin is the greatest scavenger of ROS and RNS [58]. Its antioxidant capacity is approximately six times higher than that of ascorbic acid [59]. Its protective effect against oxidative stress results in beneficial effects on the heart and lungs, which help reduce risks of lung cancer [60], asthma [61] and coronary artery diseases [62]. ROS are also involved in promotion of inflammatory processes since they induce the production of cytokines such as tumor necrosis factor alpha (TNF- α) via activation of transcription factors nuclear factor kappa-B (NF- κ B) and activator protein 1 (AP-1) [63,64]. By scavenging ROS, quercetin inhibits cytokine production in the lungs [65], brain [66] and macrophages [67], and therefore reduces inflammation. Studies performed with cell lines have shown that quercetin possesses anti-fibrotic [68], anti-bacterial [69], anti-coagulative [70], anti-atherogenic [71],

anti-hypertensive [72] and anti-proliferative effects [73,74]. However, most of those effects have not yet been confirmed by epidemiological studies [58].

Protective effects of quercetin on glial cells have been extensively studied, mostly by using C6 cells and astrocyte cultures as models for *in vitro* experiments. Exposure of C6 cells to oxidative stress results in lipid peroxidation, glutathione (GSH) depletion, increased breaking of DNA strains, and increased influx of calcium ions. However, these adverse effects are mitigated if cells are treated with quercetin [75]. Anti-inflammatory effects of quercetin on C6 cells have also been reported. In this cell line, quercetin decreases the expression of cytokines TNF α and interleukin (IL) 1 α . Consequent inhibition of inflammatory processes leads to diminished apoptosis of neurons [66]. In addition, quercetin inhibits apoptosis of C6 cells via induction of heme oxygenase 1 (HO-1), a protein belonging to the family of heat shock proteins (HSPs) [76]. Effects of quercetin on HSPs are not uniform and there are cases in which this flavonoid acts as an inhibitor. For instance, in primary culture of astrocytes, quercetin inhibits expression of three HSPs—c-fos protein, HSP70 and glial fibrillary acidic protein (GFAP)—which are involved in injury response. Their expression leads to the formation of an astrocytic scar that replaces injured neurons and represents a hallmark of brain damage [77]. Quercetin may therefore reduce the extent of brain damage caused by trauma. According to Panickar *et al.* [78], in C6 cells quercetin reduces ischemia-induced cell swelling following brain trauma. It also attenuates two other processes that accompany ischemic injury and are implicated in brain edema, namely the increase in intracellular calcium ions and production of free radicals. However, Volk *et al.* [79] reported that quercetin inhibited lactate transport in C6 cells and rat astrocytes, causing a significant accumulation of intracellular lactate, decrease of intracellular pH, and cell swelling due to increased osmotic pressure caused by lactate accumulation.

Despite inhibiting apoptosis of neurons and C6 glial cells, quercetin induces apoptosis in human glioblastoma multiforme T98G cells by activating the mitochondrial death pathway. Exposure of T98G cells to quercetin leads to activation of caspases 3 and 9, release of cytochrome c from the mitochondrion and a decrease in the mitochondrial membrane potential. Quercetin also inhibits heat shock proteins HSP27 and HSP72, which are involved in the mitochondrial apoptotic pathway [80]. Braganhol *et al.* [81] compared quercetin effects in the U138MG glioma cell line and hippocampal organotypic cultures. In U138MG cells, quercetin decreased cell proliferation and viability, induced necrotic and apoptotic cell death, arrested the cell cycle and decreased the mitotic index, while it prolonged the survival of hippocampal cells in the face of ischemic damage. Kim *et al.* [82] reported that treatment of human A172 glioma cells with quercetin caused rapid reduction in phosphorylation of extracellular signal-regulated kinase (ERK) and Akt, resulting in decreased cell viability. Moreover, quercetin increased glioma cell apoptosis by stimulating caspase activity and decreasing expression of survivin, an antiapoptotic protein. In a study by Siegelin *et al.* [83], quercetin exposure resulted in proteasomal degradation of survivin in the A172, U87-MG and U251 cell lines, but not in U373 glioma cells. Quercetin-induced degradation of survivin increased apoptosis mediated by TNF-related apoptosis-inducing ligand (TRAIL). However, Kim *et al.* [84] reported that quercetin-induced apoptosis in U373 cells by increasing the expression of tumor suppressor protein p53, which in turn released cytochrome c from mitochondria to the cytosol, resulting in the activation of the mitochondrial pathway. According to this study, quercetin also induces protective autophagy. This finding has been challenged by Jakubowicz-Gil *et al.* [80], who found no connection between quercetin and autophagy in T98G cells, while in the grade III astrocytoma cell line MOGGCCM quercetin stimulated autophagy only when combined with a low dose of temozolomide [85].

In T98G and U87 glioblastoma cells, quercetin inhibits the release of IL-6, an important cancer-related cytokine, which in turn activates the pro-oncogene STAT3 signaling pathway. In addition, the quercetin-mediated inhibition of IL-6 decreases proliferative and migratory properties of glioblastoma cells. Quercetin also modulates the expression of two target genes regulated by STAT3, *i.e.*, cyclin D1 and matrix metalloproteinase 2 (MMP-2) [86]. Isoquercitrin, a glycosylated derivative of quercetin, inhibits proliferation of glioblastoma cells via reduction in Wnt/ β -catenin signaling activity [87].

Kaempferol is a flavonol closely structurally related to quercetin, from which it is distinguished only by its lack of a hydroxyl group at C-3' (Figure 1). Several mechanisms of its anti-glioma activity have been reported. Kaempferol facilitates apoptosis of glioma cells by reducing levels of anti-apoptotic protein survivin and sensitizing the cells to the effects of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [88]. It also suppresses migration and invasion of GBM8401 glioblastoma cells by blocking pro-oncogene signaling cascades mediated by mitogen-activated protein kinase (MAPK), NF- κ B and Akt [89] (Table 2).

Flavones differ from flavonols by lacking a hydroxyl group at C-3 (Table 1). Luteolin, apigenin and hispidulin are examples of flavones with anti-glioma activity. Luteolin suppresses migration of GBM8401 cells in a manner similar to that of kaempferol, described above [89]. Apigenin triggers apoptosis of human glioblastoma cells T98G and U87MG, without affecting non-malignant human astrocytes [90]. Hispidulin inhibits proliferation of GBM cells by inducing their apoptosis and arresting cell cycles [91].

4. Catechins

Catechins are derivatives of the eponymous flavonoid catechin, which belongs to the class of flavan-3-ols (Figure 2), characterized by a single bond between C-2 and C-3, dihydroxyphenyl substituent at C-2, hydroxyl group at C-3, and lack of a keto group at C-4 [92]. Catechin derivatives commonly found in food and dietary supplements are epicatechin (EC) and epigallocatechin (EGC), as well as their respective esters with gallic acid: epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (Figure 2). Epicatechin is a common name for the two diastereoisomers of catechin, while the name epigallocatechin is used for the diastereoisomers of gallocatechin, a catechin derivative with a trihydroxyphenyl group at C-2. ECG and EGCG have a gallate moiety attached at the C-3 hydroxyl group [93,94]. All the catechin derivatives may appear in food in the form of glycosides [95].

The arguably most important dietary source of catechins is cocoa and its derivatives such as chocolate [96]. Other fruits, vegetables and beverages with high content of catechins are black and green tea, red wine, broad beans, custard apples, strawberry tree fruits, apples, peaches, cherries and plums [97–99]. Isolated green tea catechins are also available as dietary supplements [100]. Pharmacokinetics of catechins is characterized by their low bioavailability, caused by slow absorption, extensive first pass metabolism and wide tissue distribution [101,102]. The most common metabolites of catechins are glucuronate conjugates [103]. However, the both gallates (ECG and EGCG) are less extensively metabolized during their absorption in the systemic circulation, thus they are found in the bloodstream mostly in their original forms [14,104]. In enterocytes, catechins are subjected to transporter-mediated efflux, which additionally lowers their bioavailability [105]. Catechins are able to pass the BBB, but during their transport across endothelial cells composing the BBB, they are subjected to metabolic transformations and efflux [18,106]. Their transport efficiency is therefore low: according to Faria *et al.* [106] who used

RBE4 cells as a BBB model, 28% of blood epicatechin and 15% of blood catechin is transported to the brain, while according to research in rats performed by Wu *et al.* [107], these percentages are 11% and 7% for epicatechin and catechin, respectively. Catechin glucuronides and gallates are also able to pass the BBB, but their transport efficiency is even lower [106,108].

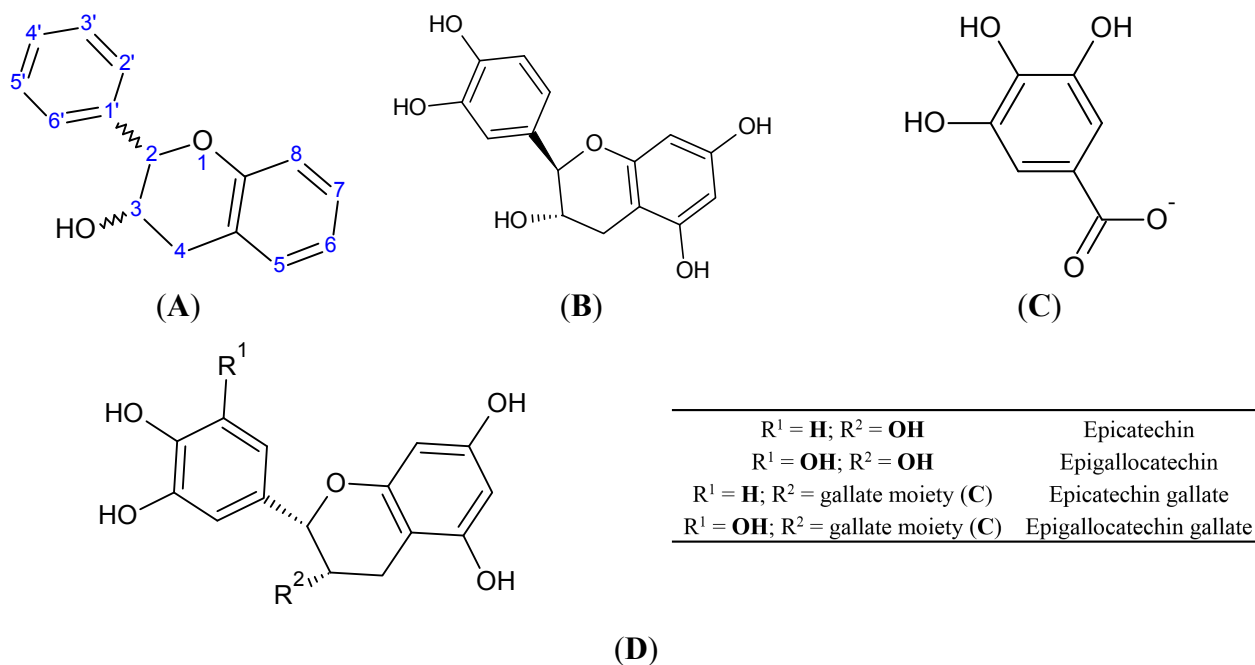


Figure 2. (A) General structural formula of flavan-3-ols; (B) (+)-catechin (2*R*,3*S*), the more common of the two catechin enantiomers; (C) gallate moiety; (D) Substituents of catechin derivatives.

Catechins are powerful antioxidants and scavengers of ROS and RNS [109,110] but low bioavailability limits their protective effects on internal organs [111]. Nevertheless, after the ingestion of food or dietary supplements containing catechins, unmetabolized forms of these compounds reach sufficiently high local concentrations in the digestive tract to protect cells from oxidative stress and malignant transformation [112]. Consequently, regular ingestion of catechins reduces the risk of digestive tract cancers [113–116]. In animal models, catechins also inhibit carcinogenesis of liver [117,118], breast [119,120], prostatic [121] and lung cancers [117,122,123]. In addition, they modulate immune response [124], suppress hyperlipidemia [125], prevent hepatotoxicity [126,127] and nephropathy [128,129], and reduce risks of cardiovascular diseases due to their antithrombotic effects [130].

Catechin derivatives exert anti-inflammatory effects in the brain [131]. In glial cells, catechin and EGCG modulate kinase signaling pathways, e.g., by inhibiting the signaling cascade of mitogen-activated protein kinase (MAPK), which regulates the expression of the pro-inflammatory cytokine TNF- α and the enzyme inducible nitric oxide synthase (iNOS). Consequently, the release of TNF- α and nitric oxide (NO) is reduced, leading to attenuated neuroinflammation [132–134]. Catechin and EGCG also reduce prostaglandin synthesis by inhibiting COX-2 expression [135,136], and suppress the generation of ROS and RNS [137,138], thus providing glial cells with additional protection against inflammation and oxidative stress. Panickar *et al.* [139] reported that in C6 cells, a combination of catechin, epicatechin and EGCG attenuated ischemia-induced astrocyte swelling, which is a major component of cytotoxic

brain edema. However, each of these catechins was unable to reduce the swelling by itself. Beneficial effects of ECG have also been reported. According to Abib *et al.* [140], ECG increased glutamate uptake into C6 cells and thus attenuated the effects of glutamate on neurons. Referred to as excitotoxicity, these effects are implicated in the pathogenesis of ischemia and neurodegenerative diseases. ECG also increased secretion of S100A9, a calcium-binding protein that exerts neurotrophic effects on neighboring neurons, astrocytes and microglia.

EGCG appears to be the only catechin derivative with significant anti-GBM activity. It inhibits telomerase in the cell lines 1321N1 and U87-MG, and therefore increases sensitivity of tumor cells to anti-cancer therapy with cisplatin and tamoxifen [141]. Chen *et al.* [142] reported that EGCG increased sensitivity of the cell lines U87 and U251 to temozolomide, the drug of choice for GBM chemotherapy. In mice with implanted tumors consisting of these cell lines, EGCG decreases the expression of glucose-regulated protein (GRP) 78, an endoplasmic reticulum chaperone that contributes to the cancer cell resistance to temozolomide [142]. Moreover, EGCG induces apoptosis of U251 cells via the laminin receptor, decreases their invasiveness by reducing expression of the matrix metalloproteinases MMP-2 and MMP-9, and inhibits their proliferation by modulating the MAPK pathway [143]. EGCG also decreases the proliferation rate of U87 cells and counters the effects of overexpression of the anti-apoptotic protein survivin, which induces resistance against ionizing radiation [144]. According to Agarwal *et al.* [145], EGCG decreased invasiveness of U87-MG cells, induced their apoptosis and downregulated the levels of pro-inflammatory cytokines. The authors suggested that EGCG induced apoptosis by elevating oxidative stress via ROS generation, but this conclusion contradicts an established view of catechins as ROS scavengers [137]. In human brain microvascular endothelial cells, EGCG intensifies the effects of ionizing radiation. These effects comprise stimulation of cell necrosis and expression of CDK inhibitors p21 and p27. On the other hand, EGCG does not induce the pro-apoptotic proteins caspase-3, caspase-9 and cytochrome C in microvascular endothelial cells [146]. In GBM, these cells are adjacent to perivascular niches, which contain therapy-resistant glioblastoma stem cells [3]. EGCG therefore represents a potential agent for necrosis induction in perivascular niches.

5. Proanthocyanidins

Molecules of flavan-3-ols can form C-C and ether bonds with each other, resulting in the formation of polymers and oligomers collectively referred to as proanthocyanidins [147]. Number of monomeric units represents the degree of polymerization (DP) [148]. Oligomers are characterized by $DP \leq 10$, while polymers have $DP > 10$ [149]. Monomeric units are mainly linked with C-4→C-8 bonds (Figure 3), but the C-4→C-6 linkage also exists. An additional ether bond can be formed between C-2 and the hydroxyl group at C-7, complementing the C-C bond (Figure 3). Flavan-3-ols that figure as monomers in proanthocyanidins are the catechin derivatives discussed in the previous heading, as well as afzelechin and epiafzelechin. The latter two are characterized by a p-hydroxyphenyl substituent at C-2. Polymers and oligomers consisting of exclusively catechin, epicatechin and ECG belong to the subclass of procyanidins, while the proanthocyanidins that also contain galocatechin/EGC/EGCG or afzelechin/epiafzelechin as monomer units are respectively designated as prodelfinidins and propelargonidins. Procyanidins are frequently found in plants while the other two subclasses are less common in nature [148,150]. All

proanthocyanidins can undergo oxidative cleavage, which produces cationic monomers known as anthocyanidins [151].

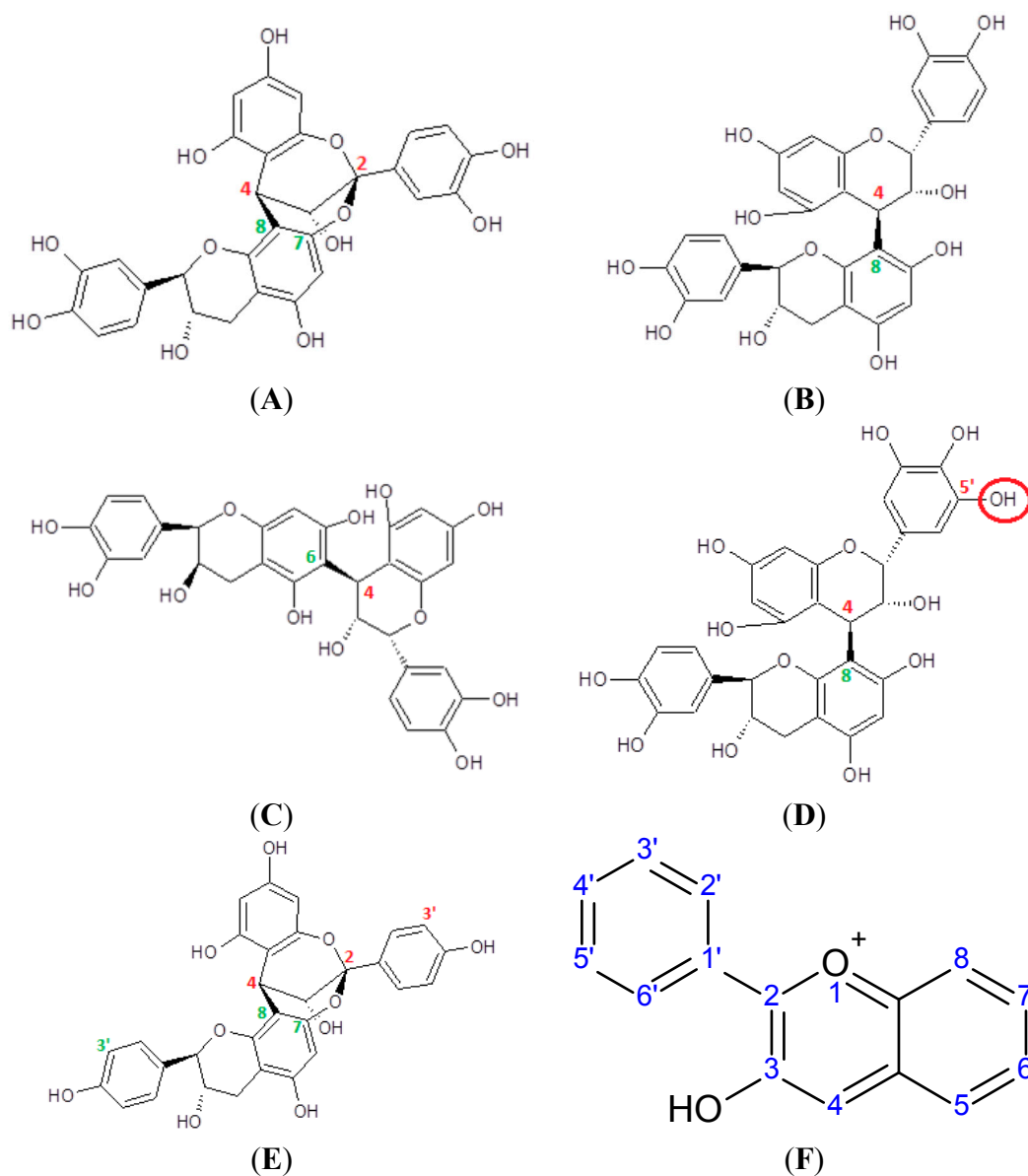


Figure 3. (A) Dimeric A type procyanidin (epicatechin-catechin dimer) with C-4→C-8 linkage and C-2→O→C-7 ether bond. Red numbering: epicatechin moiety; green: catechin moiety; (B) dimeric B type procyanidin (epicatechin-catechin) with C-4→C-8 linkage. Red: epicatechin moiety; green: catechin moiety; (C) dimeric B type procyanidin (epicatechin-epicatechin) with C-4→C-6 linkage; (D) dimeric B type prodelfphinidin (EGC-catechin) with C-4→C-8 linkage. Red: EGC; green: catechin. Additional OH group at 5' is marked; (E) dimeric A type propelargonidin (epiafzelechin-afzelechin) with C-4→C-8 and C-2→O→C-7 bonds. Red: epiafzelechin; green: afzelechin. Note the lack of OH groups at the both 3' positions; (F) general structural formula of anthocyanidins.

Many widely consumed fruits, vegetables and beverages contain both proanthocyanidin oligomers and polymers, for example, apples, grapes, wine, plums, apricots, green pears, peaches, blueberries,

strawberries, black currant, cocoa, several sorts of beans, almonds, hazelnuts and pistachios. In some cases, only oligomers are present, such as in cherries, bananas, mangos, avocados, kiwi fruits, peanuts and beer [149,152]. An extract produced from the bark of maritime pine contains 65%–75% proanthocyanidins, which have their DPs between 2 and 12. This extract is commercially available as a dietary supplement [153,154]. The grape seed extract (GSE) is another example of a supplement rich in proanthocyanidins [155].

Human gastric acid and enzymes cannot metabolize proanthocyanidins into their monomeric units [156], and the bioavailability of unmetabolized oligomers and polymers is very low [14]. However, intestinal bacteria can metabolize some of these compounds. The products of bacterial metabolism are phenolic acids such as phenylacetic, phenylpropionic, phenylvaleric and benzoic acid [157,158], which are absorbed easier than unmetabolized proanthocyanidins [159]. Lactons of phenolic acids can also be formed [160]. According to Deprez *et al.* [161], proanthocyanidin dimers and trimers can be absorbed from the intestinal lumen in a significant extent, likely via paracellular diffusion, while the absorption of polymers is negligible. Absorbed proanthocyanidins are subjected to *O*-methylation by the enzyme catechol-*O*-methyl transferase (COMT) [162]. Enzymes in enterocytes are also likely responsible for cleaving oligomers into their monomeric units, as well as for the conjugation and methylation of resulting monomers [162,163]. However, in a study by Donovan *et al.* [164], no procyanidins were detected in plasma after oral administration of procyanidin dimers to rats. To our best knowledge, there has been only one report about proanthocyanidins being able to cross the BBB [165]. On the other hand, Robert *et al.* [166] reported that proanthocyanidin oligomers decreased the permeability of BBB. Metabolism of proanthocyanidins produces monomeric catechins that are able to penetrate into the brain. Prasain *et al.* [167] conclusively detected catechin and epicatechin in the brain of rats fed with GSE.

The main beneficial health effect of proanthocyanidins is their antioxidative activity [150,168]. Results of human clinical studies [169] show that total plasma antioxidant capacity is increased after oral administration of proanthocyanidins. The increased antioxidant capacity decreases the extent of lipid peroxidation [169] and platelet aggregation [170], and reduces oxidation susceptibility of low density lipoproteins (LDLs) [171]. Proanthocyanidins also attenuate ischemic damage to myocardium [172] by neutralizing free radicals [173] and inhibiting apoptotic processes in cardiomyocytes [174]. Moreover, Ramirez and Roa [175] suggested that proanthocyanidins possessed gastroprotective and anti-ulcerogenic effects. Some proanthocyanidins-rich extracts demonstrate additional health effects; for example, those of cranberry juice prevent adhesion of pathogenic bacteria to epithelial cells in the urinary tract [176,177], those found in cinnamon improve the metabolism of glucose and lipids [178], and those of cocoa inhibit diabetes-induced cataract formation [179]. In rats, GSE proanthocyanidins increase bone formation [180] and exert neuroprotective effects in the brain [181], while in mice they attenuate hyperuremia [182]. Animal models also indicate that GSE proanthocyanidins prevent photocarcinogenesis induced by ultraviolet B (UVB) radiation [183], and may inhibit carcinogenesis of breast and colon cancers [184,185]. A study by Yamagishi *et al.* [186] indicates that the scope of anticarcinogenic effects may not be shared by all proanthocyanidins. This study reported that cocoa proanthocyanidins exerted chemopreventive effects in rat lungs, but not in the digestive tract and kidney.

Few studies have been published about effects of proanthocyanidins on glial cells. One of them [187] deals with the effects of a particular proanthocyanidin trimer isolated from a water soluble cinnamon extract. In C6 cells, this trimer attenuated an ischemia-caused increase in intracellular calcium ions,

which has been linked to cell swelling. The trimer also attenuated post-ischemic excitotoxicity of glutamate by stimulating its uptake into C6 cells. Other relevant studies analyze the effects of GSE. Roychowdhury *et al.* [188] reported that GSE proanthocyanidins improved viability of rat glial cell cultures subjected to oxidative stress caused by hydrogen peroxide. According to these authors, GSE also stimulated iNOS and therefore increased low-level NO production. The latter claim is controversial since monomeric units of GSE proanthocyanidins include catechin, which has been reported to inhibit NO release [138]. Fujishita *et al.* [189] reported that in primary culture of human astrocytes, GSE proanthocyanidins increased production of IL-6, which was released from the astrocytes as a neuroprotective paracrine and prevented the death of adjacent neurons. However, the mechanisms by which IL-6 protects neurons are also responsible for survival of GBM cells [86].

In the U87 glioblastoma cell line, cranberry juice proanthocyanidins arrest cells in G1 phase of the cell cycle and induce cell death within 48 h of exposure. In animal models, they also slow the growth of explant tumors consisting of U87 cells [190]. This cell line was used by Zhang *et al.* [191] to perform experiments on oligomer procyanidins (DP 2–15) from GSE. The oligomers significantly inhibited growth of glioblastoma cells, induced their cell-cycle arrest and a non-apoptotic cell death, and inhibited their chemotaxis, which has been implied in tumor cell invasion and metastasis. The oligomers also inhibited phosphorylations in the pro-oncogene MAPK signaling cascade. In another study [192], the same authors reported that the oligomers induced ROS production in both U87 cells and rat glioma C6 cells. Their conclusion contravenes the findings of other studies [193,194], which reported antioxidative activity of proanthocyanidins. However, Agarwal *et al.* [145] reported a similar pro-oxidative activity in U87-MG cells treated with EGCG, a catechin derivative. Increase in ROS production is a potential mechanism by which proanthocyanidins inhibit proliferation of GBM cells and induce their death.

6. Conclusions

Herbal supplements containing flavonoids have already been introduced into clinical therapy of some non-malignant brain diseases. For instance, *Hypericum perforatum* (Saint John's wort) extract is used for treatment of major depression. It is safer and at least as effective as selective serotonin reuptake inhibitors, which are the drugs of choice for this condition [195]. The major components of hypericum extract are hypericin (a polycyclic aromatic dianthraquinone), hyperforin (a prenylated phloroglucinol derivative) and various flavonoids including quercetin [196]. While hypericin is unable to pass the BBB in monkeys [197], low concentrations of both hyperforin [198] and quercetin [199] have been detected in rodent brains after oral administration of hypericum extract. These results suggest that flavonoids—alongside hyperforin—contribute to the anti-depressant activity of hypericum.

Supplements with flavonoids have also been used for therapy of malignant diseases outside the brain. PC-SPES, a preparation containing extracts of eight herbs mostly used in Chinese and Ayurvedic traditional medicines, has been used as second-line treatment for progressive androgen-independent prostatic cancer, and as an alternative to conventional anti-androgen therapy in case of hormone-responsive prostatic tumors [200,201]. Its major active components are the phytosterols β -sitosterol and stigmasterol which possess estrogenic activity, and the flavonoids baicalin and licochalcone A [200]. Baicalin induces cell cycle arrest and apoptosis of prostatic cancer cells [202], while licochalcone A acts as a phytoestrogen and modulates the expression of the pro-apoptotic protein Bcl-2 [203]. In clinical trials [201], PC-SPES

reduced serum levels of prostate-specific androgen (PSA), a marker of prostatic cancer, in patients with both the androgen-dependent and androgen-independent type of this malignancy, although some patients of the both types subsequently experienced disease progression. In patients with the androgen-dependent type it also reduced testosterone production.

Examples of hypericum and PC-SPES demonstrate that flavonoids can be effectively used in therapy of both brain diseases and cancer, thus their use for GBM prevention and treatment could be envisaged. Reduction of oxidative stress appears to be a common mechanism by which they protect glial cells. Quercetin, catechins and proanthocyanidins all possess antioxidative activity, although proanthocyanidins and EGCG may also act as pro-oxidants. In addition, quercetin and some catechin derivatives, *i.e.*, catechin and EGCG, protect glial cells and adjacent neurons against inflammation, while the catechin derivative ECG and a proanthocyanidin trimer from cranberry juice reduce glutamate-induced excitotoxicity. With respect to GBM cells, most of the discussed flavonoids, *i.e.*, quercetin, EGCG, proanthocyanidins from GSE and cranberry juice, induce the arrest of their cell cycle and eventually their cell death by both apoptotic and non-apoptotic mechanisms. In addition, quercetin and EGCG inhibit the pro-oncogene MAPK signaling pathway, and EGCG also intensifies the effects of both ionizing radiation and chemotherapeutic drugs on GBM cells.

However, most of these effects have been observed only *in vitro* or in animal models, while clinical studies on humans are lacking. The effects observed *in vitro* may not necessarily appear *in vivo* due to low bioavailability of the discussed flavonoids, as well as their limited ability to cross the BBB. The ability of proanthocyanidins to cross the barrier is particularly questionable. Given their limited bioavailability, the intake of flavonoids in normal Western diet is too low to produce brain concentrations in the same range as in the studies that reported their beneficial effects. Therefore, regular use of dietary supplements containing flavonoids is needed to increase systemic and brain concentrations of flavonoids, but even such supplementation may not be sufficient to induce protective and anti-carcinogenic effects.

Acknowledgments

M.V. was supported by a grant for young researchers from the Slovenian Research Agency.

Author Contributions

M.V. did the bibliographic research and wrote the manuscript. D.R. and R.K. chose the topic of the review and proofread the manuscript before submission.

Conflicts of Interest

The authors declare no conflict of interest.

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