Phytochem Rev (2016) 15:591–625
DOI 10.1007/s11101-016-9457-1

The antigenotoxic potential of dietary flavonoids

Vlad Simon Luca · Anca Miron · Ana Clara Aprotosoaie

Received: 13 December 2015 / Accepted: 18 February 2016 / Published online: 2 March 2016

Abstract Human exposure to genotoxic agents has dramatically increased. Both endogenous (reactive species generated during physiological and pathological processes) and exogenous (UV light, ionizing radiation, alkylating agents, antimetabolites and topoisomerase inhibitors, air, water and food pollutants) factors can impair genomic stability. The cumulative DNA damage causes mutations involved in the development of cancer and other disorders (neuromuscular and neurodegenerative diseases, immune deficiencies, infertility, cardiovascular diseases, metabolic syndrome and aging). Dietary flavonoids have protective effects against DNA damage induced by different genotoxic agents such as mycotoxins, food processing-derived contaminants (polycyclic aromatic hydrocarbons, *N*-nitrosamines), cytostatic agents, other medications (estrogenic and androgenic hormones), nicotine, metal ions (Cd^{2+}, Cr^{6+}), radiopharmaceuticals and ionizing radiation. Dietary flavonoids exert their genoprotection by reducing oxidative stress and modulation of enzymes responsible for bioactivation of genotoxic agents and detoxification of their reactive metabolites. Data on structure–activity relationship is sometimes contradictory. Free hydroxyl groups on the B ring (catechol moiety) and C-3 position of the C ring are important structural features for the antigenotoxic activity. As dietary flavonoids are extensively metabolized, more in vivo studies are needed for a better characterization of their antigenotoxic potential.

Keywords Genotoxic damage · Oxidative stress · Quercetin · Epigallocatechin gallate · Cyanidin

Abbreviations

2-AA	2-Aminoanthracene
2-AAAF	2-Acetoxyacetylaminofluorene
4,8-diMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-f] quinoxaline
8-MeIQx	2-Amino-3,8-dimethylimidazo[4,5-f] quinoxaline
^{99m}Tc-MIBI	Technetium-99 m methoxy-isobutyl-isonitrile
AC	Acridine
AFB1	Aflatoxin B1
ARE	Antioxidant responsive element
B(a)P	Benzo(a)pyrene
*t*BHP	*tert*-Butylhydroperoxide
BLM	Bleomycin
CAT	Catalase
COL	Colchicine
CPA	Cyclophosphamide
CYP450	Cytochrome P450
DEB	Diepoxybutane
DEN	Diethylnitrosamine

V. S. Luca · A. Miron (✉) · A. C. Aprotosoaie
Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy Grigore T. Popa-Iasi, Universitatii Str. 16, 700115 Iasi, Romania
e-mail: ancamiron@yahoo.com; anca.miron@umfiasi.ro

DMBA	Dimethylbenz(a)anthracene
DXR	Doxorubicin
ED_{50}	Efficient dose 50
EGCG	Epigallocatechin-3-gallate
EMS	Ethyl methanesulfonate
Endo III	Endonuclease III
EpRE	Electrophile responsive element
EtE	Ethinylestradiol
Fpg	Formamidopyrimidine-N-glycosylase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSC	Glutamylcysteine synthetase
GSH	Reduced glutathione
GST	Glutathione *S*-transferase
GSTalpha2	Glutathione *S*-transferase alpha 2
HO-1	Heme oxygenase 1
IARC	International Agency for Research on Cancer
IQ	2-Amino-3-methylimidazo[4,5-f] quinoline
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
MMC	Mitomycin C
MMS	Methyl methanesulfonate
MNU	Methyl nitrosourea, *N*-nitroso-*N*-methylurea
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NDBA	*N*-Nitrosodibutylamine
NDEA	*N*-Nitrosodiethylamine
NDMA	*N*-Nitrosodimethylamine
NNK	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone
NorA	Norethandrolone
NPIP	*N*-Nitrosopiperidine
NPYR	*N*-Nitrosopyrrolidine
NQO	4-Nitroquinoline 1 oxide
NQO1	NAD(P)H-quinone oxidoreductase 1
Nrf2	Nuclear factor E 2-related factor 2
OTA	Ochratoxin A
OxA	Oxandrolone
PAT	Patulin
PhIP	2-Amino-1-methyl-6-phenylimidazo [4,5-b]pyridine
QR	Quinone reductase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TEAC	Trolox equivalent antioxidative activity test
UDP	Uridyl diphosphate
UGT	UDP-glucuronyltransferase
URE	Urethane

Introduction

Within the human body, DNA is continuously exposed to damaging agents. It is estimated that tens of thousands of DNA lesions occur daily in each cell of the human body (Jackson and Bartek 2009). DNA damage is induced by both endogenous (reactive species produced during physiological and pathological processes) and exogenous agents (UV light, ionizing radiation, anticancer drugs such as alkylating agents, antimetabolites and topoisomerase inhibitors, air, water and food pollutants) (Swift and Golsteyn 2014). To a lesser extent, DNA lesions may occur occasionally during replication or as result of abortive topoisomerase activity (Jackson and Bartek 2009). There are several lesions affecting DNA: base depurination, base deamination, base alkylation, base oxidation, DNA adducts, DNA single- and double-strand breaks, DNA–DNA and DNA–protein cross-links (Swift and Golsteyn 2014). Among the DNA lesions, DNA double-strand breaks are highly cytotoxic (Jackson and Bartek 2009). Cells have developed multiple pathways to detect and repair DNA damage. In case DNA lesions are not properly repaired or are not repaired, they lead to gene mutations and chromosomal aberrations which play an important role in cancer development (Jackson and Bartek 2009; Swift and Golsteyn 2014). DNA damage is also associated with several neuromuscular and neurodegenerative disorders, immune deficiencies, infertility, cardiovascular diseases, metabolic syndrome and aging (Jackson and Bartek 2009).

There are several types of tests to evaluate both genotoxicity and antigenotoxicity. Tests on non-mammalian cells use either prokaryotic (*Salmonella typhimurium*, *Escherichia coli*) or eukaryotic organisms (*Saccharomyces cerevisiae*, *Aspergillus nidulans*). The Ames test, a bacterial reverse mutation

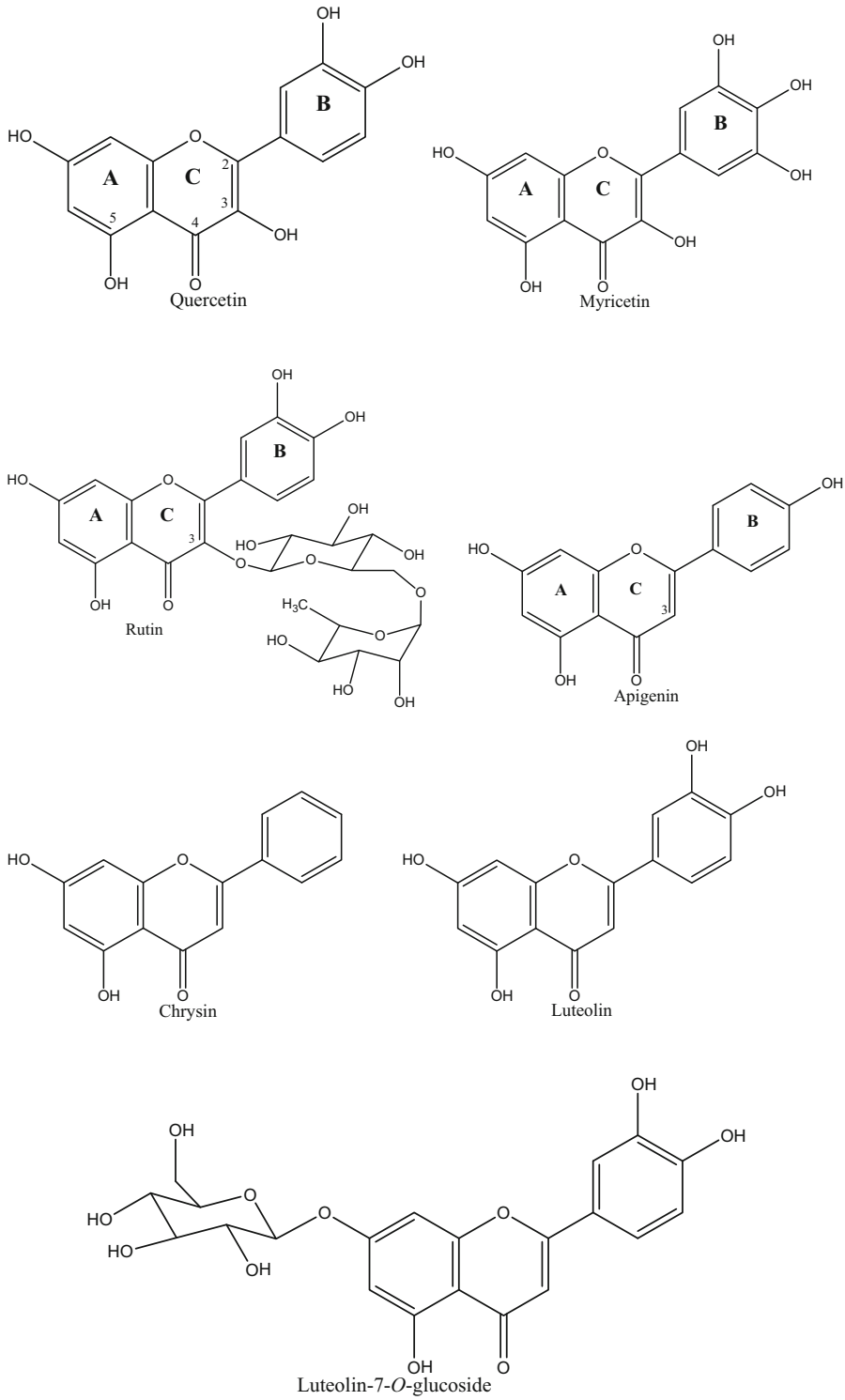
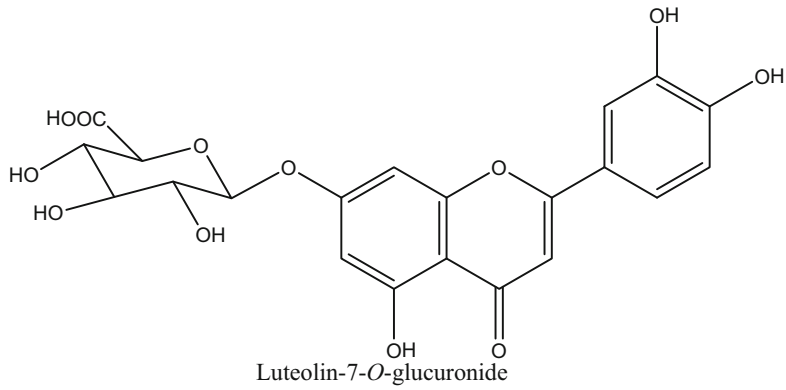


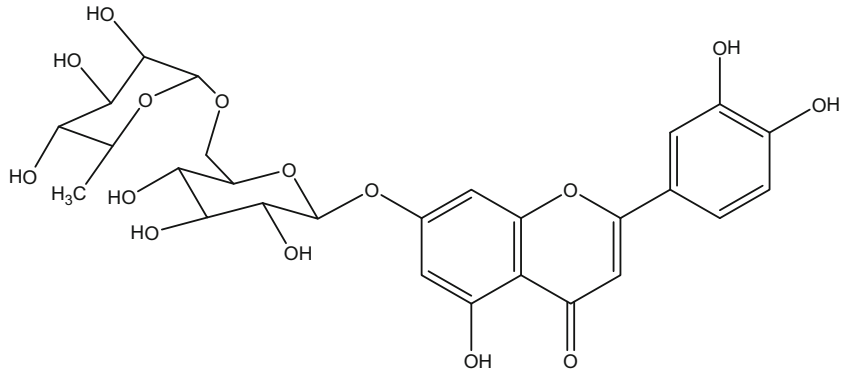
Fig. 1 Dietary flavonoids with antigenotoxic properties

assay, is the most widely used non-mammalian cell model (Ouedraogo et al. 2012; Vedmaurthy et al. 2012). Micronucleus, unscheduled DNA synthesis, sister chromatid exchanges, mouse lymphoma, single-cell gel electrophoresis (comet) and γH2AX assays are other in vitro models used to detect

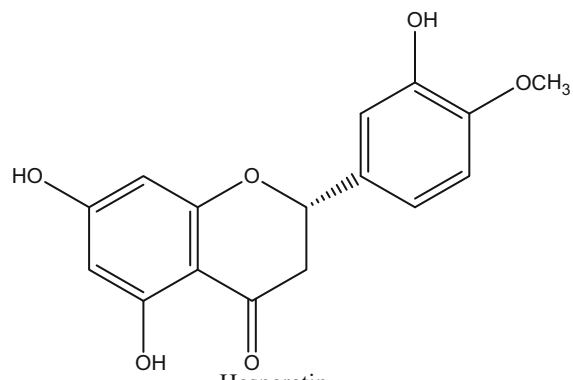
Fig. 1 continued



Luteolin-7-*O*-glucuronide



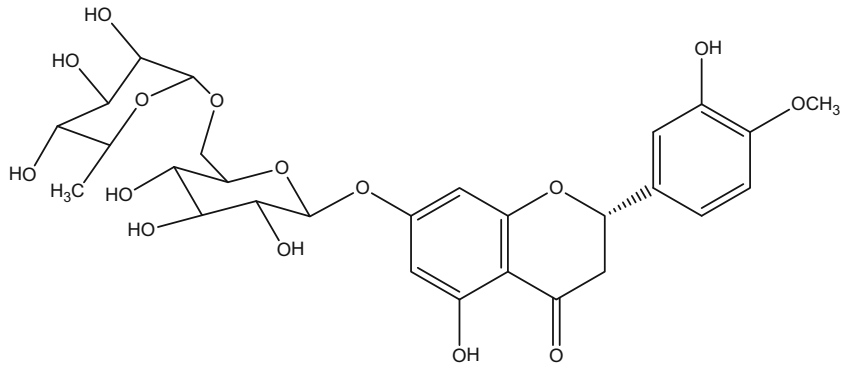
Luteolin-7-*O*-rutinoside



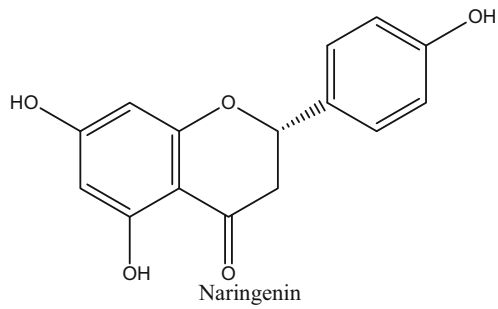
Hesperetin

different types of DNA damage in mammalian cells. Some of these assays may also be performed in vivo as well as the mouse spot, transgenic rodent mutation and somatic mutation and recombination tests. The micronucleus and comet assays are rapid and sensitive methods for assessing DNA alterations: chromosomal aberrations (micronucleus assay) and DNA lesions such as strand breaks, alkali-labile sites, DNA–DNA and DNA–protein crosslinks (comet assay) (Ouedraogo et al. 2012; Patil et al. 2014). Many genotoxic agents such as ochratoxin A (Pfohl-Leszkowicz and Manderville 2012), aflatoxin B1 (Bahari et al. 2014), benzo(a)pyrene (Delgado et al. 2008; Hassan et al. 2011), diethylnitrosamine (Gupta et al. 2010) undergo CYP450-mediated bioactivation. In this regard, cell-based assays can also be performed in the presence of rat liver S9 fraction, containing phase I and phase II metabolizing

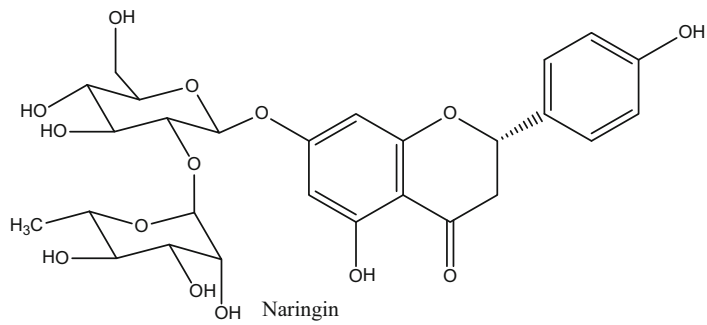
Fig. 1 continued



Hesperidin



Naringenin

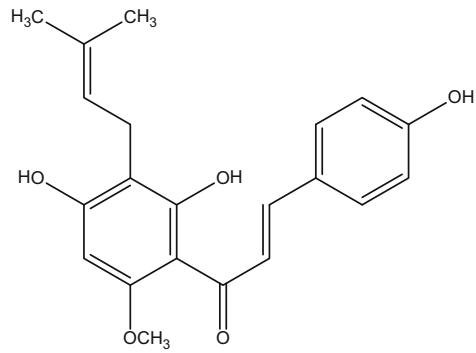


Naringin

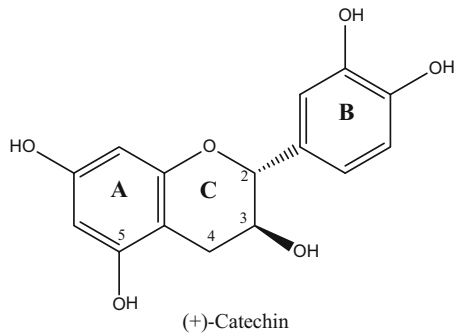
enzymes (metabolic activation) (Duffus et al. 2007; Ouedraogo et al. 2012). Pre-, simultaneous and post-treatment protocols (treatment with antigenotoxic agent before, during and after exposure to the genotoxicant, respectively) are usually used in the antigenotoxic assays. Regarding pre-treatment protocols, antigenotoxic activity is determined due to a direct or indirect inactivation of the genotoxic agent or its products of metabolization. In case of application of a post-treatment protocol, the detection of antigenotoxic activity is based on the up-regulation of mechanisms involved in DNA protection and reversal of genotoxic effects while in the simultaneous treatment all the mechanisms mentioned above might occur (Barcelos et al. 2011).

Human exposure to genotoxic agents has dramatically increased in the last decades. Mycotoxins, nitrosamines, heterocyclic aromatic amines and polycyclic aromatic hydrocarbons in foods, nicotine in tobacco smoke, UV light are only few examples of genotoxic agents people are exposed to every day (Jackson and Bartek 2009; Gupta et al. 2010; Barcelos et al. 2011; Hassan et al. 2011; Ramyaa and Padma 2013). The degree of UV exposure is enhanced due to the depletion of the stratospheric ozone layer (Norval et al. 2011). Besides, cancer

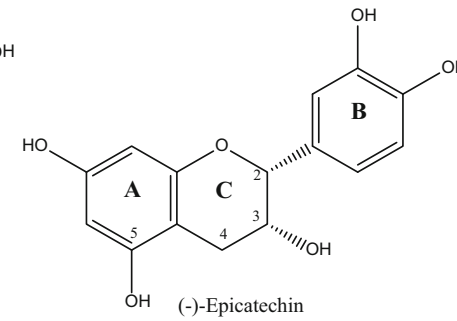
Fig. 1 continued



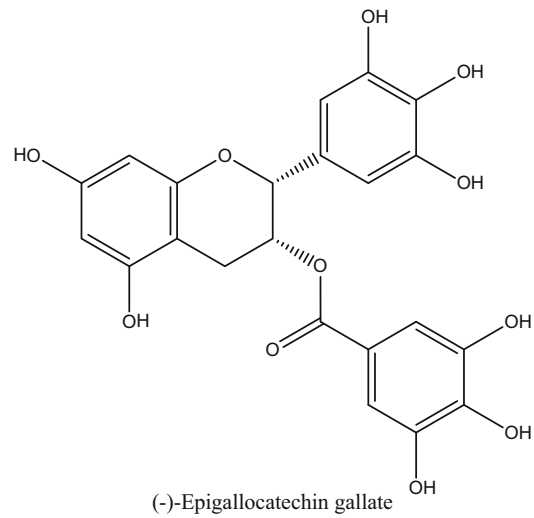
Xanthohumol



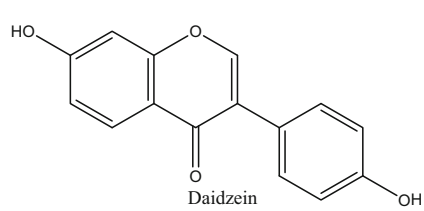
(+)-Catechin



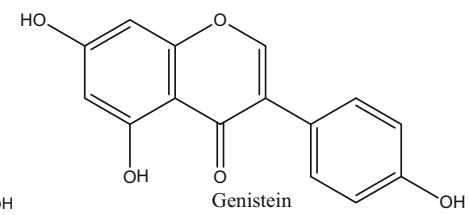
(-)-Epicatechin



(-)-Epigallocatechin gallate

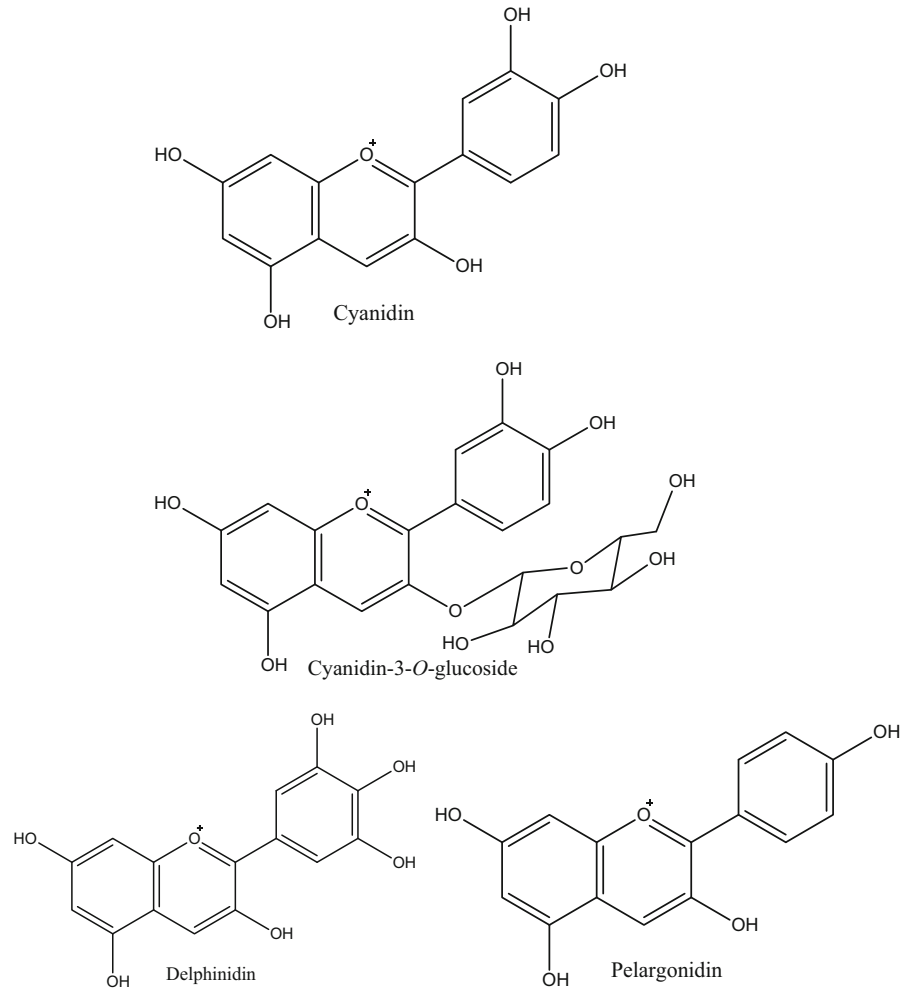


Daidzein



Genistein

Fig. 1 continued



patients undergoing radiotherapy and chemotherapy are overexposed to genotoxic agents (Attia 2012; Patil et al. 2014; Swift and Golsteyn 2014). Therefore, identification of substances, especially in dietary and medicinal plants, that can protect against genotoxic damage, is of great interest. A large number of studies have been performed in this respect and many phytochemicals have been investigated. This review summarizes data on the antigenotoxic potential of major dietary flavonoids. The mechanisms of antigenotoxic activity, structure–activity relationship and in vivo antigenotoxicity with respect to bioavailability are reviewed for different classes of flavonoids.

Flavonols and flavonol glycosides

Quercetin (Fig. 1; Table 1) is ubiquitously present in plant foods (Boots et al. 2008; D'Andrea 2015). The major dietary sources of quercetin are capers, peppers, onions, berries, cherries, apples, grapes, tea and wine (Erlund 2004; D'Andrea 2015). Regarding the mean daily intake of quercetin, the values reported by national cohort assessments vary from <5 to 40 mg (Gupta et al. 2010). Daily values of 200–500 mg can be reached in case of a high intake of fruits and vegetables (D'Andrea 2015). Quercetin has been reported to possess antioxidant (Boots et al. 2008; D'Andrea 2015), anti-inflammatory (Boots

Table 1 The antigenotoxic potential of flavonols and flavonol glycosides

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Quercetin	OTA	CBMN SCGE	HepG2	OTA (10 μM, 48 h); quercetin (10 μM) pre-treatment (24 h)	Reduction of % DNA in tail, OTM and MN frequency	Ramyaa et al. (2014)
		CBMN SCGE	Vero	OTA (10 μM, 24 h); quercetin (10 μM) pre-treatment (24 h)	Reduction of % DNA in tail, OTM and MN frequency	Ramyaa and Padma (2013)
	AFB1	SCGE	HepG2	AFB1 (1 μM, 2 h); quercetin (0.1–5 μg/ml, 2 h) pre-; simultaneous; post-treatment	Reduction of comet score	Barcelos et al. (2011)
		CBMN	Wistar rats	AFB1 (4 mg/kg, i.p.); quercetin treatment (300 mg/kg, i.p., before and simultaneous with AFB1)	Reduction of MN frequency in bone marrow	Kohli et al. (2002)
	B(a)P	^{32}P-postlabelling	HPBLC	B(a)P (1 μM, 18 h); quercetin (1–100 μM) pre-treatment (1 h)	Dose-dependent reduction of BPDE-DNA adduct level	Wilms et al. (2005)
		SCGE	HepG2	B(a)P (50 μM, 24 h); quercetin (1–10 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III sensitive site formation at all tested concentrations and Fpg sensitive site formation at 5–10 μM	Delgado et al. (2008)
	NDMA	SCGE	HepG2	NDMA (27 mM with Endo III or Fpg and 135 mM without enzymes, 24 h); quercetin (0.1–5 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III sensitive site formation	Delgado et al. (2008)
	NPIP	SCGE	HepG2	NPIP (44 mM, 24 h); quercetin (0.1 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III and Fpg sensitive site formation	Delgado et al. (2009)
	NPYR	SCGE	HepG2	NPYR (5 mM with Endo III or Fpg and 50 mM without enzymes, 24 h); quercetin (0.1–5 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III sensitive site formation at 1–5 μM and Fpg sensitive site formation at all tested concentrations	Delgado et al. (2008)
	DXR MMS	SCGE	HepG2	DXR (0.3 μM, 2 h) or MMS (400 μM, 2 h); quercetin (0.1–5 μg/ml, 2 h) pre-; simultaneous; post-treatment	Reduction of comet score	Barcelos et al. (2011)
	MMC	SCGE	HPBLC	MMC (0.1 μg/ml); quercetin (0.03–3 mM) simultaneous treatment (30 min)	Reduction of comet score	Ündĕger et al. (2004)
	H_2O_2	SCGE	Hep G2 Caco-2	H_2O_2 (50 μM, 30 min); quercetin (10–200 μM) pre-treatment (24 h)	Reduction of comet score	Aherne and O'Brien (1999, 2000a)
		SCGE	V79	H_2O_2 (50 μM, 30 min); quercetin (50 μM) pre-treatment (10 h)	Reduction of DNA strand breaks	Aherne and O'Brien (2000a)

Table 1 continued

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Quercetin	H_2O_2	SCGE	HPBLC	H_2O_2 (25 μM, 1 h); quercetin (1–100 μM) pre-treatment (1 h)	Dose-dependent reduction of TM	Wilms et al. (2005)
		SCGE	HPBLC	H_2O_2 (100 μmol/l, 5 min on ice); quercetin (7.6–279.4 μmol/l) pre-treatment (30 min)	Dose-dependent reduction of comet score	Noroozi et al. (1998)
	*t*BHP	SCGE	HepG2	*t*BHP (200 μM, 1 h); quercetin (12.5–50 μM) pre- (24 h); simultaneous treatment (1 h)	Reduction of DNA strand breaks	Ramos et al. (2008)
		SCGE	Caco-2	*t*BHP (50 μM, 30 min); quercetin (50 μM) pre-treatment (24 h)	Reduction of DNA strand breaks	Aherne and O'Brien (2000b)
	Menadione	SCGE	Caco-2	Menadione (10 μM, 30 min); quercetin (50 μM) pre-treatment (24 h)	Reduction of DNA strand breaks	Aherne and O'Brien (2000b)
	DEN	SCGE	SD rats	DEN (200 mg/kg, i.p.); quercetin (10–100 mg/kg, i.p.) post-treatment (5 days)	Dose-dependent reduction of TL, TM and % DNA in tail in hepatocytes	Gupta et al. (2010)
	Nicotine	SCGE	Wistar rats	Nicotine (2.5 mg/kg, p.o., 5 days a week); quercetin (50 mg/kg, p.o.) simultaneous treatment (22 weeks)	Reduction of TL, TM, OTM and % DNA in tail in peripheral blood	Muthukumaran et al. (2008)
	Gamma radiation	CA CBMN SCGE	Swiss albino mice	3 Gy whole-body gamma irradiation; quercetin (20 mg/kg, p.o.) pre-treatment (5 days)	Reduction of % DNA in tail and OTM in blood leukocytes; reduction of CA and MN frequency in bone marrow	Patil et al. (2014)
		SCGE	Murine splenocytes	3 Gy gamma irradiation; quercetin (100 μM) pre-treatment (2 h)	Reduction of TL, TM, OTM and % DNA in tail	Richi et al. (2012)
	Chromium	CBMN	CD-1 mice	CrO_3 (20 mg/kg, i.p.); quercetin (100 mg/kg, i.p.) pre-treatment (4 h)	Reduction of MN frequency in peripheral blood	García-Rodríguez et al. (2014)
	Alloxan	SCGE	CBA inbred mice	Alloxan (75 mg/kg, i.v.); quercetin (50 mg/kg, i.p.) post-treatment (7 days, started 2 days after alloxan injection)	Reduction of TM and TI in lymphocytes	Oršolić et al. (2011)
	NNK	SCGE	A549	NNK (700 μM, 4 h); quercetin (23 μM) pre-treatment (1 h)	Reduction of TM	Yeh et al. (2006)
Myricetin	H_2O_2	SCGE	HepG2 Caco-2	H_2O_2 (50 μM, 30 min); myricetin (10–200 μM) pre-treatment (24 h)	Reduction of comet score	Aherne and O'Brien (1999, 2000a)
		SCGE	HPBLC	H_2O_2 (100 μmol/l, 5 min on ice); myricetin (7.6–279.4 μmol/l) pre-treatment (30 min)	Dose-dependent reduction of comet score	Noroozi et al. (1998)

Table 1 continued

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Myricetin	NPIP	SCGE	HepG2	NPIP (44 mM, 24 h), myricetin (0.1–5 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks at 0.1 μM and Fpg sensitive site formation at 5 μM	Delgado et al. (2009)
	NDBA	SCGE	HepG2	NDBA (3 mM, 24 h); myricetin (0.1 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III and Fpg sensitive site formation	Delgado et al. (2009)
	NPYR	SCGE	HepG2	NPYR (5 mM with Endo III or Fpg and 50 mM without enzymes, 24 h); myricetin (0.1–5 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III sensitive site formation at 1–5 μM and Fpg sensitive site formation at all tested concentrations	Delgado et al. (2008)
	NDMA B(a)P	SCGE	HepG2	NDMA (27 mM with Endo III or Fpg and 135 mM without enzymes, 24 h) or B(a)P (50 μM, 24 h); myricetin (0.1–5 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III sensitive site formation	Delgado et al. (2008)
Rutin	AFB1 DXR MMS	SCGE	HepG2	AFB1 (1 μM, 2 h), DXR (0.3 μM, 2 h) or MMS (400 μM, 2 h); rutin (0.1–5 μg/ml, 2 h) pre-; simultaneous; post-treatment	Reduction of comet score	Barcelos et al. (2011)
	MMC	SCGE	HPBLC	MMC (0.1 μg/ml, 30 min); rutin (0.08–0.82 mM) simultaneous treatment (30 min)	Reduction of comet score	Ündeğer et al. (2004)
	B(a)P	CBMN	HTC	B(a)P (80 μM); rutin (90–810 μM) simultaneous treatment (24 h)	Reduction of MN frequency	Marcarini et al. (2011)
	*t*BHP	SCGE	Caco-2	*t*BHP (50 μM, 30 min); rutin (50 μM) pre-treatment (24 h)	Reduction of DNA strand breaks	Aherne and O'Brien (2000b)
	H_2O_2	SCGE	HepG2 Caco-2	H_2O_2 (50 μM, 30 min); rutin (10–200 μM) pre-treatment (24 h)	Reduction of comet score	Aherne and O'Brien (1999, 2000a)
		SCGE	V79	H_2O_2 (50 μM, 30 min); rutin (50 μM) pre-treatment (10 h)	Reduction of DNA strand breaks	Aherne and O'Brien (2000a)
		SCGE	HPBLC	H_2O_2 (100 μmol/l, 5 min on ice); rutin (7.6–279.4 μmol/l) pre-treatment (30 min)	Dose-dependent reduction of comet score	Noroozi et al. (1998)

Table 1 continued

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
	Gamma radiation	CA CBMN SCGE	Swiss albino mice	3 Gy whole-body gamma irradiation; rutin (10 mg/kg, p. o.) pre-treatment (5 days)	Reduction of % DNA in tail and OTM in blood leucocytes; reduction of CA and MN frequency in bone marrow	Patil et al. (2014)
Rutin	Chromium	CBMN	CD-1 mice	CrO_3 (20 mg/kg, i.p.); rutin (625 mg/kg, i.p., two repetead doses at 24 h interval) pre-treatment (4 h)	Reduction of MN frequency in peripheral blood	García-Rodríguez et al. (2014)
	NNK	SCGE	A549	NNK (700 μM, 4 h); rutin (23 μM) pre-treatment (1 h)	Reduction of TM	Yeh et al. (2006)

A549 human adenocarcinomic alveolar basal epithelial cells, *AFB1* aflatoxin B1, *B(a)P* benzo(a)pyrene, *BPDE* benzo(a)pyrene diolepoxide, *CA* chromosomal aberrations, *Caco-2* human intestinal cells, *CBMN* cytokinesis-block micronucleus, *CrO_3* chromium trioxide, *DEN* diethylnitrosamine, *DNA* deoxyribonucleic acid, *DXR* doxorubicin, *Endo III* endonuclease III, *Fpg* formamidopyrimidine-DNA glycosylase, *H_2O_2* hydrogen peroxide, *HepG2* human hepatocellular liver carcinoma cells, *HPBLC* human peripheral blood lymphocyte cells, *HTC* rat hepatoma cells, *i.p.* intraperitoneal, *MMC* mitomycin C, *MMS* methyl methanesulfonate, *MN* micronuclei, *OTA* Ochratoxin A, *OTM* olive tail moment, *NDBA N*-nitrosodibutylamine, *NDMA N*-nitrosodimethylamine, *NNK* 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *NPIP N*-nitrosopiperidine, *NPYR N*-nitrosopyrrolidine, *p. o.* orally, *SCGE* single cell gel electrophoresis, *SD* Sprague–Dawley, *tBHP tert*-butylhydroperoxide, *TI* tail intensity, *TL* tail length, *TM* tail moment, *V79* Chinese hamster lung fibroblast cells, *Vero* African green monkey kidney cells

et al. 2008; Chirumbolo 2010), antibacterial (Cushnie and Lamb 2005), anticoagulant (Yu et al. 2013), antiatherogenic (Kleemann et al. 2011), antihypertensive (Pérez-Vizcaíno et al. 2009), antifibrotic (Marcolin et al. 2012) and antiproliferative effects (Wu et al. 2011; Delgado et al. 2014).

Antigenotoxic activity of quercetin is based, in part, on its ability to protect against oxidative stress. In cell-free based assays, quercetin showed excellent free radical scavenging and transition metal ion binding activities. Quercetin scavenged both reactive oxygen species (ROS; superoxide anion and hydroxyl radicals, H_2O_2, singlet oxygen and lipid peroxyl radicals) and reactive nitrogen species (RNS; NO and peroxinitrite anion) (Boots et al. 2008; Gupta et al. 2010). In the Trolox equivalent antioxidative activity test (TEAC), quercetin was reported to have an antioxidant activity of 4.7 ± 0.1 mM. Among other phenolic phytochemicals tested in this assay, only epigallocatechin gallate and epicatechin gallate showed higher activity (4.75 ± 0.06 and 4.93 ± 0.02 mM, respectively) (Rice-Evans et al. 1996). The most important structural features for the antiradicalar properties of quercetin are the *o*-diphenolic structure of the B ring, 2,3-double bond in conjunction with 4-keto group in the C ring, both of them facilitating electron delocalization, free 3- and 5-hydroxyl groups in the C and A ring, respectively (Sroka 2005). Quercetin strongly binds transition metal ions (Cu^+, Fe^{2+}). The *o*-diphenolic (catechol) moiety, 3- and 5-hydroxyl groups along with 4-keto group are important for metal chelation. Thus, quercetin suppresses Fe^{2+}-triggered generation of very harmful ROS such as hydroxyl radical and final products of lipid peroxidation [4-hydroxynonenal, malondialdehyde (MDA)] (Guo et al. 2007; Delgado et al. 2009). Quercetin showed significant protective effects against oxidative stress in cell-based and animal models by activating cellular defense systems such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and reduced glutathione (GSH) (Han et al. 2007; Ramyaa and Padma 2013; Ramyaa et al. 2014). Antioxidant-responsive element/electrophile-responsive element (ARE/EpRE) plays a key role in the cellular defense as it mediates the expression of antioxidant and phase II detoxifying enzymes (Kluth et al. 2007; Granado-Serrano et al. 2012). Quercetin has been reported to up-regulate nuclear factor E2-related factor 2 (Nrf2) in human hepatoblastoma HepG2 cells; Nrf2 binds to ARE/EpRE with a subsequent increase in the expression of several enzymes such as NAD(P)H-quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1), GPx, GR and glutamylcysteine synthetase (GSC), the latter being involved in GSH synthesis (Tanigawa et al. 2007; Granado-Serrano et al. 2012). p38 Mitogen-activated protein kinase (p38 MAPK) was found to play a key role in Nrf2/ARE-modulation of GSH-related enzymes (Granado-Serrano et al. 2012).

Quercetin showed antigenotoxic effects in cell-based assays as well as in animal studies. In different cell cultures, quercetin protected against the genotoxicity induced by some food contaminants. Ochratoxin A (OTA) and aflatoxin B1 (AFB1) are very common food-borne mycotoxins with a high carcinogenic potential. According to International Agency for Research on Cancer (IARC), they belong to group 2B (OTA) and group 1 (AFB1) human carcinogens. OTA is produced by several *Aspergillus* and *Penicillium* species while AFB1 is a secondary metabolite of *Aspergillus flavus* and *Aspergillus parasiticus*. They both cause cancer in different organs: OTA in kidney and liver, AFB1 in liver, lungs and colon (Bahari et al. 2014; Ramyaa et al. 2014). Their genotoxic potential is due to both direct and indirect effects. OTA bioactivation products (electrophilic species generated by CYP450 isoenzymes) bind covalently to DNA generating adducts. Additionally, these electrophilic species reduce GSH level and increase ROS production and oxidative DNA damage (Pfohl-Leszkowicz and Manderville 2012). Besides, in Vero cells (normal African green monkey kidney cell line) and HepG2 cells, OTA increased the intracellular level of calcium and down-regulated the expression of Nrf2, transcription factor responsible for the induction of genes encoding antioxidant enzymes. These events led to an increase in ROS followed by oxidative DNA damage (Ramyaa and Padma 2013; Ramyaa et al. 2014). According to Ramyaa and Padma (2013), the protective activity of quercetin against OTA-induced DNA damage is due to its antioxidant potential but also to the ability to bind to DNA thus blocking the reaction between DNA and free radicals. Quercetin also counteracted the effects induced by mycotoxin [increase in the

intracellular calcium level, down-regulation of Nrf2, NO release, ROS generation, elevation of lipid peroxidation, GSH depletion, decrease of antioxidant enzymes such as SOD, CAT, GPx and glutathione-S-transferase (GST)] (Ramyaa and Padma 2013; Ramyaa et al. 2014). AFB1 undergoes CYP-mediated metabolic activation to its epoxide; the *exo*-isomer of AFB1-epoxide leads to DNA damage by irreversible binding to N7-guanine residue. Several CYP isoenzymes (CYP3A4, CYP1A1, CYP1A2, CYP1B1, CYP2A13, CYP2A6) are involved in this activation (Bahari et al. 2014). The metabolic activation of AFB1 is accompanied by a significant production of ROS (H_2O_2, hydroxyl radical) and other secondary free radicals responsible for DNA damage (Towner et al. 2003; Barcelos et al. 2011). Quercetin protected against AFB1-induced DNA damage in both cell- and animal-based studies (Kohli et al. 2002; Barcelos et al. 2011). The antigenotoxic activity of quercetin seems to be based on its antioxidant effects but also on its ability to inhibit CYP450 isoenzymes. Quercetin has been reported to down-regulate several CYP450 isoenzymes, including those involved in the bioactivation of AFB1 such as CYP3A4 (Choi et al. 2011), CYP1A2 (Yeh and Wu 2006), CYP1A1 and CYP1B1 (Choi et al. 2012).

Benzo(a)pyrene (B(a)P), a polycyclic aromatic hydrocarbon found in grilled foods, cigarette smoke but also in automobile and industrial emissions, is a systemic and local carcinogen whose genotoxicity significantly increases by bioactivation (Delgado et al. 2008; Hassan et al. 2011). Different CYP450 isoenzymes (CYP1A1, CYP1A2, CYP1B1) metabolize B(a)P to diol epoxides and ROS that damage DNA by alkylation and oxidation, respectively (Hassan et al. 2011). In cells exposed to B(a)P, quercetin reduced the level of DNA adducts as assessed by ^{32}P-postlabelling assay. In HepG2 cells, the reduction of DNA adducts was found to be related to quercetin ability to inhibit CYP1A1 gene expression with a consequent decrease in B(a)P bioactivation. Other mechanisms (antioxidant activity, inactivation of B(a)P metabolites) have also been suggested for DNA adduct reduction induced by quercetin (Kang et al. 1999; Wilms et al. 2005). Quercetin (pre-treatment followed by simultaneous treatment) attenuated purine and pyrimidine oxidation induced by B(a)P in HepG2 cells (Delgado et al. 2008).

N-Nitrosamines have been detected in a wide variety of foods and smoked tobacco. Their carcinogenic potential substantially increases after CYP450 (CYP2E1, CYP2A6, CYP1A1)-mediated transformation to DNA alkylating reactive metabolites; concomitantly, ROS are generated. The metabolites and ROS are responsible for the generation of alkali labile adducts, abasic sites, DNA strand breaks and oxidative DNA damage. Quercetin attenuated pyrimidine oxidation induced by *N*-nitrosodimethylamine (NDMA) and both purine and pyrimidine oxidation induced by *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR) in HepG2 cells but showed no protection against *N*-nitrosodibutylamine (NDBA)-induced DNA damage. ROS scavenging activity of quercetin and also CYP2E1 inhibition might be involved in DNA protection (Delgado et al. 2008, 2009). Quercetin has been reported to suppress ethanol-stimulated CYP2E1 expression through HO-1 induction (Tang et al. 2013).

In cell-based assays, quercetin reduced the genotoxic effects of other DNA destabilizing agents such as intercalating (doxorubicin) and alkylating (mitomycin C, methyl methanesulfonate) agents (Ündeğer et al. 2004; Barcelos et al. 2011). Both doxorubicin (DXR) and mitomycin C (MMC) are used in cancer chemotherapy (Lenglet and David-Cordonnier 2010) whereas methyl methanesulfonate (MMS) is commonly used in experimental research (Nicolella et al. 2014). DXR (anthracycline antibiotic originally isolated from *Streptomyces pencetius*) induces DNA damage by different mechanisms: intercalation between DNA bases thus interfering with DNA synthesis, inhibition of topoisomerase II with subsequent DNA strand breakages and stimulation of ROS production. DXR generates superoxide anion radicals (reduction catalyzed by NADPH-dependent reductases followed by redox cycling), H_2O_2 and other ROS (iron-mediated redox reactions) (Quiles et al. 2002; Yurtcu et al. 2014). MMC (aziridine antibiotic initially obtained from *Streptomyces caespitosus*) induces significant DNA damage after bioactivation to DNA alkylating and cross-linking metabolites and ROS (superoxide anion and hydroxyl radicals, H_2O_2). The process is mainly catalyzed by NADPH-cytochrome P450 (oxido)reductase (Gustafson and Pritsos 1993; Wang et al. 2007). To our knowledge, the mechanisms by which quercetin protects against the genotoxic effects of DXR and MMC have not

been elucidated yet. However, its antioxidant effects (free radical scavenging, iron chelation) might play a major role.

The involvement of antioxidant activity is supported by the protective effects of quercetin against genotoxic agents that cause major oxidative DNA injury such as H_2O_2 (Noroozi et al. 1998; Aherne and O'Brien 1999), *tert*-butylhydroperoxide (Aherne and O'Brien 2000b; Ramos et al. 2008) and menadione (Aherne and O'Brien 2000b). H_2O_2 damages DNA both directly and indirectly through hydroxyl radicals generated in a Fenton-type reaction (iron-dependent reaction) and causes DNA single-strand breaks, chromosomal aberrations and gene mutations (Aherne and O'Brien 1999). *tert*-Butylhydroperoxide (*t*BHP) decomposes to more reactive species such as H_2O_2, alkoxyl and peroxyl radicals (transition metal catalyzed decomposition), causes GSH depletion and DNA strand breaks. Menadione (vitamin K_3) interacts with thiol groups of essential proteins and undergoes redox-cycling with the generation of ROS (superoxide anion radical, H_2O_2). Ferric ions have been reported to play an important role in menadione genotoxicity (Aherne and O'Brien 2000b). In several cell lines, quercetin reduced the extent of DNA damage induced by H_2O_2, *t*BHP and menadione. This protection may be attributed, at least in part, to ROS scavenging activity of quercetin. Metal chelation might also be involved as transition metal ions play a key role in DNA damage caused by all three genotoxic agents (Noroozi et al. 1998; Aherne and O'Brien 1999, 2000b).

Quercetin acted as a protector against tobacco carcinogens involved in lung cancer development (Yeh et al. 2006; Muthukumaran et al. 2008). 4-(Methylnitrosamino)-l-(3-pyridyl)-l-butanone (NNK), a tobacco specific carcinogen, is bioactivated by CYP450 isoenzymes (CYP1A1, CYP1A2, CYP2B1, CYP2D6, CYP2E1) to reactive metabolites (O6- and N7-methylguanine, O6- and N7-pyridyloxobutylguanine) and ROS which cause DNA damage (DNA adducts, oxidative lesions). Quercetin markedly reduced DNA strand breaks in A549 (human lung cancer) cells exposed to NNK via decreasing ROS level but showed no significant effect on NNK bioactivation (Yeh et al. 2006).

Normally, DNA lesions are detected and repaired. Cells developed several pathways (O^6-methylguanine-DNA methyltransferase, nucleotide excision repair, base excision repair, DNA single and double strand break repair) to repair DNA damage (Iyama and Wilson III 2013). When the repair mechanisms fail and damaged cells divide, genetic alterations, that may promote carcinogenesis, are triggered. The ability of quercetin to enhance DNA repair mechanisms was evaluated in Caco-2 (human colonic adenocarcinoma), HepG2 and V79 (Chinese hamster lung fibroblast) cells exposed to H_2O_2. An enhancement in DNA repair causes a reduction in DNA strand breaks due to strand break rejoining. Surprisingly, no influence on DNA repair mechanisms was detected. In view of these data, it is obvious that quercetin protects against DNA damage but lacks the capacity to enhance DNA repair mechanisms (Aherne and O'Brien 2000a). Further in vitro and in vivo studies are needed to confirm this lack of activity.

The antigenotoxic potential of quercetin has been confirmed in animal models against potent genotoxic agents: diethylnitrosamine (Gupta et al. 2010), nicotine (Muthukumaran et al. 2008), AFB1 (Kohli et al. 2002), alloxan (Oršolić et al. 2011), CrO_3 (García-Rodríguez et al. 2014) and gamma radiation (Patil et al. 2014). There are several sources of exposure to diethylnitrosamine (DEN): food products (especially cheese, soybean, meat and meat products undergoing curing, baking or frying), drinking water having high nitrate levels, tobacco smoke and metabolization of certain drugs (Gupta et al. 2010). DEN can also be generated from protein-rich foods in the acidic medium of the stomach (Bingül et al. 2013). It is a potent hepatocarcinogen belonging to group 2A human carcinogens (Gupta et al. 2010). DEN is metabolized by CYP450 isoenzymes (CYP2E1) to reactive metabolites which bind covalently to DNA. Additionally, ROS are generated. DEN produces DNA adducts, DNA strand breaks and chromosomal aberrations (Gupta et al. 2010; Ali et al. 2014). In animals exposed to DEN, quercetin (post-treatment) minimized DNA damage in hepatocytes and other liver toxic effects such as decrease in GSH (antioxidant, detoxification agent) and increase in MDA (marker of lipid peroxidation) levels. DEN increased the oxidative stress in vivo and this effect was efficiently counteracted by quercetin. The DNA protective effect might be due to the antioxidant activity of quercetin but also to CYP2E1 inhibition and a consequent reduction in DEN bioactivation (Gupta et al. 2010).

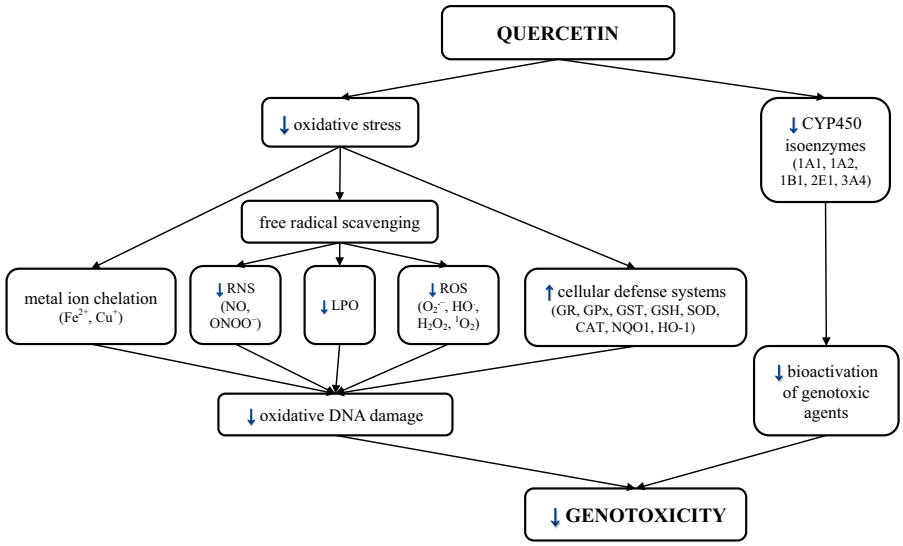
The genotoxicity of nicotine is mainly due to its pro-oxidant effects. Lipid peroxidation products of polyunsaturated fatty acids are important mediators of nicotine genotoxic effects: lipid hydroperoxides, alkoxyl and peroxyl radicals produce DNA strand breaks and guanine hydroxylation, MDA and 2-alkenals cause DNA single-strand breaks, sister chromatid exchanges, chromosome fragmentation and micronuclei; MDA also reacts with DNA to generate adducts. Nicotine exposure results in oxidative stress and severe DNA damage (Burcham 1998; Muthukumaran et al. 2008). The extent of DNA damage in the peripheral blood of rats exposed to nicotine was significantly reduced in case of simultaneous treatment with quercetin. The results of additional biochemical investigations suggested that DNA protective activity of quercetin was based on enhancement of antioxidant defense system (GSH level, SOD, CAT and GPx activities) with a consequent decrease in oxidative stress parameters such as thiobarbituric acid-reacting substances (TBARS), hydroperoxides and NO levels (Muthukumaran et al. 2008).

Cr(VI) is a potent genotoxic agent. Inside cells, Cr (VI) is reduced to Cr(III), the process being accompanied by a high production of free radicals and ROS leading to genetic damage. Pre-treatment with quercetin reduced the frequency of CrO_3-induced micronucleated polychromatic erythrocytes in mouse peripheral blood by 80.9 and 46.3 % at 24 and 48 h, respectively after treatment with CrO_3. Free radical and ROS scavenging and also metal chelation have been proposed as possible mechanisms for quercetin protective effects against genotoxicity of CrO_3 in mice (García-Rodríguez et al. 2014).

Gamma radiation is used for both diagnostic and therapeutic purposes. Gamma rays induce DNA damage through an increase in the production of ROS and lipid peroxidation; ROS react with DNA bases and deoxyribose generating base and deoxyribose radicals and consequently, single- and double-strand breaks (Srinivasan et al. 2007; Hosseinimehr et al. 2009; Patil et al. 2014). Quercetin was efficient in protecting murine splenocytes against gamma radiation-induced DNA damage (Richi et al. 2012). Protective effects against genotoxicity induced by gamma radiation were also detected in in vivo studies. Pre-treatment with quercetin before whole body gamma irradiation of mice significantly decreased the frequency of chromosomal aberrations (dicentrics, dicentrics plus rings), micronucleated polychromatic and normochromatic erythrocytes and DNA breakage in the bone marrow and peripheral blood leukocytes, respectively. The radioprotective activity of quercetin has been ascribed to its capacity to decrease oxidative stress (Patil et al. 2014).

In light of these literature data, it appears that quercetin exerts its genoprotective effect mainly by alleviating oxidative stress and inhibiting enzymes responsible for bioactivation of genotoxic agents (Fig. 2).

Myricetin (Fig. 1; Table 1), flavonol occurring in many vegetables, fruits and beverages (red wine, tea) (Li and Ding 2012; Hobbs et al. 2015), has numerous health benefits as it is endowed with antioxidant, hypoglycemic, anti-platelet, antimicrobial, antiviral and antitumor effects (Li and Ding 2012). Surprisingly, the presence of a third hydroxyl group in the B ring (galloyl moiety) does not improve the antiradicalar properties of myricetin; myricetin is a weaker free radical scavenger than quercetin. This strange behavior of myricetin was attributed to an increased susceptibility to oxidation due to its higher degree of hydroxylation (Rice-Evans et al. 1996; Sroka 2005). Myricetin possesses antigenotoxic effects. In HepG2 cells, there were detected no significant differences in protective effects against H_2O_2-induced DNA damage between myricetin and quercetin (Aherne and O'Brien 1999). In human lymphocytes exposed to H_2O_2, the DNA protective effect of myricetin was lower than that of quercetin ($ED_{50} = 64$ vs. 47 μmol/l) (Noroozi et al. 1998). Apart from quercetin, myricetin protected HepG2 cells against NPIP-induced DNA strand breaks and mitigated the oxidation of purines and pyrimidines caused by NDBA and NPYR but also NPIP-induced purine oxidation and NDMA- and B(a)P-induced pyrimidine oxidation (Delgado et al. 2008, 2009). Studies on myricetin genoprotective activity clearly show the lack of a direct relationship between the free radical scavenging and antigenotoxic effects. Other biological activities (transition metal ion chelation, modulation of enzymes involved in nitrosamine bioactivation and detoxification of reactive metabolites) might be involved in the antigenotoxicity of myricetin. In addition, the different susceptibilities to genetic alterations of cell lines (high sensitivity to H_2O_2 of V79 cells due to low CAT activity and strand break



$^1O_2$ singlet oxygen, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GSH* reduced glutathione, *GST* glutamylcysteine synthetase, H_2O_2 hydrogen peroxide, *HO* hydroxyl radical, *HO-1* heme oxygenase-1, *LPO* lipid peroxidation, *NO* nitric oxide, *NQO1* NAD(P)H- quinone oxidoreductase 1, $O_2^{\cdot-}$ superoxide anion radical, $ONOO^-$ peroxynitrite anion, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *SOD* superoxide dismutase

Fig. 2 Mechanisms of antigenotoxic activity of quercetin

rejoining capacity) must be taken into consideration too (Aherne and O'Brien 1999, 2000a; Delgado et al. 2009). Similar to quercetin, myricetin had no effect on DNA strand break rejoining after H_2O_2 exposure in Caco-2, HepG2 and V79 cells (Aherne and O'Brien 2000a) but induced DNA excision-repair mechanisms in primary rat hepatocytes exposed to ferric nitrilotriacetate (ROS-inducing genotoxic agent) via activation of DNA polymerase beta (Abalea et al. 1999).

Rutin (quercetin-3-rutinoside, rutoside) (Fig. 1; Table 1), one of the most common dietary flavonol glycosides, is present in many fruits (grapes, apples, figs, berries, citrus fruits) (Veberic et al. 2005, 2008; Iacopini et al. 2008; Chua 2013), vegetables (caper, onion, asparagus) (Makris and Rossiter 2001; Musallam et al. 2012), pseudocereals (buckwheat) (Kreft et al. 2006) and beverages (unfermented rooibos tea) (Bramati et al. 2003). In different in vitro and in vivo experimental models, rutin showed antioxidant, anti-inflammatory, antidiabetic, lipid-lowering, neuroprotective, vasoprotective, cardioprotective and anticancer effects (Chua 2013). The antigenotoxic potential of rutin was also investigated. In cell-based assays, rutin reduced the DNA damage induced by several genotoxic agents such as AFB1, DXR and MMS (Barcelos et al. 2011), MMC (Ündeğer et al. 2004), NNK (Yeh et al. 2006), B(a)P (Marcarini et al. 2011), *t*BHP (Aherne and O'Brien 2000b) and H_2O_2 (Noroozi et al. 1998; Aherne and O'Brien 1999). Except MMS which is a direct monofunctional alkylating agent (Nicolella et al. 2014), DNA damaging effects of H_2O_2 (Aherne and O'Brien 1999, 2000a), *t*BHP (Aherne and O'Brien 2000b), AFB1 (Towner et al. 2003), DXR (Quiles et al. 2002; Yurtcu et al. 2014), MMC (Gustafson and Pritsos 1993; Wang et al. 2007), NNK (Yeh et al. 2006) and B(a)P (Delgado et al. 2008; Hassan et al. 2011) are mediated, in part, by generation of ROS. Besides, AFB1 (Bahari et al. 2014), B(a)P (Delgado et al. 2008; Hassan et al. 2011) and MMC (Gustafson and Pritsos 1993; Wang et al. 2007) are bioactivated by phase I enzymes to reactive metabolites. The antigenotoxic activity of rutin might involve ROS scavenging and down-regulation of bioactivating enzymes. Due to glycosylation of 3-hydroxyl group, rutin has weaker free radical scavenging and transition metal ion chelating effects than quercetin (Rice-Evans et al. 1996; Sroka 2005). Rutin has been reported to inhibit CYP1A1 and CYP1B1, enzymes involved in the bioactivation of AFB1 and B(a)P

(Hassan et al. 2011; Choi et al. 2012) but surprisingly, it did not influence CYP450-mediated bioactivation of NNK in A549 cells (Yeh et al. 2006). A comparison between the antigenotoxic effects of rutin and quercetin revealed contradictory aspects. Thus, there were no significant differences between the protective activities of the two phytochemicals in HepG2 cells exposed to H_2O_2 (Aherne and O'Brien 1999). On the other hand, in human lymphocytes, rutin showed considerably weaker protective effects against H_2O_2-induced DNA damage than quercetin ($ED_{50} = 43$ mmol/l vs. 47 μmol/l) (Noroozi et al. 1998). A similar result was obtained in Caco-2 cells exposed to *t*BHP: rutin reduced DNA damage by 18 % whereas quercetin exhibited 50 % protection (Aherne and O'Brien 2000b). Rutin afforded less DNA protection most probably due to its weaker free radical scavenging and metal chelating effects in comparison with quercetin. Alike quercetin and myricetin, rutin lacked the ability to stimulate the DNA repair mechanisms in Caco-2, HepG2 and V79 cells (Aherne and O'Brien 2000a).

The antigenotoxic potential of rutin was confirmed by in vivo studies. In animal models, rutin protected against gamma radiation and CrO_3 induced-genomic instability (García-Rodríguez et al. 2014; Patil et al. 2014). Rutin decreased the frequency of micronucleated polychromatic erythrocytes in mouse peripheral blood by 83.3 and 82.9 % at 24 and 48 h, respectively after treatment with CrO_3. As treatment doses for rutin and quercetin were different (rutin: 625 mg/kg, dose repeated after 24 h, 20 mg/kg CrO_3 after 4 h; quercetin: 100 mg/kg, 20 mg/kg CrO_3 after 4 h) (García-Rodríguez et al. 2014), a comparison of their protective effects against genotoxicity induced by CrO_3 is not feasible.

Flavones and flavone glycosides

Apigenin (Fig. 1; Table 2) is one of the most common flavones in vegetables, spices and fruits; it is found in parsley, celery, peppermint, oregano, basil, tarragon, cilantro, lemon, apples and berries (Noel et al. 2006; Siddique et al. 2008). Both in vitro and in vivo studies have demonstrated antioxidant, anti-inflammatory and antitumor properties for apigenin (Ali et al. 2014). Apigenin's capacity to protect against DNA damaging agents has been widely investigated in bacterial, mammalian cell-based and animal assays. Mammalian cell-based assays showed protective effects against H_2O_{2-} (Noroozi et al. 1998; Siddique and Afzal 2009a), MMC- (Siddique et al. 2008, Siddique and Afzal 2009b), cyclophosphamide (CPA)- (Siddique et al. 2008) and ethinylestradiol (EtE)-induced genotoxicity (Siddique et al. 2010). Alike MMC, CPA is used in cancer chemotherapy. CPA is an alkylating agent which undergoes CYP450 (CYP2B6, CYP3A4, CYP2C9, CYP2C19)-mediated metabolic activation to phosphoramide mustard (Siddique et al. 2008). Widely used in combination with progestogens in oral contraceptive formulations, EtE has been reported to induce aneuploidy, polyploidy, unscheduled DNA synthesis, DNA adduct formation, chromosomal aberrations, sister chromatid exchanges and micronucleus formation (in vitro and in vivo studies) (Hundal et al. 1997; Siddique et al. 2010). ROS might be involved in EtE-induced genotoxicity (Wellejus et al. 2004; Siddique et al. 2005). EtE becomes genotoxic after CYP450-mediated bioactivation (Wellejus et al. 2004; Siddique et al. 2005, 2010). The antigenotoxic effects of apigenin were assessed in both absence (MMC) and presence (CPA, EtE) of metabolic activation (rat liver S9 homogenate activation system containing CYP450 isoenzymes). Apigenin showed antigenotoxic effects against MMC as well as CPA and EtE. The DNA protective activity of apigenin probably involves inactivation of CYP450 isoenzymes but also free radical scavenging effects (Siddique et al. 2008, 2010). Due to lack of *o*-dihydroxy structure/3-hydroxyl group in the B/C ring, apigenin is a weaker free radical scavenger than quercetin (TEAC = 1.45 ± 0.08 vs. 4.7 ± 0.1 mM) (Rice-Evans et al. 1996).

Several in vivo studies confirmed the DNA protective effects of apigenin and revealed possible mechanisms responsible for the antigenotoxic activity (Khan et al. 2006; Siddique and Afzal 2009b; Ali et al. 2014). In Swiss albino mice exposed to B(a)P, apigenin significantly reduced DNA damage in the liver. Apigenin also down-regulated enzymes responsible for B(a)P bioactivation (aryl hydrocarbon hydroxylase, CYP450 isoenzymes), induced phase II detoxifying enzymes such as quinone reductase (QR) and GST and restored the normal level of GSH. It appears from this study that in vivo antigenotoxic activity of apigenin is based on its ability to suppress the metabolic bioactivation, induce detoxification mechanisms and protect against oxidative stress

Table 2 The antigenotoxic potential of flavones and flavone glycosides

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Apigenin	H_2O_2	CBMN SCE	HPBLC	H_2O_2 (150 μM); apigenin treatment (5–20 μM)	Reduction of SCE and MN frequency	Siddique and Afzal (2009a)
	EtE	CA CCK SCE	HPBLC	EtE (10 μM, 24 h); apigenin (5–20 μM) post-treatment (6 h); S9 mix (0.5 ml, 6 h)	Reduction of SCE, gaps, chromatid and chromosome breaks; increase in RI	Siddique et al. (2010)
		SCGE	HPBLC	H_2O_2 (100 μmol/l, 5 min on ice); apigenin (7.6–279.4 μmol/l) pre-treatment (30 min)	Dose-dependent reduction of comet score	Noroozi et al. (1998)
	CPA MMC	CA CCK SCE	HPBLC	CPA (0.16 μg/ml) with S9 mix (0.5 ml, 6 h) or MMC (6 μM); apigenin (1–20 μM) simultaneous treatment (24 h)	Reduction of SCE, gaps, chromatid and chromosome breaks; increase in RI	Siddique et al. (2008)
	MMC	CA SCE	Swiss albino mice	MMC (2 mg/kg, i.p.); apigenin (10–40 mg/kg, i.p.) simultaneous treatment	Reduction of SCE and CA frequency in bone marrow	Siddique and Afzal (2009b)
	NDEA	CBMN SCGE	Wistar rats	NDEA (0.1 mg/ml, p.o.); apigenin (10–40 mg/ml p.o.) simultaneous treatment (21 days)	Reduction of TL and MN frequency in liver	Ali et al. (2014)
	B(a)P	SCGE	Swiss albino mice	B(a)P (125 mg/kg, p.o.); apigenin (2.5 and 5 mg/kg, p.o.) pre-treatment (7 days)	Reduction of DNA fragmentation in liver	Khan et al. (2006)
		Ames	*ST* TA100	B(a)P (2 μg/plate); apigenin (100 μg/plate)	Reduction of mutagenicity	Uhl et al. (2003)
Chrysin	B(a)P PhIP	Ames	*ST* TA98 and TA100	B(a)P or PhIP (2 μg/plate); chrysin (25–100 μg/plate or 20–50 μg/plate, respectively)	Dose-dependent reduction of mutagenicity	Uhl et al. (2003)
		CBMN	HepG2	B(a)P or PhIP (0.6 mM, 24 h); chrysin (5–35 μg/ml) pre-treatment (24 h)	Reduction of MN frequency	Uhl et al. (2003)
Luteolin	H_2O_2	SCGE	HPBLC	H_2O_2 (100 μmol/l, 5 min on ice); luteolin (7.6–279.4 μmol/l) pre-treatment (30 min)	Dose-dependent reduction of comet score	Noroozi et al. (1998)
		SCGE	Caco-2	H_2O_2 (75 μM, 5 min on ice); luteolin (10 and 20 μM) pre-treatment (2 h)	Reduction of % DNA in tail	Ramos et al. (2010)
	B(a)P	Ames	*ST* TA100	B(a)P (2 μg/plate); luteolin (100 μg/plate)	Reduction of mutagenicity	Uhl et al. (2003)
L-7-Gc L-7-Gu L-7-Ru	AC EMS	DEL recombinant	*SC* RS112	AC or EMS (100 μg/ml); L-7-Gc or L-7-Gu or L-7-Ru (2–16 μM/ml)	Reduction of DEL and ICR events	Orhan et al. (2013)

AC acridin, *B(a)P* benzo(a)pyrene, *CA* chromosomal aberrations, *Caco-2* human intestinal cells, *CBMN* cytokinesis-block micronucleus, *CCK* cell cycle kinetics, *CPA* cyclophosphamide, *DEL* deletion, *DNA* deoxyribonucleic acid, *EMS* ethyl methanesulfonate, *EtE* ethinylestradiol, *H_2O_2* hydrogen peroxide, *HepG2* human hepatocellular liver carcinoma cells, *HPBLC* human peripheral blood lymphocyte cells, *ICR* intrachromosomal recombination, *i.p.* intraperitoneal, *L-7-Gc* luteolin-7-*O*-glucoside, *L-7-Gu* luteolin-7-*O*-glucuronide, *L-7-Ru* luteolin-7-*O*-rutinoside, *MMC* mitomycin C, *MN* micronuclei, *NDEA* *N*-nitrosodiethylamine, *p.o.* orally, *PhIP* 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, *RI* replication index, *SC Saccharomyces cerevisiae, SCE* sister chromatid exchange, *SCGE* single cell gel electrophoresis, *ST Salmonella typhimurium, TL* tail length

(Khan et al. 2006). In male Wistar rats, apigenin mitigated DNA damage induced by *N*-nitrosodiethylamine (NDEA). This is mainly present in fried meals but also in meat and milk products, preserved juices, cigarette smoke and nitrate-rich water (Ali et al. 2014). In food stuffs, NDEA is generated by protein and lipid oxidation. As many other genotoxic agents, NDEA undergoes CYP450-mediated bioactivation to a toxic ethyl radical metabolite; this process is accompanied by a high production of ROS

responsible for DNA damage. Possible mechanisms for the protective activity of apigenin against NDEA-induced genotoxicity have been proposed to be direct scavenging of ethyl radical and up-regulation of antioxidant enzymes (Ali et al. 2014).

Antigenotoxic assays on **chrysin** (Fig. 1; Table 2), a flavone contained in fruits, vegetables and beverages, showed contradictory results. Chrysin has been reported to be highly active against several genotoxic agents (AFB1, B(a)P) in bacterial assays. Chrysin, in the presence of metabolic activation, protected against B(a)P and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) in *S. typhimurium* strains (TA98, TA100) showing higher protection against B(a)P in *S. typhimurium* TA100 than apigenin and luteolin. The antigenotoxic activity was assessed as reduction in the number of histidine revertant colonies in *Salmonella* strains exposed to genotoxic agents (Uhl et al. 2003). PhIP is one of the most abundant heterocyclic amines in cooked meat. Heterocyclic amines, produced during heating of protein-rich products, are highly mutagenic and carcinogenic after bioactivation. They are metabolized by CYP1A (CYP1A1, CYP1A2), *N*-acetyltransferases and sulfotransferases to reactive derivatives which further bind to C-8 of guanine generating DNA adducts (Alaejos et al. 2008; Haza and Morales 2011). In bacterial assays with exogenous activation mix, chrysin inhibited CYP1A1/2. Surprisingly, in HepG2 cells, chrysin showed DNA protection against B(a)P- and PhIP-induced damage in a very narrow and low dose range. Moreover, it was mutagenic at doses which proved high protection in bacterial assays. The mechanisms involved in DNA protection in mammalian cells differ from the ones chrysin protects bacterial cells. In HepG2 cells, chrysin inhibited enzymes involved in PhIP bioactivation (sulfotransferases) and activated enzymes responsible for detoxification of B(a)P and PhIP reactive metabolites (NAD-quinone reductase, UDP-glucuronosyl transferase-1, *N*-acetyltransferase-1). Other antigenotoxic mechanisms (ROS inactivation, epoxide hydrolase induction) have also been proposed. In addition, chrysin induced CYP1A in HepG2 cells (Uhl et al. 2003).

Luteolin (Fig. 1; Table 2), a common dietary flavone present in carrots, celery, peppers, peppermint, oregano, thyme, rosemary (López-Lázaro 2009) but also in red wine and tea (Ramos et al. 2010), is known to possess antioxidant, anti-inflammatory, anticancer and antimicrobial effects (López-Lázaro 2009; Ramos et al. 2010; Orhan et al. 2013). With regard to its antigenotoxic potential, in mammalian cell-based assays, luteolin protected DNA from H_2O_2-induced oxidative damage (Noroozi et al. 1998; Ramos et al. 2010). The ability of luteolin to protect cells against oxidative DNA damage might be due, at least in part, to its antioxidant effects. Luteolin has strong free radical scavenging effects (Sroka 2005). Additionally, luteolin has been reported to attenuate the decrease in antioxidant enzyme (GPx, GR, GST, SOD, CAT) activities and GSH level induced by toxic agents (Ramos et al. 2010). Moreover, in Caco-2 cells, luteolin specifically protected against DNA base oxidation by reducing the generation of 8-oxo-7,8-dihydroguanine under exposure to photosensitizer Ro 19-8022 and visible light. In pre-treatment protocol, luteolin increased the rate of rejoining of H_2O_2-induced strand breaks while as post-treatment it had no influence on cellular repair mechanisms. It is most likely that luteolin acted by inducing repair mechanisms not activating them (Ramos et al. 2010).

Several luteolin derivatives (luteolin-7-*O*-glucoside, luteolin-7-*O*-rutinoside, luteolin-7-*O*-glucuronide) isolated from horse mint have been reported to protect against deletion and interchromosomal recombination caused by ethyl methanesulfonate (EMS) and acridine (AC) in *S. cerevisiae* strain RS112. EMS is an alkylating agent which also causes DNA depurination and DNA strand breaks. AC binds reversibly to DNA (intercalation); at high concentrations, it induces DNA strand breaks. Both EMS and AC decreased the level of antioxidants (GSH, GPx) in yeast cells. This effect was efficiently counteracted by luteolin derivatives (Orhan et al. 2013). Overall, the antigenotoxic potential of the three luteolin derivatives might be attributed to their capacity to interfere with DNA alkylation and intercalation processes, reduce DNA strand breaks formation and increase antioxidant activity.

Flavanones and flavanone glycosides

Flavanones are almost exclusively found in citrus fruits, principally in the peel and membranous parts but they can reach high levels (hundreds of mg/l) in the juice as well. Hesperidin (hesperetin-7-rutinoside) and naringin (naringenin-7-neohesperidoside) are the

major flavanone glycosides in oranges, mandarins and grapefruit, respectively. Low amounts of naringenin are present in tomatoes and tomato-based products. Mean daily intake values for hesperetin and naringenin have been estimated at 28.3 and 8.3 mg, respectively (Erlund 2004).

Antioxidant, anti-inflammatory and anticarcinogenic effects have been reported for both **hesperidin** (Kalpana et al. 2009, 2011) and its aglycone, **hesperetin** (Trivedi et al. 2011) (Fig. 1; Table 3). Hesperetin also has anti-atherogenic, antihypertensive and neuroprotective properties (Trivedi et al. 2011) whereas hesperidin is an efficient vasoprotector (Kalpana et al. 2009, 2011). Few studies have investigated the antigenotoxic effects of hesperetin and hesperidin. The major disadvantage of chemotherapy is the toxicity to non-target tissues. In case of DXR treatment, the structure and function of reproductive organs, mainly male ones, are highly impaired. Increase in oxidative stress and subsequent oxidative DNA damage are involved, in part, in DXR-induced toxicity in testes. Hesperetin protected against DXR-induced DNA damage in both mouse sperm and testicular cells due to its ability to reduce DXR-induced oxidative stress (increase in lipid peroxidation, decrease in GSH level) (Trivedi et al. 2011). Hesperidin efficiently attenuated DNA damage induced by technetium-99 m methoxy-isobutyl-isonitrile (^{99m}Tc-MIBI) and gamma radiation in both mammalian cell-based and animal models. Besides radiotherapy, radiopharmaceuticals, used for diagnostic and therapeutic purposes, are a source of gamma irradiation and consequently, induce genotoxic effects. Human peripheral blood lymphocytes are highly exposed to this type of irradiation as they take up the radiopharmaceuticals and transport them within the body. Hesperidin significantly reduced the increase in micronuclei frequency induced by ^{99m}Tc-MIBI in cultured human peripheral blood lymphocytes (Hosseinimehr et al. 2009). Hesperidin also attenuated genetic damage in human peripheral blood lymphocytes and mice (liver and bone marrow cells) exposed to gamma radiation. An increase in antioxidant enzyme (SOD, CAT, GPx) activities and GSH level as well as a decrease in lipid peroxidation were mechanisms by which hesperidin protected against genotoxic effects induced by gamma radiation (Kalpana et al. 2009, 2011).

Besides CYP450 inhibitory potential, **naringenin** and **naringin** (Fig. 1; Table 3) have various health promoting effects (lipid-lowering, insulin-like, antioxidant, anti-inflammatory, anticancer, antimicrobial) (Oršolić et al. 2011; Bacanli et al. 2015). Naringin has shown protective effects on DNA against damage induced by several genotoxic agents such as NNK (Yeh et al. 2006), H_2O_2 (Bacanli et al. 2015), Cd (Yılmaz et al. 2012), ifosfamide (Alvarez-González et al. 2001) and gamma radiation (Jagetia and Reddy 2002). Similar to NNK and H_2O_2, Cd induces oxidative stress and subsequent DNA damage: oxidative lesions, single strand breaks, chromosomal aberrations, sister chromatid exchanges, DNA–protein crosslinks. In addition, Cd inhibits the activity of DNA repair systems (nucleotide and base excision repair, mismatch repair). Cd is highly genotoxic being classified as a group 1 human carcinogen by IARC (Yılmaz et al. 2012). Ifosfamide, a cytostatic drug, is metabolized by CYP450 isoenzymes to ifosforamide mustard, the active DNA alkylating metabolite (Ajithkumar et al. 2007). The antigenotoxic potential of naringenin has been less studied. To the best of our knowledge, only protective effects against alloxan-induced DNA damage in peripheral lymphocytes of diabetic mice have been reported (Oršolić et al. 2011). DNA protective effects of naringenin and naringin might be attributed, in part, to their ability to decrease intracellular ROS levels. Both flavanones possess free radical scavenging properties (Oršolić et al. 2011; Yılmaz et al. 2012). Naringin has been reported to scavenge ROS (superoxide, hydroxyl and peroxyl radicals), upregulate enzymatic (GPx, GR, SOD, CAT) and non-enzymatic (GSH) antioxidants and inhibit lipid peroxidation (Yılmaz et al. 2012).

Prenylated flavonoids

Xanthohumol (Fig. 1; Table 4) is the main prenylated flavonoid in hops (female inflorescences of the hop plant) being responsible for the flavor and bitterness of beer which is the major dietary source of xanthohumol (Plazar et al. 2007, 2008). The content of xanthohumol in hops usually ranges from 0.1 to 1 % whereas in commercial beers xanthohumol does not exceed 0.2 mg/l. The low xanthohumol levels in

Table 3 The antigenotoxic potential of flavanones and flavanone glycosides

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Hesperetin	DXR	SCGE	SD rats	DXR (4 mg/kg, i.p., 1 day/week); hesperetin (25–100 mg/kg, p.o., 5 days/week) pre-treatment (5 weeks)	Reduction of TL, TM, OTM and % DNA in tail in sperm and testes	Trivedi et al. (2011)
Hesperidin	^{99m}Tc-MIBI	CBMN	HPBLC	^{99m}Tc-MIBI (200 µCi, 3 h); hesperidin (50 and 100 µmol) pre-treatment (1 h)	Reduction of MN frequency	Hosseinimehr et al. (2009)
	Gamma radiation	CA CBMN SCGE	HBPLC	1–4 Gy gamma radiation; hesperidin (16.38 µM) pre-treatment (30 min)	Reduction of TL, TM, OTM, % DNA in tail, MN and DC frequency	Kalpana et al. (2009)
		SCGE	Swiss albino mice	10 Gy whole body gamma radiation; hesperidin (12.5–100 mg/kg, p.o.) pre-treatment (7 days)	Reduction of TL, TM, OTM and % DNA in tail in liver and bone marrow	Kalpana et al. (2011)
Naringenin	Alloxan	SCGE	CBA inbred mice	Alloxan (75 mg/kg, i.v.); naringenin (50 mg/kg, i.p.) post-treatment (7 days, started 2 days after alloxan injection)	Reduction of TM and TI in lymphocytes	Oršolić et al. (2011)
Naringin	H_2O_2	CBMN SCGE	HPBLC	H_2O_2 (50 µM, 5 min on ice); naringin (50–2000 µM) pre-treatment (30 min)	Reduction of TL, TM, TI and MN frequency	Bacanli et al. (2015)
		CBMN SCGE	V79	H_2O_2 (50 µM, 5 min on ice); naringin (50–2000 µM) pre-treatment (1 h)	Reduction of TL, TM, TI and MN frequency	Bacanli et al. (2015)
	NNK	SCGE	A549	NNK (700 µM, 4 h); naringin (23 µM) pre-treatment (1 h)	Reduction of TM	Yeh et al. (2006)
	Cadmium	CA	HPBLC	$CdCl_2$ (40 µM); naringin (1 and 2 µg/ml) simultaneous treatment (24 h)	Reduction of total CA frequency	Yılmaz et al. (2012)
	Ifosfamide	CBMN	Mouse	Ifosfamide (60 mg/kg, p.o.); naringin (50–500 mg/kg, p.o.) simultaneous treatment	Dose-dependent reduction of MN frequency in blood erythrocytes	Alvarez-González et al. (2001)
	Gamma radiation	CBMN	Mouse	3 and 4 Gy gamma radiation; narigin (2 mg/kg) pre-treatment	Reduction of MN frequency in bone marrow	Jagetia and Reddy (2002)

^{99m} Tc-MIBI technetium-99m methoxy isobutyl isonitrile, *A549* human adenocarcinomic alveolar basal epithelial cells, *CA* chromosomal aberrations, *CBMN* cytokinesis-block micronucleus, *$CdCl_2$* cadmium chloride, *DC* dicentric chromosomes, *DXR* doxorubicin, *H_2O_2* hydrogen peroxide, *HPBLC* human peripheral blood lymphocyte cells, *i.p.* intraperitoneal, *i.v.* intravenous, *MN* micronuclei, *NNK* 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *OTM* olive tail moment, *p.o.* orally, *SCGE* single cell gel electrophoresis, *SD* Sprague–Dawley, *TI* tail intensity, *TL* tail length, *TM* tail moment, *V79* Chinese hamster lung fibroblast cells

beer cannot provide real health benefits. Therefore, different methods for enriching the xanthohumol content of beer have been developed. Although the daily intake of xanthohumol is estimated to be low, it can induce systemic effects as it is reasonably bioavailable due to its lipophilicity (Liu et al. 2015). Xanthohumol has been reported to have numerous biological effects: anticancer, anti-inflammatory, hypoglycemic, anti-hyperlipidemic, anti-adipogenic, bone remodeling, central nervous system modulating, liver protective, anti-platelet, antimicrobial and antiparasite effects (Plazar et al. 2007, 2008; Liu et al. 2015).

Xanthohumol is a very efficient chemopreventive agent due to its antigenotoxic effects. Xanthohumol showed protective effects against DNA damage induced by menadione (Dietz et al. 2005), B(a)P, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and *t*BHP (Plazar et al. 2007, 2008). Menadione is known to be a strong inducer of ROS causing severe oxidative DNA damage. In Hepa 1c1c7 cells, xanthohumol (pre-treatment) was able to reduce menadione-induced DNA damage by up-regulating QR (Dietz et al. 2005). IQ, a heterocyclic amine found in cooked foods and cigarette smoke, acts similar as PhIP. IQ undergoes enzyme (CYP1A2, *N*-

Table 4 The antigenotoxic potential of prenylated flavonoids

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Xanthohumol	B(a)P IQ	SCGE	HepG2	B(a)P (50 μM) or IQ (1 mM); xanthohumol (0.01–10 μM) simultaneous treatment (24 h)	Reduction of % DNA in tail	Plazar et al. (2007)
		SCGE	Wistar rat liver slices	B(a)P (100 μM) or IQ (1 mM); xanthohumol (0.001–10 μM) simultaneous treatment (24 h)	Reduction of % DNA in tail	Plazar et al. (2008)
	*t*BHP	SCGE	HepG2	*t*BHP (0.5 μM, 20 min); xanthohumol (0.01–10 μM) pre-treatment (24 h)	Dose-dependent reduction of % DNA in tail	Plazar et al. (2007)
		SCGE	Wistar rat liver slices	*t*BHP (0.5 mM, 20 min); xanthohumol (0.01–1 μM) pre-treatment (24 h)	Reduction of % DNA in tail	Plazar et al. (2008)
	Menadione	SCGE	Hepa 1c1c7	Menadione (10 μM, 30 min); xanthohumol (0–8 μM) pre-treatment (48 h)	Dose-dependent reduction of DNA strand breaks	Dietz et al. (2005)

B(a)P benzo(a)pyrene, *DNA* deoxyribonucleic acid, *Hepa 1c1c7* murine hepatoma cells, *HepG2* human hepatocellular liver carcinoma cells, *HPBLC* human peripheral blood lymphocyte cells, *IQ* 2-amino-3-methylimidazo[4,5-f]quinoline, *SCGE* single cell gel electrophoresis, *tBHP tert*-butylhydroperoxide

acetyltransferase-2)-mediated bioactivation to reactive metabolites (*N*-hydroxy- and *N*-acetoxy-derivatives) that covalently bind DNA generating adducts. Additionally, the reactive metabolites induce DNA strand breaks, chromosomal aberrations and sister chromatid exchanges. By spontaneous dissociation, *N*-acetoxy-derivative forms a nitrenium ion with a high DNA binding capacity (National Toxicology Program 2002; Plazar et al. 2007). Xanthohumol significantly protected HepG2 cells against B(a)P, IQ and *t*BHP. As the genotoxicity of B(a)P and IQ is strongly dependent on CYP1A-mediated biotransformation, the protective effects of xanthohumol against B(a)P- and IQ-induced DNA damage have initially been attributed to its capacity to inhibit CYP1A isoenzymes (CYP1A1, CYP1A2). With respect to *t*BHP, an induction of cellular mechanisms involved in antioxidant defense might, at least in part, explain the DNA protective effects of xanthohumol (Plazar et al. 2007). Unexpectedly, further assays performed in fresh liver tissue (cells from rat liver slices) showed protective effects against B(a)P, IQ and *t*BHP but no effect on CYP1A1, CYP1A2 and QR expression and CYP1A activity. It appears that the antigenotoxic effects of xanthohumol are not dependent on CYP1A and QR modulation (Plazar et al. 2008).

Flavanols and flavanol derivatives

(+)-**Catechin** and (−)-**epicatechin** (Fig. 1; Table 5), the most common dietary flavanols, are present in large amounts in fruits (grapes, apples, berries, cherries, apricots, pears), broad beans, beverages (tea, red wine), cocoa and cocoa based products (chocolate). The reported values for estimated dietary intake (9.5–50 mg/day) vary greatly due to different lifestyles (diet, smoking) and distinct methods used for intake estimation (Arts et al. 2001; Mullie et al. 2007; Gadkari and Balaraman 2015). High intake of catechin-rich foods reduces the risk of mortality by coronary heart disease. In addition to this, catechins have shown many biological properties (antioxidant, antibacterial, antifungal, anti-inflammatory, anti-hypercholesterolemic, anticarcinogenic, antiviral), some of them accounting for the prevention of chronic diseases; antigenotoxic effects have also been

Table 5 The antigenotoxic potential of flavanols and flavanol derivatives

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
(+)-Catechin	8-MeIQx 4,8-diMeIQx	SCGE	HepG2	8-MeIQx (500 μM, 24 h) or 4,8-diMeIQx (200 μM, 24 h); catechin (10–25 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Fpg sensitive site formation	Haza and Morales (2011)
	PhIP	SCGE	HepG2	PhIP (300 μM, 24 h); catechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks at 10 μM, Endo III and Fpg sensitive site formation at all tested concentrations	Haza and Morales (2011)
	NDBA	SCGE	HepG2	NDBA (3 mM, 24 h); catechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks at 10 μM, Endo III sensitive site formation at 5–10 μM and Fpg sensitive site formation at 25–50 μM	Delgado et al. (2009)
	NPIP	SCGE	HepG2	NPIP (44 mM, 24 h); catechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks and Fpg sensitive site formation at 10 μM, Endo III sensitive site formation at 5–10 μM	Delgado et al. (2009)
	NDMA NPYR	SCGE	HepG2	NDMA (27 mM with Endo III or Fpg and 135 mM without enzymes, 24 h) or NPYR (5 mM with Endo III or Fpg and 50 mM without enzymes, 24 h); catechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks, Endo III and Fpg sensitive site formation	Delgado et al. (2008)
	B(a)P	SCGE	HepG2	B(a)P (50 μM, 24 h); catechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks at 25 and 50 μM and Endo III sensitive site formation at all tested concentrations	Delgado et al. (2008)
	Ketoprofen	8-OHdG	SD rats	Ketoprofen (50 mg/kg, p.o.); catechin (14 and 35 mg/kg, p.o.) pre-treatment (21 days)	Dose-dependent reduction of 8-OHdG levels in intestinal mucosa tissue	Cheng et al. (2013)
(−)-Epicatechin	8-MeIQx 4,8-diMeIQx	SCGE	HepG2	8-MeIQx (500 μM, 24 h) or 4,8-diMeIQx (200 μM, 24 h; epicatechin (50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Fpg sensitive site formation	Haza and Morales (2011)
	PhIP	SCGE	HepG2	PhIP (300 μM, 24 h); epicatechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of Endo III sensitive site formation	Haza and Morales (2011)
	NDBA	SCGE	HepG2	NDBA (3 mM, 24 h); epicatechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks at 10 μM, Fpg and Endo III sensitive site formation at all tested concentrations	Delgado et al. (2009)
	NPIP	SCGE	HepG2	NPIP (44 mM, 24 h); epicatechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction: of DNA strand breaks at 10–25 μM, Fpg and Endo III sensitive site formation at all tested concentrations	Delgado et al. (2009)
	NDMA NPYR	SCGE	HepG2	NDMA (27 mM with Endo III or Fpg and 135 mM without enzymes, 24 h) or NPYR (5 mM with Endo III or Fpg and 50 mM without enzymes, 24 h); epicatechin (10–50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks, Endo III and Fpg sensitive site formation	Delgado et al. (2008)

Table 5 continued

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
(−)-Epicatechin	B(a)P	SCGE	HepG2	B(a)P (50 μM, 24 h); epicatechin (10, 25 and 50 μM) pre-treatment (24 h) followed by simultaneous treatment (24 h)	Reduction of DNA strand breaks at all tested concentrations, Endo III sensitive site formation at 10–25 μM and Fpg sensitive site formation at at 50 μM	Delgado et al. (2008)
	Etoposide	SCGE	Brown Norway rats	Etoposide (50 mg/kg, i.p., in the last 3 days); epicatechin treatment (20 and 40 mg/kg, p. o.,7 days)	Reduction of % DNA in tail in bone marrow	Papiez (2013)
EGCG	BLM	SCGE	HPBLC	BLM (20 μg/ml, 30 min); EGCG (2 and 20 μM) pre-treatment (18 h), simultaneous (30 min) and post-treatment (15 min)	Reduction of DNA strand breaks	Glei and Pool-Zobel (2006)
		SCGE	HWBLC	BLM (0.3 μg/ml); EGCG (1–100 μM) simultaneous treatment (20 min); pre-treatment (20 min) and simultaneous treatment (20 min)	Reduction of TM	Kanadzu et al. (2006)
		SCGE	HPuBLC	BLM (0.2 μg/ml); EGCG (0.01–0.1 μM) simultaneous treatment (20 min)	Reduction of TM	Kanadzu et al. (2006)
	H_2O_2	SCGE	HWBLC	H_2O_2 (0.2 μM); EGCG (1–100 μM) simultaneous treatment (20 min)	Reduction of TM	Kanadzu et al. (2006)
	CAP	SCGE	HPBLC	CAP (50 μM); EGCG (20 μM) simultaneous treatment (1 h)	Reduction of TL and % DNA in tail	Pandir (2015)
	Gamma radiation	SCGE	Murine splenocytes	3 Gy gamma irradiation; EGCG (50 and 100 μM) pre-treatment (2 h)	Reduction of TL, TM, OTM and % DNA in tail	Richi et al. (2012)
	Chromium	CBMN	CD-1 mice	CrO_3 (20 mg/kg, i.p.); EGCG (10 mg/kg, i.p.) pre-treatment (4 h)	Reduction of MN frequency in peripheral blood	García-Rodríguez et al. (2014)
	DEB MMC PAT	CBMN	HL-60	DEB (2.5 μM) or MMC (0.9 μM) or PAT (0.25 μM); EGCG (0.5 and 5 μM) simultaneous treatment (4 h)	Reduction of MN frequency	Abraham et al. (2012)

4,8-diMeIQx 2-amino-3,4,8-trimethylimidazo[4,5-f]-quinoxaline, *8-MeIQx* 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *B(a)P* benzo(a)pyrene, *BLM* bleomycin, *CAP* capsaicin, *CBMN* cytokinesis-block micronucleus, *CrO_3* chromium trioxide, *DEB* diepoxybutane, *DNA* deoxyribonucleic acid, *EGCG* (–)-epigallocatechin gallate, *Endo III* endonuclease III, *Fpg* formamidopyrimidine-N-glycosylase, *H_2O_2* hydrogen peroxide, *HepG2* human hepatocellular liver carcinoma cells, *HL-60* human promyelocytic cells, *HPBLC* human peripheral blood lymphocyte cells, *HPuBLC* human purified blood lymphocyte cells, *HWBLC* human whole blood lymphocyte cells, *i.p.* intraperitoneal, *MMC* mitomycin C, *NDBA N*-nitrosodibutylamine, *NDMA N*-nitrosodimethylamine, *NPIP N*-nitrosopiperidine, *NPYR N*-nitrosopyrrolidine, *OTM* olive tail moment, *p.o.* orally, *PAT* patulin, *PhIP* 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine, *SCGE* single cell gel electrophoresis, *SD* Sprague–Dawley, *TL* tail length, *TM* tail moment

reported (Gadkari and Balaraman 2015). As mentioned before, *N*-nitrosamines and heterocyclic amines, generated during food processing, become mutagenic and carcinogenic after bioactivation. Catechin and epicatechin reduced DNA breakage, oxidation of purines and pyrimidines in HepG2 cells exposed to *N*-nitrosamines (NDBA, NPIP, NPYR and NDMA) but selectively attenuated DNA damage induced by heterocyclic amines such as 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (8-MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-diMeIQx) and PhIP. Catechin and epicatechin protected against PhIP-induced DNA damage (strand breaks, oxidized purines and pyrimidines) and 8-MeIQx- and 4,8-diMeIQx-induced purine oxidation showing no protective effects against strand breakage and pyrimidine oxidation caused by the two quinoxalines. The DNA protective effects of catechins seem to reside, at least in part, in their ability to protect against ROS produced during bioactivation of *N*-nitrosamines and heterocyclic amines. Catechin and epicatechin protected against DNA damage (strand breaks and oxidized pyrimidines) induced by B(a)P in HepG2 cells, but only epicatechin showed protective effects against purine oxidation (Delgado et al. 2008, 2009; Haza and Morales 2011). Catechin and epicatechin exerted in vivo antigenotoxic effects against ketoprofen and etoposide, respectively (Cheng et al. 2013; Papiez 2013). Both flavanols have structural features necessary for free radical scavenging and metal ion chelation: catechol moiety in the B ring and free 3- and 5-hydroxyl groups in the C and A rings, respectively but lack 2,3-double bond and 4-keto group in the C ring, being less active (TEAC = 2.4 ± 0.05 and 2.5 ± 0.02 mM) than quercetin (TEAC = 4.7 ± 0.1 mM) in scavenging free radicals (Rice-Evans et al. 1996; Sroka 2005). Another mechanism by which catechin and epicatechin protect DNA could be modulation of enzymes responsible for bioactivation and detoxification of genotoxic agents. Both flavanols inhibited CYP1A1 and activated UDP-glucuronyltransferase 1A4 (UGT 1A4) in microsomes from baculovirus-infected cells expressing human CYP1A1 and UGT1A4 (Haza and Morales 2011). CYP1A1 is involved in the activation of *N*-nitrosamines and heterocyclic amines (Alaejos et al. 2008; Delgado et al. 2008, 2009) whereas UGT1A4 is the major enzyme involved in the detoxification of PhIP reactive intermediates (*N*-hydroxy-PhIP) (Haza and Morales 2011). Catechin was more efficient than epicatechin in suppressing CYP1A1 and inducing UGT1A4. These differences in enzyme modulating effects might explain the higher protection against PhIP-induced DNA damage afforded by catechin in comparison with epicatechin. Structural differences between (+)-catechin and (−)-epicatechin (β-OH and α-OH in the C ring, respectively conferring different torsional angles between B and C rings) are minor but might significantly affect their biological properties (Mendoza-Wilson and Glossman-Mitnik 2006; Haza and Morales 2011).

Epigallocatechin-3-gallate (EGCG) (Fig. 1; Table 5) is the most abundant polyphenol in green tea accounting for 55–70 % of total polyphenols in green tea extract (Richi et al. 2012). It is estimated that a cup of green tea contains 30–130 mg of EGCG. EGCG is also found in small amounts in berries, black currants and grapes (García-Rodríguez et al. 2014; Gadkari and Balaraman 2015). Due to eight phenolic hydroxyl groups, EGCG has a strong antioxidant potential being more active than quercetin, vitamin E and vitamin C. It also possesses anti-inflammatory, anticancer and chemopreventive effects, the latter being strongly related to its antigenotoxic properties (Rice-Evans et al. 1996; Sroka 2005; Richi et al. 2012; Gadkari and Balaraman 2015). In different cell lines, EGCG protected against DNA damage caused by gamma radiation (Richi et al. 2012), bleomycin (BLM) (Glei and Pool-Zobel 2006; Kanadzu et al. 2006), capsaicin (Pandır 2015), H_2O_2 (Kanadzu et al. 2006), MMC, diepoxybutane (DEB) and patulin (PAT) (Abraham et al. 2012). BLM, a glycopeptide-derived antitumor antibiotic, causes an increase in ROS levels and consequently, DNA damage (DNA single and double strand breaks, oxidized bases) (Glei and Pool-Zobel 2006; Aprotosoaie et al. 2016). A three-phase experimental protocol aiming to elucidate possible antigenotoxic mechanisms (induction of detoxification systems, free radical scavenging, stimulation of DNA repair processes) showed that EGCG reduced BLM-induced DNA damage (strand breaks, oxidized pyrimidines) in human leukocytes through all three types of mechanisms mentioned above (Glei and Pool-Zobel 2006). Increase in GST and SOD activities, GSH level and reduction in oxidative stress determined in terms of TBARS have been reported to be involved in EGCG protection against gamma radiation-induced DNA damage in murine splenocytes. Within the last study, EGCG showed stronger

antigenotoxic effects than quercetin and vitamin C (Richi et al. 2012). The genotoxic effects of capsaicin, major pungent component in hot red and chili peppers, usually occur at high doses, for instance, 50, 100 and 200 μM in human erythrocytes and leukocytes (Pandir 2015), 1.94 mg/kg/day i.p. in mice during a 32-day treatment (Díaz Barriga Arceo et al. 1995) and are mediated by an increase in oxidative stress (Pandir 2015). Capsaicin promotes DNA double strand breaks, micronuclei formation and sister chromatid exchanges. In human erythrocytes and leukocytes, EGCG attenuated capsaicin-induced DNA damage and other effects (decrease in SOD, CAT and GPx activities, increase in oxidative stress assessed as MDA level) (Pandir 2015). EGCG also exhibited antigenotoxic effects in vivo by reducing the frequency of CrO_3-induced micronucleated polychromatic erythrocytes in mouse peripheral blood by 78.6, 9.8 and 42.9 % at 24, 48 and 72 h, respectively after treatment with CrO_3 (García-Rodríguez et al. 2014). As mentioned before, in this assay, a comparison of quercetin, rutin and EGCG potencies is not feasible due to different treatment doses.

Anthocyanidins and their glycosides

Anthocyanidins and anthocyanins, their glycosides, are predominantly present in fruits. **Cyanidin**, the most common anthocyanidin in higher plants, is found in berries, cherries, red apples, peaches and nectarines while **delphinidin** and **perlargonidin** are present mostly in berries (Fig. 1; Table 6). They have numerous health benefits attributed, in great part, to their strong antioxidant effects (free radical scavenging, activation of GSH-related enzymes) (Khandelwal and Abraham 2014). Recently, their antigenotoxic potential has been extensively investigated.

Cyanidin showed genoprotective effects against H_2O_2– and cisplatin-induced oxidative DNA damage in normal human lymphocytes (ex vivo) and HK-2 proximal tubular cells, respectively (Duthie 2007; Gao et al. 2013). Alike other chemotherapeutic drugs, the use of cisplatin is limited due to its severe adverse effects, especially nephrotoxicity. DNA damage (oxidative lesions, DNA adducts) play a major role in cisplatin cytotoxicity in kidney proximal tubules. Cyanidin reduced ROS overproduction induced by cisplatin thus preventing DNA damage in HK-2 proximal tubular cells (Gao et al. 2013). Pelargonidin showed significant protective effects against DNA damage induced by NQO (4-nitroquinoline 1 oxide) (Abraham et al. 2007), DEB, MMC and PAT (mycotoxin produced by *Penicillium*, *Aspergillus* and *Byssochlamys*) in human promyelocytic HL-60 cells (Abraham et al. 2012). Cyanidin-3-glucoside, the major anthocyanin in red orange juice, significantly reduced oxidative DNA damage caused by different genotoxic agents: H_2O_2, EMS and colchicine (COL) in normal human lymphocytes (Fimognari et al. 2004; Duthie 2007), OTA in human immortalized gingival fibroblasts HF1 (Russo et al. 2005). Free radical scavenging, metal ion chelation and a direct protection based on binding to DNA and generation of a stable copigmentation complex have been proposed as possible mechanisms by which cyanidin-3-glucoside exerts its genoprotective activity (Russo et al. 2005). Surprisingly, cyanidin-3-glucoside was more efficient than cyanidin in reducing the percentage of DNA strand breaks caused by H_2O_2 in normal human lymphocytes (59 vs. 63 % DNA strand breakage) (Duthie 2007). In vivo studies showed that anthocyanidins (pelargonidin, cyanidin, delphinidin) can protect against DNA damage induced by CPA and environmental genotoxicants such as DEB and urethane (URE) (Azevedo et al. 2007; Khandelwal and Abraham 2014). DEB, a bifunctional alkylating agent forming DNA–DNA crosslinks and exocyclic adenine adducts, is a very toxic metabolite of 1,3-butadiene found in cigarette smoke and automobile waste gases. URE, a group 2A human carcinogen, is generated during fermentation processes and occurs in yeast breads and alcoholic beverages; it undergoes bioactivation to vinyl carbamate, the reactive metabolite. Cyanidin was more potent than pelargonidin in reducing DEB- and URE-induced micronucleus formation in mouse bone marrow. This might be due to the presence of an additional phenolic hydroxyl group in cyanidin (Khandelwal and Abraham 2014). Highly carcinogenic *N*-nitrosamines, generated during processing of protein containing foods, can be also produced in the acidic conditions of the stomach through a reaction involving nitrites and secondary amines or *N*-substituted amides present in foods (Bingül et al. 2013; Khandelwal and Abraham 2014). Generation of a

Table 6 The antigenotoxic potential of anthocyanidins and their glycosides

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Cyanidin	Cisplatin	SCGE	HK-2	Cisplatin (8 μg/ml, 24 h); cyanidin (40 μg/ml) pre-treatment (3 h)	Reduction of OTM	Gao et al. (2013)
	DEB Urethane	CBMN	Swiss albino mice	DEB (40 mg/kg, i.p.) or urethane (800 mg/kg i.p.); cyanidin (1–4 mg/kg, p.o.) pre-treatment (30 min)	Reduction of MN frequency in bone marrow	Khandelwal and Abraham (2014)
	Irinotecan	SCGE	Wistar rats	Irinotecan (100 mg/kg, i.p.); cyanidin (100 mg/kg, p.o.) pre-treatment (2 h)	Reduction of % DNA in tail in colon mucosal cells	Esselen et al. (2013)
C-3-Gc	OTA	SCGE	HF1	OTA (50 μM); C-3-Gc (0.125 and 0.250 mM) simultaneous treatment (72 h)	Reduction of TM	Russo et al. (2005)
		SCGE	SD rats	OTA (200 ppb, p.o.); C-3-Gc (1 g/kg feed, p.o.) simultaneous treatment (4 weeks)	Reduction of DNA fragmentation in kidney, liver and brain	Di Giacomo et al. (2007)
	EMS COL	CBMN	HPBLC	EMS (120 μg/ml, 24 h) or COL (0.1 μg/ml, 24 h); C-3-Gc (50–100 μg/ml, 24 h) pre-; simultaneous; post-treatment	Reduction of MN frequency	Fimognari et al. (2004)
	H_2O_2	CBMN	HPBLC	H_2O_2 (3.4 μg/ml, 30 min); C-3-Gc (25–100 μg/ml, 30 min) pre-; simultaneous; post-treatment	Reduction of MN frequency	Fimognari et al. (2004)
	Irinotecan	SCGE	Wistar rats	Irinotecan (100 mg/kg, i.p.); C-3-Gc (150 mg/kg, p.o.) pre-treatment (2 h)	Reduction of % DNA in tail in colon mucosal cells	Esselen et al. (2013)
Delphinidin	CPA	MN assay	Swiss albino mice	CPA (50 mg/kg, i.p.); delphinidin (2, 10 and 20 mg/kg, p.o.) pre-treatment (14 days)	Reduction of MN frequency in bone marrow	Azevedo et al. (2007)
Pelargonidin	NQO	CBMN	HL-60	NQO (2.63 μM); pelargonidin (0.5 μM) simultaneous treatment (4 h)	Reduction of MN frequency	Abraham et al. (2007)
	DEB MMC PAT	CBMN	HL-60	DEB (2.5 μM) or MMC (0.9 μM) or PAT (0.25 μM); pelargonidin (0.05 and 0.5 μM) simultaneous treatment (4 h)	Reduction of MN frequency	Abraham et al. (2012)
	DEB Urethane	CBMN	Swiss albino mice	DEB (40 mg/kg, i.p.) or urethane (800 mg/kg, i.p.); pelargonidin (2.5–20 mg/kg, p.o.) pre-treatment (30 min)	Reduction of MN frequency in bone marrow	Khandelwal and Abraham (2014)

CPA cyclophosphamide, *C-3-Gc* cyanidin 3-*O*-glucoside, *CBMN* cytokinesis-block micronucleus, *COL* colchicine, *DEB* diepoxybutane, EMS ethyl methanesulfonate, *HF1* human gingival fibroblast cells, *HK-2* normal human renal tubular cells, *HL-60* human promyelocytic cells, *HPBLC* human peripheral blood lymphocyte cells *i.p.* intraperitoneal, *MMC* mitomycin C, *MN* micronuclei, *NQO* 4-nitroquinoline 1-oxide, *OTA* ochratoxin A, *OTM* olive tail moment, *p.o.* orally, *PAT* patulin, *SCGE* single cell gel electrophoresis, *SD* Sprague–Dawley, *TM* tail moment

highly genotoxic and carcinogenic *N*-nitrosamine, methyl nitrosourea (MNU), was induced in vivo by administration of a nitrosation reaction mixture (sodium nitrite and methyl urea). Both anthocyanidins significantly reduced the genotoxic damage caused by MNU generation (Khandelwal and Abraham 2014). Cyanidin and its 3-glucoside reduced DNA strand breaks in colon mucosal cells of male Wistar rats treated with irinotecan. Irinotecan, a topoisomerase I poison, is widely used in association with 5-florouracil in the treatment of colorectal cancer; it is also a treatment option in other types of cancer (pancreatic, lung and cervical cancer). Irinotecan is a prodrug being bioactivated to the reactive derivative SN-38. The latter stabilizes the topoisomerase I-DNA covalent complex causing DNA double strand breaks, chromosomal aberrations and sister chromatid exchange thus leading to cell death. The antigenotoxic effects of both phytochemicals might reduce the gastrointestinal toxicity of

irinotecan but also its effectiveness in colon cancer treatment. Further studies are needed to evaluate the interactions between irinotecan and anthocyanin-rich food supplements (Esselen et al. 2013). Cyanidin-3-glucoside also protected against genotoxicity induced by OTA by reducing oxidative stress and increasing HO-1 expression (Di Giacomo et al. 2007).

Isoflavonoids

Isoflavonoids are found exclusively in plants belonging to the Fabaceae family, soybean seeds being the major dietary source of isoflavonoids. In Asia, where the diet is based on soybean and soybean products, the isoflavonoid intake can reach 100 mg/day while in European western countries it is below 1 mg/day (Raschke et al. 2006). **Genistein** and **daidzein** (Fig. 1; Table 7), found in soybean seeds as glycosides, are undoubtedly the most important dietary isoflavonoids (Zhang et al. 1999; Beg et al. 2008). Genistein has been reported to protect against different types of cancer and disorders associated with estrogen deficiency such as cardiovascular diseases, osteoporosis and menopausal symptoms (Polkowski and Mazurek 2000; Ali et al. 2015). All these effects are based on the ability of genistein to act on different molecular targets (estrogen receptors, tyrosin kinases, phosphatidylinositol kinases, topoisomerase II, ABC transporters) and modulate cellular processes (cell cycle, apoptosis, cell differentiation and proliferation) (Polkowski and Mazurek 2000; Polívková et al. 2006). Besides the above mentioned biological effects, genistein has been reported to possess antigenotoxic properties. In cell-based assays, genistein showed protective effects against several genotoxic agents such as norethandrolone (NorA) (Beg et al. 2008), oxandrolone (OxA) (Beg et al. 2008), CPA (in the presence of metabolic activation) (Beg et al. 2007), MMS (in the absence of metabolic activation) (Beg et al. 2007), DXR (Lepri et al. 2013), 2-aminoanthracene (2-AA) (Lepri et al. 2013) and H_2O_2 (Raschke et al. 2006; Kim et al. 2008). NorA and OxA are anabolic androgenic steroids recommended for treatment of androgen deficiency in men, aplastic anemia (rare forms responsive to anabolic androgens) and catabolic states. They belong to group 2A human carcinogens and are genotoxic as they bind DNA to form adducts and generate free radicals that induce DNA damage (Beg et al. 2008; Meireles et al. 2013). Genistein might scavenge free radicals generated by the genotoxic agents mentioned above thus mitigating DNA damage (Beg et al. 2007). Genistein prevented DNA damage in hepatoma cells exposed to DXR and 2-AA by modulating GSTs, a major phase II detoxification system; genistein increased total cytoplasmic GST activity and up-regulated GSTalpha2 isoenzymes (Lepri et al. 2013). Genistein showed a significant protection against oxidative DNA damage in H_2O_2-treated NIH/3T3 fibroblasts (reduction of pyrimidine and purine base oxidation) (Kim et al. 2008). Oxidative stress plays a major role in the development of prostate cancer (Raschke et al. 2006; Khandrika et al. 2009). In Asian countries, where soybean and soybean based products represent a major component of the diet, the risk of developing prostate cancer is lower than in western Europe and USA. It was hypothesized that genistein, the main isoflavone in soybean, could protect against prostate cancer. The result of a mammalian cell-based assay supports this hypothesis: genistein protected LAPC-4 prostate carcinoma cells against H_2O_2-induced DNA damage by up-regulating enzymatic and non-enzymatic antioxidants such as GR, microsomal GST and metallothionein 1X (Raschke et al. 2006). Among the genotoxic agents used in in vivo models, NDEA and dimethylbenz(a)anthracene (DMBA) are present in many food products. They both are activated by CYP450 isoenzymes in the liver. NDEA produces ROS and electrophile species that damage DNA (Ali et al. 2015) while DMBA is metabolized to a diol epoxide that oxidizes DNA bases and deoxyribose (Pugalendhi et al. 2009). The antigenotoxic effects of genistein against AFB1, IQ and MNU, proven in the Ames test, were confirmed by in vivo studies. Genistein significantly decreased the frequency of micronuclei in the bone marrow cells of mice exposed to each of the three genotoxicants (Polívková et al. 2006). In vivo antigenotoxic effects of genistein might be due to a restoration of the cellular antioxidant defense system (SOD, CAT, GPx, GR, GSH) depleted by genotoxic agents (Pugalendhi et al. 2009; Ali et al. 2015).

Daidzein contributes to health effects of soybean but the longer elimination half-time of genistein results in a higher efficacy of the latter (Zhang et al. 1999). With

Table 7 The antigenotoxic potential of isoflavonoids

Compound	Genotoxic agent	Antigenotoxic assay	Cell culture/ animals	Experimental protocol	Outcome	References
Genistein	NorA OxA	CA SCE	HPBLC	NorA or OxA (30 and 40 μM); genistein (30 and 40 μM) simultaneous treatment (48 h)	Reduction of SCE and total CA (gaps, chromatid and chromosome breaks) frequency	Beg et al. (2008)
	MMS CPA	CA SCE	HPBLC	MMS (60 μM) or CPA (0.16 μg/ml); genistein (25 and 40 μM) simultaneous treatment (48 h)	Reduction of SCE and CA (total breaks without gaps) frequency	Beg et al. (2007)
	H_2O_2	SCGE	LAPC-4	H_2O_2 (19 μM, 5 min at 4 °C); genistein (1–30 μM) pre-treatment (24 h)	Reduction of TI	Raschke et al. (2006)
		SCGE	NIH/3T3	H_2O_2 (50 μM); genistein (2.5 μg/ml) simultaneous treatment (30 min)	Reduction of OTM	Kim et al. (2008)
	AFB1 IQ MNU	Ames test	*ST* (TA98, TA100)	AFB1 (0.1–10 μg/plate) or IQ (TA98: 0.001–0.1 μg/plate; TA100: 0.1–10 μg/plate) or MNU (10–1000 μg/plate); genistein (0.3–300 μg/plate)	Dose-dependent reduction of mutagenicity	Polívková et al. (2006)
		CBMN	Balb/c mice	AFB1 (5 mg/kg, p.o.) or IQ (20 mg/kg, p.o.) or MNU (50 mg/kg, p.o.); genistein (20 mg/kg, p.o.) pre-treatment (3 days)	Reduction of MN in bone marrow	Polívková et al. (2006)
	NDEA	CBMN SCGE	Wistar rats	NDEA (0.1 mg/ml, p.o.); genistein (25–100 mg/ml p.o.) simultaneous treatment (21 days)	Reduction of TL and MN frequency in hepatocytes	Ali et al. (2015)
	DMBA	CA CBMN	Wistar rats	DMBA (30 mg/kg, i.p.); genistein (20 mg/kg, p.o.) pre-treatment (5 days)	Reduction of MN and CA frequency in bone marrow	Pugalendhi et al. (2009)
	2-AA DXR	CBMN	HTC	2-AA (13 μM) or DXR (0.2 μM); genistein (10 μM) simultaneous treatment (26 h)	Reduction of MN frequency	Lepri et al. (2013)
Daidzein	2-AAAF	SCGE	CHO	2-AAAF (800 nM); daidzein (50–400 μg/ml) simultaneous treatment (2 h)	Dose-dependent reduction of TM	Plewa et al. (2001)
	H_2O_2	SCGE	NIH/3T3	H_2O_2 (50 μM); daidzein (2.5 μg/ml) simultaneous treatment (30 min)	Reduction of OTM	Kim et al. (2008)

2-AA 2-aminoanthracene, *2-AAAF* 2-acetoxyacetylaminofluorene, *AFB1* aflatoxin B1, *CA* chromosomal aberrations, *CBMN* cytokinesis-block micronucleus, *CHO* Chinese hamster ovary cells, *CPA* cyclophosphamide, *DMBA* 7,12-dimethylbenz[a]anthracene, *DXR* doxorubicin, H_2O_2 hydrogen peroxide, *HPBLC* human peripheral blood lymphocyte cells, *HTC* rat hepatoma cells, *i.p.* intraperitoneal, *IQ* 2-amino-3-methylimidazo[4,5-f]quinoline, *LAPC-4* human prostate carcinoma cells, *MMS* methyl methanesulfonate, *MN* micronuclei, *MNU N*-nitroso-*N*-methylurea, *NDEA N*-nitrosodiethylamine, *NIH/3T3* mouse fibroblast cells, *NorA* norethandrolone, *OTM* olive tail moment, *OxA* oxandrolone, *p.o.* orally, *SCE* sister chromatid exchange, *SCGE* single cell gel electrophoresis, *ST Salmonella typhimurium*, *TI* tail intensity, *TL* tail length, *TM* tail moment

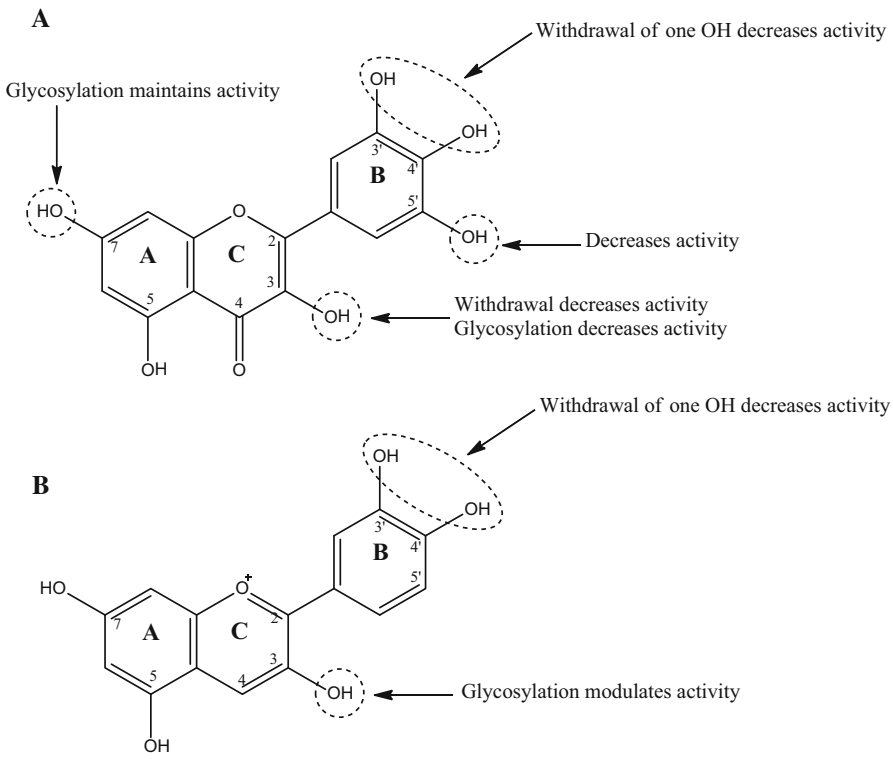


Fig. 3 Structure-antigenotoxic activity relationships of flavonoids (**A** Flavonols, **B** Anthocyanidins)

respect to daidzein, it protected against DNA damage induced by DXR, 2-AA, 2-acetoxyacetylaminofluorene (2-AAAF) and H_2O_2 (Plewa et al. 2001; Kim et al. 2008). Free radical scavenging appears to be less involved in the antigenotoxicity of genistein and daidzein. Both isoflavones showed weak free radical scavenging effects in comparison with vitamin C or trolox. At 7.5 μg/ml, vitamin C and trolox scavenged diphenylpicrylhydrazyl radical by 43.9 and 39.5 %, respectively; at the same concentration, the scavenging effects of genistein and daidzein were below 5 %. Genistein and daidzein showed higher activity in lipid peroxidation inhibition, namely inhibition of Cu^{2+}-mediated low density lipoprotein oxidation. At 7.5 μg/ml, vitamin C, trolox, genistein and daidzein inhibited lipid peroxidation by 39.2, 51.4, 30.6 and 27.3 %, respectively (Kim et al. 2008). Lipid peroxidation inhibition capacity of genistein and daidzein is more likely to be due to metal chelation than free radical scavenging effects.

In conclusion, flavonoids have protective effects against DNA damage induced by various genotoxic agents: mycotoxins, food processing-derived contaminants (polycyclic aromatic hydrocarbons, *N*-nitrosamines), cytostatic agents, other medications (estrogenic and androgenic hormones), nicotine, metal ions (Cd^{2+}, Cr^{6+}), radiopharmaceuticals and ionizing radiation. Based on some literature reports (Noroozi et al. 1998; Duthie 2007; Khandelwal and Abraham 2014), several structural features affecting the antigenotoxic potential can tentatively be highlighted. The *o*-diphenolic (catechol) moiety seems to be essential; an increase or a decrease in the number of free hydroxyls on the B ring reduces the activity. The free 3-hydroxyl group is also important; its withdrawal or glycosylation causes a decrease in activity. The activity decreases as the length of the sugar chain bound to hydroxyl group in position 3 increases. Besides, the nature of the sugar moiety (glucose, rhamnose) also affects the activity. In case of anthocyanidins, glycosylation may increase or decrease the antigenotoxic potency. These structure–activity relationships are summarized in Fig. 3. However, there is also literature data reporting the same degree of DNA protection for structurally different flavonoids (quercetin, rutin, myricetin) (Aherne and O'Brien 1999). Other studies found catechol-type flavonoids to be less active than non-catechol type ones against certain genotoxicants. For instance, in HepG2 cells, quercetin (catechol-type

flavonoid) was more efficient than myricetin (pyrogallol-type flavonoid) in protecting against NPIP-induced oxidative DNA damage but less efficient in affording protection against NDBA (Delgado et al. 2009). Similar behavior was detected for catechins. Within the same cell line, (−)-epicatechin showed higher protection against both NPIP- and NDBA-induced oxidative DNA damage than (+)-catechin (Delgado et al. 2009) whereas the latter was more active in reducing PhIP-induced DNA damage (Haza and Morales 2011). The mechanisms responsible for these differences in effectiveness need to be clarified.

In vitro investigations on antigenotoxic potential of flavonoids have to be followed by in vivo studies as flavonoids undergo an extensive metabolization and have a relatively low oral bioavailability. Flavonoid metabolism is very complex involving a wide array of reactions: deglycosylation, glucuronidation, sulfation, methylation, ring fission which take place in the gastrointestinal lumen, intestinal wall and liver (Erlund 2004). The metabolites and their precursors develop different effects. Although free radical scavenging and metal chelation have been proposed as possible mechanisms for DNA protective effects of flavonoids, both mechanisms cannot be predominant in vivo due to conjugation of free hydroxyl groups responsible for free radical scavenging and metal chelation. In vivo antigenotoxicity of flavonoids is based on other mechanisms such as modulation of enzymes involved in the bioactivation of genotoxic agents and detoxification of their reactive metabolites and up-regulation of antioxidant defense systems which inactivate ROS generated during the bioactivation of genotoxicants. Except anthocyanidins, antigenotoxic effects of other flavonoids are concentration-dependent both in vitro and in vivo. Flavonoids are antigenotoxic in a well-defined concentration range becoming genotoxic at higher concentrations and sometimes, surprisingly, at low concentrations, most probably due to pro-oxidant effects.

Overall, flavonoids have the ability to reduce the genotoxic stress but further in vivo investigations are needed for a better characterization of their metabolic transformations, relation dose-biological response and mechanisms involved in genetic protection.

Acknowledgments This work was supported by University of Medicine and Pharmacy Grigore T. Popa-Iasi, Internal Research Grant No. 1639/01.02.2013 (Investigations on the radioprotective potential of some vegetal extracts).

References

Abalea V, Cillard J, Dubos MP et al (1999) Repair of iron-induced DNA oxidation by the flavonoid myricetin in primary rat hepatocyte cultures. Free Radic Biol Med 26:1457–1466

Abraham SK, Schupp N, Schmid U et al (2007) Antigenotoxic effects of the phytoestrogen pelargonidin chloride and the polyphenol chlorogenic acid. Mol Nutr Food Res 51:880–887

Abraham SK, Eckhardt A, Oli RG et al (2012) Analysis in vitro chemoprevention of genotoxic damage by phytochemicals, as single agents or as combinations. Mutat Res 744:117–124

Aherne SA, O'Brien NM (1999) Protection by the flavonoids myricetin, quercetin, and rutin against hydrogen peroxide-induced DNA damage in Caco-2 and HepG2 cells. Nutr Cancer 34:160–166

Aherne SA, O'Brien NM (2000a) Lack of effect of the flavonoids, myricetin, quercetin, and rutin, on repair of H_2O_2-induced DNA single strand breaks in Caco-2, HepG2, and V79 cells. Nutr Cancer 38:106–115

Aherne SA, O'Brien NM (2000b) Mechanism of protection by the flavonoids, quercetin and rutin, against *tert*-butylhydroperoxide- and menadione-induced DNA single strand breaks in Caco-2 cells. Free Radic Biol Med 29:507–514

Ajithkumar T, Parkinson C, Shamshad F et al (2007) Iphosphamide encephalopathy. Clin Oncol 19:108–114

Alaejos MS, Pino V, Afonso AM (2008) Metabolism and toxicology of heterocyclic aromatic amines when consumed in diet: influence of the genetic susceptibility to develop human cancer. A review. Food Res Int 41:327–340

Ali F, Rahul Naz F et al (2014) Protective effect of apigenin against *N*-nitrosodiethylamine (NDEA)-induced hepatotoxicity in albino rats. Mutat Res 767:13–20

Ali F, Rahul Naz F et al (2015) Protective effect of genistein against *N*-nitrosodiethylamine (NDEA)-induced hepatotoxicity in Swiss albino rats. J Pharm Anal 5(1):51–57

Alvarez-González I, Madrigal-Bujaidar E, Dorado V et al (2001) Inhibitory effect of naringin on the micronuclei induced by ifosfamide in mouse, and evaluation of its modulatory effect on the Cyp3a subfamily. Mutat Res 480–481:171–178

Aprotosoaie AC, Mihai CT, Voichita G et al (2016) Antigenotoxic and antioxidant activities of a polyphenolic extract from European *Dracocephalum moldavica* L. Ind Crop Prod 79:248–257

Arts ICW, Hollman PCH, Feskens EJM et al (2001) Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen elderly study. Am J Clin Nutr 74:227–232

Attia SM (2012) Influence of resveratrol on oxidative damage in genomic DNA and apoptosis induced by cisplatin. Mutat Res 741:22–31

Azevedo L, Alves de Lima PL, Gomes JC et al (2007) Differential response related to genotoxicity between eggplant (*Solanum melanogena*) skin aqueous extract and its main purified anthocyanin (delphinidin) in vivo. Food Chem Toxicol 45:852–858

Bacanli M, Başaran AA, Başaran N (2015) The antioxidant and antigenotoxic properties of citrus phenolics limonene and naringin. Food Chem Toxicol 81:160–170

Bahari A, Mehrzad J, Mahmoudi M et al (2014) Cytochrome P450 isoforms are differently up-regulated in aflatoxin B1-exposed human lymphocytes and monocytes. Immunopharmacol Immunotoxicol 36:1–10

Barcelos GRM, Grotto D, Angeli JP et al (2011) Evaluation of antigenotoxic effects of plant flavonoids quercetin and rutin on HepG2 cells. Phytother Res 25:1381–1388

Beg T, Siddique YH, Ara G et al (2007) Antimutagenic evaluation of genistein, a polyphenol, in cultured human peripheral blood lymphocytes. Biomed Res 18(2):139–143

Beg T, Siddique YH, Ara G et al (2008) Antigenotoxic effect of genistein and gingerol on genotoxicity induced by norethandrolone and oxandrolone in cultured human lymphocytes. Int J Pharmacol 4(3):177–183

Bingül İ, Başaran-Küçükgergin C, Tekkeşin MS et al (2013) Effect of blueberry pretreatment on diethylnitrosamine-induced oxidative stress and liver injury in rats. Environ Toxicol Pharmacol 36:529–538

Boots AW, Haenen GRMM, Bast A (2008) Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol 585:325–337

Bramati L, Aquilano F, Pietta P (2003) Unfermented rooibos tea: quantitative characterization of flavonoids by HPLC-UV and determination of the total antioxidant activity. J Agric Food Chem 51:7472–7474

Burcham PC (1998) Genotoxic lipid peroxidation products: their DNA damaging properties and role in formation of endogenous DNA adducts. Mutagenesis 13:287–305

Cheng YT, Wu CH, Ho CY et al (2013) Catechin protects against ketoprofen-induced oxidative damage of the gastric mucosa by up-regulating Nrf2 in vitro and in vivo. J Nutr Biochem 24:475–483

Chirumbolo S (2010) The role of quercetin, flavonols and flavones in modulating inflammatory cell function. Inflamm Allergy Drug Targets 9:263–285

Choi JS, Piao YJ, Kang KW (2011) Effects of quercetin on the bioavailability of doxorubicin in rats: role of CYP3A4 and P-gp inhibition of quercetin. Arch Pharm Res 34:607–613

Choi EJ, Kim T, Kim GH (2012) Quercetin acts as an antioxidant and downregulates CYP1A1 and CYP1B1 against DMBA-induced oxidative stress in mice. Oncol Rep 28:291–296

Chua LS (2013) A review on plant-based rutin extraction methods and its pharmacological activities. J Ethnopharmacol 150:805–817

Cushnie TP, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26:343–356

D'Andrea G (2015) Quercetin: a flavonol with multifaceted therapeutic applications? Fitoterapia 106:256–271

Delgado ME, Haza AI, Arranz N et al (2008) Dietary polyphenols protect against *N*-nitrosamines and benzo(a) pyrene-induced DNA damage (strand breaks and oxidized purines/pyrimidines) in HepG2 human hepatoma cells. Eur J Nutr 47:479–490

Delgado ME, Haza AI, García A et al (2009) Myricetin, quercetin, (+)-catechin and (–)-epicatechin against *N*-nitrosamines-induced DNA damage in human hepatoma cells. Toxicol In Vitro 23:1292–1297

Delgado L, Fernandes I, González-Manzano S et al (2014) Anti-proliferative effects of quercetin and catechin metabolites. Food Funct 5:797–803

Di Giacomo C, Acquaviva R, Piva A et al (2007) Protective effect of cyanidin 3-*O*-β-D-glucoside on ochratoxin A-mediated damage in the rat. Brit J Nutr 98:937–943

Díaz Barriga Arceo S, Madrigal-Bujaidar E, Calderón Montellano E et al (1995) Genotoxic effects produced by capsaicin in mouse during subchronic treatment. Mutat Res 345:105–109

Dietz BM, Kang YH, Liu G et al (2005) Xanthohumol isolated from *Humulus lupulus* inhibits menadione-induced DNA damage through induction of quinone reductase. Chem Res Toxicol 18:1296–1305

Duffus JH, Nordberg M, Templeton DM (2007) Glossary of terms used in toxicology, 2nd edition (IUPAC recommendations 2007). Pure Appl Chem 79:1153–1344

Duthie SJ (2007) Berry phytochemicals, genomic stability and cancer: evidence for chemoprotection at several stages in the carcinogenic process. Mol Nutr Food Res 51:665–674

Erlund I (2004) Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr Res 24:851–874

Esselen M, Barth SW, Winkler S et al (2013) Anthocyanins suppress the cleavable complex formation by irinotecan and diminish its DNA-strand-breaking activity in the colon of Wistar rats. Carcinogenesis 34:835–840

Fimognari C, Berti F, Cantelli-Forti G et al (2004) Effect of cyanidin-3-*O*-β-glucopyranoside on micronucleus induction in cultured human lymphocytes by four different mutagens. Environ Mol Mutagen 43:45–52

Gadkari PV, Balaraman M (2015) Catechins: sources, extraction and encapsulation: a review. Food Bioprod Process 93:122–138

Gao S, Chen T, Choi MK et al (2013) Cyanidin reverses cisplatin-induced apoptosis in HK-2 proximal tubular cells through inhibition of ROS-mediated DNA damage and modulation of the ERK and AKT patways. Cancer Lett 333:36–46

García-Rodríguez MC, Nicolás-Méndez T, Montaño-Rodríguez AR et al (2014) Antigenotoxic effects of (−)-epigallocatechin-3-gallate (EGCG), quercetin, and rutin on chromium trioxide-induced micronuclei in the polychromatic erythrocytes of mouse peripheral blood. J Toxicol Environ Health Part A 77:324–336

Glei M, Pool-Zobel BL (2006) The main catechin of green tea (–)-epigallocatechin-3-gallate (EGCG), reduces bleomycin-induced DNA damage in human leucocytes. Toxicol In Vitro 20:295–300

Granado-Serrano AB, Martín MA, Bravo L et al (2012) Quercetin modulates Nrf2 and glutathione-related defenses in HepG2 cells: involvement of p38. Chem Biol Interact 195:154–164

Guo M, Perez C, Wei J et al (2007) Iron-binding properties of plant phenolics and cranberry's bioeffects. Dalton Trans 43:4951–4961. doi:10.1039/b705136k

Gupta C, Vikram A, Tripathi DN et al (2010) Antioxidant and antimutagenic effect of quercetin against DEN induced hepatotoxicity in rat. Phytother Res 24:119–128

Gustafson DL, Pritsos CA (1993) Kinetics and mechanism of mitomycin C bioactivation by xanthine dehydrogenase under aerobic and hypoxic conditions. Cancer Res 53:5470–5474

Han X, Shen T, Lou H (2007) Dietary polyphenols and their biological significance. Int J Mol Sci 8:950–988

Hassan AM, Alam SS, Abdel-Aziem SH et al (2011) Benzo-a-pyrene induced genotoxicity and cytotoxicity in germ cells of mice: intervention of radish and cress. J Genet Eng Biotechnol 9:65–72

Haza AI, Morales P (2011) Effects of (+) catechin and (−) epicatechin on heterocyclic amines-induced oxidative DNA damage. J Appl Toxicol 31:53–62

Hobbs CA, Swartz C, Maronpot R et al (2015) Genotoxicity evaluation of the flavonoid, myricitrin, and its aglycone, myricetin. Food Chem Toxicol 83:283–292

Hosseinimehr SJ, Ahmadi A, Beiki D et al (2009) Protective effects of hesperidin against genotoxicity induced by ^{99m}Tc-MIBI in human cultured lymphocyte cells. Nucl Med Biol 36:863–867

Hundal BS, Dhillon VS, Sidhu IS (1997) Genotoxic potential of estrogens. Mutat Res 389:173–181

Iacopini P, Baldi M, Storchi P et al (2008) Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: content, in vitro antioxidant activity and interactions. J Food Compos Anal 21:589–598

Iyama T, Wilson DM III (2013) DNA repair mechanisms in dividing and non-dividing cells. DNA Repair 12:620–636

Jackson SP, Bartek J (2009) The DNA damage response in human biology and disease. Nature 461:1071–1078

Jagetia GC, Reddy TK (2002) The grapefruit flavanone naringin protects against the radiation-induced genomic instability in the mice bone marrow: a micronucleus study. Mutat Res 519:37–48

Kalpana KB, Devipriya N, Srinivasan M et al (2009) Investigation of the radioprotective efficacy of hesperidin against gamma-radiation induced cellular damage in cultured human peripheral blood lymphocytes. Mutat Res 676:54–61

Kalpana KB, Devipriya N, Srinivasan M et al (2011) Evaluating the radioprotective effect of hesperidin in the liver of Swiss albino mice. Eur J Pharmacol 658:206–212

Kanadzu M, Lu Y, Morimoto K (2006) Dual function of (–)-epigallocatechin gallate (EGCG) in healthy human lymphocytes. Cancer Lett 241:250–255

Kang ZC, Tsai SJ, Lee H (1999) Quercetin inhibits benzo[a] pyrene-induced DNA adducts in human Hep G2 cells by altering cytochrome P-450 1A1 gene expression. Nutr Cancer 35:175–179

Khan TH, Jahangir T, Prasad L et al (2006) Inhibitory effect of apigenin on benzo(a)pyrene-mediated genotoxicity in Swiss albino mice. J Pharm Pharmacol 58:1655–1660

Khandelwal N, Abraham SK (2014) Intake of anthocyanidins pelargonidin and cyanidin reduces genotoxic stress in mice induced by diepoxybutane, urethane and endogenous nitrosation. Environ Toxicol Pharmacol 37:837–843

Khandrika L, Kumar B, Koul S et al (2009) Role of oxidative stress in prostate cancer. Cancer Lett 282:125–136

Kim NY, Song EJ, Kwon DY et al (2008) Antioxidant and antigenotoxic activities of Korean fermented soybean. Food Chem Toxicol 46:1184–1189

Kleemann R, Verschuren L, Morrison M et al (2011) Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models. Atherosclerosis 218:44–52

Kluth D, Banning A, Paur I et al (2007) Modulation of pregnane X receptor-and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds. Free Radic Biol Med 42:315–325

Kohli E, Raj HG, Kumari R et al (2002) Comparison of the prevention of aflatoxin B1-induced genotoxicity by quercetin and quercetin pentaacetate. Bioorg Med Chem Lett 12:2579–2582

Kreft I, Fabjan N, Yasumoto K (2006) Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. Food Chem 98:508–512

Lenglet G, David-Cordonnier M-H (2010) DNA-Destabilizing agents as an alternative approach for targeting DNA: Mechanisms of action and cellular consequences. DOI, J Nucleic Acids. doi:10.4061/2010/290935

Lepri SR, Luiz RC, Zanelatto LC et al (2013) Chemopreventive activity of the isoflavones, genistein and daidzein on mutagenicity induced by direct and indirect mutagens in cultured HTC cells. Cytotechonology 65:213–222

Li Y, Ding Y (2012) Minireview: therapeutic potential of myricetin in diabetes mellitus. Food Sci Human Wellness 1:19–25

Liu M, Hansen PE, Wang G et al (2015) Pharmacological profile of xanthohumol, a prenylated flavonoid from hops (*Humulus lupulus*). Molecules 20:754–779

López-Lázaro M (2009) Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem 9:31–59

Makris DM, Rossiter JT (2001) Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): effect on flavonol content and antioxidant status. J Agric Food Chem 49:3216–3222

Marcarini JC, Tsuboy MSF, Luiz RC et al (2011) Investigation of cytotoxic, apoptosis-inducing, genotoxic and protective effects of the flavonoid rutin in HTC hepatic cells. Exp Toxicol Pathol 63:459–465

Marcolin E, San-Miguel B, Vallejo D et al (2012) Quercetin treatment ameliorates inflammation and fibrosis in mice with nonalcoholic steatohepatitis. J Nutr 142:1821–1828

Meireles JRC, Oliveira SV, Costa-Neto AO et al (2013) Genotoxic and cytotoxic effects of testosterone cypionate (deposteron®). Mutat Res 753:72–75

Mendoza-Wilson AM, Glossman-Mitnik D (2006) Theoretical study of the molecular properties and chemical reactivity of (C)-catechin and (K)-epicatechin related to their antioxidant ability. J Mol Struct (Theochem) 761:97–106

Mullie P, Clarys P, Deriemaeker P et al (2007) Estimation of daily human intake of food flavonoids. Plant Foods Hum Nutr 62:93–98

Musallam I, Duwayri M, Shibli R et al (2012) Investigation of rutin content in different plant parts of wild caper (*Capparis spinosa* L.) populations from Jordan. Res J Med Plant 6:27–36

Muthukumaran S, Sudheer AR, Menon VP et al (2008) Protective effect of quercetin on nicotine-induced prooxidant

and antioxidant imbalance and DNA damage in Wistar rats. Toxicology 243:207–215

National Toxicology Program (2002). Report on Carcinogens, Tenth Edition; U.S. Department of Health and Human Services, Public Health Service, Research Triangle Park, North Carolina. http://ntp.niehs.nih.gov/pubhealth/roc/listings/a/iq/summary/index.html

Nicolella HD, de Oliveira PF, Munari CC et al (2014) Differential effect of manool—A diterpene from Salvia officinalis, on genotoxicity induced by methyl methanesulfonate in V79 and HepG2 cells. Food Chem Toxicol 72:8–12

Noel S, Kasinathan M, Rath SK (2006) Evaluation of apigenin using in vitro cytochalasin blocked micronucleus assay. Toxicol In Vitro 20:1168–1172

Noroozi M, Angerson WJ, Lean ME (1998) Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. Am J Clin Nutr 67:1210–1218

Norval M, Lucas RM, Cullen AP et al (2011) The human health effects of ozone depletion and interactions with climate change. Photochem Photobiol Sci 10:199–225

Orhan F, Gulluce M, Ozkan H et al (2013) Determination of the antigenotoxic potential of some luteolin derivatives by using a eukaryotic cell system, *Saccharomyces cerevisiae*. Food Chem 141:366–372

Oršolić N, Gajski G, Garaj-Vrhovac V et al (2011) DNA-protective effects of quercetin or naringenin in alloxan-induced diabetic mice. Eur J Pharmacol 656:110–118

Ouedraogo M, Baudoux T, Stévigny C et al (2012) Review of current and "omics" methods for assessing the toxicity (genotoxicity, teratogenity and nefrotoxicity) of herbal medicines and mushrooms. J Ethnopharmacol 140:492–512

Pandir D (2015) Protective effect of—epigallocatechin-3-gallate on capsaicin-induced DNA damage and oxidative stress in human erythrocytes and leucocytes in vitro. Cytotechnology 67:367–377

Papiez MA (2013) The influence of curcumin and (–)-epicatechin on the genotoxicity and myelosuppression induced by etoposide in bone marrow cells of male rats. Drug Chem Toxicol 36(1):93–101

Patil SL, Rao NB, Somashekarappa HM et al (2014) Antigenotoxic potential of rutin and quercetin in Swiss mice exposed to gamma radiation. Biomed J 37(5):305–313

Pérez-Vizcaíno F, Duarte J, Jimenez R et al (2009) Antihypertensive effects of the flavonoid quercetin. Pharmacol Rep 61:67–75

Pfohl-Leszkowicz A, Manderville RA (2012) An update on direct genotoxicity as a molecular mechanism of ochratoxin A carcinogenicity. Chem Res Toxicol 25:252–262

Plazar J, Žegura B, Lah TT et al (2007) Protective effects of xanthohumol against the genotoxicity of benzo(a)pyrene (BaP), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and *tert*-butyl hydroperoxide (*t*-BOOH)in HepG2 human hepatoma cells. Mutat Res 632:1–8

Plazar J, Filipič M, Groothuis GMM (2008) Antigenotoxic effect of xanthohumol in rat liver slices. Toxicol in Vitro 22:318–327

Plewa MJ, Berhow MA, Vaughn SF et al (2001) Isolating antigenotoxic components and cancer cell growth suppressors from agricultural by-products. Mutat Res 480–481:109–120

Polívková Z, Langová M, Šmerák P et al (2006) Antimutagenic effect of genistein. Czech J Food Sci 24:119–126

Polkowski K, Mazurek AP (2000) Biological properties of genistein. A review of in vitro and in vivo data. Acta Pol Pharm Drug Res 57:135–155

Pugalendhi P, Manoharan S, Panjamurthy K et al (2009) Antigenotoxic effect of genistein against 7,12-dimethylbenz[a]anthracene induced genotoxicity in bone marrow cells of female Wistar rats. Pharmacol Rep 61:296–303

Quiles JL, Huertas JR, Battino M et al (2002) Antioxidant nutrients and adriamycin toxicity. Toxicology 180:79–85

Ramos AA, Lima CF, Pereira ML et al (2008) Antigenotoxic effects of quercetin, rutin and ursolic acid on HepG2 cells: evaluation by the comet assay. Toxicol Lett 177:66–73

Ramos AA, Pereira-Wilson C, Collins AR (2010) Protective effects of ursolic acid and luteolin against oxidative DNA damage include enhancement of DNA repair in Caco-2 cells. Mutat Res 692:6–11

Ramyaa P, Padma VV (2013) Ochratoxin-induced toxicity, oxidative stress and apoptosis ameliorated by quercetin—modulation by Nrf2. Food Chem Toxicol 62:205–216

Ramyaa P, Krishnaswamy R, Padma VV (2014) Quercetin modulates OTA-induced oxidative stress and redox signaling in HepG2 cells—up regulation of Nrf2 expression and down regulation of NF-kB and COX-2. Biochim Biophys Acta 1840:681–692

Raschke M, Rowland IR, Magee PJ et al (2006) Genistein protects prostate cells against hydrogen peroxide-induced DNA damage and induces expression of genes involved in the defence against oxidative stress. Carcinogenesis 27:2322–2330

Rice-Evans CA, Miller JM, Paganga G (1996) Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956

Richi B, Kale RK, Tiky AB (2012) Radio-modulatory effects of green tea catechin EGCG on pBR322 plasmid DNA and murine splenocytes against gamma-radiation induced damage. Mutat Res 747:62–70

Russo A, La Fauci L, Acquaviva R et al (2005) Ochratoxin A-induced DNA damage in human fibroblast: protective effect of cyanidin 3-*O*-β-D-glucoside. J Nutr Biochem 16:31–37

Siddique YH, Afzal M (2009a) Protective effect of apigenin against hydrogen peroxide induced genotoxic damage on cultured human peripheral blood lymphocytes. J Appl Biomed 7:35–43

Siddique YH, Afzal M (2009b) Antigenotoxic effect of apigenin against mitomycin C induced genotoxic damage in mice bone marrow cells. Food Chem Toxicol 47:536–539

Siddique YH, Beg T, Afzal M (2005) Genotoxic potential of ethinylestradiol in cultured mammalian cells. Chem Biol Interact 151:133–141

Siddique YH, Beg T, Afzal M (2008) Antigenotoxic effect of apigenin against anti-cancerous drugs. Toxicol In Vitro 22:625–631

Siddique YH, Ara G, Beg T et al (2010) Anticlastogenic effect of apigenin in human lymphocytes treated with ethinylestradiol. Fitoterapia 81:590–594

Srinivasan M, Sudheer AR, Pillai KR et al (2007) Modulatory effects of curcumin on γ-radiation-induced cellular

damage in primary culture of isolated rat hepatocytes. Environ Toxicol Pharmacol 24:98–105
Sroka Z (2005) Antioxidative and antiradicalar properties of plant phenolics. Z Naturforsch 60c:833–843
Swift LH, Golsteyn RM (2014) Genotoxic anti-cancer agents and their relationship to DNA damage, mitosis, and checkpoint adaptation in proliferating cancer cells. Int J Mol Sci 15:3403–3431
Tang Y, Tian H, Shi Y et al (2013) Quercetin suppressed CYP2E1-dependent ethanol hepatotoxicity via depleting heme pool and releasing CO. Phytomedicine 20:699–704
Tanigawa S, Fujii M, Hou D-X (2007) Action of Nrf2 and Keap1 in ARE-mediated NQO1 expression by quercetin. Free Radic Biol Med 42:1690–1703
Towner RA, Qian SY, Kadiiska MB et al (2003) In vivo identification of aflatoxin-induced free radicals in rat bile. Free Radic Biol Med 35:1330–1340
Trivedi PP, Tripathi DN, Jena GB (2011) Hesperetin protects testicular toxicity of doxorubicin in rat: role of NFkB, p38 and capsase-3. Food Chem Toxicol 49:838–847
Uhl M, Ecker S, Kassie F et al (2003) Effect of chrysin, a flavonoid compound, on the mutagenic activity of 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhIP) and benzo(a)pyrene (B(a)P) in bacterial and human hepatoma (HepG2) cells. Arch Toxicol 77:477–484
Ündeğer Ü, Aydın S, Basaran AA et al (2004) The modulating effects of quercetin and rutin on the mitomycin C induced DNA damage. Toxicol Lett 151:143–149
Vedmaurthy RB, Padmanabhan S, Vijayan M et al (2012) Compatibility of different solvents with *Salmonella typhimurium* mutant strains in bacterial reverse mutation assay. Int J Pharm Pharm Sci 4:283–284
Veberic R, Trobec M, Herbinger K et al (2005) Phenolic compounds in some apple (*Malus domestica* Borkh) cultivars of organic and integrated production. J Sci Food Agric 85:1687–1694
Veberic R, Colaric M, Stampar F (2008) Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. Food Chem 106:153–157
Wang S-L, Han J-F, He X-Y et al (2007) Genetic variation of human cytochrome P450 reductase as a potential biomarker for mitomycin C-induced cytotoxicity. Drug Metab Dispos 35:176–179
Wellejus A, Bornholdt J, Vogel UB et al (2004) Cell-specific oxidative DNA damage induced by estrogen in rat testicular cells in vitro. Toxicol Lett 150:317–323
Wilms LC, Hollman PC, Boots AW et al (2005) Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. Mutat Res 582:155–162
Wu LC, Lu IW, Chung CF et al (2011) Antiproliferative mechanisms of quercetin in rat activated hepatic stellate cells. Food Funct 2:204–212
Yeh SL, Wu SH (2006) Effects of quercetin on beta-apo-8′-carotenal-induced DNA damage and cytochrome P1A2 expression in A549 cells. Chem Biol Interact 163:199–206
Yeh SL, Wang WY, Huang CS et al (2006) Flavonoids suppresses the enhancing effect of β-carotene on DNA damage induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A549 cells. Chem Biol Interact 160:175–182
Yılmaz D, Aydemir NC, Vatan Ö et al (2012) Influence of naringin on cadmium-induced genomic damage in human lymphocytes in vitro. Toxicol Ind Health 28:114–121
Yu P, Zhou Q, Zhu W et al (2013) Effects of quercetin on LPS-induced disseminated intravascular coagulation (DIC) in rabbits. Thromb Res 131:e270–e273
Yurtcu E, Íşeri ÖD, Sahin FI (2014) Genotoxic and cytotoxic effects of doxorubicin and silymarin on human hepatocellular carcinoma cells. Hum Exp Toxicol 33:1269–1276
Zhang Y, Wang G-J, Song TT et al (1999) Urinary disposition of the soybean isoflavones daidzein, genistein and glycitein differs among humans with moderate fecal isoflavone degradation activity. J Nutr 129:957–962