

Epigallocatechin gallate: a review

L. BARTOSIKOVA*, J. NECAS

Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

*Corresponding author: lenka.bartosikova@upol.cz

ABSTRACT: Epigallocatechin gallate is the major component of the polyphenolic fraction of green tea and is responsible for most of the therapeutic benefits of green tea consumption. A number of preclinical *in vivo* and *in vitro* experiments as well as clinical trials have shown a wide range of biological and pharmacological properties of polyphenolic compounds such as anti-oxidative, antimicrobial, anti-allergic, anti-diabetic, anti-inflammatory, anti-cancer, chemoprotective, neuroprotective and immunomodulatory effects. Epigallocatechin gallate controls high blood pressure, decreases blood cholesterol and body fat and decreases the risk of osteoporotic fractures. Further research should be performed to monitor the pharmacological and clinical effects of green tea and to more clearly elucidate its mechanisms of action and the potential for its use in medicine.

Keywords: epigallocatechin-3-*O*-gallate; pharmacokinetics; toxicity; biological activity

List of abbreviations

ADME = absorption, distribution, metabolism, excretion; AMP = activated protein kinase (AMPK); AUC (0–∞) = area under the concentration-time curve from 0 h to infinity; BAX = apoptosis regulator (pro-apoptotic regulator, known as bcl-2-like protein 4); Bcl-2 = B-cell lymphoma 2; BCL-XL = B-cell lymphoma-extra large; CA Prop 65 = California Proposition 65; cccDNA = covalently closed circular DNA; C_{max} = peak plasma concentration; COMT = catechol-*O*-methyltransferase; DHFR = dihydrofolate reductase; DNMT = DNA methyltransferase; EBV = Epstein-Barr virus; EC = (–)-epicatechin; ECG = (–)-epicatechin-3-*O*-gallate; EGC = (–)-epigallocatechin; EGCG = (–)-epigallocatechin-3-*O*-gallate; EGFR = epidermal growth factor receptor; ERK = extracellular signal-regulated kinases; GIT = gastrointestinal tract; HBV = hepatitis B virus; HCV = hepatitis C virus; HER2 = human epidermal growth factor receptor 2; HIV = human immunodeficiency virus; HIF-1α = hypoxia-inducible factor 1-alpha; HPV = human papilloma virus; HSV = herpes simplex virus; IGFIR = insulin-like growth factor I receptor; IL = interleukin; LC/ESI-MS2 = liquid chromatography-electrospray ionization-mass spectrometry; LD₅₀ = median lethal dose; LDLo = the lowest dose causing lethality; MAO = monoamine oxidase; MAPK = mitogen-activated protein kinases; MC3T3 = osteoblast precursor cell line (derived from *Mus musculus*); MIC₅₀ = minimum inhibitory concentration (inhibits the growth of 50% of organisms); MMPs = matrix metalloproteinases; mRNA = messenger RNA; NF-κB = nuclear factor kappa B; NOAEL = no-observed adverse effect level; NOS = nitric oxide synthase; PTEN = phosphatase and tensin homolog; ROS = reactive oxygen species; Runx2 = runt-related transcription factor-2; Saos = sarcoma osteogenic; SAPK/JNK = stress-activated protein kinases/Jun amino-terminal kinases; SULT = sulfotransferases; TDLo = toxic dose low (the lowest dose causing a toxic effect); THF = tetrahydrofolate; TLR-4 = toll like receptor; T_{max} = time to reach peak plasma concentration; TNFα = tumour necrosis factor alpha; UGT = glucuronosyltransferase

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1. Introduction

Tea is one of the most popular non-alcoholic beverages and is consumed by more than one third of the world's population due to its stimulant effects, attractive aroma, refreshing taste and health benefits. Green tea belongs to the Theaceace family and comes from two main varieties: *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* (Yang et al. 2016). The main bioactive constituents of green tea leaves are catechins, which account for 25% to 35% of their dry weight. The main catechin group consists of eight polyphenolic flavonoid-type compounds, namely, (+)-catechin (C), (–)-epicatechin (EC), (+)-gallocatechin (GC), (–)-epigallocatechin (EGC), (+)-catechin gallate (CG), (–)-epicatechin gallate (ECG), (+)-gallocatechin gallate (GCG) and (–)-epigallocatechin gallate (EGCG) (Sang et al. 2011; Min and Kwon 2014).

Many medicinal benefits of green tea extracts are dependent on the content of polyphenols and its derivatives. (–)-epigallocatechin gallate is the main and essential tea catechin and is thought to be responsible for the majority of the biological activity of green tea (Nagle et al. 2006; Sutherland et al. 2006; Mereles and Hunstein 2011; Das et al. 2014). The structure of EGCG is depicted below in Figure 1.

1.1 Chemical and physical identification

Molecular formula: $C_{22}H_{18}O_{11}$; molecular weight: 458.40; CAS registry number: 989-51-5.

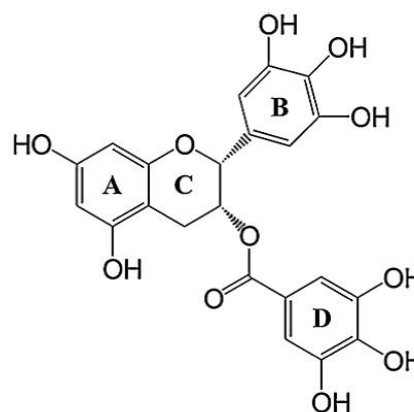


Figure 1. Structure of (–)-epigallocatechin gallate (adopted from Min and Kwon 2014)

1.1.1 Chemical and physical properties

In its pure form, EGCG takes the form of an odourless white, faint pink, or cream-coloured powder or crystals. It is soluble in water (clear, colourless solution at 5 mg/ml), acetone, ethanol, methanol,

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pyridine and tetrahydrofuran. It has a melting point of 218 °C (Merck 1997; Alexis corporation, www.enzolifesciences.com; Sigma-Aldrich 2000, www.sigmaaldrich.com/catalog/substance/epigallocatechingallate4583798951511?lang=en®ion).

1.1.2 Synonyms and trade names

Epigallocatechin gallate; epigallocatechin-3-gallate; (–)-epigallocatechin-3-*O*-gallate; 3,4,5-trihydroxybenzoic acid, (2*R*-*cis*)-3,4-dihydro-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-2*H*-1-benzopyran-3-yl ester; (2*R*,3*R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1(2*H*)-benzopyran-3,5,7-triol 3-(3,4,5-tri-hydroxybenzoate) (Merck 1997; Alexis corporation, www.enzolifesciences.com; Sigma-Aldrich 2000, www.sigmaaldrich.com/catalog/substance/epigallocatechingallate4583798951511?lang=en®ion).

1.1.3 Structure-activity relationship

EGCG has three aromatic rings (A, B and D) that are linked together by a pyran ring (C) (Figure 1). The health-promoting function of EGCG is attributed to its structure. The antiradical effects of catechins are achieved by oxidation of phenolic groups with atomic or single electron transfer in the B- and D-rings by semiquinone and quinone production (Lambert and Elias 2010; Min and Kwon 2014). Landis-Piwowar et al. (2005) found that the B- and D-rings are associated with an inhibition of proteasome activity. This inhibition of proteasome activity is exhibited only by protected analogues. Dehydroxylation of the B- and/or D-ring decreases proteasome-inhibitory activity *in vitro*. Furthermore, these protected analogues induced apoptotic cell death in a tumour cell-specific manner. These data suggest that the B-ring/D-ring peracetate-protected EGCG analogues have great potential to be developed into novel anti-cancer and cancer-preventive agents (Landis-Piwowar et al. 2005). Khandelwal et al. (2013) described and evaluated the first structure-activity relationships between EGCG and heat-shock protein 90. The results obtained suggest that phenolic groups on the A-ring are beneficial for heat-shock protein 90 inhibition, while phenolic substituents on the D-ring are detrimental (Khandelwal et al. 2013). Finally,

the hydroxyl group at the 5'-position in the B-ring showed 35–104-fold urease inhibition compared with the catechins without the 5'-hydroxyl group and inhibits the growth of *Helicobacter pylori* in the stomach (Matsubara et al. 2003).

1.1.4 Analytical methods for tea constituents

A considerable number of common analytical methods including chromatography and gel chromatography, HPLC, HPLC/electrochemical detection technique, LC-MS/MS, HPLC/MS and/or others have been validated for separation and quantification of tea catechins (Baumann et al. 2001; Sano et al. 2001; Pomponio et al. 2003; Wu et al. 2003; Wu et al. 2012). HPLC, UV spectrum and electrochemical detection is the standard and most widely used method for detection of tea constituents. The LC method for the analysis of tea polyphenols was first reported in 1976 by Hoefler and Coggon (Dalluge and Nelson 2000).

LC with coulometric electrode array detection was first reported by Lee et al. (1995). These authors analysed tea catechins in human body fluids (plasma, urine); limits of detection for EC, EGCG and EGCG were 1.0 ng/ml.

A number of other detection methods were used to measure the levels of tea constituents in biological samples, including chemiluminescence (detection limit of 20 ng/ml) (Ho et al. 1995), capillary electrophoresis (CE), capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MEKC) and non-aqueous capillary electrophoresis (NACE) (Horie et al. 1997; Dalluge and Nelson 2000; Wright et al. 2001; Weiss and Anderton 2003; Weiss et al. 2006).

2. Pharmacokinetics

The main pharmacokinetic parameters of green tea polyphenols, particularly EGCG, have been well investigated both in animals (Unno and Takeda 1995; Chen et al. 1997; Nakagawa and Miyazawa 1997; Kim and Lim 1999; Zhu et al. 2000; Swezey et al. 2001; Crowell et al. 2005) and humans (Lee et al. 2002; Chow et al. 2003; Clifford et al. 2013). Absorption, distribution, metabolism and excretion (ADME) experiments in rats and dogs have

shown that EGCG is rapidly absorbed by the intestinal system, distributed, metabolised in the liver and colon and can be reabsorbed from the intestine through enterohepatic re-circulation. The resulting metabolites are excreted through both biliary and urinary pathways. However, only traces of EGCG are detected in urine after oral administration (Lee et al. 1995; Lee et al. 2002; Meng et al. 2002). In addition, considerable differences were observed in pharmacokinetic parameters in repeated experiments and among individual subjects. Many factors act to enhance plasma levels and thus EGCG bioavailability (cool and dry storage, fasting conditions, albumin, soft water vitamin C, fish oil, piperine), while others diminish EGCG bioavailability (air contact oxidation, gastrointestinal inactivation, Ca^{2+} , Mg^{2+} , metals, COMT polymorphisms, sulfation, glucuronidation) (Mereles and Hunstein 2011).

2.1 Absorption

In animal experiments, EGCGs show poor bioavailability after oral administration: the absolute bioavailability of EGCG in CF-1 mice and Sprague-Dawley rats was found to be only 26.5 and 1.6%, respectively. Following a single oral administration to Beagle dogs, absorption was rapid with a maximal concentration in plasma at approximately one hour (Crowell et al. 2005). The low bioavailability of catechins may, in part, be caused by first pass effects, which causes drug loss via gastrointestinal metabolism and/or extraction by the liver immediately after absorption (Zhu et al. 2000). The bioavailability for humans is assumed to be in the same range (Chen et al. 1997; Lee et al. 2002; Lambert et al. 2007).

Pharmacokinetic parameters of orally administered EGCG were recently assessed in healthy subjects. Ullmann et al. (2003) examined the pharmacokinetic parameters of single dose administration of EGCG ranging from 50 mg to 1600 mg. The pharmacokinetic parameters of both free EGCG and total EGCG were measured at time intervals for a period of 26 hours following medication. However, maximal plasma concentrations greater than $1\ \mu\text{M}$ were detected after administration of more than 1 g EGCG (1600 mg dose, $C_{\text{max}} = 3392\ \text{ng/ml}$, range: 130–3392 ng/ml). Onset of peak plasmatic levels ranged from 1.3 to 2.2 hours, $\text{AUC}_{(0-\infty)}$ was 442 to 10 368 $\text{ng} \cdot \text{h/ml}$ and $t_{1/2z}$ was in the range of 1.9 to 4.6 hours.

The safety and plasma parameters of EGCG and polyphenon E (green tea extract) were evaluated over a four-week period in once-daily and twice-daily dosing regimens (Chow et al. 2003). The serum levels of EGCG were variable and bioavailability was 20.3% as compared to *i.v.* administration. Doses ranging from 800 to 1600 mg were safe and well-tolerated.

2.2 Distribution

The EGCG levels found in tissues corresponded to 0.0003–0.45% of ingested EGCG (Nakagawa and Miyazawa 1997). Despite this low absorption, EGCG is rapidly distributed in the body and/or is rapidly converted to metabolites (Chen et al. 1997). Indeed, EGCG and its metabolites have been found in serum, plasma, saliva, liver, small intestinal mucosa, colon mucosa (faeces), kidneys (urine), prostate cells, cancer cells and foetuses and placenta of pregnant rats, they can also penetrate the brain by crossing the blood-brain-barrier (Nakagawa and Miyazawa 1997; Zhu et al. 2000; Lee et al. 2002; Yashin et al. 2012; Clifford et al. 2013).

2.3 Metabolism

Catechins are enzymatically metabolised in the human body into many biologically active substances. Methylation, glucuronidation, sulfation and ring-fission biotransformation represent the main metabolic pathways for tea catechins.

Methylation. Catechol-*O*-methyltransferase (COMT) is one human enzyme involved in the catabolism of various catecholic compounds and substances with catechol-like structures. The general function of the COMT metabolic system is to eliminate potentially active or toxic endogenous and/or exogenous catechol compounds such as dietary phytochemicals. The following methylated catechin products have been observed in rat liver homogenates: 3'- and 4'-*O*-methyl-EC, 4'-*O*-methyl EGC, 4''-*O*-methyl ECG and EGCG and 4',4''-di-*O*-methyl-EGCG (Sang et al. 2011).

Glucuronidation. UGT-catalysed glucuronidation is metabolic pathway which increases water-solubility and reduces the toxicity of endogenous and endogenous substances, and, in this way, supports their excretion from the body through urine or

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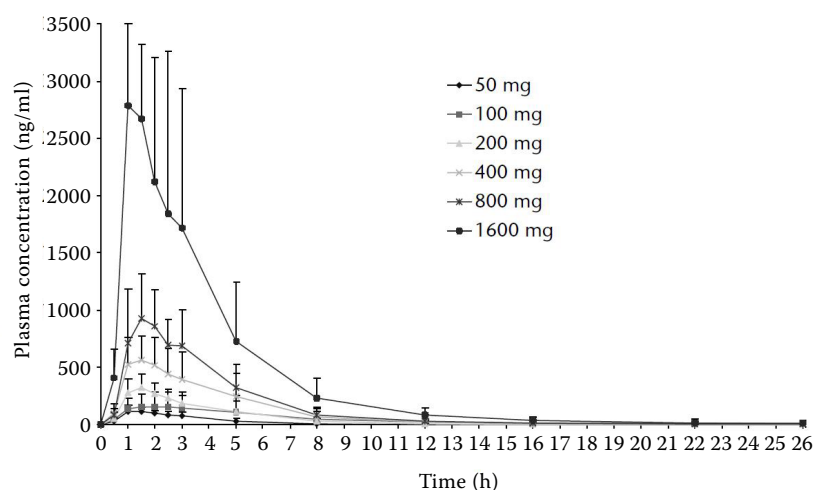


Figure 2. Plasma concentrations of increasing doses of (–)-epigallocatechin-3-*O*-gallate over time (adopted from Ullmann et al. 2003) Mean + SD plasma concentration-time curves for total epigallocatechin gallate in the different dosage groups ($n = 8$ for each group)

faeces. The major product of EGCG glucuronidation is EGCG-4''-*O*-glucuronide (Lambert et al. 2007).

Sulfation. Sulfation is the transfer of a sulfate group to a amine or alcohol substrate. The reaction is catalysed by sulfotransferase enzymes (SULT) (Sang et al. 2011). LC/MS analysis has been used to characterise the EC, EGC and EGCG sulfates in rodent and human samples (Lambert et al. 2007; Sang et al. 2011).

Glucosidation. Glucosidation in positions 7 of the A-ring and 4' of the B-ring generates a new EGCG metabolite, 7-*O*-beta-D-glucopyranosyl-EGCG-4''-*O*-beta-D-glucopyranoside, which was detected with the analytical LC/ESI–MS2 method (Sang et al. 2011).

Thiol conjugation. Mono-, bi-, and triglutathione conjugates of a (+)-catechin dimer are formed by glutathione from quinone derivatives (Sang et al. 2011).

Microbial metabolism. The gut microbiota catalyses the metabolic conversion of most polyphenols into the bioactive compounds which are responsible for the protective effects of tea drinking (Sang et al. 2011).

2.4 Excretion

EGCG is mainly excreted through bile, and EGC and EC are excreted through both the bile and urine (Chen et al. 1997; Clifford et al. 2013). A plasma concentration time curve is shown below (see Figure 2).

The main pharmacological properties are summarised in Table 1.

3. Toxicity

3.1 Acute toxicity

Acute toxicity of epigallocatechin-3-gallate is summarised in Table 2.

3.2 Subchronic and chronic toxicity

No significant changes were observed in the body weights or haematological and biochemical parameters of rats when 15 or 75 mg/kg of a green tea extract was administered orally for 28 days (Borzelleca

Table 1. The main pharmacological properties of total (–)-epigallocatechin-3-*O*-gallate dosages (50–1600 mg) studied in healthy volunteers (summarised from Ullmann et al. 2003)

Parameter	Result
Relative bioavailability	1.6% at low dose (75 mg/kg body weight); 13.9% at higher doses (250 mg/kg and 400 mg/kg body weight)
Maximum plasma concentration (C_{max})	130–3392 ng/ml
Time to reach C_{max} (T_{max})	60–115 min
AUC (0– ∞)	442–10 368 ng · h/ml
Apparent terminal elimination half-life	2.2 h after <i>i.v.</i> and 5–6 h after oral administration
Safety and tolerability	safe and tolerable at dosages of up to 1600 mg

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Table 2. Acute toxicity of epigallocatechin-3-gallate

Doses	Route	Species	References
LD₅₀			
2000–2170 mg/kg bw	oral	mice	Borzelleca 2004; Isbrucker et al. 2006b; NLM 2000; RTECS 1988; Yamani et al. 1996
3000–5000 mg/kg bw	oral	rat	
> 1860 mg/kg	skin	rat	
TDLo			
40 mg/kg	<i>i.p.</i>	rat	Borzelleca 2004; Isbrucker et al. 2006b; NLM 2000; RTECS 1988; Yamani et al. 1996
25 mg/kg	<i>s.c.</i>	guinea pig	
1 mg/kg	<i>i.v.</i>	rabbit	
120 mg/kg	<i>p.o.</i>	dog	
LDLo			
500 mg/kg	<i>s.c.</i>	rat	

bw = body weight, LDLo = the lowest dose causing lethality, NLM 2000 (www.nlm.nih.gov/databases/download/ccris.html), RTECS 1988 (<https://www.cdc.gov/niosh/docs/97-119/default.html>), TDLo = toxic dose low (the lowest dose causing a toxic effect)

2004). Male and female Sprague-Dawley rats received EGCG by gavage at daily doses of 0, 45, 150 or 500 mg/kg body weight/day for consecutive days. The reported NOELs in this study were 45 mg/kg body weight/day for males (based on reduced body weight gain and decreased absolute and relative thymus weights at the middle dose) and 150 mg/kg

body weight/day for females based on early death, suppression of body weight gain, GIT pathology and necrosis/atrophy of the thymus in both sexes at the highest dose (Borzelleca 2004).

Other authors (Isbrucker et al. 2006a) reported that dietary administration of an EGCG preparation to rats for 13 weeks was not toxic at doses of up to 500 mg/kg/day. Similarly, no adverse effects were noted when 500 mg EGCG preparation/kg/day was administered to pre-fed dogs in divided doses. This dose caused morbidity when administered to fasted dogs as a single bolus dose, although this model was considered an unrealistic comparison to the human condition. From these studies, a no-observed adverse effect level of 500 mg EGCG preparation/kg/day was established. Subchronic and chronic toxicity of green tea polyphenols is summarised in Table 3.

3.3 Carcinogenicity

No epidemiological studies or case reports investigating the association of exposure to EGCG and cancer risk in humans were identified in the available literature (SDCS 2000, ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/epigallocatechingallate_508.pdf).

Table 3. Subchronic and chronic toxicity of green tea polyphenols (toxic dose low)

Substance	Doses/duration	Route and species	References
EGCG	16.8 g/kg/12W-C and 4539.6 mg/kg/90D-C	<i>p.o.</i> mouse	RTECS 1988
	50 mg/kg/2D-I	<i>p.o.</i> mouse	
	175 and 350 mg/kg/7D-I	<i>i.p.</i> mouse	
	7200 mg/kg/12W-I	skin mouse	
	70 mg/kg/7D-I	<i>i.p.</i> rat	
	10 pph/17D-I and 5 pph/22D-I	<i>o.s.</i> of guinea pig	
	12 000 mg/kg/30DI, 3600 mg/kg/9D-I and 36 400 mg/kg/13W-I	<i>p.o.</i> dog	
Catechin	2400 mg/kg/12D-I	<i>s.c.</i> rat	Raw Material 2010; Raw Material 2011
	800 mg/kg/4W-I	<i>i.p.</i> rat	
	90 mg/kg/9D-I	<i>i.p.</i> mouse	
	400 mg/kg/10D-I	<i>p.o.</i> mouse	
Epicatechin	525 mg/kg/5W-I	<i>i.p.</i> rat	Raw Material 2010
Epigallocateco-1,3-galate	16800 mg/kg/W-C	<i>p.o.</i> mouse	RTECS 1988

C = continuous, D = day, I = intermittent, W = week

Raw Material 2010 (www.centerchem.com/Products/DownloadFile.aspx?FileID=7188), Raw Material 2011 (www.centerchem.com/Products/DownloadFile.aspx?FileID=7311), RTECS 1988 (<https://www.cdc.gov/niosh/docs/97-119/default.html>)

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Epigallocatechin 3-gallate is not listed as a carcinogen by Environmental Protection Agency, Occupational Safety and Health Administration, American Conference of Governmental Industrial Hygienists, International Agency for Research on Cancer, National Toxicology Program or CA Prop 65 (Material Safety Data Sheet, www.labor-service-bb.de/pdf/312/291945_en.pdf, www.caymanchem.com/msdss/70935m.pdf). Indeed, EGCG offers several advantages as a possible anti-cancer agent given its ubiquitous distribution in nature and its low toxicity (Rao and Pagidas 2010). It is well documented that EGCG has anti-tumour effects on a number of carcinomas and human cancer cell lines (see Table 4) (Lambert and Yand 2003; Khan et al. 2006; Spinella et al. 2006; Khan et al. 2008). Hence, EGCG is a potential therapeutic agent for cancer therapy because of its anti-proliferative and pro-

differentiation properties and its ability to cause apoptosis.

3.4 Teratogenicity and reproductive toxicity

In a guideline-directed teratogenicity study reported by Isbrucker et al. (2006b), there was no indication of teratogenic effects when EGCG was administered in the diets of rats during organogenesis at doses representing a nominal 1000 mg EGCG/kg/day. The results from this pilot study provide an indication that EGCG remains non-teratogenic when plasma concentrations reach levels as high as 191 µg/ml. Subsequent reproductive performance in these same animals was not affected by this delay. Due to reduced growth rates of the offspring in the two-generation study, the no-observable adverse

Table 4. Studies investigating the tumour inhibition properties of (–)-epigallocatechin-3-*O*-gallate (adopted from: SDCS: Summary of data for chemical selection 2000, ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/epigallocatechingallate_508.pdf)

Species	Strain/sex	Dose/route of administration/duration	Carcinogen/route/dose	Result	Ref.
Mouse	C57BL/male	0.15 mg/mouse/day in drinking water for 12 weeks	<i>N</i> -Ethyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine, 100 mg/l for 4 weeks in drinking water	inhibited incidence of duodenal tumours	NLM 2000
Mouse	C57BL/male	0.005% in diet for 12 weeks	<i>N</i> -Ethyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine, 100 mg/l for 4 weeks in drinking water	inhibited incidence of duodenal tumours	NLM 2000
Mouse	Strain A/female	560 ppm in drinking water for 13 weeks	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 11.65 mg/kg, 3 ×/week for 10 weeks, gavage	inhibited multiplicity of lung tumours	NLM 2000
Mouse	SENCAR/female	5 µmol/0.2 ml acetone, 1 ×/day for 7 days prior to carcinogen, dermal	<u>initiator</u> : 7,12-dimethylbenz- <i>[a]</i> -anthracene, 10 nmol/0.2 ml acetone, 1 ×, dermal <u>promoter</u> : 12- <i>O</i> -tetradecanoylphorbol-13-acetate, 3.2 nmol/0.2 ml acetone, 2 ×/week, dermal	inhibited multiplicity of skin tumours	NLM 2000
Mouse	C3H/HENCRJ male and female	0.05% in drinking water for 58 weeks	none	inhibited incidence and multiplicity of spontaneous tumours	NLM 2000
Rat	Wistar/male	0.05% in drinking water for 15 weeks	MNNG, 80 mg/l for 28 weeks in drinking water	inhibited incidence and multiplicity of stomach tumours	Yamani et al. 1996
Rat	Wistar/male	0.05% in diet for 16 weeks	<i>N</i> -Ethyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine, 80 mg/l for 28 weeks in drinking water	inhibited incidence of stomach tumours	Yamani et al. 1996
Rat	Sprague-Dawley/female	0.5% (58.4–81% epigallocatechin gallate) in diet for 23 weeks	7,12-dimethylbenz- <i>[a]</i> -anthracene, 50 mg/kg, 1 ×, gavage	promotion of or no effect on mammary gland tumours	NLM 2000

NLM (www.nlm.nih.gov/databases/download/ccris.html)

effect level (NOAEL) might be set at the target dose level of 100 mg/kg/day EGCG. However, during the crucial phase of lactation the EGCG intake by dams was at least 200 mg/kg/day and this may be considered as a more appropriate NOAEL in rats at all life stages (Isbrucker et al. 2006b).

3.5 Genotoxicity

The oral administration of 500, 1000 or 2000 mg EGCG/kg to mice did not induce micronuclei formation in bone marrow cells. Similarly, administration of 400, 800 or 1200 mg EGCG/kg/day in their diet for ten days did not induce bone marrow cell micronuclei and resulted in plasma EGCG concentrations comparable to those reported in human studies. The intravenous injection of 10, 25 or 50 mg EGCG/kg/day to rats resulted in much higher plasma concentrations and demonstrated an absence of genotoxic effects (Isbrucker et al. 2006c). Genotoxicity studies of EGCG and tea constituents are summarised in Table 5.

3.6 Specific target organ toxicity

The recent medical literature includes many cases of marked liver toxicity associated with alteration

of liver enzyme function (Duenas Sadornil et al. 2004; Garcia-Moran et al. 2004; Abu el Wafa et al. 2005; Bonkovsky 2006; Galati et al. 2006; Jimenez-Saenz and Martinez-Sanchez 2007; Lambert et al. 2010; Rahmani et al. 2015).

The causal association between green tea and liver damage was reviewed Mazzanti et al. (2009) on the basis of 34 cases of hepatitis (between 1999 and October 2008). Laboratory tests showed high values of transaminases (values up to 140-fold higher than normal), alkaline phosphatase levels that varied from normal to 8.3-fold higher than the normal values, gamma glutamyl transpeptidase levels of up to 394 U/l and bilirubin levels up to 25-fold above the normal values. According to the RUCAM scale, liver injury in the 32 assessable cases was classified as hepatocellular (62.50%), cholestatic (18.75%) or mixed (18.75%) (Mazzanti et al. 2009).

Cai et al. (2015) observed that high doses of EGCG (500 and 1000 mg/kg/day) could induce cardiac fibrosis. This effect appears to be related to the inhibition of AMPK-dependent signalling pathways in mice hearts. The doses at which toxicity were observed are much higher than those typically resulting from normal tea consumption, but they are more readily achievable in the context of tea-based dietary supplements (Cai et al. 2015).

Table 5. Genotoxicity studies of epigallocatechin gallate (EGCG) and tea (adapted and cited from SDCS: Summary of data for chemical selection 2000, ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/epigallocatechingallate_508.pdf)

Preparation tested	Test system/strain or cell line	Dose; study details (activation, solvent, schedule)	Result	References
Endpoint: mutation				
EGCG	<i>S. typhimurium</i> TA100	Ames test w S-9, dose not reported	high dose-positive; low dose-negative	Weisburger 1996
Endpoint: sister chromatid exchange				
EGCG	Chinese hamster cells	20 mg/ml	potentiated mitomycin C-induced sister chromatid exchange	Imanishi et al. 1991
Endpoint: chromosomal aberrations				
EGCG	Chinese hamster cells	20 mg/ml	potentiated mitomycin C-induced chromosomal aberration	Imanishi et al. 1991
Endpoint: DNA damage				
EGCG	pBR322 plasmid	0–0.1 mM	potentiated DNA single strand breaks by diethanolamine NONOate	Ohshima et al. 1998
Endpoint: microsomal degranulation				
Green tea infusion	Isol. Wistar rat liver microsomes	40 mg/ml <i>in vitro</i>	positive	Minocha et al. 1986
Green tea infusion	Wistar rats (<i>in vivo</i> study)	160 mg/kg body weight, 1 × <i>s.c.</i> , rats sacrificed after 10 h and microsomes prepared	positive	Minocha et al. 1986

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3.7 Interactions

Concomitant consumption of green tea with folic acid is not recommended in pregnant women, those with megaloblastic anaemia or when a reduction in folic acid may have clinical consequences. A folate transporter interaction has been described, leading to decreases in the bioavailability of folic acid. Epidemiological studies suggest a detrimental effect of tea drinking on iron status, but the data are inconsistent with multiple confounding influences. Dose-dependent inhibition of intestinal non-haem iron absorption was demonstrated in a controlled clinical trial using epigallocatechin gallate. Other published studies report that black tea reduces iron absorption to the largest extent and green tea to the smallest extent, suggesting that the type of polyphenol in tea is an important factor. Co-administration of tea extracts and iron products is not advised, and patients with poor iron status should avoid drinking tea with meals, because iron absorption may be impaired. Conversely, a beneficial effect of drinking tea with meals was suggested in a trial of patients with genetic haemochromatosis (Green tea 2015, accurateclinic.com/wp-content/uploads/2016/02/Green-tea-University-of-Maryland-Medical-Center.pdf). Other possible interactions are summarised in Table 6.

3.7.1 Synergistic effects

The use of EGCG could enhance the effect of conventional cancer therapies through additive or synergistic effects as well as through amelioration of deleterious side effects (Lecumberri et al. 2013). EGCG combined with 0.5 µmol/l vardenafil was required to induce significant cell death in chronic lymphocytic leukaemia cells that were otherwise resistant to EGCG-induced cell death (Kumazoe et al. 2015). Fujiki et al. (2015) collected the results of numerous investigators as follows: combinations of EGCG and 37 anticancer compounds, which include NSAIDs, phytochemicals and anti-cancer drugs, generally induced *in vitro* synergistic anti-cancer effects on 54 human cancer cell lines derived from various cancer tissues, and combinations of EGCG or green tea extract and 13 anticancer compounds elicited a reduction in tumour volumes in xenograft mouse models implanted using various human cancer cell lines (see Table 7).

3.8 Adverse reactions

The most commonly observed side effects in subjects consuming green tea polyphenol include headache, stomach ache, abdominal pain and

Table 6. Possible interactions of green tea components (adapted from Green tea 2015, accurateclinic.com/wp-content/uploads/2016/02/Green-tea-University-of-Maryland-Medical-Center.pdf)

Medications	Effects
Adenosine	Green tea may inhibit the action of adenosine, a medication given in the hospital for an irregular and usually unstable heart rhythm.
Beta-lactam	Increases the effectiveness of beta-lactam antibiotics by making bacteria less resistant to treatment.
Benzodiazepines	Caffeine from green tea may reduce the sedative effects of these medications commonly used to treat anxiety, such as diazepam and lorazepam.
Beta-blockers, propranolol, metoprolol	Caffeine from green tea may increase blood pressure in people taking propranolol and metoprolol.
Chemotherapy	Increased the effectiveness of these medications in laboratory tests.
Clozapine	The effects of clozapine may be reduced if taken within 40 minutes after drinking green tea.
Ephedrine	When taken with ephedrine, green tea may cause agitation, tremors, insomnia and weight loss.
Monoaminoxidase inhibitors	Green tea may cause a severe increase in blood pressure.
Quinolone antibiotics	Green tea may make these medications more effective and also increase the risk of side effects.
Other medications	Green tea, especially caffeinated green tea, may interact with a number for medications, including acetaminophen, carbamazepine, dipyridamole, oestrogen, fluvoxamine, methotrexate, mexiletine, phenobarbital, theophylline and Verapamil.

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Table 7. List of human cancer cell lines and anticancer compounds that have shown synergistic anticancer effects in combination of epigallocatechin gallate (EGCG) or other green tea catechins (adapted from Fujiki et al. 2015)

Human cancer tissues	Human cancer cell lines used in experiments	Effective anticancer compounds in combination with EGCG, or other green tea catechins
Head, neck and lung	A549, ChaGo K-1, H292, H358, H460, H2122, NCI-H460, PC-9, SQCCY1, Tu177, Tu212, YCU-N861, YCU-H891, 38, 886LN	celecoxib, curcumin, erlotinib, 5-fluorouracil, luteolin, sulindac, tamoxifen
Breast	MDA-MB-231, HS578T, MCF-7	curcumin, 4-hydroxytamoxifen, raloxifene, resveratrol, tamoxifen, γ -tocotrienol, tricostatin A
Prostate	ALVA-41, CWR22Rv1, IBC-10a, LNCaP, PC-3, PC-3 AP-1, PC-3ML, PCa-20a, RPMI8226 MM, Cancer sten cells of PC-3	bortezomib, docetaxel, doxorubicin, genistein, NS398, paclitaxel, quercetin, resveratrol, sulforaphane
Liver	BEL-7404/DOX, Hep3B	doxorubicin, 5-fluorouracil
Colon	HCT-116, HT-29, RKO	sodium butyrate, sulforaphane
Ovaries	A2780, A2780(<i>cis</i> R), SKOV-ip1(paclitaxel-sensitive), SKOVTR-ip2(paclitaxel-resistant)	cisplatin, sulforaphane, <i>trans</i> -palladiums
Malignant neuroblastoma	SH-SY5Y, SK-N-BE2	retinoids (ATRA, 13- <i>cis</i> -RA, 4-HPR), SU5416
Leukemia	B-cell chronic leukemia, HL-60, Jurkat T leukemia, K-562, Myelogenous leukemia	benzyl isothiocyanate, celastrol, curcumin, cytosine arabinoside, H_2O_2
Pancreas	Colo357, PANC-1, MIA PaCa-2	celecoxib, thymoquinone, TRAIL
Cervix	HeLa, TMCC-1	retinoic acid
Melanoma	A-375, G-361, Hs-294T	vorinostat
Skin	A431, SCC-13	3-deazaneplanocin
Stomach	BGC-823	docetaxel

nausea without significant haematological and biochemical changes in blood after four weeks of green tea treatment (Chow et al. 2003).

4. Mode of action and biological activity

The biological activity of green tea polyphenols is directly related to their pharmacokinetic properties and/or their bioavailability – see the ADME section (Yashin et al. 2012). EGCG directly interacts with plasma membrane proteins and phospholipids which stimulate intracellular signalling pathways (Singh et al. 2011). In addition, EGCG is transported to intracellular compartments, the cytosol, mitochondria, lysosomes and nuclei where it mediates additional biological actions. These various effects are dependent on cell type, stress conditions and concentrations of EGCG (Kim et al. 2014a).

A number of *in vitro* and *in vivo* preclinical studies as well as clinical trials have shown that polyphenolic compounds harbour a wide range of biological and pharmacological properties, which include antimicrobial (Steinmann et al. 2013),

anti-carcinogenic (anti-tumourigenic) (Wang et al. 2011), anti-oxidative, anti-allergic, anti-cardiovascular (Tachibana 2011), anti-diabetic (Babu et al. 2013), anti-inflammatory (Gu et al. 2013), anti-hypercholesterolaemic (lipid clearance) (Zhou et al. 2014), anti-atherosclerosis, anti-hypertensive (Tachibana 2011) anti-mutagenic (Schramm 2013), anti-aging (Cooper et al. 2005), decreased risk of osteoporotic fractures (Shen et al. 2009), neuroprotective (Parmar et al. 2012) and immunomodulatory effects (Gu et al. 2013).

4.1 Antimicrobial effects

4.1.1 Antibacterial activity of EGCG

Several studies have reported that epigallocatechin-3-gallate (EGCG), the main constituent of green tea, has anti-infective properties (Steinmann et al. 2013). The effects of EGCG are generally studied against bacterial strains such as methicillin-resistant *Staphylococcus aureus* and *Stenotrophomonas maltophilia*, *Mycobacterium tuberculosis*, *Helicobacter*

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pylori and *Streptococci* spp. The activity of beta-lactams (antibiotics such as methicillin) was found to be enhanced by EGCG. The biological activity of EGCG was investigated against clinical isolates of *S. aureus* and an *in vitro* study suggested that binding of negatively charged EGCG to positively charged lipids of the cell membrane damages the membrane structure or fragments the lipid bilayer causing intramembranous leakage. It has also been reported that EGCG inhibited (MIC_{50} , 10 µg/ml) penicillinase activity of the peptidoglycan of the bacterial cell membrane by binding to it either directly or indirectly, hence preventing the inactivation of penicillin. EGCG was also found to decrease or inhibit biofilm production by *S. aureus* (Das et al. 2014). By binding to the ATP binding site of the gyrase B subunit, catechins inhibit bacterial DNA gyrase, an essential bacterial enzyme whose inactivation leads to bacterial death (Gradisar et al. 2007). Moreover, EGCG shows anti-folate activity against dihydrofolate reductase (key enzyme that reduces 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate) and hence leads to the disruption of DNA synthesis (Das et al. 2014). Another study indicated that EGCG was able to inhibit the attachment of bacteria to human cells and induce *S. pyogenes* cell death (Steinmann et al. 2013).

EGCG showed significant cytoprotective effects against *H. pylori*-induced gastric cytotoxicity via interference with the TLR-4 (toll-like receptor 4) signalling induced by *H. pylori* (Lee et al. 2004).

4.1.2 Antiviral activity of EGCG

Different modes of inhibitory activity have been demonstrated for EGCG against diverse families of viruses, such as *Retroviridae*, *Orthomyxoviridae* and *Flaviviridae*, which include important human pathogens like Hepatitis C virus, human immunodeficiency virus, influenza viruses, Hepatitis B virus, enteroviruses, adenoviruses, Epstein-Barr virus and herpes simplex virus. Most of these studies demonstrated antiviral properties at physiological concentrations of EGCG *in vitro*. In contrast, the minimum inhibitory concentrations against bacteria were 10–100-fold higher (Steinmann et al. 2013). Single dose administration of EGCG at doses ranging from 50–1600 mg is sufficient to inhibit HCV and is safe for human volunteers (Chen et al. 2012). EGCG directly intervenes in the HIV life cycle. It

blocks the attachment of HIV-1 virions (Kawai et al. 2003), hinders an early step in virus entry by inhibiting the docking of the virus to the cell surface (Calland et al. 2012), inhibits reverse transcriptase (RT) which catalyses the conversion of RNA into DNA (Yamaguchi et al. 2002) and blocks the replication of virus strains (Fassina et al. 2002).

It has previously been shown that green tea extract (GTE) inhibits the infectivity of influenza viruses by preventing their adsorption into the cells; infection with both IAV and influenza B virus (IBV) was inhibited by EGCG (Imanishi et al. 2002; Xu et al. 2017). EGCG and ECG were found to be potent inhibitors of influenza virus replication in MDCK cell culture and this effect was observed in all influenza virus subtypes tested, including A/H1N1, A/H3N2 and B virus. The 50% effective inhibition concentration (EC_{50}) values of EGCG, ECG and EGC for influenza A virus were 22–28, 22–40 and 309–318 µM, respectively. EGCG and ECG exhibited hemagglutination inhibition activity, with EGCG being more effective (Song et al. 2005). Other viruses against which EGCG exerts antiviral activity are adenoviruses of the *Adenoviridae* family, non-enveloped viruses composed of a nucleocapsid and a double-stranded linear DNA genome (Das et al. 2014). The antiviral properties of EGCG are based on direct inactivation of virus particles, inhibition of intracellular virus growth and inhibition of the viral protease, adenain *in vitro* (Weber et al. 2003).

EGCG effectively suppressed the secretion of HBV-specific antigens (HBsAg, HBeAg) (He et al. 2011). It showed stronger effects than those of lamivudine in a dose- and time-dependent manner (Pang et al. 2014).

At concentrations exceeding 50 µM, EGCG inhibits the expression of Epstein-Barr virus (EBV) lytic proteins, thus blocking the EBV lytic cycle (Chang et al. 2003).

4.1.3 Antifungal activity of EGCG

The antifungal activity of EGCG is generally studied against yeast strains such as *Candida* spp. (*C. albicans*, *C. glabrata*) and dermatophytes (*Trichophyton mentagrophytes*, *T. rubrum*) (Das et al. 2014). Mechanisms of antifungal activity include impairing biofilm formation by both structural and metabolic means (Steinmann et al. 2013), disturbing osmotic integrity (Ellis 2002), anti-folate activ-

ity against DHFR through inhibition of this key enzyme in the synthesis of purines, pyrimidines and amino acids resulting in an impaired growth of fungal cells (Navarro-Martinez et al. 2006). EGCG shows synergistic antifungal effects against *C. albicans* in combination with anti-mycotic drugs (Hirasawa and Takada 2004). Moreover, an antifungal effect against *Trichophyton mentagrophytes* (MIC₅₀, 2–4 µg/ml, MIC₉₀, 4–8 µg/ml) has been reported by Park et al. (2011), while morphological changes like deformation, swelling, granular accumulation and inhibition of hyphal growth of isolates of *T. mentagrophytes* was reported an *in vitro* study by Toyoshima et al. (1994).

4.2 Immunomodulatory effects

The data regarding the immunomodulatory properties of EGCG remain somewhat contradictory. Some observations indicate that EGCG exerts strong anti-inflammatory effects on the host. For example, EGCG has been implicated in reducing ultraviolet radiation-induced inflammatory responses and infiltration of leukocytes in human skin and blocking lipopolysaccharide (endotoxin)-induced tumour necrosis factor production and lethality in BALB/c mice. Other reports, however, point to pro-inflammatory characteristics of EGCG, such as stimulation of human monocyte and polymorphonuclear cell iodination and interleukin-1 production, as well as erythrocyte-dependent B cell mitogenicity (SDCS 2000, ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/epigallocatechingallate_508.pdf).

4.3 Anti-proliferative and anti-apoptotic effects

EGCG selectively induced growth arrest and/or apoptosis in cancerous cells with a minimal effect on noncancerous cells (Zhang et al. 2015). Koh et al. (2003) showed that after hydrogen peroxide exposure in PC12 cells, EGCG inhibited many steps in the apoptotic cascade, including caspase 3, cytochrome c release, poly (ADP-ribose) polymerase cleavage and the glycogen synthase kinase-3 pathway. It also modulated cell signalling by activating the phosphatidylinositol-3 kinase (PI3K)/Akt pathway which promotes cell survival. Similarly,

treatment of prostate cancer with EGCG resulted in dose-dependent apoptosis (Gupta et al. 2000), while inhibition of cyclin-dependent kinases by EGCG blocked cell cycle progression in human breast carcinoma cells (Liang et al. 1999). Recently, Moradzadeh et al. (2017) demonstrated that EGCG could induce apoptosis in a time- and concentration-dependent manner in breast cancer cells while exerting no significant toxic effects on normal cells.

4.4 Anti-cancer effects of EGCG

Recent studies and *in vitro* and *in vivo* experiments have demonstrated that EGCG prevents tumourigenesis and shows anti-cancer effects in several types of cancer including prostate cancer (Du et al. 2012; Kim et al. 2014b), breast, lung and colorectal cancer (Du et al. 2012), melanoma (Tsukamoto et al. 2014), multiple myeloma (Shammas et al. 2006; Tsukamoto et al. 2012), acute myelogenous leukaemia (Kumazoe et al. 2013a) and chronic myelogenous leukaemia (Huang et al. 2015) without affecting normal cells.

EGCG interferes with numerous signalling pathways and biological mechanisms related to cancer development and progression. The possible mechanisms of action of green constituents in cancer prevention and progression are summarised by Granja et al. (2016) as follows: (1) suppression of DNA hypermethylation by direct inhibition of DNA methyltransferase (DNMT); (2) repression of telomerase activity; (3) inhibition of angiogenesis by repression of the transcription factors hypoxia-inducible factor 1- α (HIF-1 α) and nuclear factor kappa B (NF- κ B); (4) blocking of cell metastasis by inhibition of matrix metalloproteinases (MMPs)-2, -9 and -3; (5) promotion of cancer cell apoptosis by induction of pro-apoptotic proteins BCL-2-associated X protein (BAX) and BCL-2 homologous antagonist killer (BAK) and repression of anti-apoptotic proteins B-cell lymphoma 2 (BCL-2) and B cell lymphoma-extra large (BCL-XL); (6) induction of the tumour suppressor genes p53 and phosphatase and tensin homolog (PTEN) and inhibition of the oncogenes human epidermal growth factor receptor 2 (HER2) and epidermal growth factor receptor (EGFR); (7) inhibition of NF- κ B and the downstream processes of cell inflammation, proliferation, metastasis and angiogenesis; and (8) anti-proliferative activity by inhibition of the

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mitogen-activated protein kinase (MAPK) pathway and insulin-like growth factor I receptor (IGFIR) (Granja et al. 2016).

Recently, the 67 kDa laminin receptor has been identified as an EGCG-specific receptor (Nelson et al. 2008; Kumazoe and Tachibana 2016) that mediates the anti-cancer action of EGCG (Umeda et al. 2008). This protein is believed to be derived from a smaller precursor, the 37LRP (37 kDa laminin receptor precursor). The 67 kDa laminin receptor (67LR), also known as ribosomal protein SA (RPSA), is a cell surface receptor with high affinity for laminin (Viacava et al. 1997). 67LR is involved in adhesion in normal cells; however, several pathology studies have shown 67LR to be overexpressed in various types of cancer including breast cancer (Viacava et al. 1997; Kumazoe et al. 2013b), pancreatic cancer (Kumazoe et al. 2013b), gastric cancer (Kumazoe et al. 2013b), chronic lymphocytic leukaemia (Kumazoe et al. 2015), melanoma (Tsukamoto et al. 2014), multiple myeloma (Shammas et al. 2006; Tsukamoto et al. 2012) and acute myelogenous leukaemia (Britschgi et al. 2010). Clinical studies have shown that upregulation of the expression level of 67LR is positively correlated with poor prognosis, the histological severity of lesions and tumour progression (Viacava et al. 1997). In cancer, upregulation of 67LR plays a crucial role in the abnormally elevated expression of cyclins A and B and cyclin-dependent kinases 1 and 2 (Scheiman et al. 2009).

Moreover, 67LR is involved in adhesion-mediated drug resistance (Liu et al. 2009), plays a critical role in mediating anti-inflammatory and anti-allergic effects and modulates insulin action and inhibition of tissue factor (TF) expression (Tachibana 2011). Despite these effects, the authors of the relevant systematic reviews encompassing more than 1.6 million participants concluded that in the face of conflicting and insufficient evidence no firm recommendations can be made regarding green tea consumption for cancer prevention (Boehm et al. 2009).

4.5 Free radical scavenging/antioxidant actions of catechins

Tea catechins have shown strong antioxidant properties in many *in vitro* and *in vivo* studies and act as biological antioxidants. It has been demonstrated that EGCG, the main catechin in green tea, can scavenge both superoxide and hydroxyl radicals (Ruch

et al. 1989; Kashima 1999; Zhao et al. 2001; Forester and Lambert 2011, Kim et al. 2014a), as well as the 1,1-diphenyl-3-picrylhydrazyl radical (Nanjo et al. 1999; Zhao et al. 2001), peroxy radicals (Sang et al. 2003), NO (Kelly et al. 2001), carbon-centre free radicals, singlet oxygen and lipid free radicals (Guo et al. 1996; Zhao et al. 2001) and peroxynitrite by preventing the nitration of tyrosine (Pannala et al. 1997). In addition to its radical scavenging properties, EGCG also possesses metal chelating properties. The two structures which give this compound its property of metal chelation include the ortho-3',4'-dihydroxy moiety and the 4-keto, 3-hydroxyl or 4-keto and 5-hydroxyl moiety (Singh et al. 2016). Catechins prevent the generation of potentially damaging free radicals by chelation of metal ions (Grinberg et al. 1997). Through their abilities to chelate transition metal ions, flavonoids can complex and inactivate iron ions, thus suppressing the superoxide-driven Fenton reactions that are thought to be the most important route to the formation of reactive oxygen species (Seeram and Nair 2002). Electron transfer from catechins to ROS-induced radical sites on DNA (Anderson et al. 2001) and the formation of stable semiquinone free radicals (Guo et al. 1996) are other mechanisms by which the catechins exert antioxidant effects. The antioxidant effects of the catechins are more pronounced compared to those of vitamin C and vitamin E (Zhao et al. 1989; Terao et al. 1994, Hashimoto 2000). Moreover, the catechins can inhibit ROS-induced damage elicited by a wide array of initiators include 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH) (Terao et al. 1994; Lotito and Fraga 2000), primaquine (Grinberg et al. 1997), hydrogen peroxide (Ruch et al. 1989; Grinberg et al. 1997), azo-bisisobutyrylnitrile (Sang et al. 2003), iron (Grinberg et al. 1997; Seeram and Nair 2002), paraquat (Tanaka 2000) and radiolysis (Anderson et al. 2001).

4.6 Cardiovascular effects

Recently, there has been considerable interest in the possibility that consumption of tea reduces the risk for cardiovascular disease (Deka and Vita 2011). Some observational studies and *in vitro* experiments show that tea consumption is inversely associated with cardiovascular disease. The health effects of tea have been reviewed and investigated with respect to numerous experimental and clinical conditions and

include reduced low-density lipoprotein (LDL) oxidation, intestinal absorption of cholesterol (Hirsova et al. 2012), inhibition of cholesterol synthesis (Bursill and Roach 2006), enhanced endothelial cell function, vascular smooth muscle proliferation which is associated with atherogenesis (Locher et al. 2002; Lorenz et al. 2017), hypotensive effects, effects on atherosclerosis, platelet aggregation and improved cholesterol profiles (Babu and Liu 2008; Thielecke and Boschmann 2009). In numerous clinical trials, the cholesterol-lowering effects of tea have been investigated in healthy volunteers as well as in obese children and adults (Babu and Liu 2008; Hooper et al. 2008; Hsu et al. 2008; Matsuyama et al. 2008; Frank et al. 2009; Maki et al. 2009; Nantz et al. 2009; Momose et al. 2016).

4.7 Anti-diabetic effects

EGCG can elicit a number of changes that are associated with beneficial effects on diabetes (Babu et al. 2013). EGCG significantly potentiated glucose-stimulated insulin secretion in rat islets (at 0.1, 1 and 5 μM) and human islets (at 1 μM) and elevated insulin content within cells (at 0.1, 1, and 5 μM) and human islets (at 1 μM), ($P < 0.05$). Nutritional supplementation of EGCG (0.5% in drinking water) for 12 days in healthy rats significantly increased insulin synthesis compared to controls (Yuskavage 2008), EGCG acts on steps of the insulin signalling cascade to ameliorate beta-cell function (Cai and Lin 2009), protect beta-cells from cytokine-stimulated apoptosis (Zhang et al. 2011), has beneficial effects on fatty acid-induced insulin resistance in skeletal muscle (Deng et al. 2012), decreases oxidative stress and thus improves glucose metabolism (Yan et al. 2012), suppresses lipid accumulation via the AMPK/ACC signalling pathway (Dong 2000; Deng et al. 2012), stimulates glucose uptake in response to insulin (Lemieux et al. 2003; Green et al. 2011) and reduces stress markers (Ortsater et al. 2012).

4.8 Bone metabolism

Oxidative stress regulates increases in bone resorption, differentiation and the function of osteoclasts, so it has a significant influence on the occurrence of osteoporosis (Dudaric et al. 2015). As the main pathogenic factor, oxidative stress in-

creases the apoptosis of osteoblasts and osteocytes and suppresses osteoblastic differentiation and maturation pathways (Basu et al. 2001; Mody et al. 2001; Bai et al. 2004; Banfi et al. 2008; Fatokun et al. 2008; Hayashi et al. 2008; Manolagas 2008; Shen et al. 2008; Manolagas and Parfitt 2010). An imbalance between bone formation and bone resorption is potentiated by chronic inflammation. The antioxidant and anti-inflammatory properties of EGCG in these cases can exert beneficial effects on many regulatory cascades (Tokuda et al. 2003; Tokuda et al. 2007; Vali et al. 2007; Yamauchi et al. 2007; Kamon et al. 2009).

4.9 Neuroprotective effects

Neuronal tissue is more prone to oxidative damage than other tissues because of its high content of unsaturated fatty acids (which are sensitive targets for free radical attack leading to peroxidation), the brain's high oxygen consumption relative to its weight, its weak antioxidant defence mechanisms, higher iron levels in certain brain regions and high ascorbate levels. There are only limited data on the bioavailability of EGCG in the brain, an important prerequisite for its neuroprotective effects. A single, very high oral EGCG dose (500 mg/kg body weight) in rats yielded EGCG concentrations of 12.3 nmol/ml in plasma and 0.5 nmol/g in brain (measured by CL-HPLC) (Nakagawa and Miyazawa 1997). Catechins and epicatechin pass the blood brain barrier as shown by *in vivo* microdialysis of rat hippocampus following intravenous application of these basic monomer units of the flavanols. Briefly, EGCG acts as a powerful hydrogen-donating radical scavenger of ROS and RNS and chelates divalent transition metal ions (Cu^{2+} , Zn^{2+} and Fe^{2+}), thereby preventing the Fe^{2+} -induced formation of free radicals *in vitro*. Among 12 polyphenolic compounds, EGCG most potently inhibited Fe^{2+} -mediated DNA damage and iron ascorbate-dependent lipid peroxidation of brain mitochondrial membranes. *In vivo*, EGCG increased the expression and activity of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase but inhibited pro-oxidative ones such as monoamine oxidase (MAO)-B and nitric oxide synthase. Evidence from preclinical studies (cell and tissue cultures and animal models) and clinical trials regarding preventive and therapeutic effects of EGCG in neurodegenerative diseases are summarised in Table 8 (Mahler et al. 2013).

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4.10 Other effects

4.10.1 Effect on obesity

Recent data from human studies indicate that green tea extracts may help reduce body weight, mainly body fat, by increasing postprandial thermogenesis and fat oxidation.

In a randomised, double-blind, placebo-controlled, cross-over pilot study, six overweight men were given 300 mg EGCG per day for two days (Boschmann and Thielecke 2007). Fasting and postprandial changes in energy expenditure and substrate oxidation were assessed. Resting energy expenditure did not differ significantly between EGCG and placebo treatments, although during the first postprandial

Table 8. Neuroprotective effects of epigallocatechin-3-gallate (adapted from Mahler et al. 2013)

Preclinical studies	Epidemiological and clinical studies
Multiple sclerosis	
Inflammation, proliferation and TNF- α ; secretion of T cells \downarrow in EAE mice. Combination with glatiramer acetate: cell death \downarrow and neuronal outgrowth \uparrow in primary neurons, disease severity \downarrow in EAE mice. Th1 and Th17 \downarrow , Treg \uparrow in EAE mice.	Effects on T2 lesions in brain MRI in RRMS? (NCT00525668). Effects on brain atrophy in progressive forms? (NCT00799890). Metabolic effects in MS patients? (NCT01417312)
Alzheimer's disease	
A beta-induced death of hippocampal cells \downarrow alpha-secretase activity in Alzheimer transgenic mice \uparrow . Recovery of A beta-induced memory dysfunction in mice. Protection of microglia cells from A beta-induced \uparrow iNOS expression and NO production. Direct conversion of fibrillar species into benign protein aggregates.	Effects on the course of AD in 50 early stage patients? (NCT00951834)
Parkinson's disease	
Lipopolysaccharide-induced microglial activation \downarrow . Protection of PC12 cells against 6-hydroxydopamine-induced apoptosis. Attenuation of MPP+-induced ROS production in PC12 cells. Protection against striatal dopamine depletion and neuronal loss in MPTP mice. Loss of dopaminergic neurons \downarrow , nNOS expression \downarrow in MPTP mice. No behavioural improvements in 6-hydroxydopamine-lesioned rats. Attenuation of increased iNOS expression in MPTP mice. Inhibition of levodopa methylation by catechol-O-methyltransferase.	Inverse relation of tea drinking and PD in Chinese, Western Washington, Finnish, and Israeli cohorts Safe and efficient in <i>de novo</i> PD patients? (NCT00461942)
Huntington's disease	
Inhibition of huntingtin protein aggregation in yeast and fly models of HD. Memory impairment \downarrow in 3-NP-treated rats, glutathione level of neuronal cells \uparrow .	Efficient (changes in cognitive decline) and tolerable (1200 mg/day for 12 months) in HD patients? (NCT01357681)
Duchenne muscular dystrophy	
Elongator digitorum longus muscle necrosis \downarrow in mdx mice. Hind limb muscle necrosis \downarrow , muscle force and fatigue resistance \uparrow in mdx mice. Creatine kinase and oxidative stress \downarrow , improved histology, utrophin \uparrow in mdx mice. Glutathione synthesis \uparrow in muscle cells cultured from mdx mice. Muscle aerobic metabolism \uparrow in combination with endurance training in mdx mice. Muscle pathology in regenerating fibres \downarrow in mdx mice.	Safe and tolerable in DMD boys? (NCT01183767)
Amyotrophic lateral sclerosis	
Delayed symptom onset, prolonged life span, attenuated death signals in ALS mice. Protection of motor neurons, microglial activation \downarrow in ALS mice. Protection of motor neurons against THA-induced toxicity in rat spinal cord explants.	
Cerebral ischaemia	
Ischaemia-reperfusion brain injury \downarrow in gerbils, rats and mice.	≥ 3 cups of tea/day decrease the risk of stroke.

NOS = nitric oxide synthase, RRMS = relapsing-remitting multiple sclerosis

\downarrow = decreased, \uparrow = increased

monitoring phase, respiratory quotient values were significantly lower with EGCG treatment compared to the placebo. These findings suggest that EGCG alone has the potential to increase fat oxidation in men and may thereby contribute to the anti-obesity effects of green tea (Chacko et al. 2010).

The anti-obesity properties of EGCG are attributed to different mechanisms include inhibition of pancreatic lipase and fat anabolism and stimulation of lipid catabolism.

4.10.2 Arthritis

Ahmed et al. (2006) found that EGCG was non-toxic to *rheumatoid arthritis* synovial fibroblasts and treatment with EGCG may be potential beneficial in inhibiting joint destruction in rheumatoid arthritis.

4.10.3 Cerebral ischaemic stroke

Uchida et al. (1995) indicated that EGCG may reduce the incidence of stroke due to its radical scavenging action and inhibition of lipid peroxidation and may prolong life span of stroke-prone spontaneously hypertensive rats. Suzuki et al. (2004) investigated the protective effects of green tea catechins on cerebral ischaemic damage and suggested that daily intake of green tea catechins, mainly EGCG, efficiently protects against damage caused by cerebral ischaemia. Lim et al. (2010) evaluated the functional effects of EGCG on ischaemic stroke in a rat model and suggested that EGCG may induce functional improvement of the forelimb in a middle cerebral artery occlusion rat model with ischaemic stroke during the acute or subacute period.

4.10.4 UV protection

Green tea extracts (2% to 5%) have been evaluated for use as a topical photo-protective agent (Li et al. 2009). Clinical studies have reported minimal protective dose-independent effects (Camouse et al. 2009). A 2-year double-blind, placebo-controlled trial of 56 women aged 25 to 75 randomly receiving either 250 mg green tea polyphenols or placebo twice daily found no superiority of green tea polyphenols taken

orally over placebo in improving clinical or histologic photoaging parameters (Janjua et al. 2009).

4.10.5 Oral cavity

The positive health benefits of EGCG in the oral cavity result from the aforementioned properties of tea polyphenols. Green tea polyphenols can eliminate halitosis through modification of the organo-sulfur component methyl mercaptan and odorant sulfur component hydrogen sulphide, which is often generated secondary to dental carries or other oral pathological conditions (Narotzki et al. 2012). Tea polyphenols may reduce oxidative stress and inflammation as well as the chances of oral cancer caused by cigarette smoking (Khurshid et al. 2016).

4.11 Use in veterinary medicine

Excessive fat deposition in broiler chickens is a hot topic in the poultry industry and has been investigated for many years. To poultry farmers, excess fat is an economic burden due to its negative impact on feed efficiency, and most fat depots are lost during processing of the carcass and meat. Too much fat deposition is also undesirable for consumers who are increasingly concerned with the nutritional quality of food. For these reasons, many studies have recently focused on the lipid-lowering effects of natural compounds added to chicken feed. Huang et al. (2015) investigated the effects of EGCG (40 mg/kg body weight daily and/or 80 mg/kg body weight daily) on lipid metabolism in male broiler chickens; serum lipid profiles and abdominal fat accumulation in chickens were determined after oral administration. After four weeks of oral administration, EGCG was found to significantly reduce the level of abdominal fat deposition in broilers. The serum triglycerides and low-density lipoprotein cholesterol of chickens were also significantly decreased, and high-density lipoprotein cholesterol was notably increased at the same time.

A study from 2015 was carried out to study the healing process of extraction sockets treated with grafting materials in a scenario where sources of infection possibly remained (Hong et al. 2015). The biological activity of EGCG was evaluated after application in addition to grafting materials, at sites of induced periapical lesions treated without any

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debridement. The authors hypothesised that the adjunctive use of EGCG would decrease the inflammatory response and the severe breakdown of alveolar bone even in the infectious socket immediately grafted with collagenated bovine bone mineral, while the use of collagenated bovine bone mineral alone would be associated with pronounced destruction. Their findings suggested that the use of EGCG had a beneficial effect with respect to the control of the inflammatory response and the reduction in the extent of fibrosis at the apical area. Adjunctive use of EGCG with collagenated bovine bone mineral can be a candidate biomaterial in the grafting of extraction sockets with periapical lesion, even in the acute infection state.

Another study was aimed at enhancing periodontal tissue regeneration in dogs (Lee et al. 2016). The goal of this study was to develop a tri-layer functional chitosan membrane with epigallocatechin-3-gallate (EGCG) grafted on the outer layer for bactericidal activity and lovastatin in the middle layer for controlled release. The EGCG-chitosan-lovastatin membrane showed good biocompatibility, effective antibacterial activity, increased alkaline phosphatase activity and significantly increased new bone formation in one-walled defects of a beagle dog model. This membrane has the potential to be applied as a novel GTR membrane in periodontal regeneration surgery.

The effects of various polyphenol compounds in animal diets have recently been reviewed. These reviews have examined the use of polyphenols and their efficacy in poultry diets and mono-gastric nutrition (Surai 2013; Khan 2014; Lipinski et al. 2017). In general, the above-mentioned biological effects of polyphenols and their effects on the nutrition and health of livestock were monitored. No clear effects have been observed for nutrient digestibility and growth performance, and, therefore, more detailed studies are needed to elucidate the impact of green tea products on animal nutrition.

5. Conclusion

The consumption of green tea has been associated with various health benefits. The leaves of green tea harbour a wide spectrum of different phytochemicals that may vary according to environmental conditions and processing procedures. Approximately 30% of the green tea dry weight is

constituted by polyphenols, 60–80% of which are catechins. Catechins and, in particular, epigallocatechin-3-gallate (EGCG), which accounts for up to 80% of the catechins, are suggested to mediate the health-promoting effects of green tea. Epigallocatechin gallate (EGCG) is the ester of epigallocatechin and gallic acid. Among catechins, only EGCG is of interest in the field of medicinal chemistry. During the past decades, the health-promoting effects of green tea and its polyphenols have been intensively investigated and EGCG has been the subject of a number of basic and clinical research studies determining its potential use as a therapeutic for a broad range of disorders.

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