Food Chemistry Advances 1 (2022) 100037

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

Approaches to overcome bioavailability inconsistencies of epigallocatechin gallate, a powerful anti-oxidant in green tea

Alina Furniturewalla, Kalyani Barve*

Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM's NMIMS (Deemed to be University), Vile Parle (West), Mumbai 400056, India

ARTICLE INFO

Keywords:
EGCG
Bioavailability
Polyphenol
Green tea

ABSTRACT

Natural polyphenols, mainly found in tea, have many health benefits and have been used in traditional medicine for years; however, they have recently attracted a lot of attention in modern medicine. The present review discusses the therapeutic potential of Epigallocatechin gallate (EGCG) - its antioxidant and anticancer effects and health benefits, its structure and activity - by examining how the composition and arrangement of the molecule make it a suitable free radical scavenger, its pharmacokinetic parameters such as logP value and hydrogen bond donors and hydrogen bond acceptors and finally the main dilemma surrounding this drug molecule: its limited bioavailability - which leads to inconsistencies in its therapeutic potential. It also reviews and deliberates techniques to improve this molecule's efficacy, circumventing the obstacle of bioavailability by administering tea with different foods, administering EGCG as a double emulsion, or in the nanoparticulate form, or modifying the structure of the EGCG molecule.

1. Introduction

Green tea is a beverage that has been linked with numerous health benefits. These benefits are primarily attributed to its active compounds;(-)-epicatechin-3-gallate (ECG),

(-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG). These catechins (flavan-3-ols) are natural polyphenolic antioxidants and have more significant antioxidant activity than vitamin C (Mukai et al., 2005). Among these catechins, EGCG is the most abundant (Botten et al., 2015); and has expressed the highest antioxidant activity (Mukai et al., 2005). Studies conducted in recent years show that EGCG has beneficial effects as a prophylactic and a therapeutic agent in obesity, cancer, diabetes, neurodegenerative diseases, liver diseases, and inflammation (Miyoshi et al., 2015; Ohishi et al., 2016). On average, one cup of green tea contains approximately 50–100 mg of catechins (Jówko, 2015), of which EGCG accounts for 25–30 mg (Botten et al., 2015). Humans do not consume green tea in substantial quantities; thus, the EGCG content consumed is inadequate, and therefore it shows a variation in results for green tea consumers as no fixed dose is given. Variation also arises due to inconsistencies in brewing time and the frequency of consumption.

Additionally, EGCG is known to have low bioavailability (Lee et al., 2002), which demonstrates variable results on the efficacy of EGCG *in-vitro* and *in-vivo* studies.

Administering EGCG as an extracted bioactive and enhancing its bioavailability *in-vivo* could effectively treat the disorders mentioned above and diseases. Furthermore, a high dose of the antioxidant could be administered without the side effects associated with caffeine present in the beverage (Henning et al., 2004).

The structure of EGCG plays a significant role in its activity as shown in Table 1.

2. Structure of EGCG

The structure of EGCG is as shown in Fig. 1a and is different from the other catechins, ECG (Epicatechin gallate) and EGC (Epigallocatechin).

The structure of EGCG represents a benzenediol ring A connected to a tetrahydrophan moiety (B), a pyrogallol ring (C), and a galloyl group with D ring. It has a four-ring structure along with eight hydroxyl groups making it highly hydrophilic. EGCG is the most effective scavenger for various radical species such as superoxide anions, hydroxyl radicals, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical due to the pyrogallol ring C and the galloyl D ring (Botten et al., 2015).

EGCG acts as a preventive antioxidant due to the pyrogallol structure that gives it a strong metal-chelating capability that causes it to bind transition metal ions. Furthermore, the inhibitory effect on the microsomal enzyme system and lipid-lowering action of EGCG is linked to the galloyl group (Chen & Zhang, 2007). All these components of the structure increase the biological activity of EGCG.

* Corresponding author.
E-mail address: kalyani.barve@nmims.edu (K. Barve).

https://doi.org/10.1016/j.focha.2022.100037
Received 11 February 2022; Received in revised form 26 March 2022; Accepted 22 April 2022

Table 1
Major biological activities of EGCG.

Health benefits of EGCG	Research and findings	Refs.
Antioxidant activity	EGCG is a more powerful antioxidant than epicatechin (EC) and resveratrol. The anti-radical activity of EGCG is higher than that of resveratrol but lower than that of ascorbic acid, according to the DPPH free radical scavenging test. In comparison to stilbenes, flavonoids are more powerful antioxidants; the gallate group and the hydroxyl moiety on the B ring of flavonoids are responsible for their increased antioxidant capacity.	Boulmokh et al. (2021)
	EGCG has a high antioxidant activity (IC50 = 36.71 - DPPH assay) and can suppress ROS activity while also inhibiting advanced glycation end products, thus giving it antioxidant and anti-inflammatory characteristics.	Narmada et al. (2020)
	When compared to other catechins and caffeine, EGCG has the strongest antioxidant activity. The order of chelating properties and reducing power grew from caffeine<EGC<ECG<EGCG, and so did the strength of antioxidant activity increased from caffeine<EGC<ECG = EGCG. DPPH scavenging capacity, the chelating characteristics, reducing power, and free radical scavenging indices of EGCG also increase with its concentration.	Piechocka et al. (2021)
	The antioxidant activity of SOD and CAT, as well as the amounts of the antioxidant elements Mg, Zn, and Se, and the Zn/Cu ratio, are all increased by EGCG, thus exhibiting a neuroprotective effect. EGCG also uses its chelating capacity to effectively chelate Fe, Cu, and Pb, reducing ROS production, lipid peroxidation, and subsequent oxidative brain damage caused by Fe, Cu, and Pb.	(Lin et al., 2022)
Antiviral activity	Tea catechin administration reduces viral load, blocks entry mechanisms, and promotes apoptosis in infected cells. EGCG exhibits a low binding affinity when interacting with the three target proteins of HIV-1; it shows antiviral properties through the triple inhibitor mechanism.	Kharisma et al. (2021)
	EGCG exhibits antiviral action and is found to inhibit SARS-CoV-2 and other coronavirus infections. The less physiologically active green tea catechin, EC, on the other hand, has no inhibitory effect. EGCG demonstrates the ability to limit virus infections *in vitro* by blocking the entry of SARS-CoV-2, as well as MERS- and SARS-CoV pseudotyped lentiviral vectors.	Henss et al. (2021)
Antiobesity	EGCG demonstrates anti-obesity activity by upregulating Beclin1-dependent autophagy and catabolism of lipids in white adipose tissue through an AMPK-mediated mechanism. It decreased adipose tissue mass more significantly viscerally than subcutaneously. EGCG therapy increased glucose tolerance by lowering postprandial glucose levels via inhibiting alpha-amylase. It also demonstrated weight loss potential.	Choi et al. (2020)
	Treatment with EGCG reduces body weight gain, serum cholesterol, mesenteric fat mass, insulin resistance, fasting blood glucose and fatty liver severity. These effects are attributed to increased levels of cholesterol 7α-hydroxylase mRNA, HMG-CoA reductase and increased receptors for low-density lipoprotein and scavenger receptor B1. EGCG reduces intestinal bile acid and increases bile acid, cholesterol, and total lipid excretion.	Huang et al. (2018)
Anticancer	By modulating the TGF-β/Smad signaling pathways, EGCG significantly reduces epithelial-to-mesenchymal transition (EMT), invasion, and migration *in vitro*. EMT is linked to tumor metastasis and cancer progression; it is thought to be the fundamental mechanism for metastasis in numerous cancers.	Li et al. (2019)
	EGCG demonstrates the ability to cause apoptosis and inhibit acute promyelocytic leukemia (APL) cell proliferation.	Borutinskaitė et al. (2017)

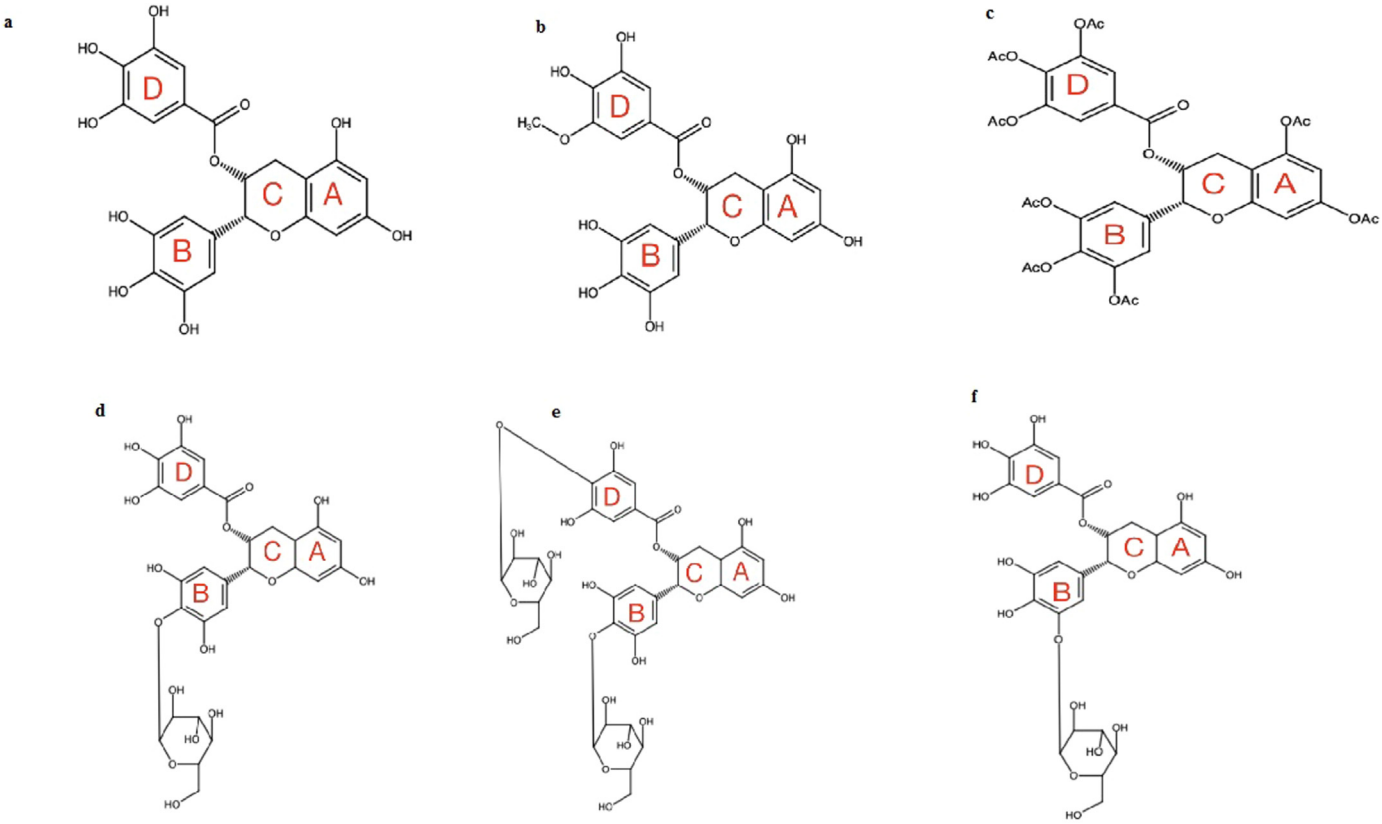

Fig. 1. (a) Structure of EGCG; (b) Structure of methylated EGCG; (c) Structure of acylated EGCG,(d). Structure of (-)-epigallocatechin gallate 4′-O-α-D-glucopyranoside (EGCG G1) (e) Structure of (-)-epigallocatechin gallate 4′,4″-O-α-D-diglucopyranoside (EGCG G2); (f)Structure of 3′-O-α-D-glucopyranoside.

3. Pharmacokinetic parameters of EGCG

It is imperative to know and comprehend the pharmacokinetics and biological effects of EGCG. Employing Lipinksi's rule of 5, the oral bioavailability of the catechin can be predicted. EGCG has a logP value < 5 (Liu et al., 2021), however, it has eight hydrogen bond donors that are the hydroxyl groups (more than 5) and a molecular weight of 458.372 g/mol (almost 500); hence it violates two criteria, and thus it is anticipated to be poorly bioavailable on oral administration.

A preclinical study on the pharmacokinetic parameters by Chen et al. (1997) shows that EGCG has low bioavailability in terms of absorption (low Cmax value) and is excreted mainly through bile (high AUC values in intestine). The study also demonstrates that when administered intravenously, EGCG tends to distribute into the peripheral compartment (K12 larger than K21) and that EGCG can stay in the body for an extended period of time (long t1/2 and small CL). However, there may be more variations in metabolism in humans than in the rat model due to species differences. EGCG has limited bioavailability because of its physical and chemical properties, which define its absorption rate, metabolism, and elimination from the body. It is classified as a BCS class III drug (hydrophilic with low oral bioavailability).

4. Bioavailability and metabolism of EGCG

Green tea catechins have extremely low stability post digestion, with only 20% remaining intact, of which EGCG is the one of the most sensitive with < 10% available for absorption (Green et al., 2007). Out of all the tea catechins, EGCG has the lowest bioavailability (Chen et al., 1997), limiting its therapeutic efficacy. Bioavailability is affected by metabolism and biotransformation as well as the stability of EGCG. Orally administered EGCG undergoes metabolic changes. The metabolism starts in the mouth where EGCG is hydrolyzed and degalloylated by salivary catechin esterase (Higdon & Frei, 2003). EGCG undergoes biotransformation in the liver; it undergoes glucuronidation by UDP-glucuronosyltransferase (UGT), sulfation by sulfotransferase (SULT) and methylation by catechol-O-methyltransferase (COMT) (Lambert et al., 2007). The gut microbiota is also important for EGCG metabolism; *in vitro* and *in vivo* investigations show that gut bacteria can de-conjugate and degrade EGCG. Using a pig cecum model, researchers discovered that EGCG was entirely metabolized by the gut bacteria within 4–8 h (van't Slot & Humpf, 2009). Additionally, ATP-dependent multidrug resistance-associated protein (MRP) efflux pumps control the quantity of EGCG and its metabolites in the cell. MRP1 effluxes EGCG from the inside of cells into the bloodstream or intestinal space, increasing bioavailability *in vivo*. MRP2 secretes EGCG absorbed from the intestine back into the intestinal lumen. EGCG that is not released into the intestinal lumen by the enterocyte is absorbed into the portal circulation thus efflux by MRP2 decreases bioavailability. Furthermore, MRP2 transcript levels in the human jejunum are nearly 10-fold greater than MRP1 and as a result, MRP2 efflux of these molecules may play a key role in reducing EGCG bioavailability *in vivo* (Hong et al., 2003).

The inherent instability of EGCG also hinders its bioavailability and overall efficacy. When *in-vitro* studies are extrapolated to *in-vivo* the poor bioavailability of this catechin should be considered. Due to the difference in its *in-vitro* and *in-vivo* studies (Krupkova et al., 2016) and its variable bioavailability, specific dose recommendations of EGCG is difficult. Thus, it is crucial to understand factors affecting its bioavailability to circumvent the problem and increase its efficacy.

Factors affecting the stability of EGCG and hence influencing its bioavailability are described below and shown in Table 2:

4.1. Temperature

Stability of EGCG decreases with increasing temperature under all pH conditions, higher temperatures leads to more degradation. Xu et al. (2019) observed that the highest degradation rate of 8.83 × 10−2 s−1 was reported at 135 °C whilst the lowest degradation rate of 1.06 × 10−7 s−1 was reported at 25 °C. The highest increases in degradation rate constants were observed between 25 and 40 °C, 80–100 °C, and 100–121 °C, demonstrating that these temperature ranges seem to have a strong influence on EGCG degradation. Higher temperatures also lead to faster epimerization between EGCG and GCG; another study found that degradation of EGCG was most significant below 44 °C and that above 44 °C the epimerization between GCG and EGCG was greater than the degradation (Wang et al., 2008).

4.2. pH

EGCG is very susceptible to degradation in alkaline pH (Zhu et al., 1997). EGCG has a pyrogallol ring B that presents a strong antioxidative activity and in high pH conditions, forms oxidized catechins containing an O-quinone structure. The oxidized catechins formed undergo auto-oxidation forming reactive oxygen species, which leads to polymerization and decomposition, and low pH leads to more excellent stability (Fangueiro et al., 2014; Ishii et al., 2011).

Temperature has a greater effect on the stability of EGCG than pH and it was found that a lower pH system served to mitigate the effects of high temperature on EGCG. Therefore, to ensure that a higher proportion of the catechin is maintained, both the pH level and the temperature should be reduced as much as possible during the processing of EGCG into appropriate oral forms (Xu et al., 2019).

4.3. Oxidation

EGCG degradation is primarily caused by oxidation of the polyphenolic structure (Cano et al., 2019). EGCG features three hydroxyl groups on the B ring and a gallate group esterified on the C ring, which allows it to exhibit its antioxidant activity. However, this structure of EGCG also makes it prone to auto-oxidation degradation - the B ring was found to be the primary site of autoxidation (Severino et al., 2009). The auto-oxidation of EGCG results in the formation of hydrogen peroxide as well as the production of multiple EGCG auto-oxidation products, which together limit its efficacy and exhibit cytotoxic effects (Wei et al., 2016).

EGCG encounters disadvantageous metabolism in the GIT and liver, which hinders its bioavailability orally. It is subjected to extreme pH from 1.5 in the stomach to 8.5 in the intestine (where maximum absorption occurs). About 80% of the total catechins are degraded in simulated human digestive conditions (Green et al., 2007).

We could circumvent these bioavailability problems by administering EGCG parenterally or using different methods to enhance its stability in the stomach, increase its absorption from the intestine and reduce its metabolism rate.

Several methods have been proposed to improve the bioavailability of EGCG which are discussed below.

5. Bioavailability enhancement of EGCG

There are various ways of enhancing the bioavailability of EGCG which are described below.

5.1. Administering tea with different foods

An *in-vitro* study using porcine pepsin, lipase, and pancreatin carried out by Green et al. (2007) showed that EGCG (administered as green tea) is more bioavailable when given with other foods.

5.1.1. Tea prepared with milk

It significantly increased tea catechin recovery from 20% to 52%. The interaction between protein and catechins provides a physical entrapment for the catechins and is speculated to stabilize the mechanism.

Table 2
Factors affecting bioavailability of EGCG.

Factors	*In vitro* studies/ *In vivo* studies
Metabolism	*In vitro* studies
Amount of EGCG prior to simulated digestion: 22.7 ± 0.43 mg/100 mL	
Amount of EGCG post simulated digestion: 2.77 ± 0.45 mg/100 mL	
Degradation due to digestive loss: 87.9 ± 2.29%	
Green et al. (2007)	
	In vivo studies
EGCG undergoes biotransformation *in vivo* by UGT, SULT and COMT (Lambert et al., 2007)	
EGCG PK parameters *in vivo* in rat model (Zhang & Zhang, 2018):	
T1/2 = 2.1 ± 0.8 h	
Cmax = 564.5 ± 121.7 ng/mL	
AUC (0–24 h) = 1042.6 ± 198.3 ng·h/mL	
MRT (0–24 h) = 5.7 ± 1.4 h	
CL = 11.2 ± 1.7 L/h/kg	
V = 5.1 ± 1.3 L/kg	
Temperature	*In vitro* studies
135 °C showed the highest degradation rate of EGCG = 8.83 × 10−2 *s* − 1	
165 °C showed the highest epimerization rate from EGCG to GCG = 1.38 × 10−1 *s* − 1 (Xu et al., 2019)	
pH	*In vitro* studies
An increase in pH values from pH 6–7, to pH 7–8 show the highest increase in the degradation rate constants by 1.30–16.0 and 1.48–18.9 times (Xu et al., 2019).	
EGCG demonstrates great stability at pH 2.0 (retention rate = 93.63 ± 2.15%) and 4.0 (retention rate = 95.23 ± 2.53%), but is unstable at pH 6.0 and 8.0.	
T1/2 of EGCG in phosphate buffer solution (pH 7.4) = 30 min (Wang et al., 2021).	
Conversion pathways	*In vitro* studies
Following pathways contribute to conversion of EGCG ultimately leading to loss of EGCG (Chen et al., 2021)	
Hydrolysis = 14.46%	
Epimerization = 9.43%	
Oxidation = 51.95%	

Per contra, this mechanism masks the antioxidant activity of the catechin molecule EGCG (Arts et al., 2002). Additionally, in the same study a significant reduction in EGCG levels at higher milk concentrations is seen; this is further elucidated and supported by a clinical study by Lorenz et al. (2007), which corroborates that milk inhibits the effects of green tea catechins presumptively by the formation non-covalent crosslinks between EGCG and the milk proteins casein (Jöbstl et al., 2006). However, Xie et al. (2013) proposed that the addition of milk increases the catechin bioavailability by increased intestinal permeability demonstrated in the Caco-2 model compared to taken without milk - they suggest that milk proteins may play a role in improving the intestinal transport. They also determined that green tea brewed at higher temperatures improved bioaccessibility.

5.1.2. Tea prepared with ascorbic acid

It showed a significant upturn of EGCG recovery from 20% to 54%. This outcome is in accordance with the stabilizing effects of ascorbic acid (Chen et al., 1998) in an alkaline medium (intestinal pH) where EGCG is extremely unstable (Zhu et al., 1997). Ascorbic acid can hinder metabolic pathways of EGCG in the small intestine, such as the formation of the semiquinone radical, thus countering the degradation of EGCG before absorption. It could also work by acting as a reductant, recycling the free radical form of EGCG, reducing the oxygen level, and preventing oxidation of EGCG. Peters et al. (2010) suggested that absorption of EGCG significantly increases when green tea is formulated with ascorbic acid and sucrose and that this formulation enhances bioavailability by increasing bioaccessibility and intestinal uptake. However, sucrose might be problematic for people with diabetes. Hence sucrose needs a substitution which can be xylitol. A study by Islam (2011) in the Journal of Medicinal food proposes that xylitol is better than sucrose as a sweetener for people with diabetes. Another study by Shim et al. (2012) suggested substituting sucrose with xylitol, which is metabolized slowly, resulting in low blood insulin levels. It was found that the intestinal uptake of catechins increased by 11 times when green tea was taken with xylitol and ascorbic acid compared to brewed green tea.

5.1.3. Tea prepared with juices

When prepared with juices such as orange, grapefruit, and lemon, it showed the most amelioration of 56–76% (Green et al., 2007). This may be due to phytochemicals such as flavanols and terpenes, which may act synergistically by working with ascorbic acid present in the juices to scavenge catechin free radicals or by preventing the oxidative damage on ascorbic acid.

5.1.4. Tea prepared with black pepper

It also increased absorption of EGCG as per the study by Lambert et al. (2004). It demonstrated that piperine, a component of black pepper, improved the bioavailability of EGCG by inhibiting the intestinal glucuronidation of EGCG, thus inhibiting its degradation and increasing its absorption. In the study, when EGCG was co-administered with piperine, Cmax was 1.1 times higher, and AUC values were 1.7 times higher compared to EGCG only.

EGCG inhibits CYP450 enzymes and OATPs (Misaka et al., 2013; Yang et al., 2016). Therefore, it is important to be careful when administering drugs and to prevent interactions.

5.2. Administering EGCG as a double emulsion

Hu et al. (2015) developed a self- double-emulsifying drug delivery system (SDEDDS) in solid form to enhance the oral absorption of EGCG.

The *in vitro* and *in vivo* experiments demonstrated increased uptake of EGCG in the form of solid SDEDDS compared to pure EGCG. The double emulsion comprises EGCG, oil phase (medium-chain triglyceride, glycerin monostearate, polyglycerol polyricinoleate), glycerol, water and surfactant (Tween 80). It formed a w/o/w double emulsion to develop a solid SDEDDS formulation. The apparent permeability coefficient (Papp) of EGCG in EGCG solution was 0.28 × 106 cm/s, which increased to 0.55 × 10 6 cm/s for solid EGCG-SDEDDS in a similar concentration when incubated with Caco-2 monolayers for up to 2 h. The AUC of EGCG significantly improved after oral administration of solid EGCG-SDEDDS. A 1.93 fold increase in AUC from 84.61 μg.h/mL (EGCG) to 163.29 μg.h/mL (EGCG-SDEDDS) and a 1.44 fold increase

was seen in Cmax value from 12.92 μg/mL (EGCG) to 17.69 μg/mL (EGCG-SDEDDS). This showed that solid EGCG-SDEDDS could enhance the oral bioavailability and Cmax of EGCG.

5.3. Administering EGCG as nanoparticles

Nanosystems present a high loading capacity for drugs, and they also propose the opportunity of attaching molecules to their surface for targeting, thus exhibiting an optimal alternative for administering drugs (Cano et al., 2019).

5.3.1. Dual drug EGCG/AA loaded PEGylated PLGA nanoparticles

Ascorbic acid (AA) acts as a reducing agent. It avoids almost 80% of EGCG degradation by providing it protection and preventing it from auto-oxidation (Fangueiro et al., 2014). Furthermore, ascorbic acid also adds anti-inflammatory properties to the formulation (Sorice et al., 2014). Cano et al. (2019) demonstrated in their study that when EGCG was formulated as EGCG/AA in polyethylene glycolated poly(lactic-co-glycolic acid) (PEGylated PLGA) nanoparticles, stability of EGCG increased. Since AA is a potent antioxidant, it helps reverse the effect of oxidation and prevents the catechin's degradation. PEGylation of the nanoparticle is employed to enhance the *in-vivo* half-life as PEG reduces aggregation, improves aqueous solubility, and reduces opsonization, thus improving the nanosystem's stability by reducing immunogenicity. It also improves penetration across the intestinal barrier because PEGylation increases the permeability coefficient of solid lipid nanoparticles and bioavailability (Yuan et al., 2013). PLGA is used considering its biocompatibility, biodegradability, non-toxicity, and fewer peripheral side effects. This nanosystem was able to increase drug permeance into the bloodstream.

A big asset of the polymeric controlled drug delivery system was the sustained release of the encapsulated drug. After 10 h, 23% EGCG was released, and after 24 h, 50% drug was released. In this study, EGCG showed an adequate and sustained release profile from the polymeric nanosystem and demonstrated enhanced stability.

5.3.2. EGCG loaded chitosan nanoparticles (Chit-nanoEGCG)

Chitosan (CS) is a natural biopolymer with mucoadhesive properties, thus forming nanoparticles using CS provides an increased retention time in the GIT and an improved penetration into the mucus layer (Takeuchi et al., 2001). A study by Khan et al. (2013), Chit-nanoEGCG nanoparticles with a diameter of <200 nm were synthesized. The release profile showed:

10% EGCG was released at the pH representing the stomach (pH 1.5), and 50% was released at the pH representing the intestine (pH 6.8) from the nanoparticles. The release rate was faster at pH 6.8, and then there was a steady release of EGCG.

Thus encapsulation of EGCG in CS escapes degradation in the stomach, and this may contribute to enhanced bioavailability.

5.3.3. EGCG loaded nanoparticles prepared from chitosan and polyaspartic acid

The study by Hong et al. (2014) examined the efficacy of EGCG when it was encapsulated into self-assembled nanoparticles of chitosan (CS) and aspartic acid (PAA).

Since the pH in the gastric and intestinal fluid varies, the release of EGCG from EGCG-CS-PAA nanoparticles at various pH was studied, and the following results were found:

25% EGCG was released at the pH representing the stomach (pH 2.5 and 4.0), whereas 41% EGCG was released at the pH representing the duodenum (pH 6.6) from the nanoparticles.

At the pH representing the jejunum (pH 7.0), the proximal ileum, and the pH representing the distal ileum and the intercellular body fluid (pH 7.4), the percentage EGCG released increased rapidly, which indicated that the nanoparticles become unstable and rapidly disintegrate to release the EGCG.

Furthermore, in simulated gastric fluid (pH 2.1), the loss of EGCG from the nanoparticles was 10–15% less than that of the free EGCG.

This study showed that EGCG-CS-PAA nanoparticles were stable in the stomach; at the same time, quick release in the intestine could improve the uptake of EGCG in the intestine and hence the bioavailability.

5.3.4. EGCG loaded chitosan–casein phosphopeptides nanoparticles

In the study conducted by Hu et al. (2012), EGCG was encapsulated within homogeneously dispersed CS–CPP nanoparticles with a particle size of 150 ± 4.3 nm. The intestinal permeability of EGCG was improved significantly.This can be explained by increased cellular uptake of these nanoparticles by Caco-2 cells. The study affirmed cellular internalization of EGCG-loaded CS–CPP nanoparticles and that the nanoparticles did not show any cellular toxicity to Caco-2 cell (based on MTT assay). The intestinal permeability and absorption of EGCG were enhanced, thus improving bioavailability.

5.3.5. EGCG nanoliposomes

As demonstrated in the experiment conducted by Zou et al. (2014) EGCG nanoliposomes (EN) showed high stability during simulated *in vitro* digestion studies. It did not show drastic change after incubation in simulated gastric fluid for 3 h; however, after incubating in simulated intestinal fluid for 1.5 h, it showed significant change compared to the EGCG solution. EGCG content dropped in the intestinal fluid from 97.9% to 3.4% for EGCG solution, but for EN, it dropped to 47.7% only - this demonstrates that the nanoliposomes show more stability during digestion. EN was prepared using the dynamic high-pressure micro fluidization method by formulating an EGCG liposome by an ethanol injection and then applying it to a microfluidizer.

5.4. Modifying the EGCG molecule

Chemical modifications can help improve the physical and chemical properties of EGCG by reducing its biotransformation and thus may help improve its bioavailability and retain its therapeutic efficacy simultaneously.

5.4.1. Methylation

Methylation of EGCG was achieved by converting the hydroxyl group on the benzene rings to a methyl ether. This can also be referred to as etherification. A study carried out by Oritani et al. (2013), illustrated that EGCG3"Me (methylation of the hydroxyl group at 3″position on the D ring) showed enhanced bioavailability and modified distribution. EGCG4"Me did not show much effect. Thus, EGCG3"Me (Fig. 1b) was the derivative of choice.

EGCG3"Me can not only be synthetically made but there is a natural source for this compound, and it is 'Benifuuki' tea from *Camellia Sinensis* L. It is rich in EGCG3″Me and can be used to extract EGCG3″Me (Maeda-Yamamoto et al., 2007).

EGCG3"Me showed higher absorption into circulating blood than EGCG upon oral administration. Its absorption ratio in the body was 6.4 times higher than EGCG (Maeda-Yamamoto et al., 2007).

The AUC values after oral and intravenous administration of EGCG3"Me were about 3 times higher than that of EGCG.

V_d of EGCG3"Me was about one third that of EGCG implicating that circulating blood had more EGCG3"Me retained than EGCG.

5.4.2. Acylation

Acylation of EGCG can be achieved by forming ester bonds between the acyl group and the hydroxyl groups on the benzene rings. This can also be referred to as esterification.

EGCG is highly unstable in neutral or alkaline conditions; this instability leads to low bioavailability. To overcome this, peracetate protection groups on the reactive hydroxyls were introduced and peracylated EGCG was formed by treating the EGCG with acetic anhydride

and pyridine (Lam et al., 2004). The study by Lambert et al. (2006) explained that peracylated EGCG (AcEGCG) acts as a prodrug and gets rapidly converted to EGCG by HCT116 cells. Administration of AcEGCG (Fig. 1c) produced a 2.8–30 fold greater intracellular concentration of EGCG as compared to plain EGCG due to increased uptake and intracellular conversion to EGCG in the presence of the enzyme esterase. Esterification blocks the polar side chains and makes the hydroxyl groups on the benzene rings unavailable for phase II biotransformation or oxidative degradation. It also showed better adequacy in proteasome inhibition and cell death induction, thus improving activity *in vivo* (Lam et al., 2004).

There was an overall increase in the AUC in the plasma, small intestine and colon was seen for example, the plasma area under the curve for total EGCG was 465.0 g/ml for AcEGCG and 194.6 g/ml for EGCG (Lambert et al., 2006).

AcEGCG was six times more stable than EGCG at neutral pH (by HPLC analysis) [39].

Albeit acetylation increases absorption, it is not counted upon to alter biotransformation and eliminate the deacetylated product (EGCG).

5.4.3. Glucosylation

Karakaya (2004), in a review, has suggested that sugar molecules may improve the absorption of phenolic compounds. The cytosolic glucosidase/lactase phlorizin hydrolase can absorb phenolics like EGCG with a sugar molecule, such as glucose, or xylose, through the small intestine. The chemical, physical, and biological properties are influenced by glycosylation (Scalbert & Williamson, 2000).

Kitao et al. (1995) synthesized two glucosylated analogs of EGCG using sucrose phosphorylase from *Leuconostoc mesenteroides* : (-)-epigallocatechin gallate 4′-O-α-D-glucopyranoside (EGCG G1) and (-)-epigallocatechin gallate 4′,4″-O-α-D-diglucopyranoside (EGCG G2), structures shown in Fig. 1d and e.

Their stabilities were compared in pH 7.4 solution. The results demonstrated that 0.07% of EGCG, 98.42% EGCG G1 and 99.75% of EGCG G2 remained after 3 h. A recent study by Gonzalez-Alfonso et al. (2019) suggested that another glucoside analog 3′-O-α-D-glucopyranoside (Fig. 1f) also has more pH stability at pH 6.7. EGCG was degraded 4 times faster than its monoglucoside analog. Thus, the EGCG glucosides are considered more stable forms of EGCG (Lu et al., 2003).

6. Conclusion

EGCG, a component of green tea has been used in the food industry as an antibacterial for food packaging. However it also has many beneficial uses in various diseases; but due to its low bioavailability and inadequate stability in the GIT, its use as a nutraceutical has diminished.

The bioavailability can be enhanced by different methods such as co-administering it with different foods - such as milk, vitamin C, juices or black pepper - among which vitamin C with xylitol showed the most favorable results. Developing an effective method of delivery like double emulsion, or nanoparticle formulations like EGCG/AA loaded PEGylated PLGA nanoparticles, EGCG loaded chitosan nanoparticles, EGCG loaded nanoparticles prepared from chitosan and polyaspartic acid, EGCG loaded chitosan–caseinophosphopeptides nanoparticles and EGCG nanoliposomes can enhance the bioavailability. These formulations can be designed into a nutraceutical preparation. Structurally modified EGCG can be used as an additive in some foods to derive the therapeutic benefits of EGCG due to improved bioavailability.

Declaration of Competing Interest

None.

Acknowledgment

The authors acknowledge SPPSPTM, SVKM's NMIMS for the facilities provided in executing the work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2022.100037.

References

Arts, M. J. T. J., Haenen, G. R. M. M., Wilms, L. C., Beetstra, S. A. J. N., Heijnen, C. G. M., Voss, H. P., et al., (2002). Interactions between flavonoids and proteins: Effect on the total antioxidant capacity. *Journal of Agricultural and Food Chemistry, 50*(5), 1184–1187. doi:10.1021/jf010855a.

Borutinskaitė, V., Virkšaitė, A., Gudelytė, G., & Navakauskienė, R. (2017). Green tea polyphenol EGCG causes anti-cancerous epigenetic modulations in acute promyelocytic leukemia cells. *Leukemia & Lymphoma, 59*(2), 469–478. doi:10.1080/10428194.2017.1339881.

Botten, D., Fugallo, G., Fraternali, F., & Molteni, C. (2015). Structural properties of green tea catechins. *The Journal of Physical Chemistry B, 119*(40), 12860–12867. doi:10.1021/acs.jpcb.5b08737.

Boulmokh, Y., Belguidoum, K., Meddour, F., & Amira-Guebailia, H. (2021). Investigation of antioxidant activity of epigallocatechin gallate and epicatechin as compared to resveratrol and ascorbic acid: Experimental and theoretical insights. *Structural Chemistry, 32*. doi:10.1007/s11224-021-01763-5.

Cano, A., Ettcheto, M., Chang, J. H., Barroso, E., Espina, M., Kühne, B. A., et al., (2019). Dual-drug loaded nanoparticles of Epigallocatechin-3-gallate (EGCG)/ascorbic acid enhance therapeutic efficacy of EGCG in a APPswe/PS1dE9 alzheimer's disease mice model. *Journal of Controlled Release, 301*, 62–75. doi:10.1016/j.jconrel.2019.03.010.

Chen, L., Lee, M. J., Li, H., & Yang, C. S. (1997). Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metabolism and Disposition, 25*(9), 1045–1050.

Chen, L., Wang, W., Zhang, J., Cui, H., Ni, D., & Jiang, H. (2021). Dual effects of ascorbic acid on the stability of EGCG by the oxidation product dehydroascorbic acid promoting the oxidation and inhibiting the hydrolysis pathway. *Food Chemistry, 337*, Article 127639. doi:10.1016/j.foodchem.2020.127639.

Chen, L., & Zhang, H. Y. (2007). Cancer preventive mechanisms of the green tea polyphenol (-)-epigallocatechin-3-gallate. *Molecules, 12*(5), 946–957 (Basel, Switzerland). doi:10.3390/12050946.

Chen, Z. Y., Zhu, Q. Y., Wong, Y. F., Zhang, Z., & Chung, H. Y. (1998). Stabilizing effect of ascorbic acid on green tea catechins. *Journal of Agricultural and Food Chemistry, 46*(7), 2512–2516. doi:10.1021/jf971022g.

Choi, C., Song, H. D., Son, Y., Cho, Y. K., Ahn, S. Y., Jung, Y. . S., et al., (2020). Epigallocatechin-3-gallate reduces visceral adiposity partly through the regulation of beclin1-dependent autophagy in white adipose tissues. *Nutrients, 12*(10), 3072. doi:10.3390/nu12103072.

Fangueiro, J. F., Parra, A., Silva, A. M., Egea, M. A., Souto, E. B., Garcia, M. L., et al., (2014). Validation of a high performance liquid chromatography method for the stabilization of epigallocatechin gallate. *International Journal of Pharmaceutics, 475*(1–2), 181–190. doi:10.1016/j.ijpharm.2014.08.053.

Gonzalez-Alfonso, J. L., Peñalver, P., Ballesteros, A. O., Morales, J. C., & Plou, F. J. (2019). Effect of α-Glucosylation on the stability, antioxidant properties, toxicity, and neuroprotective activity of (–)-epigallocatechin gallate. *Frontiers in Nutrition, 6*. doi:10.3389/fnut.2019.00030.

Green, R. J., Murphy, A. S., Schulz, B., Watkins, B. A., & Ferruzzi, M. G. (2007). Common tea formulations modulate *in vitro* digestive recovery of green tea catechins. *Molecular Nutrition & Food Research, 51*(9), 1152–1162. doi:10.1002/mnfr.200700086.

Henning, S. M., Niu, Y., Lee, N. H., Thames, G. D., Minutti, R. R., Wang, H., et al., (2004). Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. *The American Journal of Clinical Nutrition, 80*(6), 1558–1564. doi:10.1093/ajcn/80.6.1558.

Henss, L., Auste, A., Schürmann, C., Schmidt, C., von Rhein, C., Mühlebach, M. D., et al., (2021). The green tea catechin epigallocatechin gallate inhibits SARS-CoV-2 infection. *Journal of General Virology, 102*(4). doi:10.1099/jgv.0.001574.

Higdon, J, & Frei, B (2003). Tea Catechins and Polyphenols: Health Effects, Metabolism, and Antioxidant Functions. *Critical Reviews in Food Science and Nutrition, 43*(1), 89–143. doi:10.1080/10408690390826464.

Hong, J., Lambert, J. D., Lee, S. H., Sinko, P. J., & Yang, C. S. (2003). Involvement of multidrug resistance-associated proteins in regulating cellular levels of (–)-epigallocatechin-3-gallate and its methyl metabolites. *Biochemical and Biophysical Research Communications, 310*(1), 222–227. doi:10.1016/j.bbrc.2003.09.007.

Hong, Z., Xu, Y., Yin, J. F., Jin, J., Jiang, Y., & Du, Q. (2014). Improving the effectiveness of (−)-epigallocatechin gallate (EGCG) against rabbit atherosclerosis by EGCG-loaded nanoparticles prepared from chitosan and polyaspartic acid. *Journal of Agricultural and Food Chemistry, 62*(52), 12603–12609. doi:10.1021/jf504603n.

Hu, B., Ting, Y., Zeng, X., & Huang, Q. (2012). Cellular uptake and cytotoxicity of chitosan–caseinophosphopeptides nanocomplexes loaded with epigallocatechin gallate. *Carbohydrate Polymers, 89*(2), 362–370. doi:10.1016/j.carbpol.2012.03.015.

Hu, C., Wang, Q., Zhao, G., Yao, W., & Xia, Q. (2015). Improved oral absorption of (−)-epigallocatechin-3-gallate via self-double-emulsifying solid formulation. *European Journal of Lipid Science and Technology, 118*(8), 1115–1124. doi:10.1002/ejlt.201500316.

Huang, J., Feng, S., Liu, A., Dai, Z., Wang, H., Reuhl, K., et al., (2018). Green tea polyphenol EGCG alleviates metabolic abnormality and fatty liver by decreasing bile acid and lipid absorption in mice. *Molecular Nutrition & Food Research, 62*(4), Article 1700696. doi:10.1002/mnfr.201700696.

Ishii, T., Ichikawa, T., Minoda, K., Kusaka, K., Ito, S., Suzuki, Y., et al., (2011). Human serum albumin as an antioxidant in the oxidation of (−)-epigallocatechin gallate: participation of reversible covalent binding for interaction and stabilization. *Bioscience, Biotechnology, and Biochemistry, 75*(1), 100–106. doi:10.1271/bbb.100600.

Islam, Md. S. (2011). Effects of xylitol as a sugar substitute on diabetes-related parameters in nondiabetic rats. *Journal of Medicinal Food, 14*(5), 505–511. doi:10.1089/jmf.2010.0015.

Jöbstl, E., Howse, J. R., Fairclough, J. P. A., & Williamson, M. P. (2006). Noncovalent cross-linking of casein by epigallocatechin gallate characterized by single molecule force microscopy. *Journal of Agricultural and Food Chemistry, 54*(12), 4077–4081. doi:10.1021/jf053259f.

Jówko, E. (2015). Green tea catechins and sport performance. *Antioxidants in sport nutrition*. CRC Press/Taylor & Francis.

Karakaya, S. (2004). Bioavailability of phenolic compounds. *Critical Reviews in Food Science and Nutrition, 44*(6), 453–464. doi:10.1080/10408690490886683.

Khan, N., Bharali, D. J., Adhami, V. M., Siddiqui, I. A., Cui, H., Shabana, S. M., et al., (2013). Oral administration of naturally occurring chitosan-based nanoformulated green tea polyphenol EGCG effectively inhibits prostate cancer cell growth in a xenograft model. *Carcinogenesis, 35*(2), 415–423. doi:10.1093/carcin/bgt321.

Kharisma, V. D., Widyananda, M. H., Ansori, A. N. M., Nege, A. S., Naw, S. W., & Nugraha, A. P. (2021). Tea catechin as antiviral agent via apoptosis agonist and triple inhibitor mechanism against HIV-1 infection: A bioinformatics approach. *Journal of Pharmacy & Pharmacognosy Research, 9*(4).

Kitao, S., Matsudo, T., Saitoh, M., Horiuchi, T., & Sekine, H. (1995). Enzymatic syntheses of two stable (–)-epigallocatechin gallate-glucosides by sucrose phosphorylase. *Bioscience, Biotechnology, and Biochemistry, 59*(11), 2167–2169. doi:10.1271/bbb.59.2167.

Krupkova, O., Ferguson, S. J., & Wuertz-Kozak, K. (2016). Stability of (−)-epigallocatechin gallate and its activity in liquid formulations and delivery systems. *The Journal of Nutritional Biochemistry, 37*, 1–12. doi:10.1016/j.jnutbio.2016.01.002.

Lam, W. H., Kazi, A., Kuhn, D. J., Chow, L. M. C., Chan, A. S. C., Ping Dou, Q., et al., (2004). A potential prodrug for a green tea polyphenol proteasome inhibitor: Evaluation of the peracetate ester of (−)-epigallocatechin gallate [(−)-EGCG]. *Bioorganic & Medicinal Chemistry, 12*(21), 5587–5593. doi:10.1016/j.bmc.2004.08.002.

Lambert, J. D., Hong, J., Kim, D. H., Mishin, V. M., & Yang, C. S. (2004). Piperine enhances the bioavailability of the tea polyphenol (−)-epigallocatechin-3-gallate in mice. *The Journal of Nutrition, 134*(8), 1948–1952. doi:10.1093/jn/134.8.1948.

Lambert, J. D., Kim, D. H., Zheng, R., & Yang, C. S. (2006a). Transdermal delivery of (-)-epigallocatechin-3-gallate, a green tea polyphenol, in mice. *Journal of Pharmacy and Pharmacology, 58*(5), 599–604. doi:10.1211/jpp.58.5.0004.

Lambert, J. D., Sang, S., Hong, J., Kwon, S. J., Lee, M. J., Ho, C. T., et al., (2006b). Peracetylation as a means of enhancing *in vitro* bioactivity and bioavailability of epigallocatechin-3-gallate. *Drug Metabolism and Disposition, 34*(12), 2111–2116. doi:10.1124/dmd.106.011460.

Lambert, J. D., Sang, S., & Yang, C. S. (2007). Biotransformation of green tea polyphenols and the biological activities of those metabolites. *Molecular Pharmaceutics, 4*(6), 819–825. doi:10.1021/mp700075m.

Lee, M.J., Maliakal, P., Chen, L., Meng, X., Bondoc, F., Prabhu, S. et al. (2002). Pharmacokinetics of tea catechins after ingestion of green tea and (ℵ)-epigallocatechin-3-gallate by humans: Formation of different metabolites and individual variability. Cancer Epidemiology, Biomarkers & Prevention,11 (10),1025-32. doi:aacrjournals.org/cebp/article/11/10/1025/165926.

Li, T., Zhao, N., Lu, J., Zhu, Q., Liu, X., Hao, F., et al., (2019). Epigallocatechin gallate (EGCG) suppresses epithelial-mesenchymal transition (EMT) and invasion in anaplastic thyroid carcinoma cells through blocking of TGF-β1/Smad signaling pathways. *Bioengineered, 10*(1), 282–291. doi:10.1080/21655979.2019.1632669.

Liu, B., Kang, Z., & Yan, W. (2021). Synthesis, Stability, and Antidiabetic Activity Evaluation of (−)-Epigallocatechin Gallate (EGCG) Palmitate Derived from Natural Tea Polyphenols . *Molecules, 26*(2), 393. doi:10.3390/molecules26020393.

Lorenz, M., Jochmann, N., von Krosigk, A., Martus, P., Baumann, G., Stangl, K., et al., (2007). Addition of milk prevents vascular protective effects of tea. *European Heart Journal, 28*(2), 219–223. doi:10.1093/eurheartj/ehl442.

Lu, H., Meng, X., Li, C., Sang, S., Patten, C., Sheng, S., et al., (2003). Glucuronides of tea catechins: Enzymology of biosynthesis and biological activities. *Drug Metabolism and Disposition, 31*(4), 452–461. doi:10.1124/dmd.31.4.452.

Maeda-Yamamoto, M., Ema, K., & Shibuichi, I. (2007). *In vitro* and *in vivo* anti-allergic effects of “benifuuki” green tea containing O-methylated catechin and ginger extract enhancement. *Cytotechnology, 55*(2–3), 135–142. doi:10.1007/s10616-007-9112-1.

Misaka, S., Kawabe, K., Onoue, S., Werba, J. P., Giroli, M., Tamaki, S., et al., (2013). Effects of green tea catechins on cytochrome P450 2B6, 2C8, 2C19, 2D6 and 3A activities in human liver and intestinal microsomes. *Drug Metabolism and Pharmacokinetics, 28*(3), 244–249. doi:10.2133/dmpk.dmpk-12-rg-101.

Miyoshi, N., Pervin, M., Suzuki, T., Unno, K., Isemura, M., & Nakamura, Y. (2015). Green tea catechins for well-being and therapy: Prospects and opportunities. *Botanics: Targets and Therapy, 85*. doi:10.2147/btat.s91784.

Mukai, K., Mitani, S., Ohara, K., & Nagaoka, S. (2005). Structure activity relationship of the tocopherol-regeneration reaction by catechins. *Free Radical Biology and Medicine, 38*(9), 1243–1256. doi:10.1016/j.freeradbiomed.2005.01.011.

Narmada, I. B., Sarasati, A., Wicaksono, S., Rezkita, F., Wibawa, K. G. P., Hayaza, S., et al., (2020). Phytochemical screening, antioxidant activity, functional groups and chemical element characterization analysis of (-)-epigallocatechin-3-gallate (EGCG) in east javanese green tea methanolic extract: An experimental *in vitro* study. *Systematic Reviews in Pharmacy, 11*(5), 511–519.

Ohishi, T., Goto, S., Monira, P., Isemura, M., & Nakamura, Y. (2016). Anti-inflammatory action of green tea. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry, 15*(2), 74–90. doi:10.2174/1871523015666160915154443.

Oritani, Y., Setoguchi, Y., Ito, R., Maruki-Uchida, H., Ichiyanagi, T., & Ito, T. (2013). Comparison of (−)-epigallocatechin-3-O-gallate (EGCG) and O-methyl EGCG bioavailability in rats. *Biological and Pharmaceutical Bulletin, 36*(10), 1577–1582. doi:10.1248/bpb.b13-00349.

Peters, C. M., Green, R. J., Janle, E. M., & Ferruzzi, M. G. (2010). Formulation with ascorbic acid and sucrose modulates catechin bioavailability from green tea. *Food Research International, 43*(1), 95–102. doi:10.1016/j.foodres.2009.08.016.

Piechocka, J., Gramza-Michałowska, A., & Szymandera-Buszka, K. (2021). The changes in antioxidant activity of selected flavonoids and caffeine depending on the dosage and form of thiamine. *Molecules, 26*(15), 4702 (Basel, Switzerland). doi:10.3390/molecules26154702.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of Nutrition, 130*(8) 2073S2085S. doi:10.1093/jn/130.8.2073s.

Severino, J. F., Goodman, B. A., Kay, C. W. M., Stolze, K., Tunega, D., Reichenauer, T. G., et al., (2009). Free radicals generated during oxidation of green tea polyphenols: Electron paramagnetic resonance spectroscopy combined with density functional theory calculations. *Free Radical Biology and Medicine, 46*(8), 1076–1088. doi:10.1016/j.freeradbiomed.2009.01.004.

Shim, S. M., Yoo, S. H., Ra, C. S., Kim, Y. K., Chung, J. O., & Lee, S. . J. (2012). Digestive stability and absorption of green tea polyphenols: Influence of acid and xylitol addition. *Food Research International, 45*(1), 204–210. doi:10.1016/j.foodres.2011.10.016.

Sorice, A., Guerriero, E., Capone, F., Colonna, G., Castello, G., & Costantini, S. (2014). Ascorbic acid: Its role in immune system and chronic inflammation diseases. *Mini-Reviews in Medicinal Chemistry, 14*(5), 444–452. doi:10.2174/1389557514666140428112602.

Takeuchi, H., Yamamoto, H., & Kawashima, Y. (2001). Mucoadhesive nanoparticulate systems for peptide drug delivery. *Advanced Drug Delivery Reviews, 47*(1), 39–54. doi:10.1016/s0169-409x(00)00120-4.

van't Slot, G., & Humpf, H. U. (2009). Degradation and metabolism of catechin, epigallocatechin-3-gallate (EGCG), and related compounds by the intestinal microbiota in the pig cecum model. *Journal of Agricultural and Food Chemistry, 57*(17), 8041–8048. doi:10.1021/jf900458e.

Wang, R., Zhou, W., & Jiang, X. (2008). Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range. *Journal of Agricultural and Food Chemistry, 56*(8), 2694–2701. doi:10.1021/jf0730338.

Wei, Y., Chen, P., Ling, T., Wang, Y., Dong, R., Zhang, C., et al., (2016). Certain (−)-epigallocatechin-3-gallate (EGCG) auto-oxidation products (EAOPs) retain the cytotoxic activities of EGCG. *Food Chemistry, 204*, 218–226. doi:10.1016/j.foodchem.2016.02.134.

Xie, Y., Kosińska, A., Xu, H., & Andlauer, W. (2013). Milk enhances intestinal absorption of green tea catechins in *in vitro* digestion/caco-2 cells model. *Food Research International, 53*(2), 793–800. doi:10.1016/j.foodres.2012.07.063.

Xu, Y. Q., Yu, P., & Zhou, W. (2019). Combined effect of pH and temperature on the stability and antioxidant capacity of epigallocatechin gallate (EGCG) in aqueous system. *Journal of Food Engineering, 250*, 46–54. doi:10.1016/j.jfoodeng.2019.01.016.

Yang, W., Zhang, Q., Yang, Y., Xu, J., Fan, A., Yang, C. S., et al., (2016). Epigallocatechin-3-gallate decreases the transport and metabolism of simvastatin in rats. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems, 47*(1), 86–92. doi:10.3109/00498254.2016.1159747.

Yuan, H., Chen, C. . Y., Chai, G., Du, Y. Z., & Hu, F. Q. (2013). Improved transport and absorption through gastrointestinal tract by PEGylated solid lipid nanoparticles. *Molecular Pharmaceutics, 10*(5), 1865–1873. doi:10.1021/mp300649z.

Zhang, G., & Zhang, J. (2018). Enhanced oral bioavailability of EGCG using pH-sensitive polymeric nanoparticles: Characterization and *in vivo* investigation on nephrotic syndrome rats. *Drug Design, Development and Therapy, 12*, 2509–2518 Volume. doi:10.2147/dddt.s172919.

Zhu, Q. Y., Zhang, A., Tsang, D., Huang, Y., & Chen, Z. Y. (1997). Stability of green tea catechins. *Journal of Agricultural and Food Chemistry, 45*(12), 4624–4628. doi:10.1021/jf9706080.

Zou, L., Peng, S., Liu, W., Gan, L., Liu, W., Liang, R., et al., (2014). Improved *in vitro* digestion stability of (−)-epigallocatechin gallate through nanoliposome encapsulation. *Food Research International, 64*, 492–499. doi:10.1016/j.foodres.2014.07.042.