Food and Beverage Consumption and Health

Green Tea and Health

Antioxidant Properties, Consumption and Role in Disease Prevention

Nicolas Powell
Editor

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ANTIOXIDANT PROPERTIES, CONSUMPTION AND ROLE IN DISEASE PREVENTION

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PREFACE

Green tea is a popular healthful beverage in many parts of the world and its medical properties have been extensively explored. Green tea originated in China and the chemistry of green tea is renowned by its presence of polyphenolic compounds, particularly catechins. This book reviews the antioxidant activities of green tea catechins, its consumption and the ways it prevents potential infections; the potential beneficial effects of green tea consumption on Diabetes mellitus and how it can be used to reduce severe brain damage as well as its use in treating obesity. Other chapters examine how catechins may assist in the suppression of heart transplant rejection; a review on the new molecular events responsible for positive effects of EGCG; a discussion on EGCG as the most abundant catechin green tea and its capability of inhibiting cell proliferation and inducing apoptosis in cancer cells; an evaluation and comparison of the phenolic composition and metal content of commercially available aromatized green tea infusions; and an investigation of the chromatographic behavior of catechins.

Chapter 1 – Green tea, originated from the leaves of *Camellia sinensis* plant, is manufactured by drying of fresh tea leaves. The most extensively renowned attributes of green tea are their antioxidant activities. The chemistry of green tea is prominent by its notable quantity of polyphenolic compounds, particularly catechins. Among these, the predominant active components are epigallocatechin gallate, epicatechin gallate, epicatechin and epigallocatechin. The antioxidant activity of green tea catechins is directly attributed to the aromatic rings and hydroxyl groups that make up their chemical structure, and is a result of binding and neutralization of free radicals by these hydroxyl groups. Green tea catechins can also bind and sequester transition metal ions, making them unavailable for the oxidation reaction. The chemical stability of green tea catechins is primarily dependent on the pH, temperature, oxygen content and the presence of metal ions. This review outlines the chemistry, antioxidant mechanism and stability of green tea catechins in food.

Chapter 2 – Infections by microorganisms are a leading cause of morbidity and mortality worldwide. Since the advent of antimicrobial use, the ability to control these infections has vastly improved. Unfortunately, the healthcare costs for these infections is still in the billions of dollars. The ideal situation would be for people to not succumb to infections, but be able to prevent them. Green tea has the ability to eradicate many infection causing microorganisms. Many of the mechanisms of green tea that make recovery from infection possible are also potential infection prevention mechanisms. The main constituents of green tea that are antimicrobial are the catechins (polyphenols). The four main catechins are: (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-

gallate (EGCG). Consumption of green tea provides availability of these compounds throughout the body, and also the possibility for prevention of infection.

Chapter 3 – Diets have attracted great interest on the account of growing evidence of their beneficial effects on human health. Green tea has been used for a very long time as a folk remedy for a wide array of diseases. The well-known green tea beverage is made from a plant *Camellia sinensis*. The healthy properties of green tea are linked closely to its content of phenolic compounds, particularly to the (-)-epigallocatechin-3-gallate. It has been proposed that green tea may have a beneficial impact on a number of brain functions, as well as on neurodegenerative disorder prevention in humans and in various animal models, including Alzheimer's disease (AD). A large number of scientific studies have supported some of these assumptions. In the case of AD, aluminium may have an important role in the disease aetiology/pathogenesis/precipitation. However, aluminium has biological effects in the green tea plant, where it is a cofactor for polyphenol biosynthesis. Consequently, leaves of green tea accumulate and store large quantities of this element during the plant growth. Thus, it was intriguing whether the unilateral intrahippocampal application of green tea leaf extract (GTLE) and aluminium chloride would have any interaction, measured by the biochemical parameters in six brain structures: the forebrain cortex, striatum, basal forebrain, hippocampus, brain stem and cerebellum, of the adult male Wistar rats. It was found that GTLE given alone demonstrated biochemical effects not only in the ipsilateral hippocampus, but also spread into the five other examined structures at the same side, as well as into the identical brain structures on the contralateral hemisphere. In fact, there were no differences in the activity of superoxide dismutase, cytochrome *c* oxidase (COX) and acetylcholinesterase (AChE) between the right and the corresponding left brain structures. Moreover, the activity of COX and AChE were significantly higher when compared to the control group. Out of the three observed parameters, the content of reduced glutathione (GSH), superoxide anion and nitrites, aluminium itself demonstrated the strongest effects towards GSH, which was significantly reduced in all structures, compared to the control group. The changes were identical in the ipsi- and contralateral corresponding structures. Howewer, the application of GTLE just before aluminium prevented the reduction of GSH induced by aluminium, and significantly increased its content compared to the control group. Also, the content of superoxide anion was significantly reduced in most structures compared to the control, and to the aluminium-treated group as well. The obtained results of GTE in the aluminium-induced neurotoxicity are in accordance with the antioxidant effects of GTLE. Also, it is clear that GTE administered alone did not demonstrate neurotoxic effects as did the solution of aluminium chloride, but, on the contrary, showed the opposite, neuroprotective effects. To sum up, GTLE has proved to manifest strong antioxidant effects in the brain of healthy rats, and in the cases of neurotoxicity induced by aluminum, as well.

Chapter 4 – Green tea catechins are key components with many biological functions. These effects are induced by the suppression of several inflammatory factors through nuclear factor-kappa B (NF-κB). While these characteristics of tea catechins have been well documented, actions of catechins on cardiac transplantation have not yet been well investigated. To test the hypothesis that catechins can attenuate ventricular remodeling and cardiac allograft vasculopathy (CAV) in cardiac transplantation, we performed oral administration of catechins into murine cardiac recipients. We revealed that catechins suppressed myocardial remodeling and CAV formation. They altered cytokine expression,

inhibited adhesion molecules and regulated NF-κB activation. Thus, catechins are potent agents for the suppression of heart transplant rejection.

Chapter 5 – The medicinal properties of tea (*Camellia sinensis* L.) have a long and interesting history, dating back to many centuries ago. Green tea has aroused considerable interest in recent years, being nowadays one of the most studied types of teas. Green tea is a complex mixture of thousands of chemical compounds, including proteins and free amino acids, polysaccharides, vitamins, organic acids, methylxanthines, and polyphenols. Catechins, caffeine and L-theanine are often reported as the main phytochemicals responsible for green tea's health benefits, namely by its antioxidant, hypoglycemic, and neuroprotective properties. Diabetes mellitus (DM) is the most common metabolic disease and its incidence is dramatically rising. In addition, DM is associated to a high risk of developing neurodegenerative diseases, since the brain is particularly susceptible to glucose fluctuations and hyperglycaemia-induced oxidative stress. Throughout this chapter we will discuss the phytochemical composition and bioactivities of green tea, especially antioxidant, antidiabetic, and neuroprotective activities. The potential beneficial effects of green tea consumption on DM and how it can be used to reduce the severe brain damage induced by this disease will be emphasized.

Chapter 6 – Accumulating evidence supports that green tea consumption is associated with reduced risk of several human malignancies. This positive effect of green tea have been attributed to polyphenol ingredients and among these epigallocatechin-3-gallate (EGCG) is recognized as a key active constituent. Some studies on EGCG are suggesting that a large set of protein targets may directly interact with EGCG and alter the physiology of diseased cells. This chapter reviews the new molecular events responsible for positive effects of EGCG.

Chapter 7 – Accounting for 50-80% of the total catechin content, EGCG is the most abundant catechin in green tea and the most potent catechins capable of inhibiting cell proliferation and inducing apoptosis in cancer cells. One important mechanism frequently overlooked in considering the biological effects of EGCG and its derivatives is their potential interaction with tyrosine kinase onco-proteins that are capable of initiating cell signalling. Here, we review and discuss the novel molecular mechanisms of EGCG on regulation onco-proteins that are clients of heat shock protein, Hsp90.

Chapter 8 – Obesity has been increasing at an alarming rate in the last several decades, both in developed and in developing countries, reaching epidemic proportions among young people and adults as well. Unfortunately, nowadays obesity has become a global health problem. It raises the risk of morbidity from a great number of diseases like: diabetes mellitus type 2, dyslipidemia, arterial hypertension, coronary heart disease, stroke, cancer and respiratory problems including sleep apnea, etc. Both the direct and indirect costs of obesity and obesity-related morbidity have strong economic impact on the whole society. Therefore, the prevention and treatment of obesity remain and should be a priority worldwide. Besides other possible ways of treatment, phytotherapy has an important role in both scientific research and traditional medicine as well. Green tea beverage made from the dried, non-fermented leaves of the plant *Camellia sinensis*, has been consumed by humans for thousands of years. It has attained high reputation as a health promoting herb. The increasing interest in the effects of green tea is directed towards its ingredients: catechins, caffeine and theanine, all of which possess various biologically and pharmacologically effects. Some of these compounds are highly attractive in drug discovery programs. It is traditionally thought that green tea consumption decreases the risks for obesity, reduces body weight and helps in

treating overweight patients. Consequently, health abnormalities related to obesity may be alleviated by green tea consumption. A number of extensive experimental research and epidemiological studies supported the anti-obesity effects of green tea and its various forms, and proposed very complicated mechanisms concerning its potential influence on the body weight and composition. At least, the modulation of lipid and carbohydrate metabolism, body energy balance and food intake could be obtain by consuming green tea. Its antioxidant effects in treating obesity are also exploited. Green tea and its commercial forms (which generally contain ingredients like catechins and caffeine in a higher concentration than the typical green tea beverage) have proved to be highly successful in controlled experiments, but they did not demonstrate identical and unambiguous effects in randomised clinical trials (RCTs). That is the reason why its safety and efficacy could not be properly judged and claimed to be a complementary and alternative medicine used to aid weight loss and weight maintenance. To overcome the problem of the insufficient number of RCTs, a lot of systematic reviews and meta-analyses have been conducted. However, in spite of some benefits shown in decreasing body weight and weight maintenance, the obtained improvement, in general, did not reach statistical significance. That could be the result of great heterogeneity of these trials. Thus, well-characterised, randomised controlled clinical trials are needed in order to assess the promising effects of different forms of green tea on health promotion in overweight and obese humans. In order to avoid possible misleading and aggressive commercial practices conducted by the advertisers, such reliable information is extraordinary important for health-care workers, and for green tea consumers as well.

Chapter 9 – The aromatized green teas are popular due to their aroma and organoleptic properties. In this study the content of flavonoids in the infusions of five commercially available teas was determined by HPLC-MS in the negative electrospray ionization mode. All types of tea contained epigallocatechin-3-gallate in the greatest amounts, followed by epicatechin and catechin. Some teas with citrus aromas or fruits contain also the glycosides of other flavonoids such as naringin and hesperidin. The mineral contents of dry teas and the infusions produced from them were also determined by inductively coupled optical emission spectrometry. Aluminium and manganese were major constituents in dry teas, followed by iron, zinc, copper and nickel. The release of metals into tea infusions depends on whether they are strongly bond to the organic matrix or more soluble in the solution. Iron exhibited the lowest efficiency of extraction by hot water from all studied teas. Copper and nickel fractions transferred into tea brew exceeded 50% in some cases.

Chapter 10 – The goal of this study was to investigate chromatographic behavior of catechins in hydrophilic interaction liquid chromatography (HILIC). Two different HILIC columns were used: cross-linked DIOL (Luna HILIC) and zwitterionic sulfoalkylbetaine (SeQuant ZIC-HILIC). Separation parameters such as content of acetonitrile (ACN) and pH of the aqueous fraction of an eluent were studied. On the ZIC column, the retention factor of catechins increased with decreasing water content in the mobile phase and the increase in pH of the aqueous component mainly affects the polarity of the analytes. DIOL stationary phase showed more or less apparent dual retention mechanism; HILIC at ACN content $\geq$ 75% (v/v) and reversed-phase (RP) with lower content of organic modifier. Retention times for catechins are longer for zwitterionic stationary phase. In the presence of ammonium acetate in the mobile phase, retention of catechins increases slightly for both columns without change in the selectivity of separation.

Significant higher sensitivity was observed under HILIC conditions due to much higher content of ACN in the mobile phase than in RP mode. The elution order increases in the order:: catechin < epicatechin < epigallocatechin gallate.

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Chapter 1

ANTIOXIDANT ACTIVITY OF GREEN TEA CATECHINS

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ABSTRACT

Green tea, originated from the leaves of *Camellia sinensis* plant, is manufactured by drying of fresh tea leaves. The most extensively renowned attributes of green tea are their antioxidant activities. The chemistry of green tea is prominent by its notable quantity of polyphenolic compounds, particularly catechins. Among these, the predominant active components are epigallocatechin gallate, epicatechin gallate, epicatechin and epigallocatechin. The antioxidant activity of green tea catechins is directly attributed to the aromatic rings and hydroxyl groups that make up their chemical structure, and is a result of binding and neutralization of free radicals by these hydroxyl groups. Green tea catechins can also bind and sequester transition metal ions, making them unavailable for the oxidation reaction. The chemical stability of green tea catechins is primarily dependent on the pH, temperature, oxygen content and the presence of metal ions. This review outlines the chemistry, antioxidant mechanism and stability of green tea catechins in food.

Keywords: Green tea, antioxidant activity, catechins, lipid oxidation, stability

1. INTRODUCTION

Green tea is a popular healthful beverage in many parts of the world and its medicinal properties have been extensively explored. Green tea originated in China and its use for

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medicinal purposes was first documented in the 3rd Century B.C. Green tea plant, derived from *Camellia sinensis* L, is a member of the *Theaceae* family. It is an evergreen shrub that grows primarily in tropical and subtropical climates and requires at least fifty inches of rainfall annually with preference for well drained soils having an acidic pH of 4.5 to 5.5. Green tea is mostly cultivated in China, India, Sri Lanka, Japan, Indonesia, and several African and South-American countries (Senanayake, 2013a). Among these, China, India, Kenya and Sri Lanka are the world leaders in tea production. Green tea is a diminutive plant that can rise to heights of 30 feet if left undisturbed, but is typically pruned to about waist height to encourage its lateral growth. Only the top 1-2 inches of the mature plant are harvested. The leaves are indeed dark green and lustrous with ragged edges, and are 2–5 cm wide and 4–15 cm long (Senanayake, 2013b). The tea flowers are white and contain bright yellow stamens. Typically, flowering is prevented during cultivation by harvesting the leaves. The immature, light-green leaves are preferably harvested for tea production. Mature leaves are deeper green in color. Different leaf ages deliver varying tea qualities as their chemical compositions are different.

Tea can be broadly categorized into three main types, based on the level of fermentation that takes place during processing: green tea being unfermented; oolong tea being semi-fermented, and black tea fully fermented (Senanayake, 2013a). Green, black and oolong teas are all originated from the leaves of *Camellia sinensis* plant. Besides the distinction between varieties of tea, the major difference between these types of teas is the method of processing. Green tea is typically produced by harvesting of the first two to three leaves and the bud, which have the unique flavor and aroma, from each plant. The harvesting of fresh tea leaves is generally repeated every one to two weeks, depending on where it grows. Once harvested, the fresh leaves are dried immediately to prevent the fermentation process, which inhibits the polyphenol oxidase activity that causes oxidation of green tea polyphenolic compounds. The inactivation of polyphenol oxidase in fresh tea leaves is generally accomplished by either firing or by steaming **(**Velayutham et al., 2008). The tea leaves are then shaped (rolled), dried, packaged, and labelled for distribution and marketing. The final quality of green tea depends on the quality of fresh tea leaves that are harvested. The natural attributes of the fresh tea leaves, including color and aroma, should be preserved during manufacturing in order to produce high quality green tea.

2. CHEMISTRY

The chemistry of green tea is renowned by its presence of polyphenolic compounds, particularly catechins. Green tea catechins belong to the biochemical family of dietary flavonoids. The basic chemical structure of flavonoid molecule consists of a diphenylpropane structure with two benzene rings linked by a three carbon chain that forms a closed heterocyclic ring containing oxygen. Hence, flavonoids molecules possess C6-C3-C6 general structural backbone. The two benzene rings present in flavonoid molecules are referred to as the A- and B-rings. Moreover, the dihydropyran heterocycle or the C-ring in the flavonoid structure may contain a hydroxyl group on the third carbon atom (Figure 1). The A-ring of the flavonoid molecule is similar to a resorcinol moiety whereas the B ring resembles a catechol moiety (Senanayake, 2013a,b). Of the biochemical family of flavonoids, catechins belong to

the group of flavan-3-ols. The parent catechin compound typically exists in two isomers; the trans-isomer and the cis-isomer. There are two chiral centers located on the molecule on carbons 2 and 3 of the dihydropyran heterocycle. Hence, most abundant catechins in green tea exist as four stereoisomers with two of the isomers are in *trans*-configuration and the other two are in *cis*-configuration. The *trans*- and *cis*-isomers are designated as the catechin and epicatechin, respectively. Among these, the most common catechin isomers is the (+)-catechin whereas the other stereoisomer is referred to as the (-)-catechin. However, the most common epicatechin isomers is the (-)-epicatechin. Moreover, the C ring of catechin molecule can be conformationally vulnerable and may adopt two types of conformations; E-conformer is the one with B-ring in the pseudo equatorial position, whereas A-conformer is the one with B-ring in the pseudo axial position. The functional groups such as an *ortho*-3'4'-dihydroxyl group or 3'4'5'-trihydroxyl group in the B-ring, a gallate group esterified at the third position of the C ring, and hydroxyl groups at 5 and 7 positions of the A-ring are appeared to be imperative for the antioxidant activities of green tea polyphenols (Rice-Evans et al., 1996; Senanayake, 2013a).

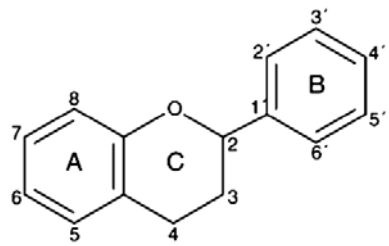

Figure 1. Chemical structure of flavonoid backbone.

The empirical formulas and molecular weights of primary components of green tea catechins are reported in Table 1. Many structure-activity relationship investigations have been previously performed on the antioxidant activity of these flavonoids, including green tea catechins (Tournaire et al., 1993; Rice-Evans et al., 1996; Guo et al., 1999; Harborne & Williams, 2000; Heim et al., 2002; Farkas et al., 2004). According to these studies, the antioxidant activity of flavonoids depends strongly on the number and position of hydroxyl groups in the molecule.

Dihydroxylated B-ring (catechol structure), the presence of unsaturation and of 4-oxo function in the C-ring are also presumed to be intensify the antioxidant activity of these flavonoids. Studies by Tournaire et al., (1993) concluded that the presence of a catechol moiety in the B-ring was the main factor contributing to the effectiveness of physical quenching of these flavonoids with the singlet oxygen. Moreover, the C-ring (in particular, the presence of a hydroxyl group activating the double bond) was the main contributing factor determining the efficiency of their chemical reactivity with singlet oxygen molecules (Tournaire et al., 1993).

Fresh green tea leaves are rich in flavan-3-ols and their gallic acid derivatives, namely, (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin, and (-)-epigallocatechin gallate.

Fresh tea leaves also contain a variety of flavor compounds including terpenes, oxygenated terpenes, sesquiterpenes, and some organic acids. Among these, catechins are exemplified by the presence of multiple hydroxyl groups on the A- and B-rings of their chemical structures. Epicatechin has an ortho-dihydroxyl group in the B-ring at carbons 30 and 40 and a hydroxyl group at carbon 3 of the C-ring (Figure 2). Epigallocatechin differs from epicatechin in that it has a trihydroxyl group at carbons 30, 40, and 50 of the B-ring (Senanayake, 2013).

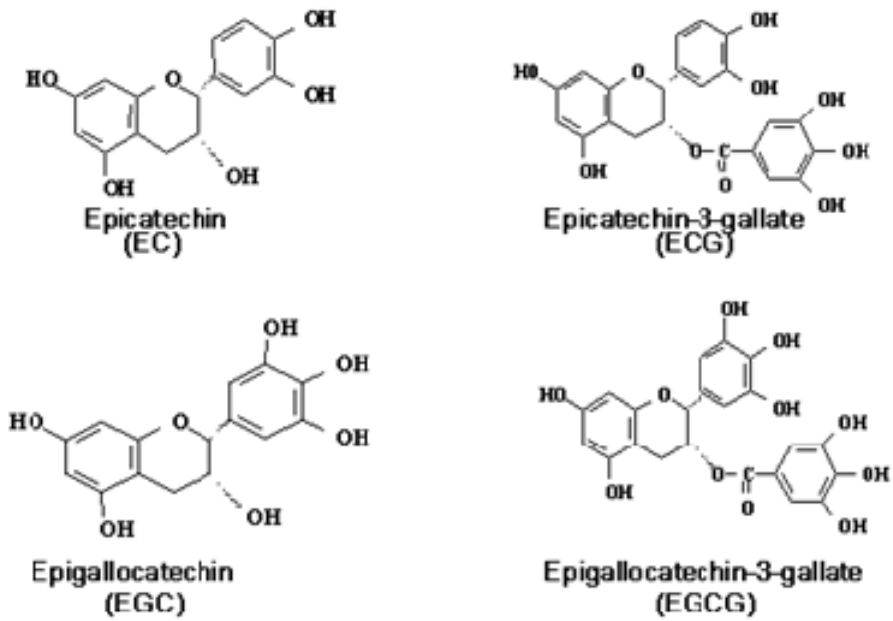

Figure 2. Chemical structures of major green tea catechins.

Epicatechin gallate differs from epicatechin in its gallate moiety esterified at carbon 3 of the C-ring. However, epigallocatechin gallate has both trihydroxyl groups at carbons 30, 40, and 50 on the B-ring and a gallate moiety esterified at carbon 3 of the C-ring (Senanayake, 2013a). A study conducted by Yilmaz (2006) documented the chemistry and application aspects of green tea, especially in relation to using their individual catechin components. The relative amount of green tea catechins depends on how the leaves are processed prior to drying.

The composition of green tea leaves is dependent on various factors such as the geographical location, climate, season, horticultural practices, variety and the age of tea leaves. Fresh tea leaves contain polyphenols, carbohydrates, lipids, proteins, amino acids, lignin, caffeine, chlorophyll, carotenoids, organic acids, minerals, and volatile flavor and aroma compounds (Table 2).

The United States Department of Agriculture has recently published a database for the flavonoid content of selected foods including green tea (USDA, 2013). The main flavonoids present in green tea include catechins or flavan-3-ols.

Table 1. Empirical formula and molecular weight of major green tea catechins

Compound	IUPAC name	Empirical formula	Molecular weight (g/mol)
Catechin	(2R,3S)-2-(3,4-dihydroxyphenyl)3,4-dihydro-2H-chromene-3,5,7-triol	$C_{15}H_{14}O_6$	290.27
Epicatechin	(2R,3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol	$C_{15}H_{14}O_6$	290.27
Epigallocatechin	(2R,3R)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol	$C_{15}H_{14}O_7$	306.27
Epicatechin gallate	((2R,3R)-2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-chromen-3-yl) 3,4,5-trihydroxybenzoate	$C_{22}H_{18}O_{10}$	442.37
Epigallocatechin gallate	(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate	$C_{22}H_{18}O_{11}$	458.37

The total catechins in fresh tea leaves comprised of approximately 30% of the dry weight of the leaves (Abdel-Rahman et al., 2011). The flavonols found in green tea are mainly quercetin, kaempferol, myricetin and their glycosides (Balentine et al., 1997). Caffeine is present at a moderate level (2.5-4.0%) along with very small quantities of the other common methyl xanthines such as theobromine and theophylline. Tea leaves also contain trace amounts of carotenoids. Among these, beta-carotene, lutein, neoxanthin and violaxanthine have been identified. Volatile compounds in green tea include alcohols, esters, carbonyls, acids and cyclic compounds. Green tea also contains many free amino acids. Theanine encompasses approximately 46% of the amino acids found in green tea. The other amino acids found in tea leaves include glutamine, asparagine, alanine, arginine and serine (Senanayake, 2013a).

Green tea contains significantly higher amounts of tea polyphenols as compared to black or oolong teas which is attributed to differences in the types of processing of these tea leaves following harvest. For green tea, freshly harvested tea leaves are steamed at high temperatures and dried to inactivate the enzymes and, as a result, prevents the oxidation of catechins. Black tea, on the other hand, is produced by extended fermentation of tea leaves which results in the development of polymeric compounds including thearubigins and theaflavins (Zaveri, 2006). Black tea contains predominantly gallates of epicatechin. Oolong tea, a partially fermented product, contains a mixture of the monomeric polyphenols and higher molecular weight theaflavins (Graham, 1992). All three varieties of tea contain significant quantities of caffeine (3–6%) which is unaffected by the different processing conditions (Chu, 1997). Furthermore, all three varieties of tea contain (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate and (-)-epigallocatechin gallate, but not catechin (Khokhar et al., 1997). Epicatechin gallate and epigallocatechin gallate are considered to be the main catechins found in black tea (Obanda et al., 2001). Epigallocatechin gallate is the most abundant catechin in the leaves of green, oolong, and black teas (Graham, 1992). The contents of epigallocatechin gallate in green and oolong teas range typically from 127 to 550 mg/L, while black teas may contain up to 300 mg/L (Balentine & Paetau-Robinson, 2000).

Table 2. Chemical constituents of green tea

Compound	Description	Reference
Flavan-3-ols	Catechin isomers, Theaflavin, Theaflavin gallate, Thearubigin	USDA, 2013; Reto et al., 2007
Flavones	Apigenin, Luteolin	
Flavonols	Kaempferol, Myricetin, Quercetin, Rutine	Peterson et al., 2005; USDA, 2013; Senanayake, 2013a
Xanthins and related compounds	Caffeine, theobromine, theophylline	Graham, 1992
Proteins and Amino Acids	Theanine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine, peptides	Senanayake, 2013a
Carbohydrates, Pectin and Fiber	Cellulose, pectin, sucrose, glucose, fructose	Cabrera et al., 2006; Adak and Gabar, 2011
Phenolic Acids	Caffeic acid, Gallic acid, Chlorogenic acid	Jeszka-Skowron and Zgoła-Grześkowiak, 2014
Vitamins	A, B_1, B_2, B_6, E, Niacin, Folic Acid, Ascorbic Acid	Adak and Gabar, 2011
Minerals	Ca, Mg, Zn, Na, K, P, F, Mn, Cr, Se, Mo, Ni, Al, Cu, Fe	Reto et al., 2007; Cabrera et al., 2006; Li et al, 2007
Pigments	Chlorophylls, Carotenoids	Graham, 1992; Cabrera et al., 2006
Lipids	Linoleic and alpha-linolenic acid	Cabrera et al., 2006
Sterols	Stigmasterol	Cabrera et al., 2006
Volatile Flavor and Aroma Compounds	Aldehydes, alcohols, ketones, esters and lactones, acids, hydrocarbons	Chaturvedula and Prakash, 2011

3. LIPID OXIDATION

Lipids, being essential structural and functional constituents of foods, have a major impact on the sensory and nutritional quality of many foods. The control of lipid oxidation and development of rancidity remain an on-going challenge in most food products. Lipid oxidation in foods is primarily mediated by the presence of reactive oxygen species (ROS). Reactive oxygen species can be categorized into oxygen-centered radicals and oxygen-centered non-radicals. Oxygen-centered radicals can be superoxide anion ($\cdot O2^-$), hydroxyl radical ($\cdot OH$), alkoxyl radical ($RO\cdot$), and peroxyl radical ($ROO\cdot$), whereas oxygen-centered non-radicals can be hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Among these, hydroxyl radical ($\cdot OH$) is the strongest oxidant and most reactive followed by singlet oxygen ($^1O_2$). Moreover, alkoxyl radical ($RO\cdot$) and peroxyl radical ($ROO\cdot$) are considered as moderately strong oxidants. The reactivity of these free radicals is derived from the presence of one or more unpaired electrons in their atomic or molecular orbitals. These oxygen radicals are typically unstable, highly reactive and energized molecules.

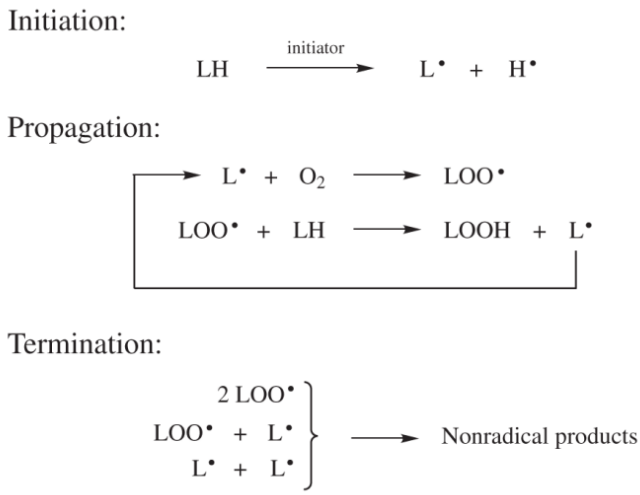

Figure 3. Classical free radical chain reaction mechanism of lipid oxidation.

Autoxidation, a quite complex process leading to oxidation of lipid-containing foods, is a radical-induced chain reaction which may be divided into the classical steps of initiation, propagation, and termination (Senanayake, 2013a). These processes typically comprised of a convoluted series of sequential and overlapping reactions (Frankel, 2005). A simplified scheme explaining the mechanism of autoxidation is shown in Figure 3. During the initiation step, lipid free radicals are generated from lipids due to spontaneous abstraction of a hydrogen atom from these lipid molecules. The generation of primary free radicals is supported by the presence of oxidation initiators such as light, heat, ionizing radiation, transition metals, metalloproteins, oxidants, various homolysis-prone substances and enzymes (Senanayake, 2013a). After initiation, propagation reactions occur in which one lipid radical is converted into a variety of lipid free radicals. Lipid hydroperoxides are recognized as the initial major products of autoxidation. Lipid hydroperoxides are typically tasteless and odorless. It should be noted that hydroperoxides are very unstable and readily decompose into secondary oxidation products. The decomposition of hydroperoxides yields aldehydes, ketones, alcohols, hydrocarbons and acids, which are known as secondary oxidation products. In many cases, these compounds are particularly important as significant contributors to flavor and aroma of oxidized oils. In the termination phase, two radicals can be combined to form non-free radical products and obstruct the cascade mode of chain reaction (Figure 3). In addition, this chain reaction is also terminated by some antioxidants or free radical scavengers.

4. FOOD ANTIOXIDANTS

Antioxidants, including green tea extracts, are of interest to the food industry because they can delay development of oxidative rancidity in food. An antioxidant is a substance that retards lipid oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals which can disseminate the oxidation reaction (Senanayake,

2013a). However, in biological systems, it can be defined as "any substance, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell, 1990). Antioxidants facilitate food preservation by delaying development of rancidity, deterioration and discoloration due to lipid oxidation. There are two main categories of antioxidants according to their mechanism of action: primary or chain breaking antioxidants and secondary or preventive antioxidants (Senanayake, 2013a). Primary or type 1 antioxidants interrupt the oxidative free radical chain reaction by contributing an electron or hydrogen from the phenolic hydroxyl groups and therefore stabilize free radicals, as a result, delay or inhibit the initiation step or interrupt the propagation step of autoxidation (Figure 4). The free radical scavenging ability of primary antioxidants can be projected from their standard one-electron potentials. When the standard one-electron potential is concerned, any compound that has a reduction potential lower than the reduction potential of a free radical (or oxidized species) is competent in donating a hydrogen atom to that free radical, producing a more stable, non-radical product (Decker, 2002). For instance, primary antioxidants have significantly lower reduction potentials than peroxyl radicals; hence, they have higher affinities for peroxyl radicals than lipids and react primarily with these peroxyl radicals. Phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary-butyl hydroquinone (TBHQ) and propyl gallate are effective chain-breaking antioxidants due to the presence of bulky alkyl groups near the hydroxyl group of the phenolic ring. When participate in oxidation reactions, these compounds are able to produce stable and relatively unreactive antioxidant radicals.

Secondary antioxidants are categorized as preventive or type II antioxidants. They exhibit their antioxidant activity through various mechanisms. Secondary antioxidants deactivate singlet oxygen, chelate metal ions (i.e., iron, copper), absorb ultraviolet radiation, scavenge oxygen, decompose hydroperoxides to non-radical species, and help regenerate primary antioxidants by providing hydrogen atoms to them (Senanayake, 2013a). However, secondary antioxidants do not convert free radicals into stable molecules.

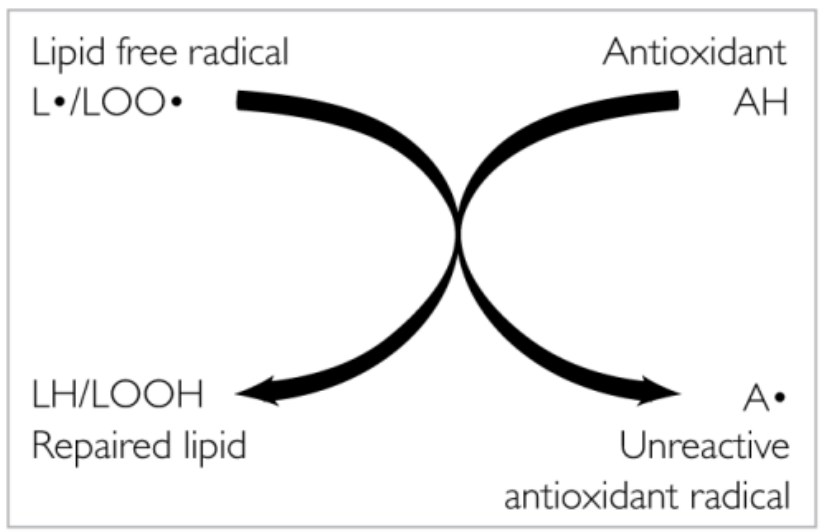

Figure 4. Antioxidant mechanism of green tea catechins.

For better effectiveness, primary antioxidants are often used in combination with secondary antioxidants. When primary and secondary antioxidants are used concurrently in foods, a synergistic effect is typically observed because they withhold both the initiation and propagation steps of lipid oxidation. Synergism is a concept in which combined antioxidant effect is significantly greater than the sum of the individual antioxidant effects. For instance, the synergistic effect of common antioxidants and metal chelators has been observed in most edible fats and oils. The most widely used antioxidants in the food industry are TBHQ, BHA, BHT, propyl gallate, tocopherols, ascorbic acid, citric acid, and polyphenol- and flavonoid-rich plant extracts. The plants from which antioxidant-enriched extracts are derived from include green tea, rosemary, oregano, sage, soybean, grape-seed, acerola, and pomegranate, among others.

5. ANTIOXIDANT MECHANISM

The antioxidant properties of green tea are based on their content of polyphenol compounds, most notably catechins. The antioxidant activity of green tea catechins is primarily attributed to the aromatic rings and hydroxyl groups that make up their chemical structure and, consequently, the binding and neutralization of lipid free radicals by these hydroxyl groups which result in non-free radical molecules (Senanayake, 2013b). The antioxidant foremost mechanism of action of green tea catechins is presumed to be *via* its free radical scavenging activity that is related to their hydrogen or electron-donating ability and to the delocalization of the resulting phenoxyl radicals within their structures. However, other mechanisms of action have been postulated such as chelation of transition metal ions, which are known to be notorious catalysts of lipid oxidation in foods. Research has demonstrated that polyphenols and tea catechins are exceptional electron donors and successful scavengers of reactive oxygen species in vitro, including superoxide anions (Nanjo et al., 1993; Guo et al., 1999; Nakagawa & Yokozawa, 2002; Michalak, 2006), peroxyl radicals, and singlet oxygen species (Guo et al., 1999; Michalak, 2006). The free radical-scavenging potential of polyphenols and tea catechins appeared to be dependent on the number and location of free hydroxyl groups on the flavonoid structure (Lupea et al., 2008). The B-ring substitution pattern is also important for free radical-scavenging ability of flavonoids. Guo et al., (1999) reported that the ability to scavenge free radicals for epigallocatechin gallate and gallocatechin gallate was stronger than epigallocatechin, gallocatechin, epicatechin and catechin due to the presence of their gallate moiety at 3-position of the C-ring. Moreover, the free radical scavenging potential of epigallocatechin and gallocatechin was higher than that of epicatechin and catechin because of a hydroxyl group at the 5′-position of the B ring.

Catechins also exhibit antioxidant activity through chelating redox active transition metal ions. Catechin molecules with multiple hydroxyl groups are more effective antioxidants than those with only fewer hydroxyl groups. The presence of the ortho-3,4-dihydroxy structure also increases the antioxidant activity of catechins (Geldof and Engeseth 2002). Flavonoids such as catechins can inhibit transition metal enhancement of oxidation by donating a hydrogen atom to them, rendering them less pro-oxidative. In addition, some flavonoid molecules can preferentially bind metal ions at the 5-hydroxyl and 4-oxo groups (Fernandez et al., 2002). Green tea polyphenols, possess hydroxyl and carboxyl groups, are able to bind

transition metal ions particularly iron and copper (Michalak, 2006) and prevent their participation in Fenton and Haber-Weiss reactions (Saewong et al, 2010). There is another mechanism underlying the antioxidant ability of plant polyphenols, including green tea catechins. Metal ions decompose lipid hydroperoxides (LOOH) by the hemolytic cleavage of the O-O bond and yield lipid alkoxyl radicals, which initiate free radical chain reaction. Phenolic antioxidants, including catechins, have the ability to inhibit lipid peroxidation by trapping these lipid alkoxyl radicals. However, this activity depends on the chemical structure of the molecules, and the number and position of hydroxyl groups in the molecules (Millic et al., 1998). Green tea catechins also demonstrate antioxidant activity *via* inhibition of pro-oxidant enzymes and stimulating the production of some antioxidant enzymes **(**Velayutham et al., 2008).

Many researchers have studied the antioxidant activity of green tea catechins using some oxidation model studies (Hirose et al., 1990; Koketsu, 1997; Zhu et al., 2000), and a number of mechanisms have been proposed. Koketsu (1997) and Hirose et al., (1990) speculated that (+)-catechins can scavenge four lipid free radicals per molecule. The antioxidant activity of individual tea polyphenols in different model systems showed a proportional relationship to the number of hydrogen radical donors of catechins. A synergistic effect has been observed between green tea catechins and ascorbic acid and α-tocopherols (Murakami et al., 2003). Catechin, a monomeric flavanol, is reported to have hydroxyl, peroxyl, superoxide and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activities (Fukumoto & Mazza, 2000; Bors & Michel, 1999). In addition, tea catechins have the ability to chelate iron in food model systems (Tang et al., 2002). When tested on microorganisms, Nakao et al., (1998) found that epicatechin gallate, epicatechin and catechin molecules have a peroxyl radical scavenging activity ten times higher than L-ascorbate and beta-carotene. In another study, Nanjo and coworkers (1996) reported that DPPH radical scavenging activities of catechin and epicatechin are less than epigallocatechin, epicatechin gallate, and epigallocatechin gallate. Epicatechin is another monomeric flavanol found naturally in green tea. Evidence has shown that epicatechin is capable of scavenging hydroxyl radicals, peroxyl radicals, superoxide radicals, and DPPH radicals (Liu et al., 2000; Fukumoto & Mazza, 2000; Bors &Michel, 1999). Nakao et al., (1998) revealed that peroxyl radical scavenging activity of epicatechin may be ten times higher than L-ascorbate or beta-carotene.

6. ANTIOXIDANT ACTIVITY

The antioxidant activity of green tea catechins and their derivatives showed a pronounced difference depending on the substrate used for their evaluation. Green tea catechins, have been reported to be effective antioxidants in bulk oils, but were shown to be prooxidants in the corresponding oil-in-water emulsions (Frankel et al., 1994; Frankel et al., 1997). Yashin and coworkers (2011) evaluated the antioxidant activity of different types of teas. The order of their antioxidant potency was green tea > oolong tea > black tea > pu'erh tea. Chen et al (1998a) reported that green tea catechin extract exhibited better antioxidant activity than rosemary extract when added to canola oil, pork lard, and chicken fat. In corn oil triacylglycerols that was oxidized at 50^{o}C, Huang & Frankel (1997) found that epigallocatechin, epigallocatechin gallate and epicatechin gallate were much more

antioxidative than epicatechin or catechin. These catechins have also been effective in retarding oxidation of polyunsaturated fatty acids-rich marine and vegetable oils (Wanasundara & Shahidi, 1998). In the oil-in-water emulsions, all catechins evaluated were pro-oxidants (Huang & Frankel, 1997). A study conducted by Roedig-Penman and Gordon (1997) indicated that components other than epigallocatechin gallate and epicatechin gallate make significant contributions to the antioxidant activity of green tea extracts in stabilizing oil-in-water emulsions at pH 5.5 during prolonged storage (40 days). Other catechins, such as epigallocatechin and epicatechin, and flavonol glycosides present in green tea extracts may make important contributions to the antioxidant properties. In addition, dimers or other oxidation products formed from epigallocatechin gallate may also provide antioxidant effects. They postulated that the solubility of catechin molecules may play a role in their effectiveness in emulsions. As epigallocatechin and epicatechin being less soluble in water than epigallocatechin gallate or epicatechin gallate and thus may be more effective in oil-in-water emulsions due to their increased concentrations at the oil-water interface. In liposomes comprising lecithin, epigallocatechin gallate was the best antioxidant, followed by epicatechin, epigallocatechin, epicatechin gallate, and catechin (Huang & Frankel, 1997). The enhanced antioxidant activity observed for tea catechins in liposomes compared to emulsions has been explained by the higher affinity of the polar catechins toward the polar surface of the liposomal membranes, thus allowing better protection against oxidation (Huang & Frankel, 1997). In another study, He and Shahidi (1997) examined the effect of crude green tea extracts and individual catechins on the oxidative stability of a fish meat model system. Progression of oxidation was monitored by measuring changes in the 2-thiobarbituric acid-reactive substances (TBARS) and headspace volatiles of samples. Their results indicated that green tea extracts and pure catechins showed excellent oxidative stability as compared with samples that contained alpha-tocopherol, BHT, BHA, and TBHQ. Tang and coworkers (2002) studied the antioxidative effect of chicken feed supplementation with green tea catechins and protection of alpha-tocopherol in chicken meat systems during frozen storage. This study showed that addition of the catechins preserved the alpha-tocopherol and controlled the degree of oxidation in chicken meat systems. When green tea catechins applied to a pork model system, Shahidi and Alexander (1998) found that green tea catechins demonstrated an antioxidative effect. The inhibitory effect of catechins on development of TBARS was concentration dependent, being highest at 200 ppm catechins. The order of their potency, at 200 ppm, in pork model system was epigallocatechin gallate = epicatechin gallate > epigallocatechin > epicatechin. In another study, Tang and coworkers (2001) compared the antioxidant activity of green tea catechins on lipid oxidation in red meat, poultry and fish. Green tea catechins added at 300 ppm significantly inhibited pro-oxidation caused by sodium chloride and controlled lipid oxidation in all cooked muscles examined. The high affinity of green tea catechins for the lipid bilayers of muscle and free radical scavenging properties of catechins may be the likely mechanism to elucidate the oxidative stability in cooked muscle foods (Tang et al., 2001).

Green tea polyphenols have also been shown to be effective against beta-carotene oxidation in food. For instance, tea catechins have been able to demonstrate an anti-discoloring effect on some selected foods containing beta-carotene (Koketsu, 1997; Unten et al., 1997). Unten et al., (1997) investigated the anti-discoloring effect of the green tea polyphenols and individual catechins on beverages containing beta-carotene and showed that green tea catechins suppressed the discoloration of beta-carotene. The anti-discoloring effect

of green tea polyphenols on beta-carotene was also investigated using margarine. The margarine containing tea catechins was found to retain the yellow color (Unten et al., 1997). Results of this study suggested that the hydroxyl group at the 5'-position of the B ring of the catechin molecule was the most contributing factor for the anti-discoloring effect. Among the individual green tea polyphenols examined, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, and gallocatechin showed strong anti-discoloring effect, while epicatechin and catechin showed almost no activity, and gallic acid showed some moderate effect (Unten et al., 1997).

7. CHEMICAL STABILITY

The chemical stability of green tea catechins is largely depends on the pH of the substrate, temperature, catechin concentration, oxygen content and the presence of metal ions. A large number of research studies have revealed that the stability of catechins is pH dependent in dilute solutions, and kinetic models for such systems have been established (Komatsu et al., 1992; Guyot et al., 1995; Chen et al., 1998b; Chen et al., 2001; Zimeri and Tong, 1999; Su et al., 2003; Li et al., 2012). In acidic solutions (pH < 4), green tea catechins exhibit remarkable stability (Li et al., 2012) whereas in alkaline solutions (pH > 8) they are extremely unstable (Zhu et al., 1997; Zimeri et al., 1999). In a study conducted by Chen et al., (1998b) examined the effect of ascorbic acid and citric acid on the stability of green tea catechins in high pH solution (pH 7.42). The addition of ascorbic acid significantly increased the stability of green tea catechins, particularly (-)-epigallocatechin gallate and (-)-epigallocatechin, whereas citric acid exhibited no effect on their stability. In another study by Zhu et al., (1997) demonstrated that (-)-epigallocatechin gallate and (-)-epigallocatechin were more unstable than (-)-epicatechin and (-)-epicatechin gallate in a basic solution, giving a plausible explanation to the fact that partial absorption of green tea catechins in red blood cells of mice is attributed to the instability of (-)-epigallocatechin gallate and (-)-epigallocatechin in the intestine where the pH is neutral or alkaline.

The stability of catechins appeared to be concentration dependent, with increasing stability evident as the concentration of catechins increased (Li et al., 2012; Li et al., 2013). Li and co-workers (2012) investigated the effect of catechin concentration *versus* their degradation in aqueous systems. Catechin degradation proceeded much more rapidly with more dilute solutions, and the rate of degradation decreased with increasing catechin concentration. Catechin stability is also affected by concentration of oxygen. Sang et al., (2005) reported that higher oxygen levels increased oxidation of tea catechins. Under low oxygen concentration (samples flushed with nitrogen), epigallocatechin gallate remained stable with only slight degradation after 6 h. In another study, Saadeh et al., (2009) indicated that oxygen in atmospheric air caused significant degradation of catechins in the extracts. Metal ions would affect antioxidant activity of catechins by their binding to the catechins. Catechins may react with metal ions to create metal complexes (Ananingsih et al., 2013). By investigating the impact of metal ions on antioxidant activity of catechins, Kumamoto et al., (2001) found that antioxidant activity of epigallocatechin gallate increased by the presence of Cu^{2+} and that its activity reduced by the Fe^{2+} ion. The reaction of epigallocatechin gallate with Cu^{2+} ions reduced its oxidation potential, which contributed to its high antioxidant

activity. By studying the effect of metal ions on antioxidant activity of epigallocatechin gallate and epicatechin gallate in oil-in-water emulsion, Roedig-Penman and Gordon (1997) reported that Fe^{3+} increased oxidation of the oil; however, Cu^{2+} had slight effect.

In a high temperature environment, green tea catechins are not very stable. By investigating degradation kinetics of green tea catechins, Li et al., (2011) developed practical shelf life models of catechin stability in green tea powders and identified the storage conditions required to maintain high catechin contents in these powdered green tea products. Catechin stability was affected by both the relative humidity (RH) and the temperature; however, the temperature was the leading factor contributing to their stability. Storing green tea powder at conditions well below glass transition temperature (T_g) was recommended by these investigators to maintain catechin stability, for instance, at ambient temperature (25^{o}C) below 43% RH or in a freezer below 60% RH. Heating may cause the conversion of green tea catechins to their corresponding isomers, a process known as epimerization. For example, as epimerization can occur at high temperature, epigallocatechin gallate in green tea extract may convert to its epimer component gallocatechin gallate. Heat treatments typically decrease the antioxidant activity of green tea catechins due to various reactions, including oxidation, thermal degradation, epimerization and polymerization (Ananingsih et al., 2013). Cabrera et al., (2006) also reported that fermentation and heating of tea leaves during the manufacturing process may result in polymerization of tea catechins, which would lead to conformational changes and thus modifying its properties.

Sharma and Zhou (2011) evaluated the stability of green tea catechins during biscuit manufacturing process. Their results showed that green tea catechins were relatively stable in dough. However, their stability decreased as the baking progressed (at 160^{o}C for 10 minutes) and increased as the concentration of green tea extract was increased in the biscuit dough. The stability of catechins also increased as pH of the dough was reduced and made less alkaline. This loss of catechins could be due to the combined effect of alkaline pH of the system, the interactions of the catechins with certain components in the dough, the epimerization or oxidation of catechins during baking, and the degradation of catechins during the various biscuit making stages, including mixing and baking. In another study, Kim et al., (2011) investigated the impact of packaging materials on the antioxidant phytochemical stability of aqueous infusions of green tea during cold storage. This study compared the performance of three packaging materials: glass, polyethylene terephthalate (PET) and retortable pouch. Among these, glass and PET are typically used for ready-to-drink (RTD) teas. Their results revealed that the green tea polyphenolics and antioxidant capacity were directly impacted by the type of packaging materials used. The quantities of the three major flavan-3-ols, epigallocatechin gallate, epigallocatechin, and epicatechin gallate were better retained in glass bottles as compared to other two packages. Moreover, antioxidant capacity was higher in glass and PET packaged green tea products as compared to that of the retortable pouch. Hence, packaging materials with different oxygen permeability are likely to be a significant shelf life predictor due to the fact that there was a distinct relationship between oxygen permeability and antioxidant polyphenolic degradation observed in study.

CONCLUSION

Green tea is well-known for its health benefits. Polyphenolic compounds, particularly catechins, are the most important components of green tea. This chapter reviewed the antioxidant activity, basic chemistry, antioxidant mechanism and chemical stability that are associated with green tea catechins. Various reports reviewed in this chapter have provided evidence that most recognized attributes of green tea are their antioxidant activities. The composition of green tea is rather complex as it contains some polyphenols, xanthins and related compounds, carbohydrates, amino acids, proteins, volatile compounds, minerals and trace elements. Among green tea polyphenols, the predominant antioxidant components are epigallocatechin gallate, epicatechin gallate, epicatechin and epigallocatechin. Various investigators have speculated the mechanism of antioxidant action of green tea catechins. According to these studies, the antioxidant activity of green tea catechins is attributed to the arrangement of aromatic rings and hydroxyl groups that assemble their chemical structure and, as a result, free radical scavenging ability by these hydroxyl groups as well as sequestering of transition metal ions. Many factors contributed to the chemical stability of green tea catechins. Catechin stability is mainly dependent on the pH of the medium, temperature, catechin concentration, oxygen content and the presence of metal ions.

REFERENCES

Adak, M. and Gabar, M.A. (2011). Green tea as a functional food for better health: A brief review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2, 645-664.

Ananingsih, V.K., Sharma, A. and Zhou, W. (2013). Green tea catechins during food processing and storage: A review on stability and detection. *Food Research International*, 50, 469–479.

Abdel-Rahman, A., Anyangwe, N., Carlacci, L., Casper, S., Danam, R.P., Enongene, E., Erives, G., Fabricant, D., Gudi, R., Hilmas, C.J., Hines, F., Howard, P., Levy, D., Lin, Y., Moore, R.J., Pfeiler, E., Thurmond, T.S., Turujman, S. and Walker, N.J. (2011). The safety and regulation of natural products used as foods and food ingredients. *Toxicological Sciences*, 123, 333-348.

Balentine, D. A. and Paetau-Robinson, I. (2000). Tea as a Source of Dietary Antioxidants with a Potential Role in Prevention of Chronic Diseases. In G. Mazza, & B.D. Oomah (Eds.), *Herbs, Botanicals, & Teas*. (pp. 265–287). Lancaster (Pennsylvania): Technomic Publishing Co., Inc.

Balentine, D.A.,Wiseman, A.S. and Bouwens, L.C.M. (1997). The chemistry of tea flavonoids. *Critical Reviews in Food Science and Nutrition*, 37, 693–704.

Bors,W. and Michel, C. (1999). Antioxidant capacity of flavanols and gallate esters: Pulse radiolysis studies. *Free Radical Biology & Medicine*, *27*, 1413–1426.

Cabrera, C., Artacho, R. and Gime´nez, R. (2006). Beneficial effects of green tea—a review. *Journal of the American College of Nutrition*, *25*, 79-99.

Chen Z.Y., Wang, L.Y., Chan, P.T., Zhang, Z., Chung, H.Y. and Liang, C. (1998a). Antioxidative activity of green tea catechin extract compared with that of rosemary extract. *Journal of the American Oil Chemists' Society*, 5, 1141–1145.

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

Chen, Z.-Y., Zhu, Q. Y., Tsang, D. and Huang, Y. (2001). Degradation of green tea catechins in tea drinks. *Journal of Agricultural and Food Chemistry*, 49, 477−482.

Chaturvedula, V.S.P. and Prakash, I. (2011). The aroma, taste, color and bioactive constituents of tea. *Journal of Medicinal Plants Research*, *5*, 2110-2124.

Chu, D.-C. (1997). Green tea—its cultivation, processing of the tea leaves for drinking materials, and kinds of green tea.; In L.R. Juneja, D.-C. Chu, & Kim, M. (Eds.), *Chemistry and Applications of Green Tea*. (pp. 1–11), Boca Raton (Florida): CRC Press.

Decker, E. Antioxidant Mechanisms. In: Editor Akoh, C.C and Min, D.B. Food Lipids: Chemistry, Nutrition and Biotechnology, New York, Marcel Deker, Inc. 2002, 517-542.

Farkas, O., Jakus, J. and Héberger, K. (2004). Quantitative structure – Antioxidant activity relationships of flavonoid compounds. *Molecules*, *9*, 1079-1088.

Fernandez, M.T., Mira, M.L., Florencio, M.H. and Jennings, K.R. (2002). Iron and copper chelation by flavonoids: an electrospray mass spectrometry study. *Journal of Inorganic Biochemistry*, 92, 105–111.

Fukumoto, L. R., and Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of Agricultural and Food Chemistry*, *48*, 3597–3604.

Frankel, E.N. (2005). Free radical oxidation. In *Lipid Oxidation*, (pp. 15-24), Bridgwater (England): The Oily Press.

Frankel, E.N., Huang, S.-W., Kanner, J. and German, J.B. (1994). Interfacial phenomena in the evaluation of antioxidants: bulk oils vs. emulsions. *Journal of Agricultural and Food Chemistry*, *42*, 1054–1059.

Frankel, E.N., Huang, S.-W. and Aescbach, R. (1997). Antioxidant activity of green teas in different lipid systems. *Journal of the American Oil Chemists' Society*, 1997, 74, 1309–1315.

Geldof, N. and Engeseth, N.J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry,* 50, 3050–3055.

Graham, H.N. (1992). Green tea composition, consumption, and polyphenol chemistry. *Preventive Medicine*, *21*, 334–350.

Guo, Q., Zhao, B., Shen, S., Hou, J., Hu, J. and Xin, W. (1999). ESR study on the structure-antioxidant activity relationship of tea catechins and their epimers. *Biochimica et Biophysica Acta*, *1427*, 13–23.

Guyot, S., Cheynier, V., Souquet, J.-M. and Moutounet, M. (1995). Influence of pH on the enzymic oxidation of (+)-catechin in model systems. *Journal of Agricultural and Food Chemistry,* 43, 2458−2462.

Halliwell, B. (1990). How to characterize a biological antioxidant, *Free Radical Research Communications*, 9, 1–32.

Harborne, J. B. and Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, *55*, 481-504.

Heim, K. E., Tagliaferro, A. R. and Bobilya D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, *13*, 572-584.

He, Y. and Shahidi. F. ((1997). Antioxidant activity of green tea and its catechins in a fish meat model system. *Journal of Agricultural and Food Chemistry,* 45, 4262-4266.

Hirose, Y., Yamamoto, H. and Nakayama, M. (1990). Oxidation product of (+)- catechin from lipid peroxidation. *Agricultural and Biological Chemistry*, *54*, 567-569.

Huang, S.W. and Frankel, E.N. (1997). Antioxidant activity of tea catechins in different lipid systems. *Journal of Agricultural and Food Chemistry*, *45*, 3033-3038**.**

Jeszka-Skowron, M. and Zgoła-Grześkowiak, A. (2014). Analysis of antioxidant activity, chlorogenic acid, and rutin content of Camellia sinensis infusions using response surface methodology optimization, *Food Analytical Methods*, 7, 2033–2041.

Khokhar, S., Venema, D., Hollman, P. C., Dekker, M. and Jonge, W. (1997). A RP-HPLC method for the determination of tea catechins. *Cancer Letters*, 114, 171–172.

Koketsu, M. (1997). In Antioxidative effects of tea polyphenols. In Yamamoto, T., Juneja, L. R., Chu, D.-C. and Kim, M., Eds., In *Chemistry and Applications of Green Tea*, CRC Press: Boca Raton, Florida, pp. 37–50.

Komatsu, Y., Suematsu, S., Yoshihiro, H., Saigo, H., Matsuda, R. and Hara, K. (1992). Effects of pH and temperature on reaction kinetics of catechins in green tea infusion. *Bioscience, Biotechnology, and Biochemistry*, 57, 907−910.

Kim, Y., Welt, B.A., and Talcott, S.T. (2011). The impact of packaging materials on the antioxidant Phytochemical Stability of Aqueous Infusions of Green Tea (Camellia sinensis) and Yaupon Holly (Ilex vomitoria) during Cold Storage. *Journal of Agricultural and Food Chemistry, 59*, 4676-4683.

Kumamoto, M., Sonda, T., Nagayama, K., and Tabata, M. (2001). Effects of pH and metal ions on antioxidative activities of catechins. *Bioscience, Biotechnology, and Biochemistry*, 65, 126−132.

Lavelli, V., Vantaggi, C., Corey, M., and Kerr, W. (2010). Formulation of a dry green tea–apple product: Study on antioxidant and color stability. *Journal of Food Science*, 75, C184−C190.

Li, T., Yu, L., Li, M. and Li, W. (2007). Comparative studies on the qualities of green teas in Karst and non-Karst areas of Yichang, Hubei Province, PR China. *Food Chemistry,* 103, 71-74.

Li, N., Taylor, L.S., and Mauer, L.J. (2011). Degradation kinetics of catechins in green tea powder: Effects of temperature and relative humidity. *Journal of Agricultural and Food Chemistry,* 59, 6082-6090.

Li, N., Taylor, L.S., Ferruzzi, M.G. and Mauer, L.J. (2012). Kinetic study of catechin stability: Effects of pH, concentration, and temperature. *Journal of Agricultural and Food Chemistry,* 60, 12531-12539.

Li, N., Taylor, L.S., Ferruzzi, M.G. and Mauer, L.J. (2013). Color and chemical stability of tea polyphenol (−)-epigallocatechin-3-gallate in solution and solid states. *Food Research International*, 53, 909-921.

Liu, Z. Q., Ma, L. P., Zhou, B., Yang, L. and Liu, Z. L. (2000). Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein. *Chemistry and Physics of Lipids*, *106*, 53–63.

Lupea, A.X., Pop, M. and Cacig S. 2008. Structure-radical scavenging activity relationships of flavonoids from Ziziphus and Hydrangea extracts. *Revista de Chimie*, 59, 309–13.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, *15*, 523-530.

Millic, B.L., Djlas, S.M. and Canadanovic-Brunet, J.M. (1998). Antioxidative activity of phenolic compounds on the metal-ion breakdown of lipid peroxidation system. *Food Chemistry*, *61*, 443-447.

Murakami, M., Yamaguchi, T., Takamura, H. and Matoba, T. (2003). Effects of ascorbic acid and α-tocopherol on antioxidant activity of polyphenolic compounds. *Journal of Food Science*, *68*, 1622- 1625.

Nakagawa, T. and Yokozawa, T. (2002). Direct scavenging of nitric oxide and superoxide by green tea. *Food and Chemical Toxicology*, *40*, 1745–1750.

Nakao, M., Takio, S. and Ono, K. (1998). Alkyl peroxyl radical scavenging activity of catechins. *Phytochemistry*, *49*, 2379–2382.

Nanjo, F., Goto, K., Seto, R., Suzuki, M., Sakai, M. and Hara, Y. (1996). Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radical Biology & Medicine*, *21*, 895–902.

Nanjo, F., Honda, M., Okushio, K., Matsumoto, N., Ishigaki, F., Ishigami, T. and Hara, Y. (1993). Effects of dietary tea catechins on alpha-tocopherol levels, lipid peroxidation, and erythrocyte deformability in rats fed on high palm oil and perilla oil diets. *Biological and Pharmaceutical Bulletin*, *16*, 1156–1159.

Obanda, M., Owuor, P. O. and Mang'oka, R. (2001). Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chemistry*, *75*, 395–404.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine*, *20*, 933-956.

Roedig-Penman, A. and Gordon M.H. (1997). Antioxidant properties of catechins and green tea extracts in model food emulsions. *Journal of Agricultural and Food Chemistry, 45*, 4267-4270.

Saadeh, R., Al-Jabari, M., Abdoh, A. and Al-Bawab, A. (2009). Stability study of green tea natural extract in aqueous solutions and its chemical kinetics. *Dirasat: Pure Sciences*, 36, 62-75.

Saewong, T., Ounjaijean. S., Mundee, Y., Pattanapanyasat, K., Fucharoen, S., Porter, J.B. and Srichairatanakool, S. (2010) Effects of green tea on iron accumulation and oxidative stress in livers of iron-challenged thalassemic mice. *Medicinal Chemistry*, 6, 57-64.

Sang, S., Lee, M. -J., Hou, Z., Ho, C. -T. and Yang, C. S. (2005). Stability of tea polyphenol (−)- epigallocatechin-3-gallate and formation of dimmers and epimers under common experimental conditions. *Journal of Agricultural and Food Chemistry*, 53, 9478−9484.

Shahidi, F. and Alexander, D.M. (1998). Green tea catechins as inhibitors of oxidation of meat lipids. *Journal of Food Lipids*, 5, 125–133.

Senanayake, S.P.J.N. (2013a). Green tea extract: Chemistry, antioxidant properties and food applications – A review. *Journal of Functional Foods, 5*, 1529-1541.

Senanayake, S.P.J.N. (2013b). Rosemary and green tea extracts as natural antioxidants: Chemistry, technology and applications. In A. Logan, U. Nienaber, & X. Pan (Eds.), Lipid Oxidation: Challenges in Food Systems, pp. 417-438, Urbana, IL, AOCS Press.

Sharma, A. and Zhou, W. (2011). A stability study of green tea catechins during the biscuit making process. *Food Chemistry,* 126, 568–573.

Su, Y. L., Leung, L. K., Huang, Y. and Chen, Z.-Y. (2003). Stability of tea theaflavins and catechins. *Food Chemistry,* 83, 189−195.

Tang, S.Z., Kerry, J.P., Sheehan, D. and Buckley, D.J. (2002). Antioxidative mechanisms of tea catechins in chicken meat systems. *Food Chemistry*, *76*, 45-51.

Tang, S., Kerry, J.P., Sheehan, D., Buckley. D.J. and Morrissey, P.A. (2001). Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation *Food Research International*, 34, 651-657.

Tournaire, C., Croux, S., Maurette, M.T., Beck, I., Hocquaux, M., Braun, A.M. and Oliveros, E., (1993). Antioxidant activity of flavonoids: efficiency of singlet oxygen (1delta g) quenching. *Journal of Photo-chemistry and Photobiology Biology*, 19, 205-215.

Unten, L., Koketsu, M. and Kim, M. (1997). Antidiscoloring activity of green tea polyphenols on β- carotene. *Journal of Agricultural and Food Chemistry*, *45*, 2009-2012.

USDA: "USDA Database for the Flavonoid Content of Selected Foods." Beltsville, Maryland: US Department of Agriculture, 2013. Release 3.1, Available from: http://www.ars.usda.gov/nutrientdata. Accessed January, 2014.

Velayutham, P., Babu, A. and Liu, D. (2008). Green tea catechins and cardiovascular health: An update. *Current Medicinal Chemistry*, *15*, 1840–1850.

Wanasundara, U.N. and Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chemistry*, *63*, 335-342.

Yashin, A., Yashin, Y. and Nemzer, B. (2011). Determination of antioxidant activity in tea extracts, and their total antioxidant content, *American Journal of Biomedical Sciences*, 3, 322-335.

Yilmaz, Y. (2006). Novel uses of catechins in foods. *Trends in Food Science & Technology*, *17*, 64–71.

Zaveri, N. T. (2006). Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. *Life Sciences*, *78*, 2073–2080.

Zhu, Q. Y., Zhang, A., Tsang, D., Huang, Y. and Chen, Z.-Y. Stability of green tea catechins. *Journal of Agricultural and Food Chemistry,* 1997, 45, 4624−4628.

Zhu, N., Sang, S., Huang, T., Bai, N., Yang**,** C.S., and Ho, C.-T. (2000). Antioxidant chemistry of green tea catechins: oxidation products of (-)-epigallocatechin gallate and (-)-epigallocatechin with peroxidase. *Journal of Food Lipids*, *7*, 275 – 282.

Zimeri, J. and Tong, C. H. (1999). Degradation kinetics of (−)-epigallocatechin gallate as a function of pH and dissolved oxygen in a liquid model system. *Journal of Food Science*, 64, 753−758.

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Chapter 2

POTENTIAL FOR PREVENTION OF INFECTION BY GREEN TEA

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ABSTRACT

Infections by microorganisms are a leading cause of morbidity and mortality worldwide. Since the advent of antimicrobial use, the ability to control these infections has vastly improved. Unfortunately, the healthcare costs for these infections is still in the billions of dollars. The ideal situation would be for people to not succumb to infections, but be able to prevent them. Green tea has the ability to eradicate many infection causing microorganisms. Many of the mechanisms of green tea that make recovery from infection possible are also potential infection prevention mechanisms. The main constituents of green tea that are antimicrobial are the catechins (polyphenols). The four main catechins are: (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG). Consumption of green tea provides availability of these compounds throughout the body, and also the possibility for prevention of infection.

INTRODUCTION

Among important diseases that plague the world, infections are a significant cause of morbidity and mortality. According to the World Health Organization's (WHO) assessment of diseases that contribute the most to morbidity and mortality, lower respiratory tract infection, diarrhoeal diseases, HIV/AIDS, and malaria are in the top ten [1]. While the advent of antimicrobial agents has helped tremendously in the fight against microorganisms, there are two major considerations: the added cost of healthcare for infectious diseases, and the

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huge problem with antimicrobial resistance. In a 2009 report from the Centers for Disease Control (CDC), the attributable cost for U.S. healthcare-associated infections alone was over $25,000 per patient [2]. A 2011 report on the healthcare costs of pneumococcal disease in the U.S. estimated total healthcare costs per year of $3.7 billion, and if costs of productivity and work loss are added, an estimated $7.7 billion [3]. The issue of antimicrobial resistance is at a crisis level. Both the CDC and WHO have expressed enormous concern over this [4, 5]. Antimicrobial resistance makes many infections difficult to treat. This also adds to the economic burden on healthcare. Various studies on added healthcare costs for methicillin-resistant *Staphylococcus aureus* (MRSA) infections show: in the U.S., over $18,000 per patient case; in Germany, nearly €9,000 per case; and in Switzerland, an average added cost of 100,177 Swiss francs [6-8]. How much better it would be for patients and healthcare costs if infectious diseases could be prevented instead. To help minimize the cost of prevention methods, scientists are looking into the possibility of finding natural plant products that have preventative capabilities. One of the products being researched is green tea.

Tea has been a very popular drink worldwide for a long time. It comes from the plant *Camellia sinensis*, and is grown in over 30 countries. The tea plants grow best in specific subtropical and tropical regions of the world [9]. There are four main produced tea varieties; white, green, Oolong, and black tea. The tea variety is based on the processing of the tea plant, and has to do with drying and fermentation methods. The least amount of processing (no fermentation) produces white tea from very young leaves and buds. Green tea is produced from mature leaves which are also unfermented. Oolong tea is produced from partially fermented leaves, and black tea from leaves that are fully fermented [9, 10]. Oolong tea is popular in Taiwan and China, black tea is most popular in the United States, and green tea is most popular in Korea, Japan, and China [11]. Scientists have been studying green tea for its potential health benefits properties, such as: anticarcinogenic, anti-inflammatory, antimicrobial, and antioxidant; and in cardiovascular and oral health.

Green tea has been found to be potentially beneficial against various types of cancer. The possible anticarcinogenic mechanisms include inhibition of angiogenesis and cell growth, and induction of apoptosis in tumor cells [12, 13]. In breast cancer the potential actions include: anti-angiogenesis, interaction with target proteins, inhibition of cell signaling pathways, inhibition of enzyme activities, and induction of cell cycle arrest and apoptosis [14]. In prostate cancer the potential actions include: inhibiting cell signaling pathways, inhibition of protein kinases, suppression of activation of transcription factors, and antagonism of androgen action [15]. Apparently green tea components have been shown to induce stabilization of p53 with a resulting activation of downstream targets, leading to apoptosis [16]. In skin cancer green tea components inhibit cancer cell viability/induce cytotoxicity through inactivation of *β*-catenin signaling, which down regulates inflammatory mediators, cell cycle regulatory proteins, cAMP levels, and cell survival signals [17]. In non-small-cell lung cancer the green tea components inhibit cell proliferation by suppressing the expression of the cell death-inhibiting gene, *Bcl-xL* [18]. In addition, green tea may be anticarcinogenic by altering epigenetic processes through DNA methylation, histone modification, and miRNA regulation [19].

Inflammation is involved in conditions such as arthritis, cardiovascular disease, aging and cancer. Certain anti-inflammatory effects of green tea may be a result of increased production of an anti-inflammatory cytokine, IL-10 [10, 12]. The anti-inflammatory effects of green tea have been studied in models of rheumatoid arthritis (RA) and osteoarthritis (OA). In human

RA fibroblasts, green tea components were shown to regulate IL-6 synthesis and signaling, and suppression of production of destruction matrix metaloproteinases via TNF-α induced phosphorylation of MAPKs [20, 21]. Research on OA showed that green tea components had an effect on suppression of chronic pain-related pro-inflammatory cytokines in dorsal root ganglia [22]. Other research has shown a more general anti-inflammatory effect against the denaturation of proteins [23].

Oxidative stress in various parts of the body, due to the damaging effects of reactive oxygen species (ROS), is closely tied to inflammation and cardiovascular disease. Recent research has shown that green tea components have an antioxidant effect by upregulating basal levels of antioxidant enzymes, and more specifically showed significant increase in activity of the antioxidant enzymes catalase, glutathione peroxidase, and glutathione reductase in the liver [24, 25]. One of the key markers of systemic inflammation and a predictor of cardiac health issues is high-sensitivity C-reactive protein (hsCRP). Green tea components have been shown to inhibit angiotensin II and IL-6 induced hsCRP expression in macrophages via interfering with reactive oxygen generation [26, 27].

In addition to the antimicrobial effects of green tea on oral microorganisms, green tea has been shown to affect oral health by increasing the activity of oral peroxidases, reducing bad breath in patients with gingivitis, preventing development and progression of chronic periodontitis, reducing dentin wear under erosive/abrasive conditions, and is a natural source of fluoride [9, 28-31].

GREEN TEA COMPOSITION

The most medically relevant components of green tea are the polyphenols; with the most important of these being flavonoids. Flavonoids are widely distributed among plants, and have a large variety of functions. In green tea the most important flavonoids are the catechins, which compose nearly 40% of the water-soluble solids in the green tea [32, 33]. The different types of tea (white, green, Oolong, black) contain varying amounts of catechins; green tea contains the most. There is an initial steaming process in the production of green tea that destroys the polyphenol oxidase enzyme, which protects the polyphenol content of the tea. There are four main catechins found in green tea: (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG). The most abundant catechin in green tea is EGCG, and represents around 59% of the total catechins present. The next most abundant catechin is EGC at 19%, then ECG at nearly 14%, then EC at around 6% [10, 11]. The amount of catechins present in tea is also affected by the geographical location of growing plants, growing conditions, where on the plant the leaves are harvested, leaf processing, and tea preparation methods [11, 34-36].

In order for any consumed product to have the possibility of affecting health, the components of the food or beverage need to be bioavailable. Green tea polyphenols may undergo metabolic processing in the human body such as methylation, glucuronidation, and sulfation [37]. To research and compute bioavailability, scientists most commonly measure levels of specific components in blood plasma, urine, and in some instances, tissue samples. Samples are quite often collected at timed intervals. A number of studies have been conducted to assess the bioavailability of green tea components; most specifically the

catechins. Consumption studies have been conducted using green tea as a normally prepared beverage [19, 37, 38], as an extract of green tea [19, 39. 40], or as the specific catechins [41-43]. These studies have shown that EGCG and ECG, and metabolites of EGC and EC can be detected and measured in blood plasma; and that metabolites of EGC and EC only can be detected and measured in urine. Peak concentrations of the catechins in blood plasma occur at roughly 2 hours after consumption; the peak concentration in urine occurs at between 4-6 hours after consumption. Table 1 shows a summary of some of these studies. Only EGCG and EGC results are included in the data as EC and ECG levels in the body are considered to be too low for therapeutic value [44]. In some studies the dosage of the catechin was varied and measured at these various dosages. The results showed that the bioavailability of the catechins was increased directly in proportion to the amount consumed [40, 41, 45-48]. The most abundant catechins, EGCG and EGC, are also the most bioavailable; EGCG in blood plasma and EGC in urine. Very few studies have been done that assess the absorption of these catechins into tissues; however, there are a few that have shown that there is absorption into a variety of tissues in mice, dogs, and humans [49-51].

Table 1. Bioavailability of green tea catechins

Catechin source	Initial dose	Plasma concentration (peak time)	EGC in urine/24 h (peak time)	References
Bottled green tea	EGCG 230-235μmol EGC 260μmol	EGCG 55*n*mol/L (1.9 h) EGC 126-205*n*mol/L (2.2 h)	33μmol	37, 38
Green tea extracts	EGCG 88-110mg EGC 82-10 mg	EGCG 119-135*n*g/ml EGC 140-148*n*g/ml	3.0mg (3-6 h)	39, 40

ANTIMICROBIAL PROPERTIES OF GREEN TEA

Integral to the potential for preventing infection is the effect that green tea catechins have on the various infectious agents. Green tea has been shown to inhibit the growth of and/or kill many microorganisms. A listing of those microorganisms can be found in Table 2. This is not meant to be an all inclusive list of affected organisms; it merely reflects those microorganisms that have actively been tested against green tea [44]. The previously discussed health benefits of green tea; anticarcinogenic, anti-inflammatory, antioxidant, cardiac and oral health; may also contribute to the antimicrobial effects. The most important known antimicrobial properties of green tea will be covered in this chapter, and include: damage to the bacterial cell membrane, inhibition of fatty acid synthesis, inhibition of other enzymes, and other inhibitory effects.

A common effect of green tea catechins on microorganisms is a result of binding to the bacterial lipid bilayer cell membrane. Binding can be inhibitory or damaging to the cell membrane [52, 53]. Studies with *Escherichia coli* measured changes in gene expression after exposure to green tea polyphenols. Changes were noted in 17 genes, 8 were downregulated and 9 were upregulated. A major outcome of these changes in gene regulation was shown to be damage to the cell membrane [54]. Green tea catechins apparently have less effect on the cell membrane of gram negative bacteria. This is probably due to the fact that the lipopolysaccharide (LPS) outer membrane of gram negative bacteria has a negative charge

[55]. When the bacterial cell membrane is damaged, there can be several results, including increased permeability of the membrane which leads to cell death. Damage to the cell membrane also inhibits the ability of the bacteria to bind to host cells [56], and to bind to each other to form biofilms [57]. Cell membrane damage decreases the ability of bacteria to be able to secrete toxins [58, 59].

Bacterial fatty acids have important functions such as being a major component of cell membranes, and as a source of energy. Recent studies have begun to look at the possibility of targeting fatty acid biosynthesis in antimicrobial drug development [60]. Other studies have shown that green tea catechins, especially EGCG, are able to inhibit certain specific bacterial reductases, such as FabG and FabI, which are involved in type II fatty acid synthesis [61, 62]. Inhibiting fatty acid biosynthesis can also result in inhibition of the production of bacterial toxic metabolites [63].

In addition to the enzymes that may be involved in fatty acid biosynthesis, green tea catechins effect other essential bacterial enzymes. Studies have shown that green tea catechins inhibit cysteine proteinases and protein tyrosine phosphatase in some anaerobic bacteria found in the oral cavity [64, 65]. Other studies have shown that green tea catechins are able to interfere with DNA replication by interacting with, and inhibiting, DNA gyrase [66]. Studies on antifolate activity in microorganisms show that green tea polyphenols inhibit the dihydrofolate reductase enzyme in bacteria and yeast. This blocks the ability of the microorganisms to synthesize folate, which is essential for many metabolic processes [67, 68]. Recently it has been found that bioflavonoids (including those found in green tea) can inhibit bacterial ATP synthetase activity. This would greatly cripple the ability of the microorganisms to produce enough energy [69].

There are many other inhibitory effects that green tea may have on bacterial functions. An example of these is inhibiting the synthesis of PBP2', the main component of the gram positive cell wall that is mutated in methicillin-resistant *Staphylococcus aureus* (MRSA), which leads to a reversal of resistance to *β*-lactam drugs [70]. Another example is inhibiting the ability of *Escherichia coli* to transfer a copy of its R plasmid via conjugation [71]. This could stop the sharing of antimicrobial genes between bacteria. Other studies using staphylococci have shown that green tea catechins have the ability to inhibit the activity of bacterial efflux pumps. One study in particular showed inhibition of the TetK efflux pump component which is involved in tetracycline resistance [72]. If green tea has this same effect on other efflux pumps it could help to enhance the ability of antimicrobial agents to destroy bacteria. Another study using *Helicobacter pylori* has shown that green tea catechins have the ability to bind to, and block the function of toll-like receptor-4 (TLR-4] located on gastric epithelial cells [73]. If the bacteria are not able to bind to these hosts cells it would greatly reduce the ability of the bacteria to cause disease.

Green tea also has been shown to have various potential preventative effects in viral infections. Studies using the HIV-1 virus have shown that EGCG is able to bind to the CD4 T-cell receptor, effectively blocking the ability of the virus to bind to those cells. This binding and blocking ability of EGCG is being considered for use in HIV-1 infection therapy [74, 75]. EGCG has also been shown to bind to the viral gp41 protein which blocks the virus from fusing with the host cell [76]. EGCG is also able to inhibit the ability of amyloid fibrils normally present in semen to bind to HIV-1. This counteracts semen-mediated enhancement of HIV infection [77]. Researchers have shown that green tea epicatechins are able to inhibit replication of the Hepatitis C virus through down-regulation of cyclooxygenase-2 [78]. Other

researchers have discovered that Epstein-Barr virus (EBV) lytic cycle infection can be inhibited by EGCG. This compound inhibited constitutive lytic infection of EBV by decreasing the phosphorylation and activation of extracellular kinase1/2 (ERK1/2) and Akt [79].

Table 2. Organisms Inhibited or Killed by Green Tea

Bacteria	Viruses	Fungi	Parasites
Acinetobacter baumannii	Epstein-Barr virus	*Actinomyces* spp.	*Trypanosoma cruzi*
Bacillus cereus	Hepatitis B	*Aspergillus niger*	
Escherichia coli (intestinal)	Hepatitis C	*Candida albicans*	
Escherichia coli (uropathogenic)	HIV-1		
	HSV-1		
Enterococcus faecalis	Influenza A H1N1		
Helicobacter pylori	Influenza A H3N2		
Listeria monocytogenes	Influenza A H5N2		
Porphyromonas gingivalis	Influenza B		
Prevotella intermedia			
Proteus mirabilis			
Pseudomonas aeruginosa			
Salmonella typhi			
Salmonella typhimurium			
Staphylococcus aureus Methicillin-resistant			
Staphylococcus aureus (MRSA)			
Staphylococcus epidermidis			
Stenotrophomonas maltophilia			
Streptococcus mutans			
Streptococcus pyogenes			
Vibrio cholerae			
Yersinia enterocolitica			

PREVENTION OF INFECTION

There is a lot of evidence to show that green tea catechins can eliminate microorganisms in the body, so any of the previously mentioned antimicrobial mechanisms should putatively be able to eliminate the microorganisms before they have a chance to establish an infection. In a study performed with urinary tract infection *E. coli isolates,* it was calculated that a cup of brewed Japanese green tea would contain 7.5 g of dry tea, and approximately 150 mg of EGC [80]. Calculated in the same way, there would be approximately 450 mg of EGCG per cup of tea. While one cup of tea per day may not contain enough catechins to eliminate all potential infections, drinking two or more cups per day might. In this section we will present results from research specifically aimed at showing prevention of infection, most of which is targeting viruses.

In a study assessing prevention of *Helicobacter felis* infection in mice, green tea was used as a beverage prior to infection. The mice were given green tea (or just water) to drink for 2 weeks before being infected, and also for the duration of the experiment. Eight weeks after infection, the mice were analyzed for *Helicobacter felis* colonization by examination of

gastric mucosa from the fundus/antrum border. The group of mice who were given green tea 2 weeks before infection had no detectable colonization. The group that had not been given green tea had a large bacterial load [81].

A study of Japanese nursery school children ages 2-6 assessed whether gargling with green tea could prevent fever and absence days due to illness. The children were to gargle at least once per day with green tea , saline, or water, and another group did no gargling. While the absence days due to sickness showed no correlation (probably due to imitations with the study), the presence of fever did. The group that gargled with green tea had 3 times fewer instances of fever than the non-gargling group, and had 1.5 to 2.5 fewer instances of fever than the saline or water gargling groups respectively [82].

A study performed using adults assessed whether green tea could have an effect on the incidence of cold or influenza symptoms. The adults were given green tea capsules, or a placebo, to take twice daily for 3 months. The group that were given the green tea capsules had over 32% fewer instances of cold or influenza symptoms, and had nearly 23% fewer illnesses that lasted for 2 days or longer [83].

A study using ferrets assessed whether green tea could prevent transmission of H3N2 Influenza A from ill animals to well animals, and also assessed whether green tea could protect well animals from becoming ill when exposed to sick animals. The ferrets were either given capsules containing green tea catechins, or a placebo. For the transmission experiment, one group of animals was infected with Influenza A and afterward were either treated with green tea capsules or given the placebo.

The infected animals were placed in with well animals who were not taking green tea capsules. There was no infection transmitted from the green tea treated infected animals to the well animals. Approximately 25% of the animals had transmission of infection from those animals not given green tea. In the other experiment, animals were infected with Influenza A and then placed in with well animals who were either receiving green tea capsules or placebo. All of the animals taking the placebo became infected, while only 57% of those animals taking green tea capsules became infected [84].

A study using healthcare workers assessed whether taking green tea capsules could prevent infection with influenza. These adults were given capsules containing green tea catechins or placebo. They were to take 6 capsules per day over a 5 month period during influenza season. The group that were given the green tea capsules had a 4% incidence of influenza during the 5 months. The group given the placebo had a 13% incidence of influenza [85].

Japanese school-age children [6-13] were surveyed (parents) during influenza season as to how many cups of green tea were consumed per day, and how many days per week. They were asked to provide information on incidences of infections with Influenza A or B. Nearly 2000 surveys were completed and assessed.

The amount of green tea consumed varied from <1 to >5 cups per day, and from <3 to ≥ 6 days per week. The results showed that the number of infections was inversely proportional to the number of cups of green tea consumed per day, and also inversely proportional to the number of days per week green tea was consumed [86].

CONCLUSION

Over the past few years the research on the antimicrobial activity of green tea has increased dramatically. In light of the failure of so many antimicrobial drugs to continue to control microorganisms, scientists are looking for alternatives. Green tea has shown great promise for treating infections of all types, and also other disorders. In an effort to improve health and save a lot of healthcare monies, researchers are now looking into prevention of disease. With what we know about the health properties of green tea it seems only logical that researchers would begin expanded testing into these areas. Much research has already been done involving the antimicrobial properties of green tea; with more studies needed that will look into these effects in human subjects directly. There is still much to be done in studying disease (and infection) prevention. These types of studies are complex and time consuming. Plus, funding may be difficult to procure. With the potential for improvement in health for future generations, encouragement from scientists and researchers will go far in bringing about the needed studies. For many parts of the world where the cost of antimicrobial drugs makes their use prohibitive, having the option of treating or preventing infections using a readily available and low cost alternative could become a reality. In areas where the possibility of repeated exposure to infectious agents is a way of life, having the ability to lessen the infectious burden could improve health conditions for multitudes of people.

REFERENCES

[1] World Health Organization, *World Health Statistics* 2014. 2014b.

[2] Scott, R.D. *The direct medical costs of healthcare-associated infections in U.S. hospitals and the benefits of prevention.* Centers for Disease Control, 2009; pub. #CS200891-A.

[3] Huang, S.S., Johnson, K.M., Ray, G.T., Wroe, P., Lieu, T.A., Moore, M.R., et al., Healthcare utilization and cost of pneumococcal disease in the United States. *Vaccine*, 2011; 29: 3398-3412.

[4] U.S. Department of Health and Human Services Centers for Disease Control and Prevention. *Antibiotic Resistance Threats in the United States,* 2013.

[5] World Health Organization. Antimicrobial resistance: Global report on surveillance, 2014. 2014a.

[6] Filice, G.A., Nyman, J.A., Lexau, C., Lees, C.H., Bockstedt, L.A., Como-Sabetti, K., et al., Excess costs and utilization associated with methicillin resistance for patients with *Staphylococcus aureus* infection. *Infect. Control. Hosp. Epidemiol.*, 2010; 31: 365-373.

[7] Hübner, C., Hübner, N.O., Hopert, K., Maletzki, S., Flessa, S. Analysis of MRSA-attributed costs of hospitalized patients in Germany. *Eur. J. Clin. Microbiol. Infect. Dis*., 2014; 33: 1817-1822.

[8] Macedo-Viñas, M., De Angelis, G., Rohner, P., Safran, E., Stewardson, A., Fankhauser, C., et al., Burden of methicillin-resistnat *Staphylococcus aureus* infections at a Swiss University hospital: excess length of stay and costs. *J. Hosp. Infect*., 2013; 84: 132-137.

[9] Gupta, D.A., Bhaskar, D.J., Gupta, R.K., Karim, B., Jain, A., Dalai, D.R. Green tea: a review on its natural anti-oxidant therapy and cariostatic benefits. *Biol. Sci. Pharm. Res*., 2014; 2:8-12.

[10] Jigisha, A., Nishant, R., Navin, K., Pankaj, G. Green tea: a magical herb with miraculous outcomes. *Int. Res. J. Pharm.* 2012; 3: 139-148.

[11] Cabrera, C., Artacho, R., Gimenez, R. Beneficial effects of green tea – a review. *J. Am. Coll. Nutr.* 2006; 25: 79-99.

[12] Serafini, M., Del Rio, D., Yao, D.N., Bettuzzi, S., Peluso, I. "Health benefits of tea", in *Herbal Medicine: Biomolecular and clinical Aspects, 2nd ed.,* Chapter 12, eds I.F.F. Benzie and S. Wachtel-Galor (Boca Raton, FL; CRC Press), 239-262, 2011.

[13] Subramani, C., Natesh, R.K., Molecular mechanisms and biological implications of green tea polyphenol, (-)-epigallocatechin-3-gallate. *Int. J. Pharm. Biosci. Tech.* 2013; 1: 54-63.

[14] Li, M.J., Yin, Y.C., Wang, J., Jiang, Y.F. Green tea compounds in breast cancer prevention and treatment. *World J. Clin. Oncol.*, 2014; 5(3): 520-528.

[15] Khan, N, Mukhtar, H. Modulation of signaling pathways in prostate cancer by green tea polyphenols. *Biochem. Pharmacol.*, 2013; 85(5): 667-672. doi:10.1016 /j.bcp.2012.09.027.

[16] Gupta, K., Thakur, V.S., Bhaskaran, N., Nawab, A., Babcook, M.A., Jackson, M.W., Gupta, S. Green tea polyphenols induce p53-dependent and p53-independent apoptosis in prostate cancer cells through two distinct mechanisms. *PloS One*. 2012; 7(12): e52572.

[17] Singh, T., Katiyar, S.K. Green tea polyphenol, (-)-epigallocatechin-3-gallate, induces toxicity in human skin cancer cells by targeting *β*-catenin signaling. *Toxicol. Appl. Pharmacol.* 2013; 273(2): 418-424.

[18] Sonoda, J.I., Ikeda, R., Baba, Y., Narumi, K., Kawachi, A., Tomishige, E., et al., Green tea catechin, epigallocatechin-3-gallate, attenuates the cell viability of human non-small-cell lung cancer A549 cells via *Bcl-xL* expression. *Exp. Ther. Med.* 2014; 8(1): 59-63.

[19] Henning, S.M., Wang, P., Carpenter, C.L., Heber, D. Epigenetic effects of green tea polyphenols in cancer. *Epigenomics*. 2013; 5(6): 729-741.

[20] Ahmed, S., Marotte, H., Kwan, K., Ruth, J.H., Campbell, P.L., Rabquer, B.J., et al., Epigallocaetchin-3-gallate inhibits IL-6 synthesis and suppresses transsignaling by enhancing soluble gp130 production. *Proc Natl Acad Sci USA*, 2008; 105(38): 14692-14697.

[21] Yun, H.J., Yoo, W.H., Han, M.K., Lee, Y.R., Kim, J.S., Lee, S.I. Epigallocatechin-3-gallate suppresses TNF-*α*-induced production of MMP-1 and -3 in rheumatoid arthritis synovial fibroblasts. *Rheumatol. Int.*, 2008; 29(1): 23-29.

[22] Leong, D.J., Choudhury, M., Hanstein, R., Hirsh, D.M., Kim, S.J., Majeska, R.J., et al., Green tea polyphenol treatment is chondroprotective, anti-inflammatory and palliative in a mouse posttraumatic osteoarthritis model. *Arthritis Res. Ther.*, 2014; 16(6): 508.

[23] Chatterjee, P., Chandra, S., Dey, P., Bhattacharya, S. Evaluation of anti-inflammatory effects of green tea and black tea: a comparative *in vitro* study. *J. Adv. Pharm. Technol. Res.*, 2012; 3(2): 136-138.

[24] Newsome, B.J., Petriello, M.C., Han, S.G., Murphy, M.O., Eske, K.E., Sunkara, M., et al., Green tea diet decreases PCB 126-induced oxidative stress in mice by upregulating antioxidant enzymes. *J. Nutr. Biochem.*, 2014; 25(2): 126-135.

[25] Tsai, C.F., Hsu, Y.W., Ting, H.C., Huang, C.F., Yen, C.C. The *in vivo* antioxidant and antifibrotic properties of green tea (*Camellia sinensis*, *Theaceae*). *Food Chem.*, 2013; 136(3-4): 1337-1344.

[26] Ohmori, R., Kondo, K., Momiyama, Y. Antioxidant beverages: green tea intake and coronary artery disease. *Clin. Med. Insights Cardiol.*, 2014; 8(Suppl 3): 7-11.

[27] Li, M., Liu, J.T., Pang, X.M., Han, C.J., Mao, J.J. Epigallocatechin-3-gallate inhibits angiotensin II and interleukin-6-induced C-reactive protein production in macrophages. *Pharmacol. Rep.*, 2012; 64: 912-918.

[28] Narotzki, B., Levy, Y., Aizenbud, D., Reznick, A.Z. Green tea and its major polyphenol EGCG increase the activity of oral peroxidases. *Adv. Exp. Med. Biol.*, 2013; 756: 99-104.

[29] Rassameemasmaung, S., Phusudswang, P., Sangalungkarn, V. Effect of green tea mouthwash on oral malodor. *ISRN Prev. Med. 2012*, 2013:975148.

[30] Kushiyama, M., Shimazaki, Y., Murakami, M., Yamashita, Y. Relationship between intake of green tea and periodontal disease. *J. Peridontol.*, 2009; 80: 372-377.

[31] Kato, M.T., Magalhães, A.C., Rios, D., Hannas, A.R., Attin, T., Buzalaf, M.A. Protective effect of green tea on dentin erosion and abrasion. *J. Appl. Oral Sci.*, 2009; 17(6): 560-564.

[32] Wang, Y., Ho, C.T. Polyphenolic chemistry of tea and coffee: a century of progress. *J. Agric. Food Chem.*, 2009; 57: 8109-8114.

[33] Roowi, S., Stalmach, A., Mullen, W., Lean, M.E., Edwards, C.A., Crozier, A. Green tea flavan-3-ols: colonic degradation and urinary excretion of catabolites by humans. *J. Agric. Food Chem.*, 2010; 58: 1296-1304.

[34] Fernandez, P.L., Pablos, F., Martin, M.J., Gonzalez, A.G. Study of catechin and xanthine tea profiles as geographical tracers. *J. Agric. Food Chem.*, 2002; 59: 1833-1839.

[35] Lin, Y.S., Tsai, Y.J., Tsay, J.S., Lin, J.K. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *J. Agric. Food Chem.*, 2003; 51: 1864-1873.

[36] Sourabh, A., Kanwar, S.S., Sud, R.G., Ghabru, A., Sharma, O.P. Influence of phenolic compounds of Kangra tea [*Camellia sinensis* (L) O Kuntze] on bacterial pathogens and indigenous bacterial probiotics of Western Himalayas. *Braz. J. Microbiol.* 2014; 44(3): 709-715.

[37] Stalmach, A., Troufflard, S., Serafini, M., Crozier, A. Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. *Mol. Nutr. Food Res.*, 2009; 53: S44-S53.

[38] Clifford, M.N., van der Hooft, J.J., Crozier, A. Human studies on the absorption, distribution, metabolism, and excretion of tea polyphenols. *Am. J. Clin. Nutr.*, 2013; 98: 1619S-1630S.

[39] Lee, M.J., Wang, Z.Y., Li, H., Chen, L., Sun, Y., Gobbo, S., et al., Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomarkers Prev.*, 1995; 4: 393-399.

[40] Yang, C.S., Chen, L., Lee, M.J., Balentine, D, Kuo, M.C., Schantz, S.P. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol. Biomarkers Prev.*, 1998; 7:351-354.

[41] Chow, H.H., Cai, Y., Alberts, D.S., Hakim, I., Dorr, R., Shahi, F., et al., Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol. Biomarkers Prev.*, 2001; 10: 53-58.

[42] Van Amelsvoort, J.M., Van Hof, K.H., Mathot, J.N., Mulder, T.P., Wiersma, A., Tijburg, L.B. Plasma concentrations of individual tea catechins after a single oral dose in humans. *Xenobiotica*, 2001; 31: 891-901.

[43] Clarke, K.A., Dew, T.P., Watson, R.E., Farrar, M.D., Bennett, S., Nicolaou, A., et al., High performance liquid chromatography tandem mass spectrometry dual extraction method for identification of green tea catechin metabolites excreted in human urine. *J. Chromatogr.B.*, 2014; 1: 972-937.

[44] Reygaert, W.C. The antimicrobial possibilities of green tea. *Front. Microbiol.*, 2014; 5: 434.

[45] Nakagawa, K., Okuda, S., Miyazawa, T. Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma. *Biosci. Biotechnol. Biochem*., 1997; 61: 1981-1985.

[46] Chow, H.H., Cai, Y., Hakim, I.A., Crowell, J.A., Shahi, F., Brooks, C.A., et al., Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin. Cancer Res.*, 2003; 9: 3312-3319.

[47] Ullmann, U., Haller, J., Decourt, J.P., Girault, N., Girault, J., Richard-Caudron, A.S., et al., A single ascending dose study of epigallocatechin gallate in healthy volunteers. *J. Int. Med. Res.*, 2003; 31 88-101.

[48] Renouf, M., Marmet, C., Guy, P.A., Beaumont, M., Lepage, M, Williamson, G, et al., Dose-response plasma appearance of green tea catechins in adults. *Mol. Nutr. Food Res.*, 2013; 57: 833-839.

[49] Suganuma, M., Okabe, S., Oniyama, M., Tada, Y., Ito, H., Fujiki, H. Wide distribution of [3H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis*, 1998; 19(10): 1771-1776.

[50] Swezey, R.R., Aldridge, D.E., LeValley, S.E., Crowell, J.A., Hara, Y., Green, C.E. Absorption, tissue distribution and elimination of 4-[3)h]-epigallocatechin gallate in beagle dogs. *Int. J. Toxicol.*, 2003; 22(3): 187-193.

[51] Rhodes, L.E., Darby, G., Massey, K.A., Clarke, K.A., Dew, T.P., Farrar, M.D., et al., Oral green tea catechin metabolites are incorporated nito human skin and protect against UV radiation-induced cutaneous inflammation in association with reduced production of pro-inflammatory eicosanoid 12-hydroxyeicosatetraenoic acid. *Br. J. Nutr.*, 2013; 110(5): 891-900.

[52] Sirk, T.W., Brown, E.F., Sum, A.K., Friedman, M. Molecular dynamics study on the biophysical interactions of seven green tea catechins with lipid bilayers of cell membranes. *J. Agric. Food Chem.*, 2008; 56: 7750-7758.

[53] Sirk, T.W., Brown, E.F., Friedman, M., Sum, A.K. Molecular binding of catechins to biomembranes: relationship to biological activity. *J.Agric. Food Chem.*, 2009; 57: 6720-6728.

[54] Cho, Y.S., Schiller, N.L., Kahng, H.Y., Oh, K.H. Cellular responses and proteomic analysis of *Escherichia coli* exposed to green tea polyphenols. *Curr. Microbiol.*, 2007; 55: 501-506.

[55] Ikigai, H., Nakae, T., Hara, Y., Shimamura, T. Bactericidal catechins damage the lipid bilayer. *Biocim. Biophys. Acta*, 1993; 1147: 132-136.

[56] Sharma, A., Gupta, S., Sarethy, I.P., Dang, S., Gabrani, R. Green tea extract: possible mechanism and antibacterial activity on skin pathogens. *Food Chem.*, 2012; 135: 672-675.

[57] Blanco, A.R., Sudano-Roccaro, A., Spoto, G.C., Nostro, A., Rusciano, D. Epigallocatechin gallate inhibits biofilm formation by ocular staphylococcal isolates. *Antimicrob. Agents Chemother.*, 2005; 49: 4339-4343.

[58] Sugita-Konishi, Y., Hara-Kudo, Y., Amano, F., Okubo, T., Aoi, N., Iwaki, M., et al., Epigallocatechin gallate and gallocatechin gallate in green tea catechins inhibit extracellular release of vero toxin from enterohemorrhagic *Escherichia coli* O157:H7. *Biochim. Biophys. Acta*, 1999; 1472: 42-50.

[59] Shah, S., Stapleton, P.D., Taylor, P.W. The polyphenol (-)-epicatechin gallate disrupts the secretion of virulence-related proteins by *Staphylococcus aureus*. *Lett. Appl. Microbiol.*, 2008; 46: 181-185.

[60] Wang, Y., Ma, S. Recent advances in inhibitors of bacterial fatty acid synthesis type II (FASII) system enzymes as potential antibacterial agents. *Chem MedChem*, 2013; 8(10): 1589-1608.

[61] Zhang, Y.M., Rock, C.O. Evaluation of epigallocatechin gallate and related plant polyphenols as inhibitors of the FabG and FabI reductases of bacterial type II fatty-acid synthesis. *J. Biol. Chem.*, 2004; 279: 30994-31001.

[62] Li, B.H., Zhang, R., Du, Y.T., Sun, Y.H., Tian, W.X. Inactivation mechanism of the beta-ketoacyl-[acyl carrier protein] reductase of bacterial type-II fatty acid synthesis by epigallocatechin gallate. *Biochem. Cell Biol.*, 2006; 84: 755-762.

[63] Sakanaka, S., Okada, Y. Inhibitory effects of green tea polyphenols on the production of a virulence factor of the periodontal-disease-causing anaerobic bacterium *Porphyromonas gingivalis*. *J. Agric. Food Chem.*, 2004; 52: 1688-1692.

[64] Okamoto, M., Leung, K.P., Ansai, T., Sugimoto, A., Maeda, N. Inhibitory effects of green tea catechins on protein tyrosine phosphatase in *Prevotella intermedia*. *Oral Microbiol. Immunol.*, 2003; 18: 192-195.

[65] Okamoto, M., Sugimoto, A., Leung, K.P., Nakayama, K., Kamaguchi, A., Maeda, N. Inhibitory effect of green tea catechins on cysteine proteinases in *Porphyromonas gingivalis*. *Oral Microbiol. Immunol.*, 2004; 19: 118-120.

[66] Grandišar, H., Pristovšek, P., Plaper, A., Jerala, R. Green tea catechins inhibit bacterial DNA gyrase by interaction with its ATP binding site. *J. Med. Chem*., 2007; 50: 264-271.

[67] Navarro-Martinez, M.D., Navarro-Peran, E., Cabezas-Herrera, J., Ruiz-Gomez, J., Garcia-Canovas, F., Rodriguez-Lopez, J.N. Antifolate activity of epigallocatechin gallate against *Stenotrophomonas maltophilia*. *Antimicrob. Agents Chemother.*, 2005; 49: 2914-2920.

[68] Navarro-Martinez, M.D., Garcia-Canovas, F., Rodriguez-Lopez, J.N. Tea polyphenol epigallocatechin-3-gallate inhibits ergosterol synthesis by disturbing folic acid metabolism in *Candida albicans*. *J. Antimicrob. Chemother.*, 2006; 57: 1083-1092.

[69] Chinnam, N., Dadi, P.K., Sabri, S.A., Ahmad, M., Kabir, M.A., Ahmad, Z. Dietary bioflavonoids inhibit *Escherichia coli* ATP synthesis in a differential manner. *Int. J. Biol.*, 2010; 46: 478-486.

[70] Yam, T.S., Hamilton-Miller, J.M., Shah, S. The effect of a component of tea (*Camellia sinensis*) on methicillin resistance, PBP2' synthesis, and beta-lactamase production in *Staphylococcus aureus*. *J. Antimicrob. Chemother.*, 1998; 42: 211-216.

[71] Zhao, W.H., Hu, Z.Q., Hara, Y., Shimamura, T. Inhibition by epigallocatechin gallate (EGCg) of conjugative R plasmid transfer in *Escherichia coli*. *J. Infect. Chemother.*, 2001; 7: 195-197.

[72] Sudano Roccaro, A., Blanco, A.R., Giuliano, F., Rusciano, D., Enea, V. Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. *Antimicrob. Agents Chemother.*, 2004; 48: 1968-1973.

[73] Lee, K.M., Yeo, M., Choue, J.S., Jin, J.H., Park, S.J., Cheong, J.Y., et al., Protective mechanism of epigallocatechin-3-gallate against *Helicobacter pylori*-induced gastric epithelial cytotoxicity via the blockage of TLR-4 signaling. *Helicobacter*, 2004; 9: 632-642.

[74] Williamson, M.P., McCormick, T.G., Nance, C.L., Shearer, W.T. Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: potential for HIV-1 therapy. *J. Allergy Clin. Immunol.*, 2006; 118: 1369-1374.

[75] Fink, R.C., Roschek, B., Jr., Alberte, R.S. HIV type-1 entry inhibitors with a new mode of action. *Antivir. Chem. Chemother.*, 2009; 19(6): 243-255.

[76] Yang, J., Li, L, Tan, S., Jin, H., Qiu, J., Mao, Q., et al., A natural theaflavins preparation inhibits HIV-1 infection by targeting the entry step: potential applications for preventing HIV-1 infection. *Fitoterapia*, 2012; 83: 348-355.

[77] Hauber, H., Hohenberg, H., Holstermann, B., Hunstein, W., Hauber, J. The main green tea polyphenol epigallocatechin-3-gallate counteracts semen-mediated enhancement of HIV infection. *Proc. Natl. Acad. Sci.*, 2009; 106(22): 9033-9038.

[78] Lin, Y.T., Wu, Y.H., Tseng, C.K., Lin, C.K., Chen, W.C., Hsu, Y.C., et al., Green tea epicatechins inhibit hepatitis C virus replication via cycloxygenase-2 and attenuate virus-induced inflammation. *PloS One*, 2013; 8(1): e54466.

[79] Liu, S., Li, H., Chen, L., Yang, L., Li, L., Tao, Y., et al., (-)-Epigallocatechin-3-gallate inhibition of Epstein-Barr virus spontaneous lytic cycle infection involves ERK1/2 and PI3-K/Akt signaling in EBV-positive cells. *Carcinogenesis*, 2013; 34(3): 627-637.

[80] Reygaert, W., Jusifi, I. Green tea as an effective antimicrobial for urinary tract infections caused by *Escherichia coli*. *Front. Microbiol.*, 2013; 4: 162.

[81] Stoicov, C., Saffari, R., Houghton, J.M. Green tea inhibits *Helicobacter* growth in vivo and in vitro. *Int. J. Antimicrob. Agents*, 2009; 33(5): 473-478.

[82] Noda, T., Ojima, T., Hayasaka, S., Murata, C., Hagihara, A. Gargling for oral hygiene and the development of fever in childhood: a population study in Japan. *J. Epidemiol.*, 2012; 22(1): 45-49.

[83] Rowe, C.A., Nantz, M.P., Bukowski, J.F., Percival, S.S. Specific formulation of *Camellia sinensis* prevents cold and flu symptoms and enhances gamma,delta T cell function: a randomized, double-blind, placebo-controlled study. *J. Am. Coll. Nutr.*, 2007; 26(5): 445-452.

[84] Oxford, J.S., Lambkin, R., Guralnik, M., Rosenbloom, R.A., Petteruti, M.P., DiGian, K., et al., In vivo prophylactic activity of QR-435 against H3N2 Influenza virus infection. *Am. J. Ther.*, 2007; 14(5): 462-468.

[85] Matsumoto, K., Yamada, H., Takuma, N., Niino, H., Sagesaka, Y.M. Effects of green tea catechins and theanine on preventing influenza infection among healthcare workers: a randomized controlled trial. *BMC Complement. Altern. Med.*, 2011; 11:15.

[86] Park, M., Yamada, H., Matsushita, K., Kaji, S., Goro, T., Okada, Y., et al., Green tea consumption is inversely associated with the incidence of influenza infection among schoolchildren in a tea plantation area of Japan. *J. Nutr.*, 2011; 141(10): 1862-1870.

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Chapter 3

THE PROTECTIVE EFFECTS OF INTRAHIPPOCAMPAL APPLICATION OF GREEN TEA LEAF EXTRACT ON ALUMINIUM-INDUCED BRAIN TOXICITY

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ABSTRACT

Diets have attracted great interest on the account of growing evidence of their beneficial effects on human health. Green tea has been used for a very long time as a folk remedy for a wide array of diseases. The well-known green tea beverage is made from a plant *Camellia sinensis*. The healthy properties of green tea are linked closely to its content of phenolic compounds, particularly to the (-)-epigallocatechin-3-gallate.

It has been proposed that green tea may have a beneficial impact on a number of brain functions, as well as on neurodegenerative disorder prevention in humans and in various animal models, including Alzheimer's disease (AD). A large number of scientific studies have supported some of these assumptions.

In the case of AD, aluminium may have an important role in the disease aetiology/pathogenesis/precipitation. However, aluminium has biological effects in the green tea plant, where it is a cofactor for polyphenol biosynthesis. Consequently, leaves of green tea accumulate and store large quantities of this element during the plant growth. Thus, it was intriguing whether the unilateral intrahippocampal application of green tea leaf extract (GTLE) and aluminium chloride would have any interaction, measured by the

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biochemical parameters in six brain structures: the forebrain cortex, striatum, basal forebrain, hippocampus, brain stem and cerebellum, of the adult male Wistar rats.

It was found that GTLE given alone demonstrated biochemical effects not only in the ipsilateral hippocampus, but also spread into the five other examined structures at the same side, as well as into the identical brain structures on the contralateral hemisphere. In fact, there were no differences in the activity of superoxide dismutase, cytochrome *c* oxidase (COX) and acetylcholinesterase (AChE) between the right and the corresponding left brain structures. Moreover, the activity of COX and AChE were significantly higher when compared to the control group.

Out of the three observed parameters, the content of reduced glutathione (GSH), superoxide anion and nitrites, aluminium itself demonstrated the strongest effects towards GSH, which was significantly reduced in all structures, compared to the control group. The changes were identical in the ipsi- and contralateral corresponding structures. Howewer, the application of GTLE just before aluminium prevented the reduction of GSH induced by aluminium, and significantly increased its content compared to the control group. Also, the content of superoxide anion was significantly reduced in most structures compared to the control, and to the aluminium-treated group as well.

The obtained results of GTE in the aluminium-induced neurotoxicity are in accordance with the antioxidant effects of GTLE. Also, it is clear that GTE administered alone did not demonstrate neurotoxic effects as did the solution of aluminium chloride, but, on the contrary, showed the opposite, neuroprotective effects. To sum up, GTLE has proved to manifest strong antioxidant effects in the brain of healthy rats, and in the cases of neurotoxicity induced by aluminum, as well.

ABBREVIATIONS FOR FIGURES 2-7

I: the brain structures ipsilateral to the injection site; C: the brain structures contralateral to the injection site; FbC: forebrain cortex; S: striatum; BFb: basal forebrain; H: hippocampus; BS: brainstem; Cer: cerebellum.

INTRODUCTION

Oxidative stress, the imbalance between the generation of free radicals and antioxidants which should efficiently defend against them, is indicated to play the central role in aging, but also in a number of acute and chronic neurological diseases. Among them are neurodegenerative diseases, one of which is Alzheimer's disease (AD) [1, 2].

Due to the increase in life expectancy, population is geting older, and the incidence of neurodegenerative diseases is growing both in the developed and in developing countries. Therefore, they represent a great health, economic and social burden. Consequently, the costs of treatments and care for the affected population are expected to rise dramatically. For these reasons, there is a need to prevent or reduce their occurrence, but also to reduce or slow down the progression of the already existing illnesses. In line with this, there is a great interest to diagnose the disease, as well as to start the treatment as early as possible. Unfortunately, both goals are quite difficult to achieve, especially due to a huge etiopathogenetic and clinical variety within these disorders.

Despite the vaguely known etiology and pathogenesis of neurodegenerative diseases, some risk factors have already been recognized. Among the most important ones are certain genetic polymorphisms and aging. More attention is also being focused on the potential environmental agents for damaging the brain. One of them is aluminium, whose uncontroversial neurotoxicity has been known for longer than a century. Moreover, recent findings have shown a more reliable link between the etiology/pathogenesis/ precipitation of AD and aluminum.

Oxidative stress has been accepted as an important factor in the initiation of specific neurodegenerative disease pathologies and/or their progression. Therefore, it represents a therapeutic target aiming to manage a very complex cross-talk between misfolded proteins and iron, as well as between misfolded proteins and free radicals in these diseases. Currently there is no effective treatment, especially if a single drug/product is applied.

There are an exceptional number of attempts to deal with the application of natural resources that could influence the course of neurodegenerative diseases. Among them are flavonoids as the most abundant natural antioxidants in dietary sources. The accumulated evidence concerning green tea, which is very rich in the polyphenolic catechins, offers promising neuroprotective effects due to its diverse biological and pharmacological activities. A number of both, human epidemiological studies and research in different animal models of neurodegenerative diseases, suggest that green tea may improve age-related cognitive decline and reduce dementia in neurodegenerative disorders [3]. Furthermore, it is suggested that green tea consumption exibits inverse correlation with the incidence of dementia and AD [4, 5].

ALZHEIMER'S DISEASE

Neurodegenerative diseases have a chronic course with a gradual and progressive deterioration of the structure and function of neurons and glial cells in the brain, until their final loss [6]. That leads to the development of a wide range of distinct neuropathological and neurological signs and symptoms. However, different neurodegenerative diseases share many similarities both in clinical manifestation, and in neuropathological findings.

Alzheimer's disease is one of the most common neurodegenerative irreversible diseases, affecting approximately 7% of people older than 65 and about 40% of people older than 80 in industrialized countries [7].

The etiopathogenesis of AD is extremely complex, heterogeneous and not fully understood. It is still difficult to recognize all biochemical pathways in this chronic CNS injury, resulting in many gaps in the understanding of AD. On the other hand, histopathological and clinical features are fairly well characterized. Protein maintenance and degradation have been shown to be highly affected in AD and abnormally generated toxic protein species have be found to be the key pathological indicators of the disease [8].

AD is manifested in the following changes:

1. The deposition of amyloid beta (Aβ): the sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase generates the toxic Aβ fragment [9]. It

is the main constituent of senile plaques placed extracellularly and in the cerebral blood vessel walls;
2. The neurofibrillary tangles that are located within neurons, are the result of hyperphosphorylated microtubule-binding tau protein;
3. The astrogliosis, microgliosis and inflammatory mediators, such as cytokines and chemokines, which are released by the activated cells (microglia, astrocytes, macrophages and lymphocytes);
4. The degeneration and loss of synapses and
5. The death of neuronal cells, which is not found globally, but primarily in the AD-vulnerable parts of the brain.

The prominent place in AD pathology is taken by the entorhinal cortex, hippocampus, forebrain cortex and basal forebrain. Each type of brain pathology shows spatio-temporal spreading during the disease progression from preclinical (prodromal, asymptomatic) stage that could last for about 20-30 years to the developed dementia, that is clinically characterized by a gradual loss of cognitive functions and by a gradual increment of behavioral disturbances.

The molecular mechanisms underlying the etiopathogenesis of AD are not unique. At the current level of knowledge, it is usually impossible to separate the causes and the consequences at the biochemical, pathological or neurochemical levels. Research findings support the theory that oxidative stress causes both the very basic changes in AD and those that occur during AD progression as well [10]. As a result of these findings, a free radical theory concerning AD has arisen in the scientific community.

The Free Radical and Oxidative Stress Theory in Alzheimer's Disease

Free radicals are highly reactive molecules of diverse chemical species. The free radical theory is very useful as it could explain some of the processes that occur in AD. It also represents a good starting point for the treatment, which is expected to slow down or even stop the progression of the disease [7]. However, in spite of the advantages, this theory is too simplistic to explain all the aspects of AD etiopathogenesis.

The theory of free radicals in Alzheimer's disease includes:

1. Oxidative stress;
2. The dysregulation of the redox homeostasis of transitional metals - metallostasis;
3. Oxidative modification of proteins.

1. Oxidative Stress

Free radicals are unavoidably formed as the by-products of aerobic respiration and other catabolic and anabolic processes, which is a mild oxidative and nitrosative stress. In physiological conditions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are made continuously in cells and are balanced by antioxidant defense to maintain the redox homeostasis involved in multiple physiological processes. Their potential damaging effects are normally counteracted by endogenous anti-oxidants. A state in which antioxidant defenses are overwhelmed and are no longer capable of protecting the cell from oxidative damage is

designated as oxidative stress that is also found in AD [11]. Walton concluded: "Oxidative damage has been called the earliest event in AD" [10].

The final result of oxidative stress is the activation of intracellular signaling cascades that may have a deleterious effect on the cellular homeostasis and the development of the oxidative damage of biomolecules like lipids, proteins and genetic structures. The biomarkers of oxidative stress are measurable substances that can indicate the level of oxidative damage of biomolecules, as well as the effects of the applied therapeutic substances. Mitochondrial dysfunction and Aβ aggregation are of particular interest in the free radical theory.

Mitochondrial dysfunction. Brain mitochondria designate both synaptic and nonsynaptic mitochondria. They play a key role in the functioning and survival of cells. Furthermore, mitochondria have been proposed to have a central place in aging and neurodegenerative diseases [12]. They are the primary intracellular site of oxygen consumption. Electron transport chain (ETC) in mitochondria is the principal site of endogenous ROS generation under physiological and pathological conditions. They are the main source of energy production (production of adenosine triphosphate - ATP) and the regulator of calcium homeostasis. Mitochondria are both the major producer and the target of free radicals. In the oxidative stress induced by a multitude of causes, including aluminum, mitochondria are a place of a vicious circle, which leads to energy depletion and mitochondrial DNA damage, followed by compromised synaptic function, synaptic degeneration and finally the cell death [13].

The activity of cytochrome *c* oxidase (COX), the complex IV and the terminal enzyme of the ETC, is decreased in AD and aluminium overload. That precisely demonstrates the damaged mitochondrial respiration that unavoidably leads to the overproduction of free radicals and the reduction of energy production [14, 15, 16, 17]. Furthermore, the localization of Aβ in mitochondrial membranes could explain mitochondrial dysfunction in AD sufferers [18].

Aβ peptide in the free radical generation. Aβ fibrils are not inert, but redox-active metalloproteins. They could bound to iron and copper ions, which is then followed by the Aβ aggregation and oligomerization. Moreover, there is accumulating evidence of the generation of free radicals in the reaction between Aβ and iron, such as nitric oxide (NO) and ROS [19]. Aβ was shown to impair mitochondrial function *via* ROS production. Both the APP and Aβ located in mitochondria, contribute to the oxidative damage, including mitochondrial respiratory enzymes and other biomolecules [20].

2. The Dysregulation of the Redox Homeostasis of Transitional Metals – Metallostasis

The prooxidant role of transition metal ions, such as iron, copper and zinc, is well documented. Under uncontrolled conditions with the excess of transition metals, a huge amount of reactive oxygen species could be generated. In line with this is the elevated iron levels in the brains of AD patients that were registered for the first time more than half a century ago. It is accumulated within, but also around amyloid plaques, neurofibrillary tangles and reactive microglia, i.e. iron is co-localized with plaques and tangles [21]. Aluminum was also found to be linked with neurodegenerative diseases much earlier, but that fact was not interpreted correctly for a long time [22].

The iron accumulation is the consequence of a large number of disorders of proteins that regulate its homeostasis. The fatigue in regulation mechanisms of metal metabolism is known

as metallostasis [22]. It could provoke the aggregation of BA and tau protein, but it could also lead to a high iron accumulation in the brain. It means that high levels of iron and other transition metals could contribute to self-aggregation of Aβ into neurotoxic fibrils and to formation of neurofibrillary tangles, which, in turn, lose their transport role of these metals. Thus, there is a two-way regulatory influence between pathologically changed proteins in AD and iron. Both of these protein-iron complexes are the sources of ROS production and oxidative stress. This suggests that metal chelators have the potential role to reduce oxidative stress during AD [23].

3. Oxidative Modification of Proteins

Several studies have shown that the oxidative damage is induced by the accumulation of conformational changed proteins, which are developed due to their oxidative modification. These modified proteins are a characteristic feature of many neurodegenerative diseases. Such misfolded proteins have increased the potential for aggregation that alters protein function, and finally leads to cell death. The accumulation of ROS/RNS is involved in multiple disturbances of proteins, such as protein fragmentation, protein misfolding and aggregation, insufficient proteasomal degradation of proteins, etc. [2, 24]. But, there is also the opposite direction: Aβ has a high affinity for transitional metals and is able to produce ROS in these oxido-reductive reactions, which increases Aβ toxicity. Furthermore, misfolded proteins induce the inappropriate compartmentalization of iron. Altogether, the interaction of misfolded proteins with iron enhances the generation of free radicals and the development of oxidative stress.

Aluminium in Alzheimer's Disease - The Metal Hypothesis

The metal hypothesis of AD is built upon the ability of some metals, like mercury, lead and aluminium, for example, to interact with certain processes that are involved in the etiopathogenesis of AD. Among them, aluminium has a very important role, as humans consume it unknowingly during the whole life, from the fetal period to death [25]. The earliest records relating to its use as a water cleaner date back to 77 AD [10].

Aluminium is widely used, particularly in industrialized societies, and people are permanently being exposed to it, generally in everyday life usually by the oral route. Sometimes there is an occupational exposure as well. It is applied for settling water pollutants, as an additive in processed foods and beverages, including infant formulas. It is an integral part of some cosmetic products, drugs, vaccines, etc. [26].

For a long time, aluminium was considered to be innocuous and harmless. However, it has been proved to be quite the opposite: in the ionic state (Al^{3+}), aluminium is highly biologically reactive and it could have deleterious effects on human health [27]. The aluminium accumulation has been associated with a variety of human pathologies, including neurotoxicity.

Aluminium is progressively accumulated in human brains over time and during the process of ageing. Unfortunately, aluminium toxicity is often not mentioned, especially by the industry, which puts it in many products [27]. That is why it is necessary to get the proper perception of its hazards to human health as early as possible and to make efforts towards the

legislative consideration of the levels of aluminium in food, drinking water and drugs, including vaccines.

The accumulation of aluminium in the brain is not uniform, but it was found to be higher in the brain regions preferentially susceptible to damage in AD: the entorhinal cortex, hippocampus, neocortex and basal forebrain [28]. Under experimental conditions, but also in post mortem analyses of human AD brains, it has been suggested that aluminium could be the trigger for all key biochemical and histopathological events found in AD.

The recent research has reinforced the potential causality between aluminium and AD, as well as dementia. This is in accordance with the first description of this connection, in 1980, which has been disputed very often and for a very long time [29]. On the basis of a great number of recently performed epidemiological and clinical research studies, and case reports, the involvement of aluminum in the etio-pathogenesis of AD and a relation between aluminum intake and the prevalence of AD, can be more reliably claimed [30]. All results are not always compliant, though. It was found that prolonged exposure to aluminium in drinking water increases the risk of the development of AD, especially in the elderly [31]. Occupational epidemiological studies also demonstrated the involvement of aluminium in AD [32, 33]. Finaly, beside other conclusions about aluminium exposure and brain toxicity, Walton stated the following: "The evidence supports the concept that AD is a human form of chronic aluminum neurotoxicity" [10].

There are multiple mechanisms that of aluminium neurotoxicity. Some of them are:

1. Pro-oxidant properties because of its propensity to be bound by oxygen-based functional groups associated with myriad biomolecules. This has been demostrated *in vitro*, *in vivo*, in acute and chronic exposure to aluminium.
   Aluminium could induce oxidative stress independently, but also in synergy with iron. Chronic exposure to aluminium was found to increase not only its own concentration in the brain, but also, the concentration of iron, copper, manganese and zinc, for example, in the hippocampus [34]. Furthermore, two antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, are particularly inhibited by aluminium. Aluminium also decreases the mitochondrial respiration and inhibits cytochrome *c* oxidase, indicating mitochondrial dysfunction [17, 35, 36].
   Aluminium competes with magnesium, iron and calcium ions for the same binding sites in/on proteins and other biomolecules. Because of its higher affinity, aluminium could substitute these essential metals, interfering with the physiological processes in which these elements are involved. Furthermore, the occupied molecules are blocked with aluminium, in contrast with the fast dissociation between them and essential metals. Because of the dysregulated metabolism of these ions and their disrupted homeostasis, a great number of disorders, including disturbances in enzymes and other regulatory factors in the cell function, may be affected. The resulting changes are similar to those occurring in AD [28].
2. Inflammation: microglia and astrocytes become activated in the presence of aluminum, indicating inflammatory processes [37, 10].
3. Under experimental conditions, the accumulation of aluminium in the brain precedes the oxidative stress that induces the misfolding of tau protein and Aβ, as well as other biomolecules. Furthermore, the mitochondrial dysfunction is potentiated by Al-Aβ complex, compared to Aβ alone [38].

4. The oxidative stress induced by aluminium is involved in the formation of Aβ plaques and further affects metabolism of Aβ in various ways, including the inhibition of Aβ clearance that leads to amyloidogenesis [39].
5. The hyperphosphorylated tau and neurofibrillary tangles are formed in aluminium rich neurons, since aluminium induces the aggregation of these proteins and retards their proteolysis. The consequences are the damage of neuronal cytoskeleton and, thus, of its physiological function for intracellular communication and transport. The final consequence is the death of neurons.

All the aforementioned changes in the brain (1-6), induced by aluminium, support the therapeutic approach with antioxidants and chelating agents [40].

The Treatment of Neurodegenerative Diseases, Includig Alzheimer's Disease

Despite the heterogeneous etiopathogenesis of neurodegenerative diseases, including AD, their clinical manifestation and outcome, it seems that the oxidative damage of biomolecules and the increased accumulation of iron take common ground in the vulnerable brain regions relevant in CNS disorders. They, therefore, represent the target for treatment with the intention to stop or delay the appearance/progression of the cognitive, functional and motor deterioration, or even to provide some sort of improvement.

It becomes evident that the neurodegenerative disease treatment, due to their complex pathogenesis, is neither easy nor simple, and that a great treatment success cannot be expected as there are no disease-modifying therapies. Therefore, multiple target therapies should be considered, if any possible therapeutic agents can be found. In spite of the lack of systematic clinical trials with green tea polyphenols in neurodegenerative diseases, the accumulated evidence from preclinical, epidemiological, and prospective studies suggests that green tea consumption inversely correlates with the incidence of dementia, AD, and PD. Thus, green tea appears to be one of the promising agents in AD and, generally, in neurodegenerative disease treatment as well.

EFFECTS OF GREEN TEA IN THE BRAIN, INCLUDING ALZHEIMER'S DISEASE

Green tea beverage is made from the *Camellia sinensins* plant leaves. There are four types of tea altogether: black which is fermented, green which is non-fermented, oolong which is partially (semi)-fermented and white which is made from leave buds that were not subjected to any fermentation.

Green tea contains a number of bioactive compounds, but it is particularly rich in flavonoids, named catechins, which have the strongest antioxidant properties. The main polyphenol constituent of green tea is (-)-epigallocatechin-3-gallate (EGCG). Several other polyphenols are found in lower abundance such as: (-)-epigallocatechin (EGC), (-)-epicatechin (EC) and (-)-epicatechin-3-gallate (ECG). Approximately 600 different molecules are found in green tea leaves. Apart from polyphenols, there are a number of other bioactive

chemicals, such as caffeine, theanine, carbohydrates, vitamins A, K, B and C, aminophylline, etc. [41].

A considerable number of studies have demonstrated the beneficial effects of green tea in the treatment of neurodegenerative disorders, including AB, Parkinson disease, Huntington's disease, multiple sclerosis, disease- and ageing-associated cognitive decline, etc. [42, 43]. A great number of research studies in animal models of these diseases demonstrated similar effects [17, 44, 9]. Thus, the implementation of green tea as a disease-modifying agent is being examined thoroughly [45, 46].

Although the potential health benefits of green tea have been partially attributed to its antioxidant properties, this aspect of the therapeutic effects of green tea as the only mechanism seems to be overdrawn. In fact, pharmacological active components in green tea exert various multifunctional properties in neuroprotection. Here are the mechanisms of action of green tea which, first of all, refer to the EGCG.

1. Antioxidant effects of polyphenols could be obtained in different pathways:
   - Scavenging of free radicals since the polyphenols are powerful hydrogen-donating compounds, which possess iron radical scavenging properties. Among all polyphenols, EGCG was shown to be more efficient in reacting with the most reactive oxygen, as well as nitrogen species, like nitric oxide and peroxynitrite ($ONOO^-$);
   - Metal-chelating activity enables the chelating of excess metal ions accumulated in the brain, like iron, copper and zinc. By forming a stable complex with a metal and thereby decreasing its toxicity, polyphenols reduce the formation of free radicals and the histopathological hallmarks of neurodegenerative diseases. Polyphenols present in tea infusions are important Al-complexing compounds, as well [47];
   - The prevention of oxidative modification of biomolecules by transferring an polyphenolic electron to ROS-induced radical sites on biomolecules;
   - The influence on antioxidant/prooxidant enzymes:
     a. They elevate the activity of some antioxidant enzymes thereby promoting endogenous antioxidant capacity. The activity of SOD, catalase, glutathione peroxidase and reductase is increased in the brain [48];
     b. They inhibit enzymes which are involved in ROS generation and whose activity may promote oxidative and nitrosative stress, like iNOS, nNOS, and monoamine oxidase B (MAO-B) [44, 45]. When all the above mentioned is taken into account, it can be reliably concluded that green tea exhibits strong antioxidant effects.
2. The green tea polyphenols interfere with the aggregation of amyloidogenic proteins and ameliorate their detrimental effects. They modulate the aggregation of proteins implicated in neurodegenerative diseases, such as huntingtin, Aβ, and α-synuclein. With regard to AD, it has an impact on both types of the fibrillar protein aggregating species (Aβ and tau).
   - The reduction of cerebral amyloidosis and senile plaques was demonstrated. The diminished ability of amyloidogenesis was the consequence of the direct binding of natural flavonoids to unfolded Aβ and stabilisation of these assembly-incompetent forms. As a result of that binding, further fibrilization is prevented.

Furthermore, the non-amyloidogenic pathway in proteolytic cleavage of amyloid precursor protein (APP) mediated via α-secretase processing is enhanced [49]. Under experimental conditions, EGCG was found to prevent the Aβ-induced cell death and cognitve decline [45]. Also, green tea has beneficial effects against beta-amyloid-induced oxidative and nitrosative stress [44];

- New data related to the impact of green tea on the tau protein misfolding demonstrated the inhibitory effects of EGCG on tau aggregation. It prevents the formation of toxic tau oligomers [50].

3. The neuroprotective effects of EGCG could be obtained by the activation of the protein kinaze C (PKC), which is directly or indirectly involved in various signaling pathways. It has an essential role in the regulation of cell survival and programmed cell death. Its activation prevents mitochondrial dysfunction and apoptosis, improves cell survival, APP metabolism, and memory function as well. All of these pathways are of great importance considering the standpoint of AD [51].
4. The neuroinflammation that is linked to the production of inflammatory substances and free radicals is suppressed by EGCG. In the etiopathogenesis of AD and other neurodegenerative diseases, central and peripheral inflammation has a very important role in its etiopathology [5].

It seems reasonable that green tea could act on multiple pathophysiological brain targets in order to provide neuroprotection/ neurorescue.

PROTECTIVE EFFECTS OF GREEN TEA LEAF EXTRACT ON ALUMINIUM-INDUCED BRAIN TOXICITY

Material and Methods

Animals and Experimental design

Male adult Wistar rats were used for these experiments. They were 12 weeks old at the beginning of the experiments. The animals were allowed free access to commercial rat food and tap water under controlled light and environmental conditions (a 12 h/12 h light/dark cycle, room temperature 23±2°C). The research protocol was approved by the local ethics committee for animal experimentation (Military Medical Academy, Belgrade, Serbia), which is in harmony with international regulations.

On a stereotaxic instrument for small animals, the solutions of the examined substances were injected by Hamilton micro syringe into the left brain hemisphere Cornu Ammonis region 1 (CA1) of the hippocampus, a limbic system structure. The position for the injection was determined relative to the lambda suture, defined from its centre: 3.1 mm dorsally, 4.3 mm laterally and 2.5 mm ventrally from the skull surface (Figure 1) [52]. During these procedures, rats were in the total anaesthesia induced by intraperitoneally applied sodium thiopental (Specia, Paris), in the dosage of 0.04 g/kg body weight. The recovery period after treatments lasted 12 days. Thereafter the rats were sacrificed by decapitation. The heads were

flash-frozen in liquid nitrogen and stored at -70 °C until brain samples were prepared for biochemical analysis.

Treatments

Four groups of rats were treated. There were 9-12 animals in each group. All examined substances were applied as solutions in the volume of 0.01 mL. The first group received 0,9 per cent w/v NaCl (NaCl, control group), the second one green tea leaf extract (GTLE) alone (Green tea group), the third - aluminium chloride (Sigma-Aldrich, USA) at $3.7x10^{-4}$ g/kg body weight dissolved in sterile deionized water (AlCl3 group), while the fourth group received GTLE just before aluminium (Green tea+AlCl3 group). The rats were randomly subjected to treatments.

GTLE preparation: deionized water (10 mL) was boiled and added to 0.5 g of commercially available dried green tea leaves (Žid Trade, Belgrade, Serbia). 30 min later, the obtained extract was filtered through a standard Whatman laboratory filter paper. Before the application, GTLE was cooled to room temperature.

Biochemical analyses

Biochemical analyses were performed in crude mitochondrial fractions from the ipsi - and contralateral brain regions kept on ice while dissecting [53]. There were six regions which were examined: the forebrain cortex (FbC), striatum (S), basal forebrain (BFb), hippocampus (H), brain stem (BS) and cerebellum (Cer). The activities of superoxide dismutase (SOD, EC 1.15.1.1) [54], cytochrome *c* oxydase (COX, EC 1.9.3.1) [55], and acetylcholinesterase (AChE, EC 3.1.1.7) [56], as well as production of superoxide radical [57], nitrate and nitrite (NOx) [58], and reduced glutathione [59] were determined in these regions.

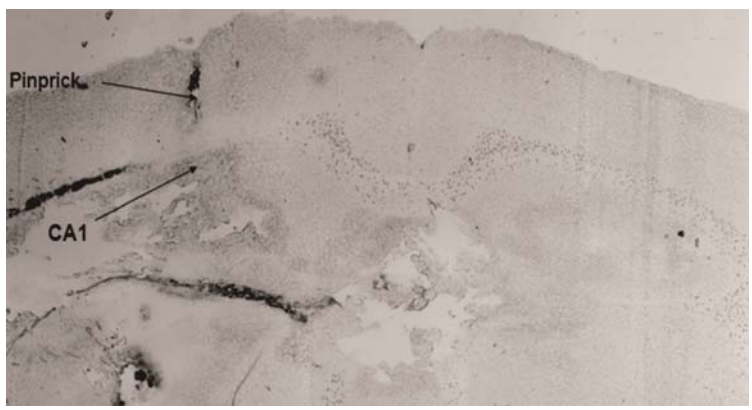

Figure 1. Native histological preparation of the hippocampus of both brain hemispheres with the site of pinprick in the CA1 hippocampal sector with ipsilateral lesion of pyramidal neurons in comparison to the contralateral side relative to the pinprick.

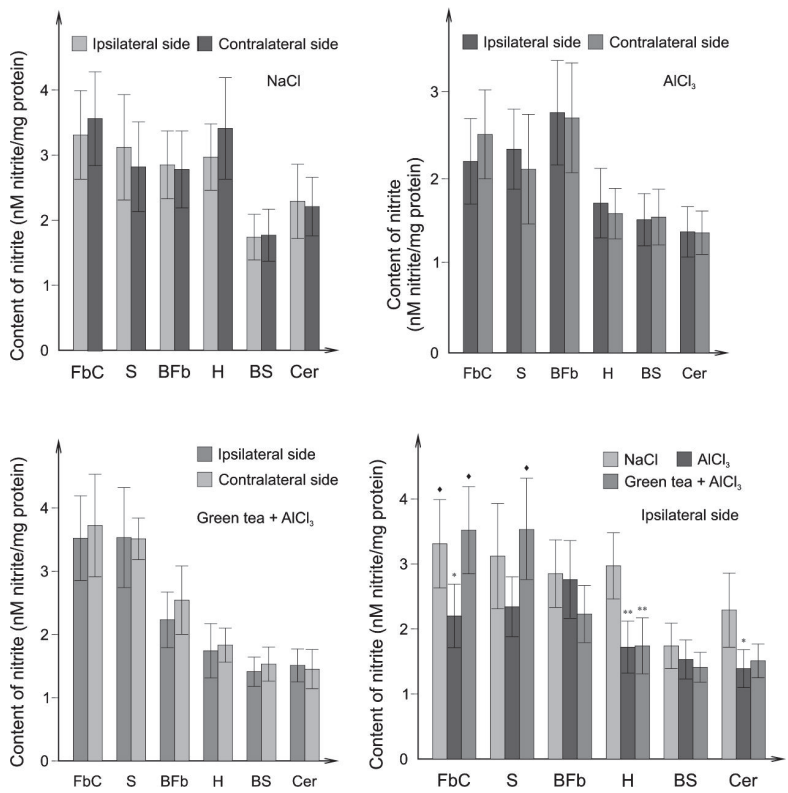

Figure 2. Content of nitrites in the brain of Wistar rats (n=9–12) intrahippocampally treated with 0.9% NaCl, aluminium chloride (AlCl3) and green tea leaf extract administered prior to AlCl3.
The values were expressed as mean ± SD. $p<0.05$, $p<0.01$ are the levels of statistical significance found versus 0.9% NaCl (*, **) and versus AlCl3-treated rats (♦, ♦♦) (Student's t-test). The rats were sacrificed 12 days after treatments.

Statistical Analysis

Data were expressed as means±SD. The Student's t-test was used for comparisons between groups. When data were given as percentages, the differences between two percentages were calculated. Statistical significance was determined as $p<0.05$.

Results and Discussion

Neurotoxic Effects of Aluminium

The acute hippocampal exposure to a huge single amount of aluminum led to changes in the chosen biochemical parameters. The decreased nitrite content in the forebrain cortex, hippocampus and cerebellum ($p<0.01$ for the hippocampus, $p<0.05$ for other two structures) was found (Figure 2). The content of superoxide radical was decreased in the forebrain cortex and basal forebrain ($p<0.05$ for both structures), while in the hippocampus and cerebellum this decrement did not rich statistical significance (Figure 3). Furthermore, GSH contents were decreased in all the examined brain structures ($p<0.05$ for all structures) (Figure 4). All the obtained results suggest the prooxidant effects of aluminium.

Not only were the toxic effects of aluminum present at the application site, but they also spread throughout the brain in the same hemisphere of the hippocampus in which aluminium was applied, as well as in the parts of brain that were more distant from the place of toxicant application. In fact, such changes were registered in the other, opposite hemisphere as well. When we compared all studied biochemical parameters in the corresponding structures of both hemispheres the differences between them could not be found (Figure 2, 3, 4). This extensive spatial propagation could be the result of a very complex anatomical and neurochemical communication within the brain. The connectivity between different brain regions within each brain hemisphere, as well as between the hemispheres themselves, is obtained through neurons organized into bundles known as the fornix and commissure, wherein the fornix is the largest efferent tract of the hippocampus [60]. Consequently, such neurological brain organization enables the spreading of the effects of GTLE and aluminum from the hippocampus, where they were applied, to other parts of the brain, both ipsi- and contralaterally.

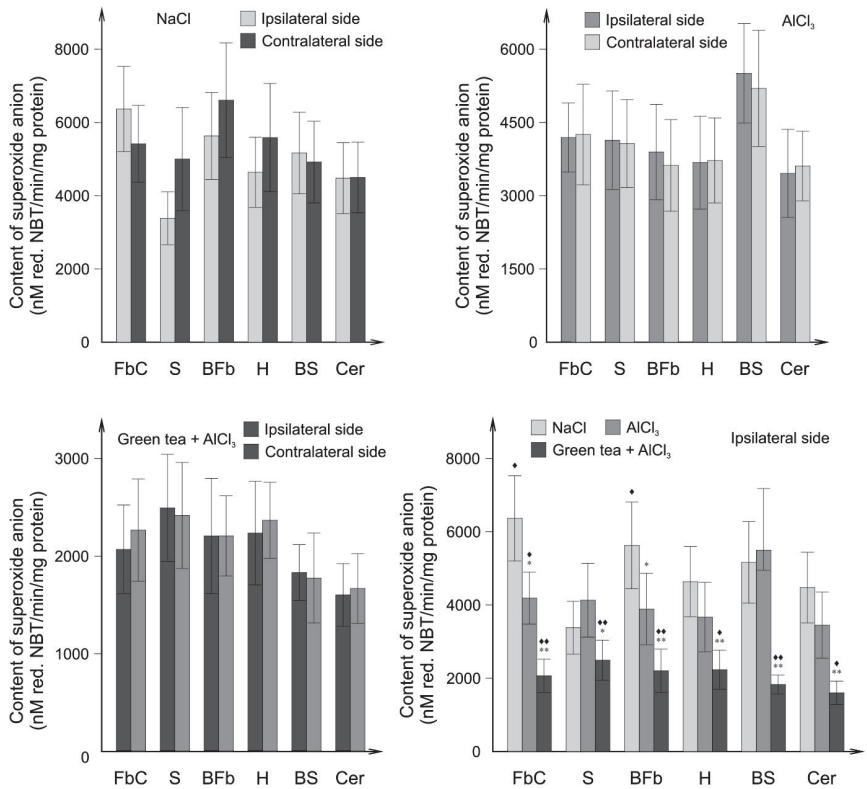

Figure 3. Content of superoxide anion in the brain of Wistar rats (n=9–12) intrahippocampally treated with 0.9% NaCl, aluminium chloride (AlCl3) and green tea leaf extract administered prior to AlCl3. The values were expressed as mean ± SD. $p<0.05$, $p<0.01$ are the levels of statistical significance found versus 0.9% NaCl (*, **) and versus AlCl3-treated rats (♦, ♦♦) (Student's t-test). The rats were sacrificed 12 days after treatments.

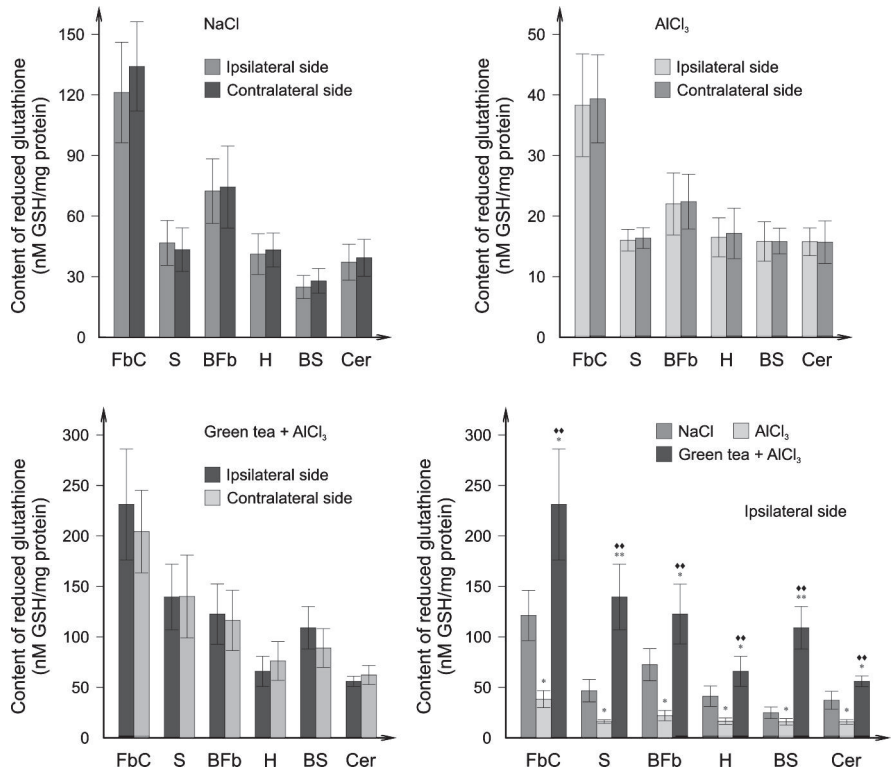

Figure 4. Content of reduced glutathione in the brain of Wistar rats (n=9–12) intrahippocampally treated with 0.9% NaCl, aluminium chloride (AlCl3) and green tea leaf extract administered prior to AlCl3.

The values were expressed as mean ± SD. $p<0.05$, $p<0.01$ are the levels of statistical significance found versus 0.9% NaCl (*, **) and versus AlCl3-treated rats (♦, ♦♦) (Student's t-test). The rats were sacrificed 12 days after treatments.

The spatial and temporal spreading of aluminum neurotoxicity was demonstrated both biochemically and immunohistologically [61]. Immunohistologically, this spreading was proved to be related to the two crucial hallmarks of AD: the generation of Aβ and the hyperphosphorilation of tau protein [62].

The hippocampus always appears to be an early target of age-related and AD structural and physiological neuropathology. The same effects are visible after the perioral alluminium intoxication. Out of all brain regions where oxidative stress is developed, the hippocampal mitochondria are the first to be affected [36]. Among the three sectors of the hippocampus (CA1, CA2 and CA3, while CA4 is disputed), the most vulnerable one is the CA1, which is suggested to be the primary output site from the hippocampus [8]. The hippocampus is essential for a range of memory functions including the acquisition of spatial reference memory tasks and the recollection of learning episodes. The hippocampal damage will disrupt the neurochemical connections between itself and other brain regions involved in learning and memory processes. In this way, the hippocampus is isolated from other brain regions, which, consequently, would have a strong impact on cognitive processes.

Memory defects due to aluminium accumulation are well documented in humans, as well as in animals treated with aluminium [10, 17]. The hippocampus and its CA1 sector are among the parts of the brain in which aluminium is particularly strongly accumulated, which was demonstrated in AD patients as well [10]. Thus, chelating agents administered in order to reduce the aluminium burden in AD patients and in aluminium intoxication under experimental conditions look promising.

Nitric oxide, a weak gaseous free radical, is a non-classical neurotransmitter. It maintains and regulates normal brain functions, such as signaling, memory and synaptic plasticity. It is included in the regulation of mitochondrial electron transport chain. The imbalance of its concentration is implicated in the pathogenesis of CNS diseases. The nitrosative stress and disturbed NO-soluble gualnilat cyclase - cyclic guanosine monophosphate (NO-cGMP) pathway are features of AD and it is attributed to amyloid-β neuropathology [63]. In our research, the decreased content of superoxide anion was almost parallel with the reduced content of NOx. Apart from being removed with SOD, this lower level of superoxide radical could indicate the yielding of a very toxic $ONOO^-$. That is the result of a rapid reaction of NO with superoxide radicals, since NO has a far higher affinity for the superoxide anion in competition with the mitochondrial SOD. $ONOO^-$, a very potent oxidant and a molecule which easily penetrates the membrane, appears to accelerate the free radical cascade, to damage the mitochondria and to have other detrimental effects on cell functions [64]. GSH reacts readily with RNS, including $ONOO^-$ and thereby protects the cells against oxidative damage. In our study, a very low level of GSH indicated the possibility of such a sequence of events (Figure 4).

Glutathione. The cells could be protected or repaired by antioxidants, in order to prevent deleterious effects of oxidative stress. Antioxidants are comprised of enzymatic and nonenzymatic compounds. Glutathione, a tripeptide antioxidant, is almost 90% located in cytosol that is the main place of its synthesis, considered to act as a major defence against ROS/RNS toxicity in the brain. Glutathione is homeostatically controlled, both inside the cell and outside. Besides that, it is an essential cofactor for many enzymes, interfering with energy and neurotransmitter synthesis, as well as with NO.

Glutathione exists in two forms: the antioxidant one is reduced glutathione (GSH), most commonly called glutathione, while the oxidized form is known as glutathione disulfide (GSSG). Physiologically, the concentration of GSH is 10-100 times higher than GSSG [65]. GSH has a great reducing power due to its high electron-donating capacity and high intracellular concentration. The deficiency of GSH contributes to the appearance of oxidative/nitrosative stress and its deterioration.

The levels of this nonenzymatic antioxidant appear to be a sensitive indicator of cell function and viability, antioxidant status of cell and its ability to resist endogene and exogene toxic attacs. According to the results of GSH content in the human brain, the decline of GSH is found in neurological diseases, including AD, in which its depletion precedes neurodegeneration [66].

In the aluminium treated animals in the present study, the GSH content was decreased in all the examined structures 12 days after the aluminium application (Figure 4). However, in the very early stage of intoxication (three hours after the aluminium application), the levels of GSH were increased in the examined forebrain cortex and striatum ($p<0.05$), and were at the control levels 30 days after the treatment, thus indicating its fluctuation during time [67].

A very large decrease of GSH levels that was obtained could be the consequence of its high consumption due to oxidative stress induced by aluminium. Also it could be due to the conditions of impaired GSH regeneration since the disrupted pentose phosphate pathway of glucose metabolism by aluminium. That was determined by the decreased glucose-6-phosphate dehydrogenase activities, which is one of the catalytic enzymes in this pathway [62]. However, the pentose phosphate pathway is the main source of the reduced form of nicotinic adenine dinucleotide phosphate (NADPH), which is necessary for GSH regeneration from GSSG, as the enzyme GSH reductase is NADPH dependent. As GSH is critical in prevention or reparation of oxidative damage during physiological and pathological conditions, mitochondrial dysfunction is quite a normal consequence in aluminium neurotoxicity [68].

Based on all the aforementioned, it can be concluded that aluminum neurotoxicity is associated with the same or similar biochemical and imunohistochemical changes in the brain which are also seen in AD.

Beneficial Effects of Green Tea in Healthy and Aluminium Treated Rats

Healthy Rats

Green tea is a rich source of aluminium. The tea plant takes it from the soil and stores it in plant leaves. It promotes the biosynthesis of polyphenols. Thus, the aluminium content in green tea is a concern, when chronic usage of this tea is in question. Whether the aluminium from this source could be neurotoxic or not, represents a controversy in the scientific community. In order to test this, we applied GTE into the CA1 part of the hippocampus of healthy rats. Instead of causing oxidative stress, which is normally registered upon aluminium chloride application, green tea itself caused just the opposite - protective effects. That was documented by a number of biochemical parameters. Furthermore, the beneficial effects of GTE were obtained in all the examined structures of both hemispheres in spite of its application into the CA1 sector of the hippocampus of one hemisphere (Figure 5, 6, 7).

The activity of COX. There were no differences between ipsi- and contralateral side of each corresponding structure, in either the control, or in the GTLE treated group (Figure 5). The GTLE treatment induced the increment of COX activity in all structures, when compared to NaCl-treated animals. The smallest increment was found in the forebrain cortex (29 and 43%, in the ipsi, and contralateral side, respectively). In all other structures it was much higher, and even reaching 116%. Except in the forebrain cortex, all the changes reached statistical significance ($p<0.05$). It could be assumed that antioxidant effects of green tea are directly in mitochondria, where it is accumulated [69].

The activity of AChE. The impaired cholinergic innervation and decreased activity of AChE, which catalyses degradation of acetylcholine, are important elements in developing dementia, since the normal function of cholinergic system is one of the key elements in the preservation of the learning and memory processes. Special attention is paid to the cholinergic connections between the basal forebrain and hippocampus, as well as the forebrain cortex. The increased supply of acetylcholine would be helpful to AD patients [70].

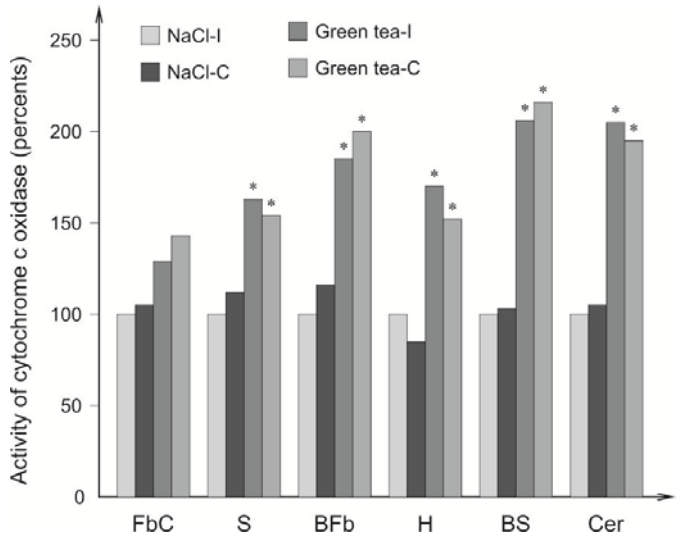

Figure 5. Activity of cytochrome *c* oxidase (COX) in the brain of Wistar rats (n=9–12) intrahippocampally treated with 0.9% NaCl and green tea leaf extract.
The measurement unit was mg cyt. c/mg prot. The value of every ipsilateral brain structures treated with 0.9% NaCl represents 100% of the enzyme activity. $p<0.05$, $p<0.01$ are the levels of statistical significance found versus ipsilateral 0.9% NaCl (*, **) (The differences between two percentages were calculated). The rats were sacrificed 12 days after treatments.

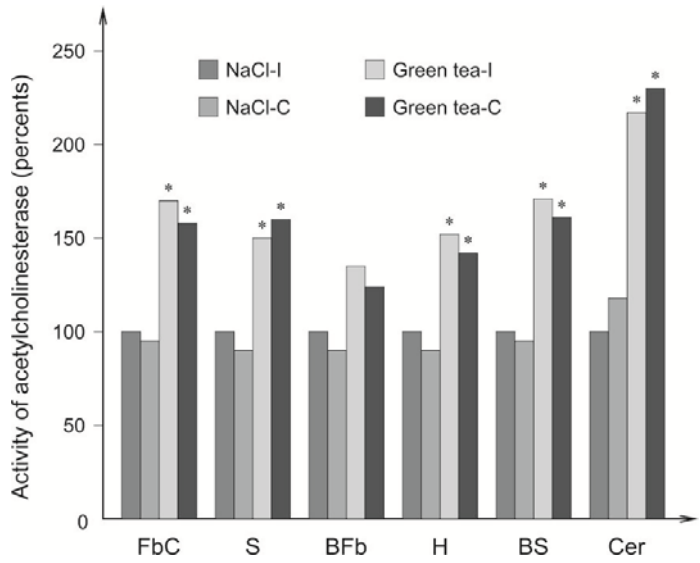

Figure 6. Activity of acetylcholinesterase (AChE) in the brain of Wistar rats (n=9–12) intrahippocampally treated with 0.9% NaCl and green tea leaf extract.
The measurement unit was mM acetylthiocholine/min./g prot. The value of every ipsilateral brain structures treated with 0.9% NaCl represents 100% of the enzyme activity. $p<0.05$, $p<0.01$ are the levels of statistical significance found versus ipsilateral 0.9% NaCl (*, **). (The differences between two percentages were calculated). The rats were sacrificed 12 days after treatments.

The activity of AChE showed no differences under the same treatment (NaCl and GTLE) within the left and right hemisphere of the corresponding structures (Figure 6). The application of GTLE increased this activity statistically significantly compared to the control ($p<0.05$), except in the basal forebrain. The increased AChE means better supply of substrate (acetylcholine) and, thus, the improved function of cholinergic synapse. Acetyl coenzyme A (acetyl-CoA), an intermediary in the oxidation process of carbohydrates, fats, and proteins, is one of the two main components necessary for the acetylcholine synthesis. It is produced in mitochondria. The improved mitochondrial function in the presence of GTLE, which is demonstrated through the increased COX activity, would be a prerequisite for increasing the production of acetylcholine and, consequently, the increased activity of AChE. These findings are in harmony with the growing evidence of the cognitive improvement which is the result of green tea treatment [46, 71, 72].

The low levels of AChE, which could also be seen in aluminium neurotoxicity, were significantly increased in the rats pretreated with GTLE [17]. This improvement indicates the improvement of the cholinergic system functions under the influence of green tea.

The activity of superoxide dismutase was similar between the corresponding structures of the left and right hemisphere under the same treatment (saline and GTLE), (Figure 7). In comparison to saline, GTLE treatment decreased this activity to same degree in all the structures (up to 35%), which was without statistical significance. This is in line with the increased activity of COX that indicates preserved mitochondrial function as the main source of the free radical generation.

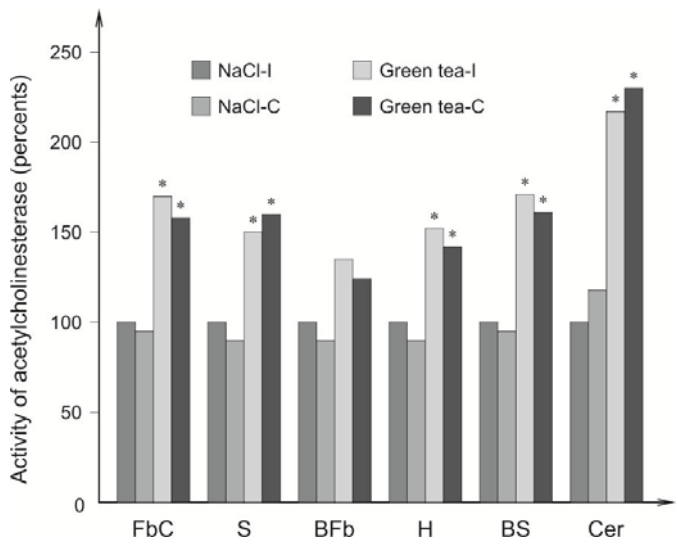

Figure 7. Activity of superoxide dismutase (SOD) in the brain of Wistar rats (n=9–12) intrahippocampally treated with 0.9% NaCl and green tea leaf extract.
The measurement unit was U/mg prot. The value of every ipsilateral brain structures treated with 0.9% NaCl represents 100% of the enzyme activity. $p<0.05$, $p<0.01$ are the levels of statistical significance found versus ipsilateral 0.9% NaCl (*, **). (The differences between two percentages were calculated). The rats were sacrificed 12 days after treatments.

Therefore, in spite of the fact that it contains aluminium, GTLE did not demonstrate to be toxic under the applied experimental conditions. A number of data showed that aluminium in leaves and beverages is not free, but it is linked with strong bounds in complexes compounds. That is thought to be the reason for low aluminium toxicity, in spite of green tea ingestion over a long period of time [47].

The Aluminium Treated Rats

GTLE, when given immediately before aluminium, attenuated the elements of the aluminium induced oxidative stress. The most prominent findings were related to the GSH content (Figure 4). In all the examined structures it was restored. In comparison with the aluminium treated group, the differences were significant in all the structures ($p<0.01$). The increment was registered in comparison with the controls, too, even though not to the same extent. Besides in the striatum and brain stem, where the $p<0.01$, in other structures significances were $p<0.05$. This increment was similar in the corresponding ipsi- and contralateral brain structures. This effect of green tea is especially important when one bears in mind that the recovery of low GSH found in AD could be the therapeutic task [73].

Furthermore, the production of superoxide anion was decreased in all the structures bilaterally after the GTLE pretreatment (Figure 3). That was obtained not only in comparison with the control group ($p<0.05$ in the striatum, in other structures $p<0.01$), but also in comparison to the group which was treated with aluminium with significance in the hippocampus and cerebellum ($p<0.05$), as well in other structures ($p<0.01$). That is in harmony with the findings of the green tea beneficial effects in oxidative and nitrosative stress [44].

The production of NOx was reverted to the control values by the pretreatment with GTLE, except in the hippocampus, where it was at the same level as in the aluminium treated rats ($p<0.05$, in comparison with the control group) (Figure 2). However, such changes are not sufficient to maintain the deteriorated learning and memory capacities, which were caused by aluminium [17].

It was demonstrated that GTLE could be so efficient that it restored all the examined deleterious effects of aluminium. The beneficial capabilities of GTLE were also demonstrated towards the almost depleted COX and AChE in the aluminium treated rats, whose activities could be not restituted, but only increased to some extent [17].

CONCLUSION

The obtained results in healthy adult rats showed that GTLE was not only able to maintain the activity of SOD, and to increase the activity of COX and AChE, but also was able to enhance GSH in aluminium treated rats, and to extremely diminish the production of superoxide anion. This emphasizes the powerful effects of GTLE on the respiratory chain in mitochondria, as well as on the cytoplasmatic activity in terms of the acetylcholine synthesis.

The protective effects of GTLE could be the result of its potent antioxidant activity obtained through metal chelation, radical scavenging and anti-inflammatory activities. Since the oxidative stress seems to be the first step in aging, and in the pathogenesis of neurodegenerative diseases as well, the results of a number of studies and our results of the

GTLE effects in both healthy and aluminium treated rats, seem to be promising for these conditions, because of the complex antioxidant and other effects of green tea. It is difficult to single out the substance that causes these effects, due to a very large number of pharmacologically active compounds in GTLE. Based on both various research studies and our own about the greatest amount of EGCG in GTLE, we can, with high probability, assume that EGCG is the one which the stated effects primarily depend on.

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REFERENCES

[1] Radi, E; Formichi, P; Battisti, C; Federico, A. Apoptosis and oxidative stress in neurodegenerative diseases. *J Alzheimers Dis.*, 2014, 42 Suppl 3, S125-52.

[2] Dasuri, K; Zhang, L; Keller, JN. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. *Free Radic Biol Med.*, 2013, 62, 170-85.

[3] Mandel, SA; Amit, T; Kalfon, L; Reznichenko, L; Youdim, MB. Targeting multiple neurodegenerative diseases etiologies with multimodal-acting green tea catechins. *J Nutr.*, 2008, 138, 1578S-1583S.

[4] Dragicevic, N; Smith, A; Lin, X; Yuan, F; Copes, N; Delic, V; et al., Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. *J Alzheimers Dis.*, 2011, 26, 507-21.

[5] Apetz, N; Munch, G; Govindaraghavan, S; Gyengesi, E Natural compounds and plant extracts as therapeutics against chronic inflammation in Alzheimer's disease-a translational perspective. *CNS Neurol Disord Drug Targets.*, 2014, 13, 1175-91.

[6] Glass, CK; Saijo, K; Winner, B; Marchetto, MC; Gage, FH. Mechanisms underlying inflammation in neurodegeneration. *Cell.*, 2010, 140, 918-34.

[7] Ferreira, ME; de Vasconcelos, AS; da Costa Vilhena, T; da Silva, TL; da Silva Barbosa, A; Gomes, AR; et al., Oxidative stress in Alzheimer's disease: should we keep trying antioxidant therapies? *Cell Mol Neurobiol.*, 2015 Jan 24. [Epub ahead of print].

[8] Xekardaki, A; Kövari, E; Gold, G; Papadimitropoulou, A; Giacobini, E; Herrmann, F; et al., Neuropathological changes in aging brain. *Adv Exp Med Biol.*, 2015, 821, 11-7.

[9] Cox, CJ; Choudhry, F; Peacey, E; Perkinton, MS; Richardson, JC; Howlett, DR; et al., Dietary (-)-epicatechin as a potent inhibitor of βγ-secretase amyloid precursor protein processing. *Neurobiol Aging.*, 2015, 36(1), 178-87.

[10] Walton, JR. Chronic aluminum intake causes Alzheimer's disease: applying Sir Austin Bradford Hill's causality criteria. *J Alzheimers Dis.*, 2014, 40, 765-838.

[11] Abdel Moneim, AE. Oxidant/antioxidant imbalance and the risk of Alzheimer's disease. *Curr Alzheimer Res.*, 2015 Mar 25. [Epub ahead of print].

[12] Srividhya, R; Zarkovic, K; Stroser, M; Waeg, G; Zarkovic, N; Kalaiselvi, P. Mitochondrial alterations in aging rat brain: effective role of (-)-epigallo catechin gallate. *Int J Dev Neurosci.*, 2009, 27(3), 223-31.

[13] Ruszkiewicz, J; Albrecht, J. Changes in the mitochondrial antioxidant systems in neurodegenerative diseases and acute brain disorders. *Neurochem Int.* 2015; Jan 6. pii: S0197-0186(15)00004-2. doi: 10.1016/j.neuint.2014.12.012. [Epub ahead of print].

[14] Mosconi, L; de Leon, M; Murray, J; E, L; Lu, J; Javier, E; McHugh, P; et al., Reduced mitochondria cytochrome oxidase activity in adult children of mothers with Alzheimer's disease. *J Alzheimers Dis*., 2011, 27, 483-90.

[15] Long, J; He, P; Shen, Y; Li, R. New evidence of mitochondria dysfunction in the female Alzheimer's disease brain: deficiency of estrogen receptor-β. *J Alzheimers Dis.*, 2012, 30, 545-58.

[16] Selfridge, JE; E, L; Lu, J; Swerdlow, RH. Role of mitochondrial homeostasis and dynamics in Alzheimer's disease. *Neurobiol Dis.*, 2013, 51, 3-12.

[17] Jelenković, A; Jovanović, MD; Stevanović, I; Petronijević, N; Bokonjić, D; Zivković, J; et al., Influence of the green tea leaf extract on neurotoxicity of aluminium chloride in rats. *Phytother Res.*, 2014, 28(1), 82-7.

[18] Hansson Petersen, CA; Alikhani, N; Behbahani, H; Wiehager, B; Pavlov, PF; Alafuzoff, I; et al., The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. *Proc Natl Acad Sci*, *U S A*. 2008, 105, 13145-50.

[19] Mandel, S; Amit, T; Bar-Am, O; Youdim, MB. Iron dysregulation in Alzheimer's disease: multimodal brain permeable iron chelating drugs, possessing neuroprotective-neurorescue and amyloid precursor protein-processing regulatory activities as therapeutic agents. *Prog Neurobiol.*, 2007, 82, 348-60.

[20] Chen, JX; Yan, SS. Role of mitochondrial amyloid-beta in Alzheimer's disease. *J Alzheimers Dis.*, 2010, 20 Suppl 2, S569-78.

[21] Wong, BX; Duce, JA. The iron regulatory capability of the major protein participants in prevalent neurodegenerative disorders. *Front Pharmacol*., 2014 Apr 21, 5, 81.

[22] Ayton, S; Lei, P; Bush, AI. Metallostasis in Alzheimer's disease. *Free Radic Biol Med.*, 2013, 62, 76-89.

[23] Ellis, G; Fang, E; Maheshwari, M; Roltsch, E; Holcomb, L; Zimmer, D; et al., Lipid oxidation and modification of amyloid-β (Aβ) in vitro and in vivo. *J Alzheimers Dis.*, 2010, 22(2), 593-607.

[24] Squier, TC. Oxidative stress and protein aggregation during biological aging. *Exp Gerontol.*, 2001, 36, 1539-50.

[25] Exley, C. What is the risk of aluminium as a neurotoxin? *Expert Rev Neurother.*, 2014, 14, 589-91.

[26] Shaw, CA; Li, D; Tomljenovic, L. Are there negative CNS impacts of aluminum adjuvants used in vaccines and immunotherapy? *Immunotherapy.*, 2014, 6, 1055-71.

[27] Exley, C; Vickers, T. Elevated brain aluminium and early onset Alzheimer's disease in an individual occupationally exposed to aluminium: a case report. *J Med Case Rep.*, 2014 Feb 10, 8, 41. doi: 10.1186/1752-1947-8-41.

[28] Walton, JR. Aluminum involvement in the progression of Alzheimer's disease. *J Alzheimers Dis.*, 2013, 35, 7-43.

[29] Perl, DP; Brody, AR. Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. *Science.*, 1980, 208, 297-9.

[30] Exley, C. Why industry propaganda and political interference cannot disguise the inevitable role played by human exposure to aluminum in neurodegenerative diseases, including Alzheimer's disease. *Front Neurol.*, 2014 Oct 27, 5:212. doi: 10.3389/fneur. 2014.00212. eCollection 2014.

[31] Rondeau, V; Jacqmin-Gadda, H; Commenges, D; Helmer, C; Dartigues, JF. Aluminum and silica in drinking water and the risk of Alzheimer's disease or cognitive decline: findings from 15-year follow-up of the PAQUID cohort. *Am J Epidemiol.*, 2009, 169, 489-96.

[32] Riihimäki, V; Aitio, A. Occupational exposure to aluminum and its biomonitoring in perspective. *Crit Rev Toxicol.*, 2012, 42, 827-53.

[33] Buchta, AM; Kiesswetter, BE; Schäper, BM; Zschiesche, CW; Schaller, DKH; Kuhlmann, AA; et al., Neurotoxicity of exposures to aluminium welding fumes in the truck trailer construction industry. *Environ Toxicol Pharmacol.*, 2005, 19, 677-85.

[34] Yu, L; Jiang, R; Su, Q; Yu, H; Yang, J. Hippocampal neuronal metal ion imbalance related oxidative stress in a rat model of chronic aluminum exposure and neuroprotection of meloxicam. *Behav Brain Funct.*, 2014 Mar 11, 10, 6. doi: 10.1186/ 1744-9081-10-6.

[35] Mohan, N; Alleyne, T; Adogwa, A. The effects of ingested aluminium on brain cytochrome oxidase activity. *West Indian Med J.*, 2009, 58(5), 422-7.

[36] Vučetić-Arsić, S; Radonjić, NV; Jovanović, M; Selaković, V; Nikolić, T; Velimirović, M; et al., Oxidative stress precedes mitochondrial dysfunction in gerbil brain after aluminum ingestion. *Environ Toxicol Pharmacol.*, 2013, 36, 1242-52.

[37] Stevanović, ID; Jovanović, MD; Colić, M; Jelenković, A; Bokonjić, D; Ninković, M, et al., N-nitro-L-arginine methyl ester influence on aluminium toxicity in the brain. *Folia Neuropathol.*, 2011, 49, 219-29.

[38] Bolognin, S; Zatta, P; Lorenzetto, E; Valenti, MT; Buffelli, M. β-Amyloid-aluminum complex alters cytoskeletal stability and increases ROS production in cortical neurons. *Neurochem Int.*, 2013, 62, 566-74.

[39] Zhao, Y; Hill, JM; Bhattacharjee, S; Percy, ME; Pogue, AI; Lukiw, WJ. Aluminum-induced amyloidogenesis and impairment in the clearance of amyloid peptides from the central nervous system in Alzheimer's disease. *Front Neurol.*, 2014 Sep 5, 5, 167.

[40] Kumar, V; Gill, KD. Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review. *Neurotoxicology*., 2014, 41, 154-66.

[41] Unachukwu, UJ; Ahmed, S; Kavalier, A; Lyles, JT; Kennelly, EJ. White and green teas (Camellia sinensis var. sinensis): variation in phenolic, methylxanthine, and antioxidant profiles. *J Food Sci*., 2010, 75, C541-8. doi: 10.1111/j.1750-3841.2010.01705.x.

[42] Kuriyama, S; Hozawa, A; Ohmori, K; Shimazu, T; Matsui, T; Ebihara, S; et al., Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. *Am J Clin Nutr.*, 2006, 83, 355-61.

[43] Noguchi-Shinohara, M; Yuki, S; Dohmoto, C; Ikeda, Y; Samuraki, M; Iwasa, K; et al., Consumption of green tea, but not black tea or coffee, is associated with reduced risk of

cognitive decline. *PLoS One.* 2014 May 14; 9(5):e96013. doi: 10.1371/journal.pone. 0096013. eCollection 2014.

[44] Kim, CY; Lee, C; Park, GH; Jang, JH. Neuroprotective effect of epigallocatechin-3-gallate against beta-amyloid-induced oxidative and nitrosative cell death via augmentation of antioxidant defense capacity. *Arch Pharm Res.*, 2009, 32, 869-81.

[45] Mähler, A; Mandel, S; Lorenz, M; Ruegg, U; Wanker, EE; Boschmann, M; et al., Epigallocatechin-3-gallate: a useful, effective and safe clinical approach for targeted prevention and individualised treatment of neurological diseases? *EPMA J.*, 2013 Feb 18, 4(1), 5. doi: 10.1186/ 1878-5085-4-5.

[46] Williams, RJ; Spencer, JP. Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radic Biol Med.*, 2012, 52, 35-45.

[47] Alberti, G; Biesuz, R; Profumo, A; Pesavento, M. Determination of the total concentration and speciation of Al(III) in tea infusions. *J Inorg Biochem.*, 2003, 97, 79-88.

[48] Choi, DY; Lee, YJ; Hong, JT; Lee, HJ. Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. *Brain Res Bull.*, 2012, 87, 144-53.

[49] Rezai-Zadeh, K; Shytle, D; Sun, N; Mori, T; Hou, H; Jeanniton, D; et al., Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J Neurosci*., 2005, 25, 8807-14.

[50] Wobst, HJ; Sharma, A; Diamond, MI; Wanker, EE; Bieschke, J. The green tea polyphenol (-)-epigallocatechin gallate prevents the aggregation of tau protein into toxic oligomers at substoichiometric ratios. *FEBS Lett.*, 2015, 589, 77-83.

[51] Ebrahimi, A; Schluesener, H. Natural polyphenols against neurodegenerative disorders: potentials and pitfalls. *Ageing Res Rev.*, 2012, 11, 329-45.

[52] Konig, JFR; Klippel, RA. The Rat Brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem. Baltimore: The Williams and Wilkins Company, 1963.

[53] Gurd, JW; Jones, LR; Mahler, HR; Moore, WJ. Isolation and partial characterization of rat brain synaptic plasma membranes. *J Neurochem.*, 1974, 22, 281-90.

[54] Sun, M; Zigman, S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Anal Biochem.*, 1978, 90, 81-9.

[55] Hess, HH; Pope, A. Intralaminar distribution of cytochrome c oxidase activity in human isocortex. *J Neurochem*., 1960, 5, 207-17.

[56] Ellman, GL; Courtney, KD; Andres, VJr; Feather-Stone, RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.*, 1961, 7, 88-95.

[57] Auclair, C; Voisin, E. Nitroblue tetrazolium reduction. In: Greenwald RA (ed.). CRC handbook of methods for oxygen radical research. Boca Raton: CRC Press; 1985. 123-132.

[58] Navarro-Gonzálvez, JA; García-Benayas, C; Arenas, J. Semiautomated measurement of nitrate in biological fluids. *Clin Chem.*, 1998, 44, 679-81.

[59] Anderson, ME. Tissue glutathione. In: Greenwald RA (ed.). CRC Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press; 1985. 317-323.

[60] Jang, SH; Kwon, HG. Perspectives on the neural connectivity of the fornix in the human brain. *Neural Regen Res.*, 2014, 9(15), 1434-6.

[61] Stevanović, ID; Jovanović, MD; Colić, M; Jelenković, A; Bokonjić, D; Ninković, M. Nitric oxide synthase inhibitors protect cholinergic neurons against AlCl3 excitotoxicity in the rat brain. *Brain Res Bull*., 2010, 81(6), 641-6.

[62] Jovanović, MD; Jelenković, A; Stevanović, ID; Bokonjić, D; Colić, M; Petronijević, N; et al., Protective effects of glucose-6-phosphate dehydrogenase on neurotoxicity of aluminium applied into the CA1 sector of rat hippocampus. *Indian J Med Res.*, 2014, 139, 864-72.

[63] Zhihui, Q. Modulating nitric oxide signaling in the CNS for Alzheimer's disease therapy. *Future Med Chem.*, 2013, 5(12), 1451-68.

[64] Malinski, T. Nitric oxide and nitroxidative stress in Alzheimer's disease. *J Alzheimers Dis.*, 2007, 11, 207-18.

[65] Aquilano, K; Baldelli, S; Ciriolo, MR. Glutathione: new roles in redox signaling for an old antioxidant. *Front Pharmacol.*, 2014 Aug 26, 5, 196. doi: 10.3389/fphar. 2014.00196.

[66] Aoyama, K; Nakaki, T. Impaired glutathione synthesis in neurodegeneration. *Int J Mol Sci.*, 2013, 14, 21021-44.

[67] Stevanović, ID; Jovanović, MD; Jelenković, A; Colić, M; Stojanović, I; Ninković, M. Effects of L-NAME, a non-specific nitric oxide synthase inhibitor, on AlCl3-induced toxicity in the rat forebrain cortex. *J Vet Sci.*, 2009, 10, 15-22.

[68] Ribas, V; García-Ruiz; C, Fernández-Checa, JC. Glutathione and mitochondria. *Front Pharmacol.* 2014 Jul 1; 5:151. doi: 10.3389/ fphar.2014.00151.

[69] Schroeder, EK; Kelsey, NA; Doyle, J; Breed, E; Bouchard, RJ; Loucks, FA; et al., Green tea epigallocatechin 3-gallate accumulates in mitochondria and displays a selective antiapoptotic effect against inducers of mitochondrial oxidative stress in neurons. *Antioxid Redox Signal.*, 2009, 11, 469-80.

[70] Dani, JA; De Biasi, M; Liang, Y; Peterson, J; Zhang, L; Zhang, T; et al., Potential applications of nicotinic ligands in the laboratory and clinic. *Bioorg Med Chem Lett.*, 2004, 14, 1837-9.

[71] Park, SK; Jung, IC; Lee, WK; Lee, YS; Park, HK; Go, HJ; et al., A combination of green tea extract and l-theanine improves memory and attention in subjects with mild cognitive impairment: a double-blind placebo-controlled study. *J Med Food*., 2011, 14, 334-43.

[72] Rendeiro, C; Guerreiro, JD; Williams, CM; Spencer, JP. Flavonoids as modulators of memory and learning: molecular interactions resulting in behavioural effects. *Proc Nutr Soc.*, 2012, 71, 246-62.

[73] Braidy, N; Zarka, M; Welch, J; Bridge, W. Therapeutic Approaches to ModulatingGlutathione Levels as a Pharmacological strategy in Alzheimer's Disease. *Curr Alzheimer Res*., 2015 Mar 24. [Epub ahead of print].

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Chapter 4

PREVENTIVE EFFECTS OF TEA CATECHINS ON CARDIAC TRANSPLANT REJECTION

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ABSTRACT

Green tea catechins are key components with many biological functions. These effects are induced by the suppression of several inflammatory factors through nuclear factor-kappa B (NF-κB). While these characteristics of tea catechins have been well documented, actions of catechins on cardiac transplantation have not yet been well investigated. To test the hypothesis that catechins can attenuate ventricular remodeling and cardiac allograft vasculopathy (CAV) in cardiac transplantation, we performed oral administration of catechins into murine cardiac recipients. We revealed that catechins suppressed myocardial remodeling and CAV formation. They altered cytokine expression, inhibited adhesion molecules and regulated NF-κB activation. Thus, catechins are potent agents for the suppression of heart transplant rejection.

1. INTRODUCTION

Heart transplantation is a common surgical procedure in humans; almost 116,000 heart transplantations have been performed worldwide over the past 40 years [1]. However, acute rejection and cardiac allograft vasculopathy (CAV) are still problems [2]. Acute rejection is characterized by myocardial remodeling such as cell infiltration, necrosis and fibrosis. These problems are enhanced by several cytokines and adhesion molecules. CAV, that is considered as chronic rejection, is characterized by intimal thickening comprised of proliferative smooth muscle cells (SMCs) [3-5].

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Catechins are key components of green tea with many biological functions, including anti-inflammatory and anti-oxidative effects [6-9]. These effects are induced by the suppression of several inflammatory factors including nuclear factor-kappa B (NF-κB), a multipotential promoter of cytokines and adhesion molecules [10]. The major tea catechins are epigallocathechin-3 gallate (EGCG), epigallocathechin (EGC), epicathechin-3 gallate (ECG). While these characteristics of tea catechins have been well documented [11], their effects on cardiac transplantation have not yet been well investigated.

NF-κB plays a pivotal role in the coordinated transcription of multiple inflammatory genes [12-14]. We have reported that decoy oligonucleotide (ODN) against the *cis* element of NF-κB prevents acute rejection and CAV by suppressing expression of multiple genes [15]. Since NF-κB decoy ODN inhibits inflammatory gene expression, we hypothesized that tea catechins could suppress myocardial remodeling and CAV formation via NF-κB and related inflammatory factor regulation. In the investigation, we showed that tea catechins reduced both myocardial remodeling and CAV formation with supression of NF-κB activation, altered Th1/Th2 cytokine balances and suppressed adhesion molecules [16].

2. Effects of Tea Catechins on Cardiac Allografts

2.1. Methods of Murine Cardiac Transplantation and Analyses

Male inbred mice (4 to 6 weeks, 20-25 g) were used in this study. A full allomismatch combination, C57BL/10Sn Slc (B/10, H2-b, Sankyo Laboratory Service Co., Tokyo, Japan) to C3H/HeN Jcl (C3H, H2-k, Clea Japan Inc., Tokyo, Japan) was used for analyzing acute rejection indicated by graft survival. C57BL/6 (B/6, H-2b) and B6.C-H-2 ^{bm12} KhEg (Bm12, H-2^{bm12}) combination was used as the class II mismatch combination for analyzing chronic rejection indicated by pathological findings such as CAV and ventricular remodeling. Isografts (B/6 to B/6) were used for negative control. Allografts were heterotopically transplanted in an intra-abdominal location using the microsurgical technique. Briefly, this technique involves anastomosing the end of the donor aorta to the side of the recipient abdominal aorta, after which the donor pulmonary artery is connected to the inferior vena cava of the recipient mice to return myocardial blood flow. Ischemic time averaged 60 minutes, and the overall success rate was greater than 90% [17-20].

The transplanted mice were assigned randomly to two groups. The recipient mice were orally supplemented with tea catechins (20 mg/kg/day, THEA-FLAN 90S (Ito-en Co. Shizuoka, Japan) which includes EGCG: 45.2% ECG: 13.7% EGC: 0.23%) daily. This ratio is the natural dose ratio in the extracts from green tea. For control, transplanted mice were supplemented with normal water without the catechins. We observed mice condition daily; we measured mice body weight (HF-3200, A&D Company Ltd., Tokyo, Japan) of each mouse and averaged. Graft function was assessed by daily palpation and graft failure was defined as the absence of detectable beating. Full allomismatch grafts were harvested on the day of the absence of detectable beating; class II cardiac allografts were harvested at day 60 after transplantation [17-19].

To evaluate the heart allografts pathologically, we harvested the grafts and measured each maximal dimension and weight. Grafts were sectioned transversely at the maximal

circumference of the ventricle. As previously described, serial sections (6 μm) from tissue embedded in OCT were stained with hematoxylin and eosin (HE), Mallory and Elastica van Gieson (EvG) to highlight the internal elastic lamina (IEL) [17-19]. The grafts were photographed and processed using an image analysis system to calculate the area of cell infiltration and fibrosis. The areas of myocardial cell infiltration (consisting of inflammatory cells and myocardial necrosis) using HE staining and fibrosis using Mallory staining were determined with a computer-assisted analyzer (Scion Image beta 4.0.2). The area ratio was calculated as (affected area X100) /whole area (%) as described [19-22]. Values for three ventricular regions were averaged for each heart, and the mean percentage of affected area for each group was calculated. The graft arteries were also photographed, videodigitized and processed using an image analysis system (NIH Image). The area encompassed by the lumen and IEL was traced carefully, and the area of luminal stenosis in each cross section was calculated according to the formula: luminal occlusion = (IEL area - luminal area) X100/IEL area (%) as described. All data were analyzed in a blind fashion by two independent investigators and averaged [3, 5, 15].

For immunohistochemistry, serial sections (6 μm) were cut and dipped in cold acetone for 10 minutes. The sections were rehydrated in PBS and incubated with 5% normal goat serum to block non-specific reactions. Sections were incubated with primary antibodies against murine intercellular adhesion molecule (ICAM)-1 (YN1/1.7), vascular cell adhesion molecule (VCAM)-1 (MK/2), CD11b (#1561-01, Southern Biotechnology Associates Inc., Birmingham, AL), monocytes/macrophages (MOMA-2, #T-2007, BMA Biomedicals AG, Rheinstrasse, Switzerland), TGF-beta (#SC-146, Santa Cruz Biotechnology Inc., Santa Cruz, CA), and NF-κB (p65, #SC372, Santa Cruz) for 12 h at 4^{o}C [19-21]. Antibody-biotin conjugate was detected with Vectastain ABC Kit (Histofine Kit, Nichirei Co., Tokyo, Japan) used according to the manufacturer's instructions. Enzyme activity was detected with diaminobenzidine (0.5 mg/ml) with 0.05% NiCl in 50 mM Tris buffer, pH 7.5. Intensity of expression was scored as follows: 0, no visible staining; 1, few cells with faint staining; 2, few cells with moderate staining; 3, some cells with moderate staining; 4, diffuse cells with moderate staining or 5, diffuse and intense staining. Scores of two independent reviewers were averaged [21].

To measure mRNA levels, we performed RNase protection assay (RPA). The harvested allografts were homogenized in Trizol reagent (Life Technologies, Grand Island, NY) and frozen at -80^{o}C. Cytokine mRNA expression was measured by RPA using mCK1 template (Riboquant kit, PharMingen, San Diego, CA). Levels of mRNA expression were quantified and normalized to GAPDH mRNA using densitometry [19].

All data are expressed as mean +/- SEM. Scores were compared among the groups using a Scheffe's ANOVA. Differences with values of $P < 0.05$ were considered significant.

2.2. Results of Murine Cardiac Transplantation

Firstly, we confirmed the effects of the catechin on acute rejection. The catechins did not prolong the graft survival statistically in the full-allomismatch combination [23]. Heart dimension and weight of the class II mismatch grafts on day 60 were measured. Although severely enlarged graft dimension and weight gain were observed in nontreated allografts, catechins markedly attenuated the heart dimension and weight gain. Histologically, severe

myocardial cell infiltration and fibrosis was observed in nontreated class II mismatch allografts at day 60. However, tea catechins significantly attenuated myocardial cell infiltration and fibrosis. Immunohistochemically, enhancement of CD4, CD8, CD11b, monocytes/macrophages, ICAM-1 and NF-κB expression was observed in nontreated allograft myocardium. However, catechin markedly attenuated expression of all these factors.

Secondly, we confirmed the effects of the catechin on chronic rejection. Heavy neointimal thickening was observed in the coronary arteries of untreated allografts at day 60. However, arterial intimal thickening was attenuated in the catechin treated group. Immunohistochemically, CD4-, CD8-, CD11b-positive cells and macrophages were accumulated in the thickened intima of nontreated allografts, while these positive cell numbers were suppressed in the catechin treated allograft arteries. VCAM-1 and NF-κB (p65) were expressed strongly and diffusely in the thickened intima of arteries of nontreated allografts, while catechin treatment suppressed the expression. However, TGF-beta was faintly expressed in the thickened intima of arteries of nontreated allografts as comparable in catechin-treated graft arteries.

To analyze molecular mechanism, we examined cytokine, cell proliferation and NF-κB binding activity. Because a Th2 cytokine IL-10 plays a pivotal role in CAV formation [24], we analyzed IL-10 mRNA levels. We revealed that IL-10 was significantly elevated in the catechin treated group compared with that of non-treated group.

3. DISCUSSION

Polyphenols, especially catechins, are the most potent component of green tea affecting cell function [25].

Many biological functions of polyphenols have been studied [26], including anti-oxidative [27], anti-carcinogenic [28, 29], anti-inflammatory [30], and anti-proliferative [31] effects. These effects were induced by several mechanisms such as the binding of NF-κB to the promoters of cytokines and adhesion molecules. While these characteristics of cathechins have been well documented, its anti-inflammatory effects on cardiac transplantation have not been investigated in detail.

3.1. Catechins Suppress NF-κB Activation

We have demonstrated that catechin intake significantly suppresses the expression of inflammatory factors, including adhesion molecules, cytokines and matrix metalloproteinases (MMPs) in experimental cardiovascular disease models. These factors are known to be regulated by NF-κB, which is central to the development of inflammatory diseases [32]. We have reported specific inhibition of NF-κB using a decoy ODN in myocardial ischemia [33], myocarditis [34] and heart transplant rejection [15]. In these studies, NF-κB decoy ODN suppressed many inflammatory factors, including adhesion molecules, cytokines and MMPs. Although catechins are not specific inhibitors of NF-κB, their effects are similar to an IKK inhibitor [35, 36]. Other investigations indicated that catechins have a significant role in the regulation of NF-κB. In cardiovascular systems, Aneja et al., reported that EGCG attenuated

myocardial ischemia reperfusion injury in rats. This beneficial effect of EGCG was associated with a reduction of NF-κB and activator protein-1 DNA binding [37]. Han et al., showed the inhibitory effects of EGCG on rat aortic SMCs via NF-κB down-modulation. In the study, EGCG treatment resulted in significant inhibition in attachment, proliferation and an appreciable cell cycle arrest at both G0/G1- and G2/M-phases in SMCs. Immunoblot analysis revealed that the constitutive expression of NF-κB/p65 nuclear protein in SMCs was lowered by EGCG in both the cytosol and the nucleus [38]. To clarify the mechanism, several investigations were performed using various types of cells and tissues. As Kim et al., demonstrated, EGCG suppresses NF-κB activation and phosphorylation of p38 MAPK and JNK in human astrocytoma U373MG cells [39]. Mackenzie et al., investigated the capacity of endothelial cells (ECs) in Hodgkin's lymphoma cells; they showed that EC inhibited NF-κB-DNA binding activity. The inhibition was not associated with EC antioxidant activity with changes in p65 phosphorylation or NF-κB nuclear translocation [40].

3.2. Catechins Regulate MMPs in Cardiovascular Systems

Since MMPs are key components in the positive feedback loop of heart remodeling, MMP inhibition is an effective therapy for the pathological condition [41-44]. It is also well known that catechins suppress several inflammatory factors, including MMPs induced by NF-κB [45]. To clarify the role of catechins on the ischemic hearts, we made a rat myocardial ischemia model by ligating the left anterior descending coronary artery. This continued for 28 days. After ischemic injury, the non-treated ischemia group showed a significant decline of blood pressure compared to non-treated sham-operated group. However, the catechin administration suppressed the decline of the blood pressure compared to that of the non-treated ischemia group. Pathologically, the anterior wall of the hearts was completely fibrotic and the remaining area showed interstitial fibrosis and cell infiltration. However, catechin treated hearts showed significantly less infarct size, infarct length, left ventricular circumference and left ventricular inner diameter than those in the non-treated ischemia group**.** To reveal the role of MMPs, the infarct region and myocardium were separated under a dissecting microscope and they were used for zymography as previously reported. This showed that increased gelatinase (MMP-2 and MMP-9) activity was observed in hearts in the non-treated ischemia group. However, this enhanced gelatinase activity was decreased by catechin administration [46].

Other investigations indicated that catechins have a pivotal role in the regulation of MMPs in cardiovascular systems. Cheng et al., revealed that EGCG blocks the activation of pro-MMP-2, MMP-2, membrane type (MT) 1-MMP in the cultured vascular SMCs. They also demonstrated that EGCG enhanced the expression of tissue inhibitor of metalloproteinase (TIMP)-2. The data from decreased TIMP-2 activity using its siRNA suggested that enhanced TIMP-2 expression is critical for inhibition of SMC invasion by EGCG [47]. Other investigators also demonstrated how the catechin effects SMCs [48-51] and ECs [52] via MMP and TIMP regulation. Yamazaki et al., reported that (-)-epicatechin had limited increases in the infarct region in rat myocardial ischemia-reperfusion injury models [53]. Catechins are known to affect not only cardiovascular cells but also other organs and cells. Ho et al., demonstrated that EGCG suppressed the invasion of human oral cancer cells via decreased production of MMPs. They used cytotoxicity, invasion, and migration

assays to investigate the effects of human oral cancer cells using gelatin and casein zymography. They revealed that EGCG showed a dose-dependent inhibitory effect on the invasion and migration of cancer cells in the absence of cytotoxicity with decreased expression of MMP-2 and MMP-9 [54]. Zhen et al., demonstrated that EGCG prevented carbon tetrachloride-induced hepatic fibrosis. In that paper, EGCG attenuated hepatic stellate cell activation as well as MMP-2 activity. It also demonstrated that the concanavalin A-induced activation of secreted MMP-2 was inhibited by EGCG through the influence of membrane type 1-MMP activity [55]. Lee et al., evaluated the effects of EGCG on the extracellular matrix changes induced by UV radiation using artificial skin. They revealed that EGCG decreased the level of MMP production and increased TIMP levels [56]. Because it is well known that extracellular matrix metabolism is tightly controlled by collagen degrading MMPs and TIMPs, catechin plays a significant role in tissue remodeling via extracellular matrix metabolism.

3.3. Catechins Alter Cytokine Balances in Cardiovascular Systems

Although cytokines play an essential role for the progression of inflammation, any established treatment has not yet been elucidated in acute myocardial inflammation, such as myocarditis [57-61]. Experimental autoimmune myocarditis (EAM) is a rat model that is characterized by myocardial damages and multinucleated giant cell infiltration. This has been used as a disease model of human acute myocarditis [62-64]. To clarify the effects of catechins on myocarditis, we administered catechins into rats after the induction of EAM. We found that the catechins significantly reduced the heart weight/body weight ratio compared to that of non-treated EAM controls. Echocardiogram revealed catechins improved the cardiac function compared to the controls. Pathologically, non-treated control EAM animals showed severe myocardial cell infiltration and fibrotic lesions. However, the catechin treatment showed significantly less myocardial cell infiltration and fibrosis areas compared to those in controls. To examine expression of cytokine mRNA in EAM hearts, RNase protection assay was used. TNF-alpha mRNA level was markedly decreased in the catechin treated group compared to the control group. On the other hand, mRNA levels of Th2 cytokines such as IL-4 and IL-10 in the catechin treated group were markedly enhanced compared to the control group. We revealed that the myocardial cell infiltration, fibrosis and proinflammatory cytokines were enhanced in the EAM progression and the catechins suppressed the development of these changes with altered cytokine expression [65].

Other investigators demonstrated that catechins have a significant role in the regulation of cytokines and chemokines in the cardiovascular system. Ahn et al., revealed that EGCG prevented TNF-alpha-mediated monocyte chemotactic protein-1 (MCP-1) production and reduced phosphorylation of Akt (Ser473) in bovine coronary artery endothelial cells. In addition, EGCG attenuated TNF-alpha mediated down-regulation of TNF-alpha receptor 1 (TNFR1), but not TNFR2 [66]. It was also reported that catechin inhibits tumor-specific angiogenesis by regulating the production pro-inflammatory cytokines. The paper showed that catechin administration differentially regulated the elevation of cytokines such as IL-1beta, IL-6, and TNF-alpha [67].

Catechin also effects cytokine expression in other organs. Armed et al., found that EGCG inhibits IL-1beta-induced IL-6 production in rheumatoid arthritis synovial fibroblasts by

inducing alternative splicing of gp130 mRNA. They concluded that the prevention was promising as a potential therapeutic agent for rheumatoid arthritis [68]. Crouvezier et al., investigated the effects of catechins on the production of pro- and anti-inflammatory cytokines by human leukocytes in vitro. EGCG decreased the production of IL-1beta and enhanced the production of IL-10, but had no effect on the production of IL-6 or TNF-alpha [69]. In contrast, EGCG treated murine colitis with a significant reduction of NF-κB and AP-1 activation. However, treatment with EGCG did not reduce plasma cytokine levels in this murine model [70]. Although these effects showed anti-inflammatory properties of the catechins, the catechins effect on cytokines is different among the experimental models and time points.

3.4. Catechins Suppress Adhesion Molecule Expression in Cardiovascular Systems

It is well known that adhesion molecules play a pivotal role on the development of cardiovascular diseases. To evaluate the effects of tea catechins for the development of atherosclerosis induced by hyperlipidemia, we administered catechins (2 or 4 % THEA-FLAN 90S contained high fat chaw) to LDL receptor knockout (LDLRKO) mice. Immunohistocemically, VCAM-1 expression was enhanced in the endothelial cells, smooth muscle cells and infiltrating cells in the aortic walls of LDLRKO mice. However, catechin administration significantly suppressed VCAM-1 expression in the atherosclerotic lesions in LDLRKO mice, although LDLRKO mice with the 2% catechins showed comparable cholesterol levels [32]. In the study, catechins prevent development in the animals through the suppression of adhesion molecules with or without changing the plasma lipid levels. Babu et al., reviewed that catechins have further effects on cell adhesion molecules. They showed that catechins prevent vascular inflammation via suppression of leukocyte adhesion to endothelium. Furthermore, subsequent transmigration through inhibition of transcriptional factor NF-κB-mediated production of adhesion molecules both in endothelial cells and inflammatory cells was also suppressed [71].

Other studies indicated that catechins play a large role in the regulation of adhesion molecules in inflammatory cardiovascular diseases. Ludwig et al., investigated the effects of various tea catechins on cytokine-induced expression of ICAM-1, VCAM-1, and endothelial leukocyte adhesion molecule-1 (E-selectin) in human umbilical vein endothelial cells (HUVECs). EGCG prevented the induction of VCAM-1 expression, whereas EC and EGC had few effects [72]. Chae et al., revealed that EGCG inhibited the angiotensin II-induced elevation of VCAM-1 and ICAM-1 in the HUVEC plasma membranes via inhibition of the p38 MAPK and the ERK1/2 signaling pathways [73]. These significant effects of catechins are not only observed in cardiovascular cells but also other organs and cells. Handa et al., reported that catechins inhibited the expression of adhesion molecules on gastric cancer cells and leukocytes [74]. Chen et al., investigated the effect of catechins on substance P-induced bladder hyperactivity. Two week-catechin pretreatment reduced bladder ICAM-1 expression and ameliorated the hyperactive bladder response [75]. Thus, these papers demonstrated that the anti-cell adhesion molecule activity of catechins was observed as a systemic anti-inflammatory effect.

3.5. Catechins Suppress Inflammatory Factors via NF-kB in Transplantation

Our results of cardiac transplantation have demonstrated that catechin intake significantly suppressed the expression of inflammatory factors, including adhesion molecules and cytokines via NF-κB. NF-κB is a key factor for the development of ventricular remodeling and CAV; we have revealed that specific inhibition of NF-κB using a decoy ODN in rejected cardiac allografts significantly suppressed the progression of tissue remodeling and CAV. In the study, the NF-κB decoy ODN suppressed many inflammatory factors, including adhesion molecules and cytokines [15]. Although catechins are not specific inhibitors of NF-κB, their effects are similar to NF-κB decoy ODN. Recently, Tripathi et al., demonstrated that green tea extract (GTE) in combination with low dose CyA significantly prolongs graft survival, as well as increases the production of immunosuppressive cytokines in the murine model of nonvascularized cardiac allografts. They concluded that GTE is useful as an adjunctive therapy in combination with CyA to prolong allograft survival and to reduce CyA induced nephrotoxicity [76].

It is noteworthy that catechins not only attenuate ventricular remodeling but also suppress CAV formation in cardiac allografts. In general, myocardial fibrosis and remodeling are the consequence of acute rejection; CAV is recognized as one of results of chronic rejection [2, 3, 15].

However, an immunosuppressant, such as FK506, suppresses myocardial cell infiltration while the drug does not suppress CAV. Thus, several therapeutic trials have been undertaken to attenuate CAV but without widespread use [77, 78]. In this study, we have demonstrated that the catechins inhibit intimal hyperplasia effectively. The prevention of neointimal formation was associated with suppressed expression of adhesion molecules; it can be deduced that NF-κB must play an important role in SMC proliferation. NF-κB is widely known to be a key enhancer of inflammation, this stimulates SMC proliferation in CAV. Therefore, suppression of NF-κB activation reduces SMC proliferation. As we have previously reported, suppression of NF-κB using decoy ODN transfection decreased CAV formation [15].

Recent results indicated that statins [79] and rapamycine derivative [80] have the potential to suppress CAV. Because statins and rapamycine also have anti-inflammatory effects via suppression of NF-κB activation, catechins have the potential to suppress CAV in the same way. Although statins and rapamycine are effective for prevention of CAV, they may have systemic adverse effects. However, tea catechins have no adverse effects because they are natural extracts and millions of people have drunk them for more than several centuries. In fact, several clinical trials have proved their safety and effects on cancer and other diseases [81, 82].

CONCLUSION

In this article, we demonstrated that tea catechins attenuate both myocardial remodeling and CAV formation by inhibition of multiple inflammatory genes without systemic adverse effects. Further studies should be conducted in other models to explore the clinical utility of catechins for prevention of ventricular remodeling and arterial diseases.

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REFERENCES

[1] Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, Dobbels F, Goldfarb SB, Levvey BJ, Meiser B, Yusen RD, Stehlik J; International Society of Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: Thirty-first Official Adult Heart Transplant Report-2014; Focus Theme: Retransplantation. *J. Heart Lung. Transplant*. 2014; 33: 996-1008.

[2] Suzuki J, Isobe M, Morishita R, Nagai R. Characteristics of chronic rejection in heart transplantation –Important elements of pathogenesis and future treatments-. *Circ. J.* 2010; 74: 233-239.

[3] Suzuki J, Isobe M, Aikawa M, Kawauchi M, Shiojima I, Kobayashi N, et al., Nonmuscle and smooth muscle myosin heavy chain expression in rejected cardiac allograft. A study in rat and monkey models. *Circulation.* 1996, 96: 1118-24.

[4] Mitchell RN. Graft vascular disease: immune response meets the vessel wall. Annu. Rev. Pathol. 2009; 4: 19-47.

[5] Suzuki J, Isobe M, Morishita R, Aoki M, Horie S, Okubo Y, et al., Prevention of graft coronary arteriosclerosis by antisense cdk2 kinase oligonucleotide. *Nature Med*. 1997, 3: 900-3.

[6] Ishikawa T, Suzukawa M, Ito T, Yoshida H, Ayaori M, Nishiwaki M, et al., Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. *Am. J. Clin. Nutr*. 1997, 66: 261-6.

[7] Junkun J, Selman SH, Swiercz R, Skrzypczak-Jankun E. Why drinking tea could prevent *cancer. Nature*. 1997, 387: 561.

[8] Beecher GR, Warden BA, Merken H. Analysis of tea polyphenols. Proc Soc. Exp. Biol. Med. 1999, 220: 267-70.

[9] Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu. Rev. Nutr*. 2001, 21: 381-406.

[10] Lin YL, Lin JK. (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB. *Mol. Pharmacol*. 1997, 52: 465-72.

[11] Benelli R, Vene R, Bisacchi D, Garbisa S, Albini A. Anti-invasive effects of green tea polyphenol epigallocatechin-3-gallate (EGCG), a natural inhibitor of metallo and serine proteases. *Biol. Chem*. 2002, 383: 101-5.

[12] May MJ, Ghosh S. Signal transduction through NF-kappa B. *Immunol. Today*. 1998, 19: 80-8.

[13] Sha WC. Regulation of immune responses by NF-kappa B/Rel transcription factor. *J. Exp. Med*. 1998, 187: 143-146.

[14] Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N. Eng. J. Med*. 1997, 336: 1066-71.

[15] Suzuki J, Morishita R, Amano J, Kaneda Y, Isobe M. Decoy against nuclear factor-kappa B attenuates myocardial cell infiltration and arterial neointimal formation in murine cardiac allografts. *Gene. Ther*. 2000, 7: 1847-52.
[16] Suzuki J, Ogawa M, Sagesaka YM, Isobe M. Tea catechins attenuate ventricular remodeling and graft arterial diseases in murine cardiac allografts. *Cardiovasc. Res*. 69: 272-279, 2006.
[17] Isobe M, Yagita H, Okumura K, Ihara A. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. *Science*. 1992, 255: 1125-7.
[18] Furukawa Y, Matsumori A, Hirozane T, Sasayama S. Angiotensin II receptor antagonist TCV-116 reduces graft coronary artery disease and preserves graft status in a murine model. A comparative study with captopril. *Circulation*. 1996, 93: 333-9.
[19] Suzuki J, Cole SE, Batirel S, Kosuge H, Shimizu K, Isobe M, et al., Tumor necrosis factor receptor -1 and -2 double deficiency reduces graft arterial disease in murine cardiac allografts. *Am. J. Transplant*. 2003, 3: 968-76.
[20] Isobe M, Suzuki J, Yagita H, Okumura K, Yamazaki S, Nagai R, et al., Immunosuppression to cardiac allografts and soluble antigens by anti-vascular cellular adhesion molecule-1 and anti-very late antigen-4 monoclonal antibodies. *J. Immunol*. 1994, 153: 5810-8.
[21] Suzuki J, Isobe M, Yamazaki S, Horie S, Okubo Y, Sekiguchi M. Inhibition of accelerated coronary atherosclerosis with short-term blockade of intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 in a heterotopic murine model of heart transplantation. *J. Heart Lung. Transplant.* 1997, 16: 1141-8.
[22] Futamatsu H, Suzuki J, Kosuge H, Yokoseki O, Kamada M, Ito H, et al., Attenuation of experimental autoimmune myocarditis by blocking activated T cells through inducible costimulatory molecule pathway. *Cardiovasc. Res*. 2003, 59: 95-104.
[23] Suzuki J, Ogawa M, Sagesaka YM, Isobe M. Catechins attenuate myocardial cell infiltration and fibrosis but do not prolong graft survival in murine cardiac allografts. *Transplant. Proc.* 2005, 37: 119-20.
[24] Furukawa Y, Becker G, Stinn JL, Shimizu K, Libby P, Mitchell RN. Interleukin-10 augments allograft arterial disease: paradoxical effects of IL-10 in vivo. *Am. J. Pathol*. 1999, 155: 1929-39.
[25] Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* 2000, 130(8S Suppl): 2073S-85S.
[26] Weisburger JH. Lifestyle, health and disease prevention: the underlying mechanisms. *Eur. J. Cancer Prev.* 2002, 11 Suppl 2: S1-7.
[27] Aviram M, Dornfeld L, Kaplan M, Coleman R, Gaitini D, Nitecki S, et al., Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. *Drugs Exp. Clin. Res*. 2002, 28: 49-62.
[28] Lambert JD, Yang CS. Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. *Mutat. Res.* 2003, 523-524: 201-8.
[29] Kudo M, Naito Z, Yokoyama M, Asano G. Effects of quercetin and sunphenon on responses of cancer cells to heat shock damage. *Exp. Mol. Pathol.* 1999, 66: 66-75.
[30] Takabayashi F, Harada N. Effects of green tea catechins (Polyphenon 100) on cerulein-induced acute pancreatitis in rats. *Pancreas.* 1997, 14: 276-9.

[31] Locher R, Emmanuele L, Suter PM, Vetter W, Barton M. Green tea polyphenols inhibit human vascular smooth muscle cell proliferation stimulated by native low-density lipoprotein. *Eur. J. Pharmacol*. 2002, 434: 1-7.

[32] Suzuki J, Ogawa M, Izawa A, Sagesaka YM, Isobe M. Dietary consumption of green tea catechins attenuate hyperlipidemia-induced atherosclerosis and systemic organ damage in mice. *Acta Cardiologica*. 2005; 60: 271-276.

[33] Morishita R, Sugimoto T, Aoki M, et al., In vivo transfection of cis element "decoy" against nuclear factor-kappaB binding site prevents myocardial infarction. *Nat. Med*. 1997; 3: 894-899.

[34] Yokoseki O, Suzuki J, Kitabayashi H, et al., cis element decoy against nuclear factor-κB attenuates development of experimental autoimmune myocarditis in rats. *Circ. Res*. 2001; 89: 899-906.

[35] Wakatsuki S, Suzuki J, Ogawa M, Masumura M, Muto S, Shimizu T, Takayama K, Itai A, Isobe M. A novel IKK inhibitor suppresses heart failure and chronic remodeling after myocardial ischemia via MMP alteration. *Expert Opin. Ther. Targets*. 2008; 12: 1469-76.

[36] Onai Y, Suzuki J, Maejima Y, Haraguchi G, Muto S, Itai A, Isobe M. Inhibition of NF-kB improves left ventricular remodeling and cardiac dysfunction after myocardial infarction. *Am. J. Physiol. Heart Circ*. Physiol. 2007; 291: H530-8.

[37] Aneja R, Hake PW, Burroughs TJ, Denenberg AG, Wong HR, Zingarelli B. Epigallocatechin, a green tea polyphenol, attenuates myocardial ischemia reperfusion injury in rats. *Mol. Med*. 2004; 10: 55-62.

[38] Han DW, Lim HR, Baek HS, Lee MH, Lee SJ, Hyon SH, Park JC. Inhibitory effects of epigallocatechin-3-O-gallate on serum-stimulated rat aortic smooth muscle cells via nuclear factor-kappaB down-modulation. *Biochem. Biophys. Res. Commun.* 2006; 345: 148-55.

[39] Kim SJ, Jeong HJ, Lee KM, Myung NY, An NH, Yang WM, Park SK, Lee HJ, Hong SH, Kim HM, Um JY. Epigallocatechin-3-gallate suppresses NF-kappaB activation and phosphorylation of p38 MAPK and JNK in human astrocytoma U373MG cells. *J. Nutr. Biochem*. 2007; 18: 587-96.

[40] Mackenzie GG, Oteiza PI. Modulation of transcription factor NF-kappaB in Hodgkin's lymphoma cell lines: effect of (-)-epicatechin. *Free Radic. Res*. 2006; 40: 1086-94.

[41] Li C, Browder W, Kao RL. Early activation of transcription factor NF-kB during ischemia in perfused rat heart. *Am. J. Physiol*. 1999; 45: H543–H552.

[42] Li Q, Verma IM, NF-kB regulation in the immune system. *Nat. Rev. Immunol.* 2002; 2: 725–734.

[43] Chandrasekar B, Freeman GL. Induction of nuclear factor B and activation protein 1 in postischemic myocardium. *FEBS Lett*. 1997; 401: 30–34.

[44] Onai Y, Suzuki J, Kakuta T, et al., Inhibition of IkB phosphorylation in cardiomyocytes attenuates myocardial ischemia/reperfusion injury. *Cardiovasc. Res*. 2004; 63: 51-59.

[45] Lin YL, Lin JK. (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB. *Mol. Pharmacol*. 1997; 52: 465-72.

[46] Suzuki J, Ogawa M, Maejima Y, et al., Tea catechins attenuate chronic ventricular remodeling after myocardial ischemia in rats. *J. Mol. Cell* Cardiol. 2007; 42: 432-440.

[47] Cheng XW, Kuzuya M, Nakamura K, Liu Z, Di Q, Hasegawa J, Iwata M, Murohara T, Yokota M, Iguchi A. Mechanisms of the inhibitory effect of epigallocatechin-3-gallate on cultured human vascular smooth muscle cell invasion. Arterioscler. *Thromb. Vasc. Biol.* 2005; 25: 1864-70.

[48] Ivanov V, Roomi MW, Kalinovsky T, Niedzwiecki A, Rath M. Anti-atherogenic effects of a mixture of ascorbic acid, lysine, proline, arginine, cysteine, and green tea phenolics in human aortic smooth muscle cells. *J. Cardiovasc. Pharmacol*. 2007; 49: 140-5.

[49] El Bedoui J, Oak MH, Anglard P, Schini-Kerth VB. Catechins prevent vascular smooth muscle cell invasion by inhibiting MT1-MMP activity and MMP-2 expression. *Cardiovasc. Res*. 2005; 67: 317-25.

[50] Kim CH, Moon SK. Epigallocatechin-3-gallate causes the p21/WAF1-mediated G(1)-phase arrest of cell cycle and inhibits matrix metalloproteinase-9 expression in TNF-alpha-induced vascular smooth muscle cells. *Arch. Biochem. Biophys*. 2005; 435: 264-72.

[51] Maeda K, Kuzuya M, Cheng XW, Asai T, Kanda S, Tamaya-Mori N, Sasaki T, Shibata T, Iguchi A. Green tea catechins inhibit the cultured smooth muscle cell invasion through the basement barrier. *Atherosclerosis.* 2003; 166: 23-30.

[52] Singh AK, Seth P, Anthony P, Husain MM, Madhavan S, Mukhtar H, Maheshwari RK. Green tea constituent epigallocatechin-3-gallate inhibits angiogenic differentiation of human endothelial cells. *Arch. Biochem. Biophys*. 2002; 401: 29-37.

[53] Yamazaki KG, Romero-Perez D, Barraza-Hidalgo M, Cruz M, Rivas M, Cortez-Gomez B, Ceballos G, Villarreal F. Short- and long-term effects of (-)-epicatechin on myocardial ischemia-reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol*. 2008; 295: H761-7.

[54] Ho YC, Yang SF, Peng CY, Chou MY, Chang YC. Epigallocatechin-3-gallate inhibits the invasion of human oral cancer cells and decreases the productions of matrix metalloproteinases and urokinase-plasminogen activator. *J. Oral Pathol. Med*. 2007; 36: 588-93.

[55] Zhen MC, Wang Q, Huang XH, Cao LQ, Chen XL, Sun K, Liu YJ, Li W, Zhang LJ. Green tea polyphenol epigallocatechin-3-gallate inhibits oxidative damage and preventive effects on carbon tetrachloride-induced hepatic fibrosis. *J. Nutr. Biochem*. 2007; 18: 795-805.

[56] Lee JH, Chung JH, Cho KH. The effects of epigallocatechin-3-gallate on extracellular matrix metabolism. *J. Dermatol. Sci*. 2005; 40: 195-204.

[57] Oakley CM. Myocarditis, pericarditis and other pericardial diseases. Heart. 2000; 84: 449-454.

[58] Batra AS, Lewis AB. Acute myocarditis. *Curr. Opin. Pediatr*. 2001; 13: 234-239.

[59] Zee-Cheng CS, Tsai CC, Palmer DC, Codd JE, Pennington DG, Williams GA. High incidence of myocarditis by endomyocardial biopsy in patients with idiopathic congestive cardiomyopathy. *J. Am. Coll. Cardiol.* 1984; 3: 63-70.

[60] Parrillo JE, Aretz HT, Palacios I, Fallon JT, Block PC. The results of transvenous endomyocardial biopsy can frequently be used to diagnose myocardial diseases in patients with idiopathic heart failure: endomyocardial biopsies in 100 consecutive patients revealed a substantial incidence of myocarditis. *Circulation.* 1984; 69: 93-101.

[61] Kodama M, Matsumoto Y, Fujiwara M, Masani F, Izumi T, Shibata A. A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. *Clin. Immunol. Immunopathol.* 1990; 57: 250-262.

[62] Kodama M, Izumi T. Experimental autoimmune myocarditis. Acta. Med. Bio. 1991; 39: 1-10.

[63] Kitabayashi H, Isobe M, Watanabe N, Suzuki J, Yazaki Y, Sekiguchi M. FTY 720 prevents development of experimental autoimmune myocarditis through reduction of circulating lymphocytes. *J. Cardiovasc. Pharmacol.* 2000; 35: 410-416.

[64] Futamatsu H, Suzuki J, Kosuge H, et al., Attenuation of experimental autoimmune myocarditis by blocking activated T cells through inducible costimulatory molecule pathway. *Cardiovasc. Res*. 2003; 59: 95-104.

[65] Suzuki J, Ogawa M, Futamatsu H, Kosuge H, Sagesaka YM, Isobe M. Tea catechins improve left ventricular dysfunction, suppress myocardial inflammation, fibrosis, and alter cytokine expression in rat autoimmune myocarditis. *Eur. J. Heart Fail*. 2007; 9: 152-159.

[66] Ahn HY, Xu Y, Davidge ST. Epigallocatechin-3-O-gallate inhibits TNFalpha-induced monocyte chemotactic protein-1 production from vascular endothelial cells. *Life Sci*. 2008; 82: 964-8.

[67] Guruvayoorappan C, Kuttan G. (+)-Catechin inhibits tumour angiogenesis and regulates the production of nitric oxide and TNF-alpha in LPS-stimulated macrophages. *Innate Immun*. 2008; 14: 160-74.

[68] Ahmed S, Marotte H, Kwan K, Ruth JH, Campbell PL, Rabquer BJ, Pakozdi A, Koch AE. Epigallocatechin-3-gallate inhibits IL-6 synthesis and suppresses transsignaling by enhancing soluble gp130 production. *Proc. Natl. Acad. Sci. USA*. 2008; 105: 14692-7.

[69] Crouvezier S, Powell B, Keir D, Yaqoob P. The effects of phenolic components of tea on the production of pro- and anti-inflammatory cytokines by human leukocytes in vitro. *Cytokine.* 2001; 13: 280-6.

[70] Abboud PA, Hake PW, Burroughs TJ, Odoms K, O'Connor M, Mangeshkar P, Wong HR, Zingarelli B. Therapeutic effect of epigallocatechin-3-gallate in a mouse model of colitis. *Eur. J. Pharmacol.* 2008; 579: 411-7.

[71] Babu PV, Liu D. Green tea catechins and cardiovascular health: an update. *Curr. Med. Chem*. 2008; 15: 1840-1850.

[72] Ludwig A, Lorenz M, Grimbo N, Steinle F, Meiners S, Bartsch C, Stangl K, Baumann G, Stangl V. The tea flavonoid epigallocatechin-3-gallate reduces cytokine-induced VCAM-1 expression and monocyte adhesion to endothelial cells. Biochem. Biophys. Res. Commun. 2004; 316: 659-65.

[73] Chae YJ, Kim CH, Ha TS, Hescheler J, Ahn HY, Sachinidis A. Epigallocatechin-3-O-gallate inhibits the angiotensin II-induced adhesion molecule expression in human umbilical vein endothelial cell via inhibition of MAPK pathways. *Cell. Physiol. Biochem*. 2007; 20: 859-66.

[74] Handa O, Naito Y, Takagi T, Ishikawa T, Ueda M, Matsumoto N, Kokura S, Ichikawa H, Yoshida N, Shimoi K, Yoshikawa T. Inhibitory effects of catechins on neutrophil-dependent gastric inflammation. *Redox. Rep*. 2002; 7: 324-8.

[75] Chen WC, Hayakawa S, Shimizu K, Chien CT, Lai MK. Catechins prevents substance P-induced hyperactive bladder in rats via the downregulation of ICAM and ROS. *Neurosci. Lett*. 2004; 367: 213-7.

[76] Tripathi S, Bruch D, Gatto LA, Kittur DS. Green tea extract prolongs allograft survival as an adjunctive therapy along with low dose cyclosporine A. *J. Surg. Res*. 2009; 154: 85-90.

[77] Uretsky BF, Murali S, Reddy PS, Rabin B, Lee A, Griffith BP, et al., Development of coronary artery disease in cardiac transplanted patients receiving immunosuppressive therapy with cyclosporine and predonisolone. *Circulation*. 1987, 76: 827-34.

[78] Pollak R, Fabrega AJ. Diltazem in the prevention of coronary artery disease in heart-transplant recipients. *N. Eng. J. Med*. 1993, 28: 1851-2.

[79] See VY Jr, DeNofrio D, Goldberg L, Chang G, Sasseen B, Kolansky DM, et al., Effect of *atorvastatin* on postcardiac transplant increase in low-density lipoprotein cholesterol reduces development of intimal hyperplasia and progression of endothelial dysfunction. *Am. J. Card*. 2003, 92: 11-5.

[80] Eisen HJ, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, Starling RC, Sørensen K, Hummel M, Lind JM, Abeywickrama KH, Bernhardt P; RAD B253 Study Group. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *N. Engl. J. Med*. 2003; 349: 847-58.

[81] Moyers SB, Kumar NB. Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials. *Nutr. Rev*. 2004, 62: 204-11.

[82] Mukhtar H, Ahmad N. Tea polyphenols: prevention of cancer and optimizing health. *Am. J. Clin. Nut.* 2003, 71: 1698S-702S.

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Chapter 5

IMPACT OF GREEN TEA (*CAMELLIA SINENSIS* L.) CONSUMPTION IN DIABETES MELLITUS-INDUCED NEURODEGENERATION

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ABSTRACT

The medicinal properties of tea (*Camellia sinensis* L.) have a long and interesting history, dating back to many centuries ago. Green tea has aroused considerable interest in recent years, being nowadays one of the most studied types of teas. Green tea is a complex mixture of thousands of chemical compounds, including proteins and free amino acids, polysaccharides, vitamins, organic acids, methylxanthines, and polyphenols. Catechins, caffeine and L-theanine are often reported as the main phytochemicals responsible for green tea's health benefits, namely by its antioxidant, hypoglycemic, and neuroprotective properties.

Diabetes mellitus (DM) is the most common metabolic disease and its incidence is dramatically rising. In addition, DM is associated to a high risk of developing neurodegenerative diseases, since the brain is particularly susceptible to glucose fluctuations and hyperglycaemia-induced oxidative stress. Throughout this chapter we will discuss the phytochemical composition and bioactivities of green tea, especially antioxidant, antidiabetic, and neuroprotective activities. The potential beneficial effects of green tea consumption on DM and how it can be used to reduce the severe brain damage induced by this disease will be emphasized.

INTRODUCTION

The tea plant, *Camellia sinensis* (L.), has been extensively used in traditional medicine and ancient cultures to prevent and treat several diseases [1, 2]. The origins of tea are mythological. The "Father of Tea", Eisai, said: "Tea is a miraculous medicine for the maintenance of health. Tea has an extraordinary power to prolong life." [2]. Tea is the infusion prepared by using *C. sinensis* leaves and is one of the most widely consumed beverages in the world [3]. The sensorial properties and stimulating effects, with potential health benefits make this a very popular drink [1, 4, 5]. Green tea is the most studied type of tea and, like white tea, has been reported to have a beneficial effect in cardiovascular diseases, cancer and reproduction [1, 6-9]. In recent years, the interest regarding the potential benefits for health of green tea intake has grown [1] due to the interest in alternative medicinal strategies. Indeed, scientific studies of this beverage and its constituents have been underway for less than three decades. Several of those studies have shown a significant association between green tea consumption and reduced rate of cardiovascular and metabolic diseases, cancer, neurodegenerative diseases, and others [1, 10]. Moreover, it is known that phenolic compounds, namely catechins, and other phytochemicals, such as methylxanthines and L-theanine, are responsible for the medicinal effects of this beverage [1, 6, 7].

Diabetes mellitus (DM) is one of the greatest threats to modern global health and is one of the most prevalent chronic diseases in western societies. It was estimated that about 300 million of people will develop DM in 2025 [11]. This is a metabolic disorder that may result from absolute deficiency of insulin, insulin resistance, or both [12]. It can be classified in two major forms: Type 1 Diabetes Mellitus (T1DM) or Type 2 Diabetes Mellitus (T2DM). T1DM results from the autoimmune destruction of the pancreatic beta cells and T2DM is characterized by impaired insulin secretion and increased insulin resistance. Although these types of DM result from different actions, the hyperglycaemic state is a common feature responsible for changes in the structure and function of several cells, tissues and organs. The brain is no exception. Indeed, high blood glucose levels are implicated in the development of cerebrovascular disease and other neurological comorbidities, such as cognitive dysfunction and dementia [13, 14]. Diabetic individuals are reported to have a higher risk of cognitive decline and neurodegeneration [15]. Some studies report that individuals with T2DM have an accelerated cognitive decline associated with a higher increase in the volume of brain ventricles [16]. Furthermore, various studies demonstrated the connection between T2DM and Alzheimer's disease (AD) [17], though the exact mechanisms by which DM affects the health of the brain remain unclear. This is of extreme relevance since DM is one of the major causes of dead in the world. In addition, neurodegenerative diseases are important medical and social challenges that modern societies face. Thus, the association of DM with an increased probability of its development must be carefully discussed. In the last years it has been discussed that new ways to reduce the damage caused by DM may arise through a modification in lifestyle, particularly by changes in diet. There is a large interest in finding an effective therapy for DM, particularly to DM-associated neurodegeneration, and tea seems to be a good candidate [18]. Green tea and its phytochemicals have gain attention from different research groups due to its interesting antidiabetic, neuroprotective and antioxidant properties [19-21]. Throughout this chapter we will discuss the phytochemical composition of green tea,

as well as its beneficial effects to DM and neurodegenerative processes caused by this metabolic disease.

ORIGIN AND PRODUCTION OF GREEN TEA

C. sinensis, commonly known as the tea plant, is an evergreen shrub of the Theaceae family, native to Southeast China [22]. Nowadays, tea is cultivated in over thirty countries across the world [2], including one single place in Europe - S. Miguel Island (Azores Archipelago, Portugal) [23]. Tea is the most ancient and widely consumed beverage in the world, with a per capita consumption of approximately 120 mL/day [24]. Notably, it has been used in traditional medicine for centuries due to its several claimed health benefits [1, 4, 20] such as the prevention and treatment of some diseases, including DM [1, 2].

Tea is the infusion prepared from the leaves or buds of the *C. sinensis*. The tea plant can originate four main types of tea, usually classified in three categories: unfermented (white and green teas), semi-fermented (oolong tea) and fully fermented (black tea) forms. This classification is based on the differences that occur in the collection and manufacture processes, resulting in different chemical compositions [25]. Upon harvesting, the leaves suffer an enzymatic oxidation process, commonly called “fermentation” [4, 20, 26]. This process occurs with exposure to air by a reaction, which involves the enzyme polyphenol oxidase (PO). As expected, according to the level of “fermentation”, the types of tea have different chemical compositions (phenolic profiles) and organoleptic properties (appearances and tastes). To produce green tea, the fresh leaves are harvested and quickly steamed to inactivate PO, preventing the oxidation of catechins, and then rolled and dried [1, 2] (Figure 1). Thus, its composition is very similar to that of *C. sinensis* leaves.

CHEMICAL COMPOSITION OF GREEN TEA

Green tea is generally prepared in a proportion of 1 g of tea leaves per 100 mL of boiling water. This preparation guarantees that the green tea has a very complex chemical composition, containing polyphenols (e.g. catechins and their derivatives), methylxanthines (e.g. caffeine, theophylline and theobromine), free amino acids (e.g. L-theanine), minerals and trace elements, organic acids, lipids, and other components [4, 20, 26]. However, the chemical composition of green tea varies according to the preparation since the tea phytochemicals are susceptible to extraction conditions (e.g. solvents, temperatures, times of extraction) [27, 28]. In addition, several other factors such as geographical origin, climate, growing conditions, harvesting practices, and manufacturing processes [20, 29] can alter the chemical and organoleptic properties of the tea.

Green Tea Polyphenols

Polyphenols seem to be the most abundant and active group present in tea leaves. In addition, several studies highlight that they are responsible for much of the health benefits

attributed to green tea [30, 31]. These phytocomponents are relatively abundant [1, 2]. In fact, 200 mL of green tea might contain up to 200 mg of polyphenols [32]. Catechins (or flavan-3-ols) constitute the most abundant class of phenolic compounds found in unfermented teas. The major catechins found in green tea are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin 3-gallate (EGCG) [1, 7, 20]. These compounds have a very high antioxidant power [31, 33]. The health benefits attributed to catechins are mainly due to its chemical structure. The major catechins are composed of three rings (two aromatic rings, A and B, linked to a dihydropyran heterocyclic ring, C) and are characterized by the presence of several hydroxyl groups [34] (Figure 2). Their chemical differences are due to the presence of different groups attached to those rings [4, 34]. EC contains an ortho-di-hydroxyl group in the B ring (at carbons 3' and 4') and a hydroxyl group in the C ring (at carbon 3); EGC has a three hydroxyl groups at carbons 3', 4', and 5' on the B-ring, while ECG has a gallate moiety esterified at carbon 3 of the C-ring and EGCG contains both the three hydroxyl groups at carbons 3', 4', and 5' on the B-ring and a gallate moiety esterified at carbon 3 on the C-ring.

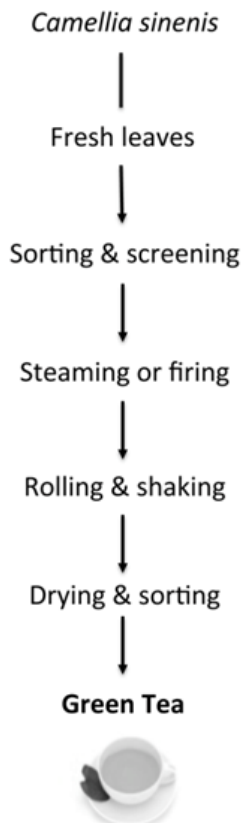
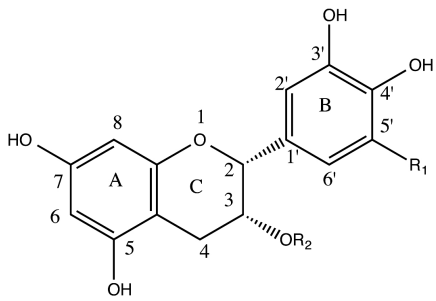
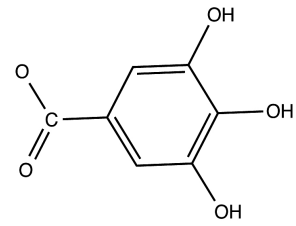


Figure 1. Schematic representation of green tea processing. In the production of green tea, the leaves are harvested and then quickly steamed or fired, to inactivate polyphenol oxidase (PO) and prevent oxidation, before drying.



Main catechin structure



Gallate group

	R1	R2
EC	H	H
EGC	OH	H
ECG	H	Gallate group
EGCG	OH	Gallate group

Figure 2. Chemical structure of the main green tea catechins. The major catechins are composed by two aromatic rings (A and B) and a dihydropyran heterocyclic ring (C). The (-)-epicatechin (EC) is constituted by an ortho-di-hydroxyl group in the B ring (at carbons 3' and 4') and a hydroxyl group in the C ring (at carbon 3). Its ester derivative, (-)-epicatechin 3-gallate (ECG), differs in this structure by possessing an additional gallate moiety esterified in the C ring, at carbon 3. On the other hand, (-)-epigallocatechin (EGC) contains three hydroxyl groups on the B ring (at carbons 3', 4' and 5') and its ester derivative (-)-epigallocatechin 3-gallate (EGCG) additionally possesses an esterified gallate at the carbon 3 of the C ring.

The concentration of catechins is different in each type of tea. It has been shown that green and white teas have the higher levels of catechins [4, 29]. EGCG is considered the most abundant and active, and has been extensively studied [2, 35]. In addition, phenolic acids, flavonols, and/or flavones and their derivatives were also consistently found in green tea extracts [6, 23, 36]. The oxidation process of catechins, catalyzed by PO, results in the formation of theaflavins and thearubigins [29]. Theaflavins are composed by a bicyclic benzotropolone ring, and result from the dimerization of catechins. Thearubigins have oligopolymeric structures and can result of the hydroxylation of theaflavins. Interestingly, these compounds are less soluble and are responsible for black and oolong teas bitter taste and dark color [2, 36, 37].

The redox properties of polyphenols, which are associated with their chemical structures, are in the basis of the reported antioxidant properties of tea. The relationship between the content of pyrogallol and hydroxyl groups, and the presence of galloyl moieties, may be involved in the superoxide anion and hydroxyl radicals scavenging ability, respectively [1, 38]. Moreover, the number and position of the hydroxyl groups influence the antioxidant ability of flavonoids [34]. However, higher total phenolic component may not always directly represent a greater antioxidant capacity, since different phenolic profiles can yield different responses [39]. Fermented teas have a lower catechins concentration comparatively to

unfermented teas but black and oolong tea have also considerable antioxidant properties, such as hydroxyl radical scavenging and nitric oxide suppressing [29]. Several studies suggest that tea catechins are effective scavengers of reactive oxygen species (ROS) [40, 41], and this is of extreme relevance for the control of oxidative stress (OS). It is know that OS is implicated in the establishment and progression of DM and its associated co-morbidities [42]. In addition, OS induces neuronal death, suggesting that it may be in the origin of DM-induced neurodegeneration [17]. Thus, there is a growing interest in the possible benefits of green tea against the neurodegeneration reported in DM individuals.

Besides the phenolic compounds, there are other phytochemicals that may contribute to the improvement of health by green tea, such as caffeine, theophylline, L-theanine, among others.

Tea Methylxanthines

Caffeine (Figure 3) is the main methylxanthine present in teas (1.0-3.5%) [29]. Theobromine and theophylline are other important methylxanthines present in tea (Figure 3).

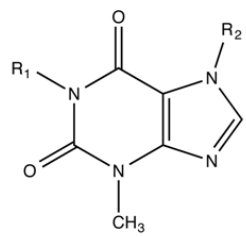
Caffeine is one of the most consumed psychoactive substances in the world [43], mainly due to its stimulant properties. Tea leaves are a major source of dietary caffeine. Due to its chemical stability, the oxidation process does not affect its levels in tea [44]. However, the caffeine content in the various types of tea is not completely consensual. Different extraction conditions and distinct analytical methods may explain the controversy between absolute values. Some authors reported that fermented teas present greater caffeine content than green tea [29]. However, a recent study conducted by our research group showed that white tea can contain a higher concentration of caffeine than green tea [7]. Interestingly, some authors proposed that the lowest caffeine content in green tea contributes to its beneficial health properties [45], but this subject remains under debate.

Pharmacokinetic studies have shown that humans easily absorb caffeine and approximately 100% of bioavailability is achieved when taken by oral route. This methylxanthine is absorbed in the stomach and small intestine within 45 minutes after intake and reaches a maximum concentration in blood after 15-120 minutes [46]. After being absorbed, caffeine is distributed to various tissues, and is reported to stimulate the central nervous system (CNS). It acts through stimulation of adenosine receptors and competitively inhibits the action of adenosine in the cells, which results in an increased release of hormones such as norepinephrine, dopamine and serotonin [47]. Moderate levels of caffeine are reported to have some benefits to health. For example, caffeine seems to be a likely candidate against memory loss [48], and has a notorious neuroprotective potential [49]. Of note, the consumption of caffeine-containing beverages, in particular tea, is associated with a lower risk of developing T2DM [19]. Caffeine, in moderate doses, is beneficial to health due to antioxidant effects. It is know that antioxidants may reduce the amount of ROS, decreasing insulin resistance and beta cell dysfunction. In addition, a study in rats showed that this methylxanthine can interact with glucose transporters in adipocytes and act as an antagonist of adenosine receptors [50]. Others have also reported that caffeine has an antioxidant role, protecting against cellular damage, by decreasing lipid peroxidation [51, 52]. For instance, it was recently shown that moderate consumption of caffeine appears to be safe to the metabolic functioning of human Sertoli cells, and male fertility in general [53]. However, when caffeine

is consumed in excess, it may lead to various deleterious health effects [54], such as coronary heart disease, reproductive disorders, and psychiatric disturbances [47]. Nevertheless, data on the role of caffeine on tea-associated health benefits remain largely unknown and more studies are needed.

Tea Amino Acids

L-Theanine (Figure 4) is a non-proteinogenic amino acid that was first isolated from green tea leaves in 1940s by Sakato [55]. This free amino acid usually constitutes about 1-3% of the dry weight of tea, but this percentage may vary according to growing location and method of cultivation, tea grade, variety, processing and collection time [55]. For instance, reduced sunlight during tea growing has been shown to induce higher concentrations of L-theanine and lower contents of catechins [55]. Moreover, tea variety is also important; for example, *C. sinensis* var. *sinensis* is known to contain higher concentrations of L-theanine than C. *sinensis* var. *assamica* [55]. Nevertheless, green, oolong and black teas are reported to contain similar levels of L-theanine [56].



	R1	R2
Caffeine	CH_3	CH_3
Theophylline	CH_3	H
Theobromine	H	CH_3

Figure 3. Chemical structure of the three methylxanthines present in tea. Caffeine (1,3,7-trimethylpurine-2,6-dione), theophylline (1,3-dimethylpurine-2,6-dione) and theobromine (3,7-dimethylpurine-2,6-dione). They are all purine derivatives, with three methyl groups at positions 1, 3 and 7 or two methyl groups at positions 1 and 3 or 3 and 7.

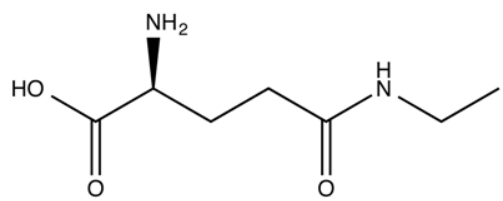


Figure 4. Chemical structure of L-theanine (N-ethyl-L-glutamine). This is an amino acid analogue of L-glutamate and L-glutamine that has an ethyl group at the amide nitrogen.

Some health benefits have been reported to this amino acid. For instance, it can be considered as a relaxing agent with antioxidant and neuroprotective effects [57, 58]. Metabolically, it is easily absorbed from the gastrointestinal tract and peak plasma concentrations are detected 30 minutes after administration [58]. In addition, it is partially transported to the brain via leucine preferring transporter system and can cross the blood brain-barrier (BBB) having protective and preventive effects on neuronal cell death [59]. Nevertheless, its pharmacology and modes of action remain relatively unknown.

Besides L-theanine, other amino acids can also be found in this beverage: L-glutamic acid, L-arginine, L-aspartic acid, L-glutamine, L-serine, L-tyrosine, L-alanine, L-asparagine, L-lysine and L-valine [60].

GREEN TEA AND DIABETES MELLITUS

DM is considered one of the leading causes of morbidity and mortality worldwide. It is described as a metabolic disorder with several long-term complications that result from physiological and morphological alterations in tissues and organs throughout the organism [20, 61]. Hyperglycaemia is a hallmark of this disease as a consequence of impaired insulin secretion, insulin resistance, or both. Consequently, body glucose metabolism becomes deregulated. Some risk factors related to lifestyle, such as overweight and unhealthy diet, may contribute to the development of this disease. Furthermore, due to the complexity of this disease, it has been established an intermediate state called prediabetes. It is characterized by elevated blood glucose levels, though not high enough to be considered DM [12]. This prodromal stage is increasing among young people and is known as a major risk for the development of T2DM. T2DM is the most common type of DM, accounting for up to 90-95% of all cases diagnosed [12]. Age, obesity, cardiovascular diseases, and lack of physical activity are some conditions that increase the risk of developing T2DM [62] and thus promote prediabetes. Noteworthy, the clinical symptoms are frequently detected only in an advanced phase of the disease, allowing the progression of functional changes in cells and tissues that may not be reverted.

DM is incurable but there are many available strategies for its treatment. However, the side effects and the loss of effectiveness of some treatments are issues that must be take into consideration when discussing the therapy to this disease. In recent years, it has been encouraged the search of more efficient and cost-effective alternatives, recurring to dietary and lifestyle changes. In that search, natural products have arisen has a possible strategy.

In Vitro Studies and Animal Models

Functional foods and nutraceuticals have been targets of great interest in the field of Food Science in order to complement or even replace current therapies. Green tea has been valued around the world due to its medicinal properties, and is being widely studied. Many health benefits have been attributed to tea [6-8, 20, 26] and green tea phytochemicals play an important role in contributing to those human health benefits.

Green tea is an excellent source of potent antioxidants, being EGCG its main and most known antioxidant phytochemical. Animal studies and *in vitro* studies report elucidative data illustrating that green tea can be a very effective treatment to DM. Traditionally, green tea has been used to control glucose levels. *In vitro* studies in H4IIE rat hepatoma cells treated with EGCG, the major catechin of green tea, showed that EGCG is insulinomimetic, decreasing not only the production of glucose by these cells but also the expression of genes that control gluconeogenesis [63]. Similarly, Wolfram and collaborators [64] studied the influence of EGCG on glucose and lipid metabolism-related genes in the same cells. It was reported that this catechin reduced the expression of genes involved in fatty acids synthesis, downregulated genes involved in gluconeogenesis, and increased genes involved in glycolysis and glucose transporter 1 (GLUT 1) [64]. Others suggested that catechins, mainly EGCG, and theaflavins help to prevent hyperglycaemia by enhancing insulin activity and possibly by preventing damage in pancreatic beta-cells [65]. Moreover, in isolated pancreatic islet cells culture, the addition of EGCG improved the survival rate [66] and protected against cytokine-induced damage to the pancreatic beta cell line RINm5F [67]. The summary of the *in vitro* studies reported herein is presented in Table 1.

Of note, some of the effects of tea components observed *in vitro* have, to some extent, been also reported *in vivo*. It has been reported that green tea reduces blood glucose levels and improves glucose metabolism in diabetic rats [80, 81]. Hyperglycaemia has been associated to oxidative stress (OS) and OS is involved in the development and progression of DM [82] illustrating a cyclic link between these events. It has been reported that DM-related hyperglycaemia amplifies OS [83] by increasing the production of free radicals and/or by declining the antioxidant defenses [42]. Notably, OS is reported as a hallmark present in the early (prediabetic state) and late phase of DM [84]. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually lead to cell death [85]. Glucose oxidation, non-enzymatic glycation of proteins, oxidative degradation of glycated proteins and the mitochondrial respiratory system form free radicals in diabetic individuals [42]. Moreover, it has been shown that reactive oxygen species (ROS) are produced in various tissues under diabetic conditions [86]. Thus, antioxidants that scavenge ROS may be of great interest to prevent the onset and /or the progression of diseases that are associated with oxidative unbalance.

The antidiabetic and antioxidant activities of green tea were reported in several animal models studies (summarized in Table 1). For instance, a study performed in streptozotocin (STZ)-induced diabetic rats with hepatic injury showed that the treatment with green tea during 8 weeks was able to reduce the blood glucose level and improve the biochemical and histopathological status of these rats [70]. In addition, green tea consumption was able to increase the levels of reduced glutathione (GSH) levels [70]. This was an important data since GSH is the major endogenous antioxidant produced by the cells, thus playing an important role in the neutralization of free radicals and ROS [70]. Thus, the daily consumption of green tea improved the antioxidant status of rats with STZ-induced DM. In another study, alloxan was used to induce a diabetic state in rats [71]. Alloxan is a glucose analogue, which accumulates in pancreatic beta cells and inhibits the secretion of insulin [87]. This substance generates ROS by a redox reaction with diluric acid, in the presence of glutathione. The auto-oxidation of dialuric acid generates free radicals. Notably, the continuous treatment with an aqueous solution of green tea polyphenols (500 mg per kg of body weight) was able to increase the glucose tolerance in normal rats, at 60 minutes [71]. In addition, a dose level of

100 mg per kg of body weight of green tea polyphenols reduced the serum glucose level in alloxan diabetic rats [71]. The lipid peroxidation was decreased and the antioxidant potential was improved, namely by improvements in superoxide dismutase and glutathione levels [71]. Diets supplemented with EGCG during 10 weeks in *db/db* mice, a model of obesity, diabetes and dyslipidemia, showed that this catechin improved glucose tolerance, increased glucose-stimulated insulin secretion and preserved the islets of Langerhans structure [72]. Very recently, our research group has reported that daily consumption of white tea improves glucose tolerance and insulin sensitivity in STZ-induced prediabetic rats [9, 18]. Moreover, tea consumption altered the glycolytic profile, improved oxidative status, and increased the antioxidant power in the cerebral cortex of prediabetic rats [18]. In addition, this type of tea was also able to improve the cardiovascular metabolic state of prediabetic rats [9]. Green and white teas are very similar, with respect to processing, although it has been reported that green tea presents lower levels of antioxidants than white tea [7]. Certainly, both types of tea cause similar effects. In this context, the dietary supplementation with the major green and white teas catechins can be a nutritional strategy in the prevention and treatment of DM.

Several works have reported that green tea phenolic compounds, namely catechins, are potent antioxidant agents, scavenging ROS [41] and metal chelators [88]. As previously referred, the chemical structure of tea catechins is associated with its antioxidant properties [34]. It has been suggested that content of pyrogallol and hydroxyl groups influences the superoxide anion radical scavenging ability [1]. Furthermore, the presence of galloyl moieties improves the ability to quench hydroxyl radicals [1]. Several structures appear to be important for these antioxidant activities of tea polyphenols, including the ortho-3',4'-dihydroxyl (catechol) group in the B-ring, that promotes the formation of a stable phenoxyl radical due to effective electron delocalization [89] or the 3',4',5'-trihydroxyl group in the B-ring, a gallate group esterified at the 3 position of the C-ring, and hydroxyl groups at the 5 and 7 positions of the A-ring [90].

Although the antioxidant capacity of green tea polyphenols has been reported, recent studies highlight that when polyphenols are present in high concentrations they can also exert pro-oxidant effects. Tea catechins also possess the ability to generate ROS due to their instability and undergo auto-oxidative reactions, in typical cell culture conditions [91]. Moreover, the stability of EGCG is dependent on the total concentration of catechins, the pH of the system, the presence of oxygen, and the temperature of the incubation [91]. All these effects have been tested and reported in animal models. A study conducted by Yun and collaborators [74] showed that an intraperitoneal treatment with EGCG (5 mg per kg per day) during 4-days, impaired the beta-cell response to high glucose in the diabetic rats. On the other hand, treatment of CF-1 mice with a single oral dose of 1500 mg per kg EGCG reduced the animals' survival by 85% and the administration of daily doses of 500 and 750 mg per kg decreased survival by 20% and 75%, respectively [73].

Table 1. Summary of the main effects of green tea and its bioactive components, as reported in *in vitro* and *in vivo* studies, to DM and neurodegeneration

		Green tea / Bioactive Component Tested	Main Remarks
***In vitro* Studies**	H4IIE rat hepatoma cells [63, 64]	EGCG	↓ Glucose production [63] ↓ Expression of genes that control gluconeogenesis [63, 64] ↓ Expression of genes involved in fatty acid synthesis [64] ↑ Genes involved in glycolysis [64] ↑ GLUT 1 [64]
	Fat cells [65]	EGCG (and theaflavins)	↑ Insulin activity [65] ↑Hepatoprotection [65]
	Pancreatic islets [66]	EGCG	↑ Survival rate [66]
	RINm5F cell line [67]		Protection against cytokine-induced damage [67]
	Hippocampal neuronal cells [68, 69]		↓ Development of dementia and neurodegenerative diseases [68] ↑ Neuronal viability [68]
***In vivo* Studies**	Rat model [70-79]	Green tea	↓ Blood glucose [70, 71] ↑ Biochemical and histopathological status [70] ↑ GSH levels [70] ↑ Glucose tolerance [71] ↓ Lipid peroxidation [71] ↑ Antioxidant potential [71] Prevent striatal dopamine depletion [75] ↓ Loss of substantia nigra dopaminergic neuron [75]
		EGCG	↑ Glucose tolerance [72] ↑ Glucose-stimulated insulin secretion [72] Preservation of islets of Langerhans structure [72] Pro-oxidant effects [73, 74] ↓ OS [76] ↑ Antioxidant defenses [76] ↑ Spatial cognition and learn ability [77] ↓ Cerebral amyloidosis [78]
		EC, EGC, ECG	↑ Redox status [79] ↓Structural damage [79]

Legend: EGCG – (-)-epigallocatechin 3-gallate; EGC – (-)-epigallocatechin; ECG – (-)-epicatechin-3-gallate; EC – (-)-epicatechin; OS – Oxidative stress; GLUT1 – Glucose transporter 1; GSH – Reduced glutathione; ↓ - Decrease; ↑ - Increase/Improve.

Epidemiological and Interventional Clinical Studies

The probability of developing DM depends on several factors, including the lifestyle behavior. Of note, an unbalanced diet highly increases the risk for developing this disease. Thus, the link between nutrition and DM has been clearly suggested and is well established [92]. Green tea has received special consideration due to the beneficial effects of its phytochemicals to health. In fact, this type of tea appears as a good antidiabetic agent. However, in studies in humans, the conclusions are not so elucidative as *in vitro* and *in vivo* studies. Some studies in humans suggest that regular consumption of green tea contributes to a protection against the development of DM [93-97]. Other studies report that the consumption of this beverage has no association with the disease [98]. These studies are very important but there are some drawbacks that must be considered. For example, the studies discussed herein were performed in very different populations, which greatly vary in terms of age, countries and lifestyles. Moreover, green tea is generally prepared by using 1 g of tea leaves per 100 ml of boiling water but differences can arise from preparation and the protocol for consumption, as well as metabolization. Moreover, the differences between the animal species subjected to research and humans may also hamper the correct interpretation, extrapolation and practical application of the results and conclusions.

A retrospective cohort study conducted by Iso and collaborators [96] evaluated the relationship between green tea consumption and the risk for T2DM. The authors concluded that people that drink 6 or more cups of green tea per day are less likely to develop T2DM than those who drink less than one cup of this beverage per week [96]. A previous cross-sectional study showed an unclear association between green tea consumption and glucose tolerance [97]. This study evaluated 3224 Japanese men and concluded that impaired fasting glucose was less frequent in those who consumed more green tea. Nevertheless, the association between green tea consumption and glucose tolerance is not clear. Another cohort study of middle-aged and older women reported that women who consumed 4 or more cups of tea per day had a 30% lower risk of developing T2DM [95]. In a double-blind randomized study of decaffeinated green tea extract performed in adults with T2DM, during 3 months, the data showed that there were no significant effects on glucose levels in adults with T2DM at the end of the treatment [98]. In addition, the intake of green tea extract containing 300 mg EGCG, during 12 weeks by healthy volunteers, showed a reduction of plasma glucose and insulin [94]. Rizvi and collaborators [93] also reported that EGCG may protect against the development of long-term complications that arise from DM, reducing OS in erythrocytes. An overview of human studies focused on the effects of green tea consumption on DM is provided in Table 2.

GREEN TEA AND NEURODEGENERATION

The mammalian brain depends upon glucose as one of its main source of energy. Thus, it is expectable that glucose dysfunction promoted by DM, namely T2DM, may be responsible for brain damage and/or dysfunction [20, 99]. In fact, DM is implicated in the development of cerebrovascular disease and other neurological comorbidities, such as cognitive dysfunction and dementia [13]. Moreover, it was reported that the risk of cognitive decline and

neurodegeneration are increased at an early stage, before the onset of disease [15] though the role for glucose dysfunction in these events remains largely unknown. Pathological alterations in CNS are also associated to both types of DM [14]. Notably, it has long been discussed that some brain areas are more vulnerable to deregulation of glucose metabolism. For example, cortical neurons and astrocytes are reported to be more susceptible than cells from striatum or hippocampus [100]. Furthermore, the cerebral cortex is particularly sensitive to deregulated metabolism [101], since it is quite vulnerable to OS due to its high consumption of oxygen, the abundance of easily oxidizable fatty acids, and the relative low presence of antioxidant defenses [102]. Interestingly, the cerebral cortex is greatly affected by AD, and several studies have demonstrated that AD and DM are connected [17]. In addition, OS is reported as an important factor that contributes to aging processes and neurodegeneration [102].

In vitro studies, animal data and human epidemiological studies provided compelling evidence that drinking tea may have pharmacological benefits in the protection of the brain. These findings are described below. *In vitro* and animal studies are summarized in Table 1, whereas human studies are summarized in Table 2.

In Vitro Studies and Animal Models

Green tea and its bioactive compounds have biological and pharmacological activities very relevant to human health. So, consumption of green tea may be an advantage in the protection of the brain, particularly against metabolic diseases. Many studies have shown that tea consumption is inversely correlated with the incidence of dementia and neurodegenerative diseases [68, 69, 107, 108] and it decreases the prevalence of cognitive impairment [77, 109, 110]. In recent years, many research groups have been trying to understand the molecular mechanisms by which green tea acts and protects the brain. Besides its antioxidant activity, neuron viability can also be improved by the modulation of signal transduction pathways, cell survival/death genes, and mitochondrial function [68]. Several authors consider the main catechin, EGCG, as one of the greatest natural antioxidants and the most pharmacologically active compound in the unfermented teas [4, 20, 26]. Several of the neuroprotective activities of tea are associated with this powerful catechin. EGCG is reported to cross the BBB and thus, it can easily reach the brain parenchyma [111] exerting its effects. The neuroprotective effect of tea polyphenols was shown in animal models of neurological disorders, through improving age-related cognitive decline and protecting against cerebral ischemia/reperfusion injuries [112]. A study performed in a mice model of Parkinson's disease (PD) induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), showed that green tea extract or isolated EGCG were able to prevent striatal dopamine depletion and the loss of substantia nigra dopaminergic neuron [75]. Another study in Wistar rats showed that continuous administration of EGCG (2 mg per kg of body weight) for 30 days was able to improve rats' antioxidant defenses, ameliorating the age-induced OS levels in their brains [76]. A long-term administration of green tea catechins or EGCG showed to improve spatial cognition and learning ability in rats [77]. Similarly, it was also able to reduce cerebral amyloidosis in AD transgenic mice [78]. Recently, with the aim of studying other bioactive compounds of green tea, some authors investigated the anti-aging effects of a catechin-rich green tea extract, free of caffeine and L-theanine, and low content of EGCG [79]. The results showed that EC, EGC and ECG are effective protectors of proteins and lipids against oxidative changes related to

aging [79]. Thus, this demonstrates that other catechins are able to protect against oxidative damage.

In vitro findings reported that neuronal cell death caused by the neurotoxins 6-hydroxydopamine (6-OHDA), and amyloid beta can be prevented by green tea catechins [69, 113]. Notably, green tea catechins are reported to interfere in the modulation of several protein kinase-signaling pathways modulating cellular functions. For example, the neuroprotective action exerted by EGCG may be due to its involvement with protein kinase C (PKC) [68]. This protein is involved in the regulation of cell survival, apoptosis, long-term potentiation, and consolidation of different types of memory [68]. EGCG is able to activate PKC, illustrating that this mechanism may be responsible for most of the neuroprotective capacity of the main tea catechin [68, 113]. The mitogen-activated protein kinases (MAPK), phosphatidylinositide 3′-OH kinase/AKT and protein kinase A signaling cascades, are other pathways reported to be activated by the action of green tea catechins [114]. These pathways may exert neuronal protection functions and are essential for neuronal differentiation and survival [115].

The pathological development of AD begins with the abnormal processing of the amyloid precursor protein (APP), leading to the excessive accumulation of the amyloid beta (Aβ) peptide in the brain, and consequent formation of senile plaques. *In vitro* observations show that EGCG inhibits OS and neurotoxicity [69], and EC reduces the formation of Aβ-fibril formation [116] illustrating possible mechanisms by which these catechins exert their protective effective against AD. Moreover, the proteolytic processing of APP can be regulated *in vivo* and *in vitro* by EGCG [117], suggesting that green tea polyphenols might be potentially promising therapeutic agents for neurodegenerative diseases.

Table 2. Epidemiological studies regarding green tea consumption or tea phytochemicals, and its effects to DM and neurodegeneration

Type of Study	Population Studied	Tested components / Amount	Main Remarks	Citation
Retrospective cohort	17413 Japan adults	≥ 6 cups green tea / day	↓ Risk of incident T2DM	[96]
Cross-sectional	3224 Japanese men	≥ 5 cups green tea /day	↓ Glucose tolerance	[97]
Cohort	38018 women	≥ 4 cups green tea /day	↓ 30% risk of developing T2DM	[95]
Double-blind randomized	49 subjects	375 mg tea (150 mg green tea catechins + 75 mg black tea theaflavins + 150 mg other tea polyphenols /day)	No effects	[98]
Case-control	23 healthy males	Green tea extract (of which 300 mg was EGCG)	↓ Plasma glucose and insulin	[94]
Case-control	31 T2DM subjects	EGCG, EGC, ECG, EC (each 10^{-5}-10^{-8} mol/L)	↓ OS in erythrocytes	[93]

Type of Study	Population Studied	Tested components / Amount	Main Remarks	Citation
Cross-sectional	1003 Ederly Japanese subjects	≥ 2 cups green tea /day	↓ Cognitive deficits	[103]
Case- control	557 subjects	≥ 2 cups green tea /day	↓ Risk of developing PD	[104]
Prospective cohort	25000 Finnish adults	≥ 3 cups green tea /day	↓ Risk of developing PD	[105]
Double-blind randomized	24 subjects	L-theanine (250 mg) + caffeine (150 mg)	↑ Cognitive performance	[106]

Legend: T2DM – Type 2 diabetes mellitus; EGCG – (-)-epigallocatechin 3-gallate; EGC – (-)-epigallocatechin; ECG – (-)-epicatechin-3-gallate; EC – (-)-epicatechin; OS – Oxidative stress; PD – Parkinson diseases; ↓ - Decrease; ↑ - Increase/Improve

L-theanine is the major free amino acid found in green tea and has been reported as antioxidant and neuroprotective against PD-related neurotoxicants and may be clinically useful for preventing PD symptoms [108].

Epidemiological and Interventional Clinical Studies

Human epidemiological studies suggest that the pharmacological benefits of tea consumption may help to protect the brain. A cross-sectional study in Japan explored the association between consumption of green tea and cognitive function in elderly Japanese subjects. It was reported that the consumption of two or more cups per day (approximately 100 mL per cup) of green tea is associated with lower prevalence of cognitive impairment [103]. In a case-control study in the United States, it was found that subjects who consumed two or more cups of tea per day have a decreased risk of developing PD [104]. Likewise, a prospective cohort study with more than 25.000 Finnish adults aged 25–74 years found that drinking three or more cups (200 mL per cup) of tea was associated with a reduced risk of PD [105]. Therefore, is extremely important to perform well-designed controlled studies to assess the probable reduction of developing neurodegenerative diseases for those who drink green tea. Undoubtedly, the biological effects of green tea consumption may benefit subjects with neurodegenerative diseases, but more studies are necessary to investigate the effectiveness of green tea in humans. Moreover, the different mechanisms of its neuroprotective function must be unveil to better understand the potential benefits and risks associated with tea drinking.

The major free amino acid found in green tea, L-theanine, has been a focus of attention in the last years. This amino acid has the ability to pass through BBB, and remains in the brain for, at least 5 hours, after administration [118]. Moreover, L-theanine may influence the secretion and function of neurotransmitters in the CNS [119]. It possesses antioxidant [108, 120] and neuroprotective properties [57, 58]. It also improves memory function [121] and prevents memory impairment induced by cerebral ischemia [122]. A study involving thirty-five individuals evaluated L-theanine effect on mental state [123]. The results showed that L-theanine has a significant effect on the general state of mental alertness or arousal. In addition, this amino acid has relaxing capacity without inducing drowsiness [123]. Besides L-theanine, other bioactive compounds, such as caffeine, are also present in considerable amounts in green tea and may play a key role for the beneficial health effects reported for this

beverage [4, 7]. A double-blind randomized study [106] evaluated the acute cognitive and mood effects of L-theanine, caffeine, and both in combination. L-theanine combined with caffeine provides better cognitive outcomes than in isolated form, but the mechanisms underlying the effects are not known [106].

All of these studies show that green tea and its bioactive compounds are associated with reduced risk of DM and consequently reduced risk of neurodegeneration. However, the mechanisms of action remain largely unknown. In sum, green tea consumption and/or the administration of its phytochemical compounds ameliorates glucose metabolism, improving insulin sensitivity and decreasing insulin resistance. Furthermore, green tea is able to protect the brain by preventing cognitive impairment, neuronal loss, neurodegeneration and dementia. Nevertheless, all these effects have not yet been sufficiently evaluated. Furthermore, *in vivo* studies are needed to clarify whether green tea and its bioactive compounds, reach the brain, at sufficient concentrations and with enough bioactivity to promote the effects reported *in vitro*. Noteworthy, *in vitro* and animals' studies consume higher doses of tea than those consumed by humans, because the experimental conditions are generally optimized for the evaluation of an effect. Moreover, it is believed that tea's consumption benefits are due to the synergistic action of several compounds. Finally, based on these observations, green tea consumption is recommended to the normal population.

CONCLUSION

DM is a pandemic disease, affecting an enormous number of people worldwide. There are also several comorbidities associated with this disease that highly increase its complexity. Compelling evidence illustrates that DM affects brain function, being hyperglycaemia and OS key mediators of DM-induced neurodegeneration. Green tea has been used in traditional medicine to prevent and treat several health problems and diseases, including DM. Currently, this pleasant, economical and popular beverage appears as a potential functional food product that contains several nutraceuticals, such as catechins, caffeine and L-theanine with documented health benefits, namely antidiabetic, antioxidant and neuroprotective properties. However, more studies are necessary to explain the molecular mechanisms involved in the protective effects of green tea and its phytochemicals. DM and associated brain damage can be prevented by modification of lifestyle, particularly by alterations in diet. Thus, green tea consumption is proposed herein as an excellent dietary habit to diabetic individuals and can protect the brain from DM-related neurodegeneration.

LIST OF ABBREVIATIONS

6-OHDA	6-hydroxydopamine
AD	Alzheimer's disease
APP	Amyloid precursor protein
Aβ	Amyloid beta
BBB	Blood brain-barrier
CNS	Central Nervous System

DM	Diabetes mellitus
EC	(-)-epicatechin
ECG	(-)-epicatechin-3-gallate
EGC	(-)-epigallocatechin
EGCG	(-)-epigallocatechin 3-gallate
GLUT1	Glucose transporter 1
GSH	Reduced glutathione
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
OS	Oxidative stress
PD	Parkinson's disease
PKC	Protein kinase C
PO	Polyphenol oxidase
ROS	Reactive oxygen species
STZ	Streptozotocin
T1DM –	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

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REFERENCES

[1] Moderno, PM; Carvalho, M; Silva, BM. Recent patents on Camellia sinensis: source of health promoting compounds. *Recent Patents on Food, Nutrition & Agriculture*, 2009, 1, 182-192.

[2] Wheeler, D; Wheeler, W. The medicinal chemistry of tea. *Drug Development Research*, 2004, 61, 45-65.

[3] Cheng, TO. All teas are not created equal: the Chinese green tea and cardiovascular health. *International Journal of Cardiology*, 2006, 108, 301-308.

[4] Dias, TR; Tomás, GD; Teixeira, NF; Alves, MG; Oliveira, PF; Silva, BM. White Tea (Camellia Sinensis (L.)): Antioxidant Properties and Beneficial Health Effects. *International Journal of Food Sciences and Nutrition*, 2013, 11, 1-15.

[5] Baptista, JA; Tavares, J; Carvalho, R. Comparative study and partial characterization of azorean green tea polyphenols. *Journal of Food Composition and Analysis*, 1999, 12, 273-287.

[6] Carvalho, M; Jerónimo, C; Valentão, P; Andrade, PB; Silva, BM. Green tea: A promising anticancer agent for renal cell carcinoma. *Food Chemistry*, 2010, 122, 49-54.

[7] Dias, TR; Alves, MG; Tomás, GD; Socorro, S; Silva, BM; Oliveira, PF. White Tea as a Promising Antioxidant Medium Additive for Sperm Storage at Room Temperature: A Comparative Study with Green Tea. *Journal of Agricultural and Food Chemistry*, 2014, 62, 608-617.

[8] Martins, AD; Alves, MG; Bernardino, RL; Dias, TR; Silva, BM; Oliveira, PF. Effect of white tea (Camellia sinensis (L.)) extract in the glycolytic profile of Sertoli cell. *European Journal of Nutrition*, 2014, 53, 1383-1391.

[9] Alves, MG; Martins, AD; Teixeira, NF; Rato, L; Oliveira, PF; Silva, BM. White tea improves cardiac glycolytic and oxidative profile of prediabetic rats. *Journal of Functional Foods*, 2015, 14, 102-110.

[10] Zaveri, NT. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sciences*, 2006, 78, 2073-2080.

[11] Agbaje, IM; Rogers, DA; McVicar, CM; McClure, N; Atkinson, AB; Mallidis, C; Lewis, SEM. Insulin dependant diabetes mellitus: implications for male reproductive function. *Human Reproduction*, 2007, 22, 1871-1877.

[12] Association, AD. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 2012, 35, S64-S71.

[13] J, SR-F; Sa-Roriz, TM; Rosset, I; Camozzato, AL; Santos, AC; Chaves, ML; Moriguti, JC; Roriz-Cruz, M. (Pre)diabetes, brain aging, and cognition. *Biochimica et Biophysica Acta*, 2009, 1792, 432-443.

[14] Biessels, GJ; Gispen, WH. The impact of diabetes on cognition: what can be learned from rodent models? *Neurobiology of Aging*, 2005, 26, 36-41.

[15] Luchsinger, JA; Tang, MX; Shea, S; Mayeux, R. Hyperinsulinemia and risk of Alzheimer disease. *Neurology*, 2004, 63, 1187-1192.

[16] Reijmer, YD; van den Berg, E; de Bresser, J; Kessels, RP; Kappelle, LJ; Algra, A; Biessels, GJ. Accelerated cognitive decline in patients with type 2 diabetes: MRI correlates and risk factors. *Diabetes Metabolism Research and Reviews*, 2011, 27, 195-202.

[17] Moreira, PI. Alzheimer's disease and diabetes: an integrative view of the role of mitochondria, oxidative stress, and insulin. *Journal of Alzheimers Disease* 2012, 30, S199-S215.

[18] Nunes, AR; Alves, MG; Tomás, GD; Conde, VR; Cristóvão, AC; Moreira, PI; Oliveira, PF; Silva, BM. Daily consumption of white tea (Camellia sinensis (L.)) improves the cerebral cortex metabolic and oxidative profile in prediabetic Wistar rats. *Bristish Journal of Nutriton* 2015, 1-11.

[19] Dieren, Sv; Uiterwaal, CS; Shouw, YTvd; A, DLvd; Boer, JM; Spijkerman, A; Grobbee, DE; Beulens, JW. Coffee and tea consumption and risk of type 2 diabetes. *Diabetologia*, 2009, 52, 2561-2569.

[20] Nunes, AR; Alves, MG; Moreira, PI; Oliveira, PF; Silva, BM. Can Tea Consumption be a Safe and Effective Therapy Against Diabetes Mellitus-Induced Neurodegeneration. *Current Neuropharmacology*, 2014, 12, 475-489.

[21] Almajano, MP; Carbó, R; Jiménez, JAL; Gordon, MH. Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, 2008, 108, 55-63.

[22] López, V; Calvo, MI. White tea (Camellia sinensis Kuntze) exerts neuroprotection against hydrogen peroxide-induced toxicity in PC12 cells. *lant Foods for Human Nutrition*, 2011, 66, 22-26.

[23] Baptista, JA; Tavares, J; Carvalho, R. Comparative Study and Partial Characterization of Azorean Green Tea Polyphenols. *Journal of Food Composition and Analysis*, 1999, 12, 273-287.

[24] Mckay, DL; Blumberg, JB. The role of tea in human health: an update. *Journal of the American College of Nutrition*, 2002, 21, 1-13.

[25] de Mejia, EG; Ramirez-Mares, MV; Puangpraphant, S. Bioactive components of tea: cancer, inflammation and behavior. *Brain, Behavior, and Immunity*, 2009, 23, 721-731.

[26] Conde, VR; Alves, MG; Oliveira, PF; Silva, BM. Tea (Camellia sinensis (L.)): A Putative Anticancer Agent in Bladder Carcinoma? *Anti-Cancer Agents in Medicinal Chemistry*, 2015, 15, 26-36.

[27] Rusak, G; Komes, D; Likić, S; Horžić, D; Kovač, M. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. *Food Chemistry*, 2008, 110, 852-858.

[28] Venditti, E; Bacchetti, T; Tiano, L; Carloni, P; Greci, L; Damiani, E. Hot vs. cold water steeping of different teas: Do they affect antioxidant activity? *Food Chemistry*, 2010, 119, 1597-1604.

[29] Lin, YS; Tsai, YJ; Tsay, JS; Lin, JK. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *Journal of Agricultural and Food Chemistry*, 2003, 51, 1864-1873.

[30] Khan, N; Mukhtar, H. Tea polyphenols for health promotion. *Life Sciences*, 2007, 81, 519-533.

[31] Aboul-Enein, HY; Berczynsk, P; Kruk, I. Phenolic compounds: the role of redox regulation in neurodegenerative disease and cancer. *Mini-Reviews in Medicinal Chemistry*, 2013, 13, 385-398.

[32] Lakenbrink, C; Lapczynski, S; Maiwald, B; Engelhardt, UH. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *Journal of Agricultural and Food Chemistry*, 2000, 48, 2848-2852.

[33] Costa, RM; Magalhães, AS; Pereira, JA; Andrade, PB; Valentão, P; Carvalho, M; Silva, BM. Evaluation of free radical- scavenging and antihemolytic activities of quince (Cydonia oblonga) leaf: A comparative study with green tea (Camellia sinensis). *Food and Chemical Toxicology*, 2009, 47, 860-865.

[34] Braicu, C; Ladomeryc, MR; Chedead, VS; Irimief, A; Berindan-Neagoea, I. The relationship between the structure and biological actions of green tea catechins. *Food Chemistry*, 2013, 141, 3282-3289.

[35] Seeram, NP; Henning, SM; Niu, Y; Lee, R; Scheuller, HS; Heber, D. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 2006, 54, 1599-1603.

[36] Stewart, AJ; Mullen, W; Crozier, A. On-line high-performance liquid chromatography analysis of the antioxidant activity of phenolic compounds in green and black tea. *Molecular Nutrition & Food Research*, 2005, 49, 52-60.

[37] Yang, CS; Hong, J; Hou, Z; Sang, S. Green tea polyphenols: antioxidative and prooxidative effects. *Journal of Nutrition*, 2004, 134, 3181s.

[38] Nanjo, F; Mori, M; Goto, K; Hara, Y. Radical scavenging activity of tea catechins and their related compounds. *Bioscience, Biotechnology, and Biochemistry*, 1999, 63, 1621-1623.

[39] Gorjanovic, S; Komes, D; Pastor, FT; Belscak-Cvitanovic, A; Pezo, L; Hecimovic, I; Suznjevic, D. Antioxidant capacity of teas and herbal infusions: polarographic assessment. *Journal of Agricultural and Food Chemistry*, 2012, 60, 9573-9580.

[40] Paquay, JB; Haenen, GR; Stender, G; Wiseman, SA; Tijburg, LB; Bast, A. Protection against nitric oxide toxicity by tea. *Journal of Agricultural and Food Chemistry*, 2000, 48, 5768-5772.

[41] Nakagawa, T; Yokozawa, T. Direct scavenging of nitric oxide and superoxide by green tea. *Food and Chemical Toxicology*, 2002, 40, 1745-1750.

[42] Maritim, AC; Sanders, RA; Watkins, JB, 3rd. Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical and Molecular Toxicology*, 2003, 17, 24-38.

[43] Hashimoto, T; He, Z; Ma, WY; Schmid, PC; Bode, AM; Yang, CS; Dong, Z. Caffeine inhibits cell proliferation by G0/G1 phase arrest in JB6 cells. *Cancer Research*, 2004, 64, 3344-3349.

[44] Li, S; Lo, CY; Pan, MH; Lai, CS; Ho, CT. Black tea: chemical analysis and stability. *Food & Function*, 2013, 4, 10-18.

[45] Lee, H; Bae, JH; Lee, SR. Protective effect of green tea polyphenol EGCG against neuronal damage and brain edema after unilateral cerebral ischemia in gerbils. *Journal of Neuroscience Research*, 2004, 77, 892-900.

[46] Sinclair, CJ; Geiger, JD. Caffeine use in sports. A pharmacological review. J*ournal of Sports Medicine and Physical Fitness*, 2000, 40, 71-79.

[47] Nawrot, P; Jordan, S; Eastwood, J; Rotstein, J; Hugenholtz, A; Feeley, M. Effects of caffeine on human health. *Food Additives & Contaminants*, 2003, 20, 1-30.

[48] Cunha, RA. Caffeine, adenosine receptors, memory and Alzheimer disease. *Medicina Clínica (Barcelona)*, 2008, 131, 790-795.

[49] Duarte, JM; Carvalho, RA; Cunha, RA; Gruetter, R. Caffeine consumption attenuates neurochemical modifications in the hippocampus of streptozotocin-induced diabetic rats. *Journal of Neurochemistry*, 2009, 111, 368-379.

[50] Steinfelder, HJ; Petho-Schramm, S. Methylxanthines inhibit glucose transport in rat adipocytes by two independent mechanisms. *Biochemical Pharmacology*, 1990, 40, 1154-1157.

[51] Grucka-Mamczar, E; Zalejska-Fiolka, J; Chlubek, D; Kasperczyk, S; Blaszczyk, U; Kasperczyk, A; Swietochowska, E; Birkner, E. The influence of sodium fluoride and caffeine on the activity of antioxidative enzymes and the concentration of malondialdehyde in rat liver. *Fluoride*, 2009, 42, 105-109.

[52] Ofluoglu, E; Pasaoglu, H; Pasaoglu, A. The effects of caffeine on L-arginine metabolism in the brain of rats. *Neurochemical Research*, 2009, 34, 395-399.

[53] Dias, TR; Alves, MG; Bernardino, RL; Martins, AD; Moreira, AC; Silva, J; Barros, A; Sousa, M; Silva, BM; Oliveira, PF. Dose-dependent effects of caffeine in human Sertoli cells metabolism and oxidative profile: Relevance for male fertility. *Toxicology*, 2015, 328, 12-20.

[54] Sepkowitz, KA. Energy drinks and caffeine-related adverse effects. *Jama*, 2013, 309, 243-244.

[55] Vuong, QV; Bowyer, MC; Roach, PD. L-Theanine: properties, synthesis and isolation from tea. *Journal of the Science of Food and Agriculture*, 2011, 91, 1931-1939.

[56] Ekborg-Ott, KH; Taylor, A; Armstrong, DW. Varietal differences in the total and enantiomeric composition of theanine in tea. *Journal of Agricultural and Food Chemistry*, 1997, 45, 353–363.

[57] Nishida, K; Yasuda, E; Nagasawa, K; Fujimoto, S. Altered levels of oxidation and phospholipase C isozyme expression in the brains of theanine-administered rats. *Biological & Pharmaceutical Bulletin*, 2008, 31, 857-860.

[58] Kakuda, T. Neuroprotective effects of theanine and its preventive effects on cognitive dysfunction. *Pharmacological Research*, 2011, 64, 162-168.

[59] Yokogoshi, H; Kobayashi, M; Mochizuki, M; Terashima, T. Effect of Theanine, r-Glutamylethylamide, on Brain Monoamines and Striatal Dopamine Release in Conscious Rats. *Neurochemical Research*, 1998, 23, 667-673.

[60] Silva, BM. *Tea Consumption and Health*. Gautam Banerjee (Unilever R &D India, Bangalore, India). United States of America: Nova Science Publishers, Inc; 2012.

[61] Alves, MG; Martins, AD; Cavaco, JE; Socorro, S; Oliveira, PF. Diabetes, insulin-mediated glucose metabolism and Sertoli/blood-testis barrier function. *Tissue Barriers*, 2013, 1, e23992.

[62] Golay, A; Ybarra, J. Link between obesity and type 2 diabetes. *Best Practice & Research: Clinical Endocrinology & Metabolism*, 2005, 19, 649-663.

[63] Waltner-Law, ME; Wang, XL; Law, BK; Hall, RK; Nawano, M; Granner, DK. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. *Journal of Biological Chemistry*, 2002, 277, 34933-34940.

[64] Wolfram, S; Raederstorff, D; Preller, M; Wang, Y; Teixeira, SR; Riegger, C; Weber, P. Epigallocatechin Gallate Supplementation Alleviates Diabetes in Rodents. *Journal of Nutrition*, 2006, 136, 2512-2518.

[65] Anderson, RA; Polansky, MM. Tea enhances insulin activity. *Journal of Agricultural and Food Chemistry*, 2002, 50, 7182-7186.

[66] Hara, Y; Fujino, M; Takeuchi, M; Li, X-K. Green-tea polyphenol (-)-epigallocatechin-3-gallate provides resistance to apoptosis in isolated islets. *Journal of Hepato-Biliary-Pancreatic Surgery*, 2007, 14, 493-497.

[67] Han, MK. Epigallocatechin gallate, a constituent of green tea, suppresses cytokine-induced pancreatic beta-cell damage. *Experimental & Molecular Medicine*, 2003, 35, 136-139.

[68] Mandel, SA; Amit, T; Kalfon, L; Reznichenko, L; Youdim, MB. Targeting multiple neurodegenerative diseases etiologies with multimodal-acting green tea catechins. *Journal of Nutrition*, 2008, 138, 1578S-1583S.

[69] Choi, YT; Jung, CH; Lee, SR; Bae, JH; Baek, WK; Suh, MH; Park, J; Park, CW; Suh, SI. The green tea polyphenol (-)-epigallocatechin gallate attenuates beta-amyloid-induced neurotoxicity in cultured hippocampal neurons. *Life Sciences*, 2001, 70, 603-614.

[70] Abolfathi, AA; Mohajeri, D; Rezaie, A; Nazeri, M. Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. *Evidence-Based Complementary and Alternative Medicine*, 2012, 2012, 740671.

[71] Sabu, MC; Smitha, K; Ramadasan, K. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *Journal of Ethnopharmacology*, 2002, 83, 109-116.

[72] Ortsäter, H; Grankvist, N; Wolfram, S; Kuehn, N; Sjöholm, A. Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in db/db mice. *Nutrition and Metabolism*, 2012, 9, 1-10.

[73] Lambert, JD; Kennett, MJ; Sang, S; Reuhl, KR; Ju, J; Yang, CS. Hepatotoxicity of high oral dose (-)-epigallocatechin-3-gallate in mice. *Food and Chemical Toxicology*, 2010, 48, 409-416.

[74] Yun, SY; Kim, SP; Song, DK. Effects of (-)-epigallocatechin-3-gallate on pancreatic beta-cell damage in streptozotocin-induced diabetic rats. *European Journal of Pharmacology*, 2006, 541, 115-121.

[75] Levites, Y; Weinreb, O; Maor, G; Youdim, MB; Mandel, S. Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. *Journal of Neurochemistry*, 2001, 78, 1073-1082.

[76] Srividhya, R; Jyothilakshmi, V; Arulmathi, K; Senthilkumaran, V; Kalaiselvi, P. Attenuation of senescence-induced oxidative exacerbations in aged rat brain by (-)-epigallocatechin-3-gallate. *International Journal of Developmental Neuroscience*, 2008, 26, 217-223.

[77] Haque, AM; Hashimoto, M; Katakura, M; Tanabe, Y; Hara, Y; Shido, O. Long-Term Administration of Green Tea Catechins Improves Spatial Cognition Learning Ability in Rats. *Journal of Nutrition*, 2006, 136, 1043-1047.

[78] Rezai-Zadeh, K; Shytle, D; Sun, N; Mori, T; Hou, H; D, J; Ehrhart, J; Townsend, K; Zeng, J; Morgan, D; Hardy, J; Town, T; Tan, J. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *Journal of Neuroscience*, 2005, 25, 8807-8814.

[79] Rodrigues, J; Assunção, M; Lukoyanov, N; Cardoso, A; Carvalho, F; Andrade, JP. Protective effects of a catechin-rich extracts on the hippocampal formation and spatial memory in aging rats. *Behavioural Brain Research*, 2013, 246, 94-102.

[80] Tang, W; Li, S; Liu, Y; Huang, M-T; Ho, C-T. Anti-diabetic activity of chemically profiled green tea and black tea extracts in a type 2 diabetes mice model via different mechanisms. *Journal of Functional Foods*, 2013, 5, 1784–1793.

[81] Tsuneki, H; Ishizuka, M; Terasawa, M; Wu, J-B; Sasaoka, T; Kimura, I. Effect of green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. *BMC Pharmacology*, 2004, 4, 18.

[82] Ceriello, A. Oxidative stress and glycemic regulation. *Metabolism*, 2000, 49, 27-29.

[83] Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature*, 2001, 414, 813-820.

[84] Su, Y; Liu, XM; Sun, YM; Jin, HB; Fu, R; Wang, YY; Wu, Y; Luan, Y. The relationship between endothelial dysfunction and oxidative stress in diabetes and prediabetes. *International Journal of Clinical Practice*, 2008, 62, 877-882.

[85] Lobo, V; Patil, A; Phatak, A; Chandra, N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 2010, 4, 118-126.

[86] Baynes, JW; Thorpe, SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*, 1999, 48, 1-9.

[87] Lenzen, S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, 2008, 51, 216-226.

[88] Atoui, AK; Mansouri, A; Boskou, G; Kefalas, P. Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry*, 2005, 89, 27–36.

[89] Wiseman, SA; Balentine, DA; Frei, B. Antioxidants in tea. *Critical Reviews in Food Science and Nutrition*, 1997, 37, 705-718.

[90] Rice-Evans, CA; Miller, NJ; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine*, 1996, 21, 417.

[91] Sang, S; Lee, MJ; Hou, Z; Ho, CT; Yang, CS. Stability of tea polyphenol (-)-epigallocatechin-3-gallate and formation of dimers and epimers under common experimental conditions. *Journal of Agricultural and Food Chemistry*, 2005, 53, 9478-9484.

[92] Riccardi, G; Aggett, P; Brighenti, F; Delzenne, N; Frayn, K; Nieuwenhuizen, A; Pannemans, D; Theis, S; Tuijtelaars, S; Vessby, B. PASSCLAIM--body weight regulation, insulin sensitivity and diabetes risk. *European Journal of Nutrition*, 2004, 4, II7-II46.

[93] Rizvi, SI; Zaid, MA; Anis, R; Mishra, N. Protective role of tea catechins against oxidation-induced damage of type 2 diabetic erythrocytes. *Clinical and Experimental Pharmacology and Physiology*, 2005, 32, 70-75.

[94] Hase, T; Komine, Y; Meguro, S; Takeda, Y; Takahashi, H; Matsui, Y; Inaoka, S; Katsuragi, Y; Tokimitsu, I; Shimasaki, H; Itakura, H. Anti-obesity effects of tea catechins in humans. *Journal of Oleo Science*, 2001, 50, 599-605.

[95] Song, Y; Manson, JE; Buring, JE; Sesso, HD; Liu, S. Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis. *Journal of the American College of Nutrition*, 2005, 24, 376-384.

[96] Iso, H; Date, C; Wakai, K; Fukui, M; Tamakoshi, A. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Annals of Internal Medicine*, 2006, 144, 554-562.

[97] Yamaji, T; Mizoue, T; Tabata, S; Ogawa, S; Yamaguchi, K; Shimizu, E; Mineshita, M; Kono, S. Coffee consumption and glucose tolerance status in middle-aged Japanese men. *Diabetologia*, 2004, 47, 2145-2151.

[98] MacKenzie, T; Leary, L; Brooks, WB. The effect of an extract of green and black tea on glucose control in adults with type 2 diabetes mellitus: double-blind randomized study. *Metabolism*, 2007, 56, 1340-1344.

[99] Alves, MG; Oliveira, PF; Socorro, S; Moreira, PI. Impact of diabetes in blood-testis and blood-brain barriers: resemblances and differences. *Current Diabetes Reviews*, 2012, 8, 401-412.

[100] Xu, L; Sapolsky, RM; Giffard, RG. Differential sensitivity of murine astrocytes and neurons from different brain regions to injury. *Experimental Neurology*, 2001, 169, 416-424.

[101] Bree, AJ; Puente, EC; Daphna-Iken, D; Fisher, SJ. Diabetes increases brain damage caused by severe hypoglycemia. *American Journal of Physiology Endocrinology and Metabolism*, 2009, 297, E194-201.

[102] Wang, X; Michaelis, EK. Selective neuronal vulnerability to oxidative stress in the brain. *Frontiers in Aging Neuroscience*, 2010, 2, 12.

[103] Kuriyama, S; Hozawa, A; Ohmori, K; Shimazu, T; Matsui, T; Ebihara, S; Awata, S; Nagatomi, R; Arai, H; Tsuji, I. Green tea consumption and cognitive function: a cross-

sectional study from the Tsurugaya Project 1. *American Journal of Clinical Nutrition*, 2006, 83, 355-361.

[104] Checkoway, H; Powers, K; Smith-Weller, T; Franklin, GM; Longstreth, WT, Jr.; Swanson, PD. Parkinson's disease risks associated with cigarette smoking, alcohol consumption, and caffeine intake. *American Journal of Epidemiology*, 2002, 155, 732-738.

[105] Hu, G; Bidel, S; Jousilahti, P; Antikainen, R; Tuomilehto, J. Coffee and tea consumption and the risk of Parkinson's disease. *Movement Disorders*, 2007, 22, 2242-2248.

[106] Haskell, CF; Kennedy, DO; Milne, AL; Wesnes, KA; Scholey, AB. The effects of L-theanine, caffeine and their combination on cognition and mood. *Biological Psychology*, 2008, 77, 113-122.

[107] Jang, S; Jeong, HS; Park, JS; Kim, YS; Jin, CY; Seol, MB; Kim, BC; Lee, MC. Neuroprotective effects of (-)-epigallocatechin-3-gallate against quinolinic acid-induced excitotoxicity via PI3K pathway and NO inhibition. *Brain Research*, 2010,1313, 25-33.

[108] Cho, HS; Kim, S; Lee, SY; Park, JA; Kim, SJ; Chun, HS. Protective effect of the green tea component, L-theanine on environmental toxins-induced neuronal cell death. *Neurotoxicology*, 2008, 29, 656-662.

[109] Rogers, PJ; Smith, JE; Heatherley, SV; Pleydell-Pearce, CW. Time for tea: mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together. *Psichopharmacology*, 2008, 195, 569-577.

[110] Smith, A. Effects of caffeine on human behavior. *Food and Chemical Toxicology*, 2002, 40, 1243-1255.

[111] Suganuma, M; Okabe, S; Oniyama, M; Tada, Y; Ito, H; Fujiki, H. Wide distribution of [3H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis*, 1998, 19, 1771-1776.

[112] Lee, S; Suh, S; Kim, S. Protective effects of the green tea polyphenol (-)-epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils. *Neuroscience Letters*, 2000, 287, 191-194.

[113] Levites, Y; Amit, T; Youdim, MB; Mandel, S. Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (-)-epigallocatechin 3-gallate neuroprotective action. *Journal of Biological Chemistry*, 2002, 277, 30574-30580.

[114] Mandel, SA; Avramovich-Tirosh, Y; Reznichenko, L; Zheng, H; Weinreb, O; Amit, T; Youdim, MB. Multifunctional activities of green tea catechins in neuroprotection. Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. *Neurosignals*, 2005, 14, 46-60.

[115] Gary, DS; Milhavet, O; Camandola, S; Mattson, MP. Essential role for integrin linked kinase in Akt-mediated integrin survival signaling in hippocampal neurons. *Journal of Neurochemistry*, 2003, 84, 878-890.

[116] Ono, K; Yoshiike, Y; Takashima, A; Hasegawa, K; Naiki, H; Yamada, M. Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. *Journal of Neurochemistry*, 2003, 87, 172-181.

[117] Levites, Y; Amit, T; Mandel, S; Youdim, MB. Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor

protein by green tea polyphenol (-)-epigallocatechin-3-gallate. *Faseb Journal*, 2003, 17, 952-954.

[118] Yokogoshi, H; Kobayashi, M; Mochizuki, M; Terashima, T. Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. *Neurochemical Research*, 1998, 23, 667-673.

[119] Terashima, T; Takido, J; Yokogoshi, H. Time-dependent changes of amino acids in the serum, liver, brain and urine of rats administered with theanine. *Bioscience, Biotechnology, and Biochemistry*, 1999, 63, 615-618.

[120] Serrano, F; Klann, E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. *Ageing Research Reviews*, 2004, 3, 431-443.

[121] Egashira, N; Hayakawa, K; Osajima, M; Mishima, K; Iwasaki, K; Oishi, R; Fujiwara, M. Involvement of GABA(A) receptors in the neuroprotective effect of theanine on focal cerebral ischemia in mice. *Journal of Pharmacological Sciences*, 2007, 105, 211-214.

[122] Yamada, T; Terashima, T; Honma, H; Nagata, S; Okubo, T; Juneja, LR; Yokogoshi, H. Effects of theanine, a unique amino acid in tea leaves, on memory in a rat behavioral test. *Bioscience, Biotechnology, and Biochemistry*, 2008, 72, 1356-1359.

[123] Nobre, AC; Rao, A; Owen, GN. L-theanine, a natural constituent in tea, and its effect on mental state. *Asia Pacific Journal of Clinical Nutrition*, 2008, 17, 167-168.

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Chapter 6

NEW CELLULAR AND MOLECULAR TARGET OF EPIGALLOCATECHIN-3-GALLATE

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ABSTRACT

Accumulating evidence supports that green tea consumption is associated with reduced risk of several human malignancies. This positive effect of green tea have been attributed to polyphenol ingredients and among these epigallocatechin-3-gallate (EGCG) is recognized as a key active constituent. Some studies on EGCG are suggesting that a large set of protein targets may directly interact with EGCG and alter the physiology of diseased cells. This chapter reviews the new molecular events responsible for positive effects of EGCG.

INTRODUCTION

Tea is one of the most consumed beverages in the world. A wide number of reports demonstrated that tea constituents possess many biological and pharmacological properties, such as anticancer, antioxidative, antiatherosclerosis, and antihypercholesterolemic activities (Martinotti et al., 2014).

These properties are principally due to the most abundant polyphenolics compound of tea, catechins. Major catechins found in tea leaves are (–)-epigallocatechin-3-O-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-O-gallate (ECG), (–)-epicatechin (EC).

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Among the green tea catechins, EGCG is the most abundant. Because EGCG is not found in any other plant than tea, EGCG is regarded as a constituent characterizing green tea.

To the best of our knowledge, the majority of clinical and epidemiological studies involving catechins (in particular EGCG) have surveyed the potential relationship between these dietary compounds and many types of cancer (Borrelli et al., 2004; Chow et al., 1999; Fujiki et al., 2015; Ranzato et al., 2012).

The effects of green tea consumption on the risk of human tumor have been explored in many epidemiological studies, but the results have been not conclusive.

The conflicting results of the epidemiological studies were probably due to different confounding factors, such as some problems in quantifying tea consumption, varied cancer etiology and population heterogeneity. When these factors were successfully managed, a clear relationship between tea consumption and cancer risk was observed (Yang et al., 2009).

Other studies have investigated the association between catechin and vascular disease prevention, in particular for the incidence of coronary heart disease. Japanese men who drank four or more cups of green tea a day displayed an inverse association with coronary atherosclerosis (Sasazuki et al., 2000).

The association between catechins and vascular diseases has been accredited, in part, to the catechin antioxidant abilities that prevent low-density lipoprotein (LDL) oxidation, reducing the occurrence of coronary pathologies or related vascular diseases.

MECHANISMS OF ACTIONS

Many of the EGCG's molecular and cellular target have been identified, but the mechanisms behind these activities have not been fully revealed.

These heterogeneous actions can be classified into two categories: the antioxidant/pro-oxidant activity and the modulation of specific signaling pathways.

Antioxidants Properties

Numerous data suggest that tea catechins, and EGCG in particular, are effective as scavengers of reactive oxygen species, which can cause DNA damage and promote carcinogenesis.

This antioxidant activity has been verified both *in vitro* and *in vivo* experiments. Bladder urothelial cells are protected against oxidative stress using EGCG (Frei and Higdon, 2003). Intriguingly, the administration of daily green tea catechin is able to decrease in plasma the level of oxidized low-density lipoprotein compared to controls (Inami et al., 2007).

Even though gallocatechins are generally known to be strong antioxidants, there is evidence that some of the effects of these compounds may be related to ROS release. Transition metals, primarily Cu and Fe, are capable of initiating phenolic oxidations and are essential catalysts in this process. These reactions yield superoxide ($O_2^{-\bullet}$) or its protonated hydroperoxyl radical ($HO_2^{\bullet}$), which can be further reduced to hydrogen peroxide (Ranzato et al., 2012). In line with these findings, it has been shown that EGCG can also inhibit cancer cell growth through pro-oxidant activities (Lambert and Elias, 2010).

In a recent work, Ranzato *et al.,* (Ranzato et al., 2012) described a new mechanism for the oxidative toxicity of EGCG, They reconstructed a selective mechanism of EGCG toxicity based on the expression of T-type Ca^{2+} channels. The mechanism starts with the induction of H_2O_2 release at the outside of cells triggered by EGCG. This H_2O_2 acts by inducing T channel opening at the plasma membrane. The Ca^{2+} leakage into the cytosol causes $[Ca^{2+}]_i$ rise and triggers ROS production. Ca^{2+} and ROS cooperate in inducing either apoptosis or necrosis, depending on damage extent (Ranzato et al., 2012).

Antiproliferative Properties

EGCG is known to exert antitumor activity in many types of cancer cells. In several cases, transformed cells have been found to be more sensitive to EGCG than their normal counterparts are. Following this body of evidence, EGCG has been proposed as a possible chemopreventive or chemotherapeutic agent in the treatment of cancer.

In a vast array of studies, it has been shown that EGCG can modify the physiology of cells by interacting with a large set of protein targets, inducing an arrest of cell cycle, modulating some growth signaling pathways, and inhibiting cell survivals pathways (Yu et al., 2014).

Following upon epidemiological evidence of catechin efficacy for cancer prevention and treatment, extensive effort has been dedicated to investigate the protective effects of EGCG against hormone related cancers, such as breast and prostate cancer.

New reports suggest an intriguingly capacity of EGCG to interfere through T-type calcium channels with $[Ca^{2+}]_i$ regulation in some cancer cells, such as breast tumor (Ranzato et al., 2014) and malignant mesothelioma (Ranzato et al., 2012). These data open up a new horizon for the use of the green tea polyphenol as a therapeutic tool in the treatment of malignancies expressing T-type Ca^{2+} channels (Ranzato et al., 2014).

In most cases, green tea catechins have synergistic effects when combined with other anticancer drugs. Ranzato and coworkers developed the idea of combining active nutrients and pharmaceutical drugs in the treatment of malignant mesothelioma (Martinotti et al., 2011; Martinotti et al., 2014; Ranzato et al., 2012; Volta et al., 2013). The organism generally better tolerates antitumor nutrients than drugs, and they can therefore help in increasing the efficiency of drugs without using higher doses, if the mixture is synergistic. Following tests have revealed a synergistic cytotoxicity of ascorbate in combination with gemcitabine and EGCG (Martinotti et al., 2011; Martinotti et al., 2014; Ranzato et al., 2012; Volta et al., 2013). Thereafter, a triple mixture of these compounds, named AND (Active Nutrients/Drug), has been found to act synergistically *in vitro* on various mesothelioma cell lines, and to arrest the growth and invasiveness of mouse mesothelioma xenografts (Volta et al., 2013).

At molecular and cellular level, the mechanism of toxicity of this triple mixture is synergistic, and involves an impairment of free cytosolic Ca^{2+}, the upregulation of DAPK2, the repression of NF-κB, and a block of cell cycle that prevents cells from entering into the G2/M phase. These observations suggest that this mixture could be profitably used as a clinical treatment for cancer, possibly yielding higher response rates in patients without scaling up drug dosages (Martinotti et al., 2014).

CONCLUSION

Based on results briefly discussed in this chapter, tea, which is readily available and widely consumed, has a high potential for application in the prevention of human malignancy. Nevertheless, the cancer-preventive activity of tea has not been regularly observed in studies in humans.

This is principally due to the relatively difficult of quantify and compare tea consumption by different human populations and the various confounding factors in epidemiological studies. More knowledge on the biological properties and activities of tea polyphenols, EGCG in particular, will be useful in the design of new studies and in the selection of the agent, dosage and biomarkers for intervention trials.

REFERENCES

Borrelli, F., Capasso, R., Russo, A., and Ernst, E. (2004). Systematic review: green tea and gastrointestinal cancer risk. *Aliment. Pharmacol. Ther., 19*, 497-510.

Chow, W.H., Blot, W.J., and McLaughlin, J.K. (1999). Tea drinking and cancer risk: epidemiologic evidence. *Proc. Soc. Exp. Biol. Med.,* 220, 197.

Frei, B., and Higdon, J.V. (2003). Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *J. Nutr.,* 133, 3275S-3284S.

Fujiki, H., Sueoka, E., Watanabe, T., and Suganuma, M. (2015). Primary cancer prevention by green tea, and tertiary cancer prevention by the combination of green tea catechins and anticancer compounds. *J. Cancer,* Prev 20, 1-4.

Inami, S., Takano, M., Yamamoto, M., Murakami, D., Tajika, K., Yodogawa, K., Yokoyama, S., Ohno, N., Ohba, T., Sano, J., et al., (2007). Tea catechin consumption reduces circulating oxidized low-density lipoprotein. *Int. Heart J.,* 48, 725-732.

Lambert, J.D., and Elias, R.J. (2010). The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. *Arch. Biochem. Biophys.,* 501, 65-72.

Martinotti, S., Calabrese, G., Ranzato, E., (2014). Polyphenols: Food Sources, Bioactive Properties and Antioxidant Effects. In Plant Polyphenols and Human Health, D.T. Cobb, ed. (Nova Science Publishers).

Martinotti, S., Ranzato, E., and Burlando, B. (2011). In vitro screening of synergistic ascorbate-drug combinations for the treatment of malignant mesothelioma. *Toxicol. In Vitro,* 25, 1568-1574.

Martinotti, S., Ranzato, E., Parodi, M., Vitale, M., and Burlando, B. (2014). Combination of ascorbate/epigallocatechin-3-gallate/gemcitabine synergistically induces cell cycle deregulation and apoptosis in mesothelioma cells. *Toxicol. Appl. Pharmacol.,* 274, 35-41.

Ranzato, E., Magnelli, V., Martinotti, S., Waheed, Z., Cain, S.M., Snutch, T.P., Marchetti, C., and Burlando, B. (2014). Epigallocatechin-3-gallate elicits Ca2+ spike in MCF-7 breast cancer cells: essential role of Cav3.2 channels. *Cell Calcium,* 56, 285-295.

Ranzato, E., Martinotti, S., Magnelli, V., Murer, B., Biffo, S., Mutti, L., and Burlando, B. (2012). Epigallocatechin-3-gallate induces mesothelioma cell death via H2 O2 -dependent T-type Ca2+ channel opening. *J. Cell Mol. Med.,* 16, 2667-2678.

Sasazuki, S., Kodama, H., Yoshimasu, K., Liu, Y., Washio, M., Tanaka, K., Tokunaga, S., Kono, S., Arai, H., Doi, Y., et al., (2000). Relation between green tea consumption and the severity of coronary atherosclerosis among Japanese men and women. *Ann. Epidemiol.,* 10, 401-408.

Volta, V., Ranzato, E., Martinotti, S., Gallo, S., Russo, M.V., Mutti, L., Biffo, S., and Burlando, B. (2013). Preclinical demonstration of synergistic Active Nutrients/Drug (AND) combination as a potential treatment for malignant pleural mesothelioma. *PLoS One,* 8, e58051.

Yang, C.S., Wang, X., Lu, G., and Picinich, S.C. (2009). Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nat. Rev. Cancer,* 9, 429-439.

Yu, Y., Deng, Y., Lu, B.M., Liu, Y.X., Li, J., and Bao, J.K. (2014). Green tea catechins: a fresh flavor to anticancer therapy. *Apoptosis,* 19, 1-18.

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Chapter 7

ROLE OF EGCG IN REGULATION TYROSINE KINASE ONCO-PROTEINS IN CANCER

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ABSTRACT

Accounting for 50-80% of the total catechin content, EGCG is the most abundant catechin in green tea and the most potent catechins capable of inhibiting cell proliferation and inducing apoptosis in cancer cells. One important mechanism frequently overlooked in considering the biological effects of EGCG and its derivatives is their potential interaction with tyrosine kinase onco-proteins that are capable of initiating cell signalling. Here, we review and discuss the novel molecular mechanisms of EGCG on regulation onco-proteins that are clients of heat shock protein, Hsp90.

1. INTRODUCTION

Since ancient times, green tea (*Camellia sinensis*) has been considered to be a health-promoting beverage. Green tea catechins (GTCs), including epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and (−)-epigallocatechin-3-gallate (EGCG), are the major polyphenolic compounds of green tea. The dry weights of EC, ECG, EGC and EGCG are 792 ± 3, 1702 ± 16, 1695 ± 1 and 8295 ± 92 mg/100 g, in green tea, and 240 ± 1, 761 ± 4, 1116 ± 24 and 1199 ± 0.12 mg/100 g, in black tea [1].Several properties of GTCs have been implicated in their anti-cancer effects including anti-mutagenic [152] and

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anti-angiogenic effects [154]. Among GTCs, EGCG is the most potent catechins capable of inhibiting cell proliferation and inducing apoptosis in cancer cells [2-5]. EGCG has been found to inhibit the development of cancer including lung [6], prostate [7], colon [8], skin [9], and breast cancers [10].Diverse molecular mechanisms have been suggested to account for the antitumor effect of GTPs/EGCG in various cancers. An important mechanism frequently overlooked in considering the biological effects of EGCG and its derivatives is their potential interaction with tyrosine kinase oncoproteins that are capable of initiating cell signalling [11-20]. EGCG inhibits the activation or expression of some receptor tyrosine kinases (RTKs) including epidermal growth factor receptor (ErbB) family members: EGFR [11,12], HER2, HER3 [13,14], vascular endothelial growth factor receptor (VEGFR) [15], PDGFR [16], fibroblast growth factor receptors (FGFR) [12], insulin-like growth factor 1 receptor (IGF-1R) [17], KIT [19] and FLT3 [20]. Many possible mechanisms have been proposed and tested to account for the effects of EGCG on RTKs. Interestingly, these RTKs are clients of the molecular chaperone, heat shock protein Hsp90 [21-23]. In this review, we will discuss a potent molecular mechanisms of EGCG on regulationFLT3 and KIT that are clients of heat shock protein Hsp90.

2. THE REGULATION ROLE OF EGCG ON FLT3, KIT AND THEIR DOWNSTREAM SIGNALING MOLECULES

2.1. EGCG Inhibited FLT3 Expression and KITactivity

FMS-like tyrosine kinase 3 *(FLT3)* is a commonly mutated gene in acute myeloid leukaemia (AML) [24]. Approximately 30 % AML patients harbour a FLT3 mutation [24]. FLT3 belongs to the class III receptor tyrosine kinase (RTK) family, along with the stem cell (Steel) factor receptor (KIT), macrophage colony-stimulating factor (M-CSF), receptor-FMS and the platelet-derived growth factor receptors PDGFRα and β. FLT3 shares approximately 30% homology with other family members. FLT3 receptor is normally expressed on the cell surface of haematopoietic progenitor cells, but its expression is lost upon cell maturation [25]. The expression of FLT3 has been also detected in the brain, bone marrow, placenta and gonads, where its function is unknown [25-27]. FLT3 expression can be found in different human and mouse cell lines of both myeloid and lymphoid lineages, but the distribution is quite different [28-30]. Many studies indicate that FLT3 has a crucial role in the development, survival and proliferation of normal stem/progenitor cells [31]. Recent studies have indicated that approximately 10%–15% AML patients display high expression of wild-type (WT) FLT3 [32]. High FLT3 expression has a negative impact on overall and event-free survival in cytogenetically normal AML (CN-AML) patients lacking FLT3 mutations [33]. Our report showed that EGCG, EGC, ECG but not Catechin induced apoptotic cell death by suppressing the expression of FLT3 protein [20].

KIT has been an excellent molecular target for treating gastrointestinal stromal tumor (GISTs) since KIT is over-expressed in almost all GISTs including imatinib-resistant cases (about 95% of GISTs overexpress KIT) [34]. EGCG treatment inhibited the proliferation of GIST-T1 cells, induced apoptosis by inhibited the phosphorylation of KIT [19].

2.2. Effect of EGCG on the Activity of Downstream Molecules in Kinase Cascades

A large number of reports demonstrated that molecules that are downstream of the kinases signalling cascades have been affected by EGCG, i.e. PI3K/AKT, Raf/MAPK and JAK/STAT molecules.

PI3K comprises a family of lipid kinases that have been linked to the control of proliferation, adhesion, motility, and viability in a variety of cell types [35]. PI3K could be activated through Ras or be activated by associating directly with phosphorylated tyrosine residue on the activated receptor through anSrc-homology 2 (SH2) domain. After activation PI3K phosphorylates the 3′-OH position of the inositol ring of phosphatidyl inositol (PtdIns), generating mainly PtdIns(3,4)P_2, PtdIns(3,5)P_2, and PtdIns(3,4,5)P_3. The resulting product, PtdIns(3,4,5)P3 (PIP3), is able to physically associate with proteins containing a pleckstrin homology (PH) domain, leading to their recruitment to plasma membrane where they can be activatedand initiated the signaling cascade (For review, see [36]).Among these kinases, the phosphotidylinositide-dependent kinase 1 (PDK1) is responsible for phosphorylation of T308 in AKT, the activated form of AKT [37]. After activation, AKT translocates to the nucleus and affects the activity of quite a few transcriptional factors, such as CREB [38], NF-κB[39], murine double minute 2 (MDM2) [40], BAD [41] and GSK-3β [42], which are involved in the regulation of cell survival, cell cycle progression and cellular growth.EGCG has been reported to regulate on AKT signallingin many previous studies that including the direct down-regulation of AKTmRNA and inhibition of AKT activation as well as theindirect inhibition of upstream signalling such as the activations of RTKs, c-Met, EGFR and IGFR[2,43-45].

Receptor tyrosine kinases (RTK) activate RAS through association with SOS, a guanine nucleotide exchange factor that facilitates exchange of GDP for GTP, leading to activation of RAS. SOS exists in the cell in a preformed complex with the adapter protein GRB2, which in turn associates via its SH2 domain to phosphorylated tyrosine residues within the consensus sequence p-YXN. These tyrosine residues exist either in the receptor or in downstream signal transduction molecules such as the protein tyrosine phosphatase SHP-2 or the adapter protein SHC [46-48]. Thus, the GRB2-SOS complex is recruited to the vicinity of the plasma membrane, where it can act on RAS. Activated RAS has the ability to interact with the serine/threonine kinase RAF, leading to its activation. The targets for RAF kinase activity are the dual-specificity kinases MEK1 and MEK2 [49], which are activated by phosphorylation. The serine/threonine kinases ERK1 and ERK2 are activated through phosphorylation by MEK1/2 [50]. Activated ERKs dimerize and are translocated to the nucleus [51], where transcription factors are phosphorylated whereby their activities are regulated, and influencing gene transcription [52] and other proteins which play a critical role in promoting cell proliferation, differentiation and regulating apoptosis.

Previous finding have shown that the activation of MAPkinase (i.e. phospho-ERK1/2), was significantly reduced intumour tissues but not in normal lung tissues after EGCGtreatment [53,54].

The JAK/STAT pathway is one of signal transduction cascades used to transduce a multitude of signals for development and homeostasis in animals. JAKs are cytoplasmic tyrosine kinases that are activated through ligand-mediated receptor multimerization. The activated JAKs subsequently phosphorylate the major substrates, STATs. STATs are latent

transcription factors that reside in the cytoplasm until activated. The seven mammalian STATs bear a conserved tyrosine residue near the C-terminus that is phosphorylated by JAKs. This phosphotyrosine permits the dimerization of STATs through interaction with a conserved SH2 domain. Phosphorylated STATs enter the nucleus by a mechanism that is dependent on importing α-5 (also called nucleoprotein interactor 1) and the Ran nuclear import pathway. Once in the nucleus, dimerized STATs bind specific regulatory sequences to activate or repress transcription of target genes. Thus the JAK/STAT cascade provides a direct mechanism to translate an extracellular signal into a transcriptional response [55].

EGCG suppressed the activity of AKT, MAPK and STAT5 and induce apoptosis in cell lines harbouring FLT3 mutation [20]. EGCG treatment inhibited the proliferation of GIST-T1 cells, induced apoptosis by inhibited the phosphorylation of KIT and KIT downstream signalling molecules including MAPK and AKT [19]

3. FLT3 AND KIT ARE CLIENTS OF THE HEAT SHOCK PROTEIN HSP90

Heat shock protein 90 (Hsp90) is one of the most abundant chaperone proteins in cells, comprising 1%–2% cellular proteins under non-stress conditions [56]. It interacts with a large and diverse group of substrateproteins; it interacts with almost 400 proteins including human kinases, transcription factors, and E3 ligases, commonly referred to as 'clients', promoting theirfolding and function[22]. Hsp90 seemingly focuses onmetastableproteins that are regulatory hubs in biological networks[21].

Mutant FLT3 has been demonstrated to be a *bona fide* client protein for Hsp90 in cell models and primary AML cells [57-60]. Hsp90 inhibitors including herbimycin A (HA); 17-allylaminodemethoxygel danamycin (17-AAG), geldanamycin were shown to disrupt the chaperone association of Hsp90 with mutant FLT3 resulting in the degradation of FLT3-ITD and apoptosis of myeloid cell lines transfected with mutant FLT3 as well as of primary AML cells expressing FLT3-ITD [20,57-61]. However, in the literature, for the chaperoning of FLT3-WT by Hsp90 is still a controversial topic. The results obtained using 32D/FLT3-WT cells [57] and AML blasts [58] showed that FLT3-WT did not bind to Hsp90. The absence of FLT3-WT from the Hsp90-immunoprecipitated complex in FLT3-WT AML blasts was assumed to be not because of its low expression but perhaps because of Hsp90 having a low affinity for FLT3-WT [58]. On the other hand, others reports indicated that Hsp90 could form a complex with FLT3-WT in SEMK2 leukaemic cells, Ba/F3 and 32D expressing FLT3-WT [59,60].

Mutant KIT was reported to be chaperoned by Hsp90 in Kasumi-1 cells [23]. The data suggested that hsp90 inhibitor - 17-AAG was effective in targeting mutated KIT, causing its degradation. 17-AAG treatment for Kasumi-1 cells also resulted in dissociation of mutant KIT from the Hsp90 complex with a subsequent reduction in phosphorylated mutant KIT level [23].

4. GTCS/EGCG DISRUPTED THE FLT3/HSP90 PROTEIN COMPLEX

EGCG has been demonstrated as an inhibitor of Hsp90 [62]. EGCG acts by binding at or near a C-terminal ATP binding site to inhibit dimerization and promote an Hsp90 conformation that interferes with its chaperone activity for client proteins [62]. EGCG also inhibits Hsp90 function by impairing its association with co-chaperones including Hsc70 and p23 in the pancreatic cancer cell line MIA PaCa-2 [63]. In 2010, Tran *et al.,* reported that in MCF-7 human breast cancer cells, EGCG specifically inhibited the expression of Hsp90 by inhibiting the promoter activity of Hsp90 [64].Our previous data have shown that GTCs/EGCG did not cause any effect on the expression of Hsp90 protein but disrupted the interaction between Hsp90 and FLT3 protein [20]. The same results were obtained in case of KIT (unpublished data).

Another study found that short-term exposure to EGCG (within 2 h) inhibited EGF-induced EGFR phosphorylation and its downstream signalling pathways but long-term exposure to EGCG (> 24 h) decreased the expression levels of EGFR in human lung cancer cells [65]. Although, EGFR is not phosphorylated in A549 lung cancer cells, EGFR-WT has been reported to interact with Hsp90 [66] and non-activated EGFR is inhibited by EGCG [65];this indicates that EGCG tends to target Hsp90 functions rather than the activity of kinase proteins. In the current study, short-term incubation (2 h) of MOLM-13 cells with 60μM EGCG did not significantly affect the phosphorylation of FLT3 (data not shown). Therefore, reduction in kinase activation along with down-regulationof kinase protein levelson long-term incubation with EGCG maybe due to the dissociation of kinase clients from Hsp90 as a result of Hsp90 functiondisruption by EGCG. These data arestronger supporting for our assumption that Hsp90 mediated the antitumor effects of EGCG.

Recent studies have reported that EGCG can bind to its receptor 67LR, which is related to cancer-metastasis [67-71]. 67LR has been originally identified as a non-intergrin cell-surface receptor for the extracellular matrix molecule laminin, over-expressed in various cancer types[72]. Further, EGCG has been shown to induce cell death in HL-60 cells by inducing the 67LR/Akt/ eNOS/cGMP pathway [73]. However, Adachi et al., indicated that EGCG may not directly bind to the cell surface receptor [74]. Moreover, when EGCG is incubated with cells, 75% radioactively labelled EGCG was found in the cytosolic compartment while some radioactivity was found in the membrane fraction [75]. This suggests that the majority of EGCG permeates into the cytoplasm, where it directly interacts with Hsp90 and other cytoplasmic molecules with a high possibility. Furthermore, only EGCG but not EGC, ECG and other GTCs was shown to bind to 67LR [67]. However, many reports found that other GTCs have anti-tumour effect like EGCG [20,76,77]. Therefore, 67LR is not the sole pathway for EGCG and other GTCs mediated its effect. To RTKs, Hsp90 but not 67LR has high potent to involve in mediating the effect of GTCs/EGCG.

CONCLUSION

In conclusion, GTCs/EGCG destabilized RTKs by disrupting their interaction with Hsp90 that lead to down-regulate RTKs expression and their downstream signalling

molecules, finally induce apoptosis cell death (Figure 1). Thus, we propose that EGCG treatment can be a useful therapeutic approach for targeting RTKs that are clients of Hsp90.

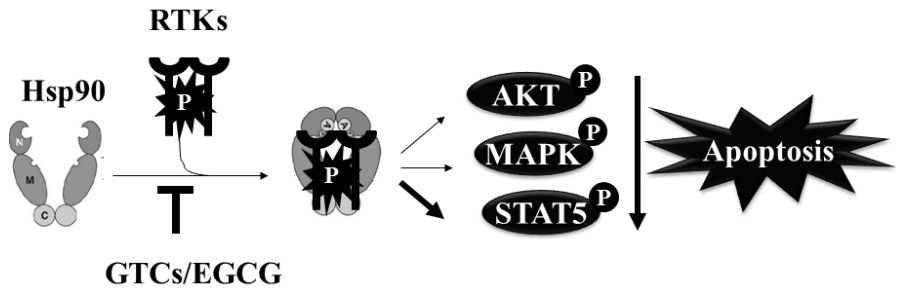

Figure1. The mechanism of down-regulated RTKs protein expression by GTCs/EGCG.

GTCs/EGCGdisrupt the interaction between RTKs and Hsp90 that lead to down-regulate of RTKs expression and their downstream signalling molecules, finally induce apoptosis.

REFERENCES

[1] Belsville M (2003) U.S. Department of Agriculture; USDA Database for the Flavonoid Content of Selected Foods, Prepared by the Nutrient Data Laboratory, Food Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service.

[2] Yang CS, Wang X, Lu G, Picinich SC (2009) Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nat. Rev.* Cancer 9: 429-439.

[3] Yang CS, Maliakal P, Meng X (2002) Inhibition of carcinogenesis by tea. *Annu. Rev. Pharmacol. Toxicol.* 42: 25-54.

[4] Shimizu M, Weinstein IB (2005) Modulation of signal transduction by tea catechins and related phytochemicals. *Mutat. Res.* 591: 147-160.

[5] Yin ST, Tang ML, Su L, Chen L, Hu P, et al., (2008) Effects of Epigallocatechin-3-gallate on lead-induced oxidative damage. *Toxicology* 249: 45-54.

[6] Zhou DH, Wang X, Yang M, Shi X, Huang W, et al., (2013) Combination of Low Concentration of (-)-Epigallocatechin Gallate (EGCG) and Curcumin Strongly Suppresses the Growth of Non-Small Cell Lung Cancer in Vitro and in Vivo through Causing Cell Cycle Arrest. *Int. J. Mol. Sci.* 14: 12023-12036.

[7] Chuu CP, Chen RY, Kokontis JM, Hiipakka RA, Liao S (2009) Suppression of androgen receptor signaling and prostate specific antigen expression by (-)-epigallocatechin-3-gallate in different progression stages of LNCaP prostate cancer cells. *Cancer Lett*. 275: 86-92.

[8] Sanchez-Tena S, Vizan P, Dudeja PK, Centelles JJ, Cascante M (2013) Green tea phenolics inhibit butyrate-induced differentiation of colon cancer cells by interacting with monocarboxylate transporter 1. *Biochim. Biophys. Acta* 1832: 2264-2270.

[9] Singh T, Katiyar SK (2013) Green tea polyphenol, (-)-epigallocatechin-3-gallate, induces toxicity in human skin cancer cells by targeting beta-catenin signaling. *Toxicol. Appl. Pharmacol.* 273: 418-424.

[10] Belguise K, Guo S, Sonenshein GE (2007) Activation of FOXO3a by the green tea polyphenol epigallocatechin-3-gallate induces estrogen receptor alpha expression reversing invasive phenotype of breast cancer cells. *Cancer Res.* 67: 5763-5770.

[11] Mocanu MM, Ganea C, Georgescu L, Varadi T, Shrestha D, et al., (2014) Epigallocatechin 3-O-gallate induces 67 kDa laminin receptor-mediated cell death accompanied by downregulation of ErbB proteins and altered lipid raft clustering in mammary and epidermoid carcinoma cells. *J. Nat. Prod. 77*: 250-257.

[12] Liang YC, Lin-shiau SY, Chen CF, Lin JK (1997) Suppression of extracellular signals and cell proliferation through EGF receptor binding by (-)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. *J. Cell Biochem*. 67: 55-65.

[13] Guo S, Lu J, Subramanian A, Sonenshein GE (2006) Microarray-assisted pathway analysis identifies mitogen-activated protein kinase signaling as a mediator of resistance to the green tea polyphenol epigallocatechin 3-gallate in her-2/neu-overexpressing breast cancer cells. *Cancer Res.* 66: 5322-5329.

[14] Pianetti S, Guo S, Kavanagh KT, Sonenshein GE (2002) Green tea polyphenol epigallocatechin-3 gallate inhibits Her-2/neu signaling, proliferation, and transformed phenotype of breast cancer cells. *Cancer Res.* 62: 652-655.

[15] Lee YK, Bone ND, Strege AK, Shanafelt TD, Jelinek DF, et al., (2004) VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG), in B-cell chronic lymphocytic leukemia. *Blood* 104: 788-794.

[16] Sachinidis A, Skach RA, Seul C, Ko Y, Hescheler J, et al., (2002) Inhibition of the PDGF beta-receptor tyrosine phosphorylation and its downstream intracellular signal transduction pathway in rat and human vascular smooth muscle cells by different catechins. *FASEB J.* 16: 893-895.

[17] Shimizu M, Shirakami Y, Sakai H, Tatebe H, Nakagawa T, et al., (2008) EGCG inhibits activation of the insulin-like growth factor (IGF)/IGF-1 receptor axis in human hepatocellular carcinoma cells. *Cancer Lett.* 262: 10-18.

[18] Vu HA, Beppu Y, Chi HT, Sasaki K, Yamamoto H, et al., (2010) Green tea epigallocatechin gallate exhibits anticancer effect in human pancreatic carcinoma cells via the inhibition of both focal adhesion kinase and insulin-like growth factor-I receptor. *J. Biomed. Biotechnol.* 2010: 290516.

[19] Chi HT, Vu HA, Iwasaki R, Thao le B, Hara Y, et al., (2009) Green tea (-)-epigalocatechin-3-gallate inhibits KIT activity and causes caspase-dependent cell death in gastrointestinal stromal tumor including imatinib-resistant cells. *Cancer Biol. Ther.* 8: 1934-1939.

[20] Ly BT, Chi HT, Yamagishi M, Kano Y, Hara Y, et al., (2013) Inhibition of FLT3 expression by green tea catechins in FLT3 mutated-AML cells. *PLoS One* 8: e66378.

[21] Taipale M, Jarosz DF, Lindquist S (2010) HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nat. Rev. Mol. Cell Biol.* 11: 515-528.

[22] Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, et al., (2012) Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. *Cell* 150: 987-1001.

[23] Yu W, Wang J, Jin J, Qian W, Qian J, et al., (2011) Heat shock protein 90 inhibition results in altered downstream signaling of mutant KIT and exerts synergistic effects on

Kasumi-1 cells when combining with histone deacetylase inhibitor. *Leuk. Res*. 35: 1212-1218.

[24] Network CGAR (2013) Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N. Engl. J. Med*. 368: 2059-2074.

[25] Maroc N, Rottapel R, Rosnet O, Marchetto S, Lavezzi C, et al., (1993) Biochemical characterization and analysis of the transforming potential of the FLT3/FLK2 receptor tyrosine kinase. *Oncogene* 8: 909-918.

[26] Rosnet O, Marchetto S, deLapeyriere O, Birnbaum D (1991) Murine Flt3, a gene encoding a novel tyrosine kinase receptor of the PDGFR/CSF1R family. *Oncogene* 6: 1641-1650.

[27] Matthews W, Jordan CT, Wiegand GW, Pardoll D, Lemischka IR (1991) A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations. *Cell* 65: 1143-1152.

[28] Brasel K, Escobar S, Anderberg R, de Vries P, Gruss HJ, et al., (1995) Expression of the flt3 receptor and its ligand on hematopoietic cells. *Leukemia* 9: 1212-1218.

[29] Turner A, Lin N, Issarachai S, Lyman S, Broudy V (1996) FLT3 receptor expression on the surface of normal and malignant human hematopoietic cells. *Blood* 88: 3383-3390.

[30] Kikushige Y, Yoshimoto G, Miyamoto T, Iino T, Mori Y, et al., (2008) Human Flt3 is expressed at the hematopoietic stem cell and the granulocyte/macrophage progenitor stages to maintain cell survival. *J. Immunol.* 180: 7358-7367.

[31] Stirewalt DL, Radich JP (2003) The role of FLT3 in haematopoietic malignancies. Nat Rev Cancer 3: 650-665.

[32] Riccioni R, Pelosi E, Riti V, Castelli G, Lo-Coco F, et al., (2011) Immunophenotypic features of acute myeloid leukaemia patients exhibiting high FLT3 expression not associated with mutations. *Br. J. Haematol.* 153: 33-42.

[33] Kuchenbauer F, Kern W, Schoch C, Kohlmann A, Hiddemann W, et al., (2005) Detailed analysis of FLT3 expression levels in acute myeloid leukemia. *Haematologica* 90: 1617-1625.

[34] Miettinen M, Lasota J (2001) Gastrointestinal stromal tumors--definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch*. 438: 1-12.

[35] Sattler M, Salgia R (2004) Targeting c-Kit mutations: basic science to novel therapies. *Leuk. Res.* 28 Suppl. 1: S11-20.

[36] Foster FM, Traer CJ, Abraham SM, Fry MJ (2003) The phosphoinositide (PI) 3-kinase family. *J. Cell Sci.* 116: 3037-3040.

[37] Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, et al., (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr. Biol.* 7: 261-269.

[38] Du K, Montminy M (1998) CREB is a regulatory target for the protein kinase Akt/PKB. *J. Biol. Chem.* 273: 32377-32379.

[39] Kane LP, Shapiro VS, Stokoe D, Weiss A (1999) Induction of NF-kappaB by the Akt/PKB kinase. *Curr. Biol*. 9: 601-604.

[40] Martelli AM, Evangelisti C, Chiarini F, Grimaldi C, Cappellini A, et al., (2010) The emerging role of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in normal myelopoiesis and leukemogenesis. *Biochim. Biophys. Acta* 1803: 991-1002.

[41] del Peso L, Gonzalez-Garcia M, Page C, Herrera R, Nunez G (1997) Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science* 278: 687-689.

[42] Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378: 785-789.

[43] Singh BN, Shankar S, Srivastava RK (2011) Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. *Biochem. Pharmacol.* 82: 1807-1821.

[44] Khan N, Mukhtar H (2008) Multitargeted therapy of cancer by green tea polyphenols. *Cancer Lett.* 269: 269-280.

[45] Bigelow RL, Cardelli JA (2006) The green tea catechins, (-)-Epigallocatechin-3-gallate (EGCG) and (-)-Epicatechin-3-gallate (ECG), inhibit HGF/Met signaling in immortalized and tumorigenic breast epithelial cells. *Oncogene* 25: 1922-1930.

[46] Rozakis-Adcock M, McGlade J, Mbamalu G, Pelicci G, Daly R, et al., (1992) Association of the Shc and Grb2/Sem5 SH2-containing proteins is implicated in activation of the Ras pathway by tyrosine kinases. *Nature* 360: 689-692.

[47] Lowenstein EJ, Daly RJ, Batzer AG, Li W, Margolis B, et al., (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 70: 431-442.

[48] Li W, Nishimura R, Kashishian A, Batzer AG, Kim WJ, et al., (1994) A new function for a phosphotyrosine phosphatase: linking GRB2-Sos to a receptor tyrosine kinase. *Mol. Cell Biol.* 14: 509-517.

[49] Kyriakis JM, App H, Zhang XF, Banerjee P, Brautigan DL, et al., (1992) Raf-1 activates MAP kinase-kinase. *Nature* 358: 417-421.

[50] Crews CM, Erikson RL (1992) Purification of a murine protein-tyrosine/threonine kinase that phosphorylates and activates the Erk-1 gene product: relationship to the fission yeast byr1 gene product. *Proc. Natl. Acad. Sci. U S A* 89: 8205-8209.

[51] Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, et al., (1998) Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* 93: 605-615.

[52] Murphy LO, Smith S, Chen RH, Fingar DC, Blenis J (2002) Molecular interpretation of ERK signal duration by immediate early gene products. *Nat. Cell Biol.* 4: 556-564.

[53] Li GX, Chen YK, Hou Z, Xiao H, Jin H, et al., (2010) Pro-oxidative activities and dose-response relationship of (-)-epigallocatechin-3-gallate in the inhibition of lung cancer cell growth: a comparative study in vivo and in vitro. *Carcinogenesis* 31: 902-910.

[54] Zhou H, Chen JX, Yang CS, Yang M, Deng Y, et al., (2014) Gene regulation mediated by microRNAs in response to green tea polyphenol EGCG in mouse lung cancer. *BMC Genomics* 15 Suppl 11: S3.

[55] Rawlings JS, Rosler KM, Harrison DA (2004) The JAK/STAT signaling pathway. *J. Cell Sci.* 117: 1281-1283.

[56] Sreedhar AS, Kalmar E, Csermely P, Shen YF (2004) Hsp90 isoforms: functions, expression and clinical importance. *FEBS Lett.* 562: 11-15.

[57] Minami Y, Kiyoi H, Yamamoto Y, Yamamoto K, Ueda R, et al., (2002) Selective apoptosis of tandemly duplicated FLT3-transformed leukemia cells by Hsp90 inhibitors. *Leukemia* 16: 1535-1540.

[58] Al Shaer L, Walsby E, Gilkes A, Tonks A, Walsh V, et al., (2008) Heat shock protein 90 inhibition is cytotoxic to primary AML cells expressing mutant FLT3 and results in altered downstream signalling. *Br. J. Haematol.* 141: 483-493.

[59] Yao Q, Nishiuchi R, Li Q, Kumar AR, Hudson WA, et al., (2003) FLT3 expressing leukemias are selectively sensitive to inhibitors of the molecular chaperone heat shock protein 90 through destabilization of signal transduction-associated kinases. *Clin. Cancer Res*. 9: 4483-4493.

[60] Oshikawa G, Nagao T, Wu N, Kurosu T, Miura O (2011) c-Cbl and Cbl-b ligases mediate 17-allylaminodemethoxygeldanamycin-induced degradation of autophosphorylated Flt3 kinase with internal tandem duplication through the ubiquitin proteasome pathway. *J. Biol. Chem.* 286: 30263-30273.

[61] George P, Bali P, Annavarapu S, Scuto A, Fiskus W, et al., (2005) Combination of the histone deacetylase inhibitor LBH589 and the hsp90 inhibitor 17-AAG is highly active against human CML-BC cells and AML cells with activating mutation of FLT-3. *Blood* 105: 1768-1776.

[62] Yin Z, Henry EC, Gasiewicz TA (2009) (-)-Epigallocatechin-3-gallate is a novel Hsp90 inhibitor. *Biochemistry* 48: 336-345.

[63] Li Y, Zhang T, Jiang Y, Lee HF, Schwartz SJ, et al., (2009) (-)-Epigallocatechin-3-gallate inhibits Hsp90 function by impairing Hsp90 association with cochaperones in pancreatic cancer cell line Mia Paca-2. *Mol. Pharm*. 6: 1152-1159.

[64] Tran PL, Kim SA, Choi HS, Yoon JH, Ahn SG (2010) Epigallocatechin-3-gallate suppresses the expression of HSP70 and HSP90 and exhibits anti-tumor activity in vitro and in vivo. *BMC Cancer* 10: 276.

[65] Ma YC, Li C, Gao F, Xu Y, Jiang ZB, et al., (2014) Epigallocatechin gallate inhibits the growth of human lung cancer by directly targeting the EGFR signaling pathway. *Oncol. Rep*. 31: 1343-1349.

[66] Ahsan A, Ramanand SG, Whitehead C, Hiniker SM, Rehemtulla A, et al., (2012) Wild-type EGFR is stabilized by direct interaction with HSP90 in cancer cells and tumors. *Neoplasia* 14: 670-677.

[67] Tachibana H, Koga K, Fujimura Y, Yamada K (2004) A receptor for green tea polyphenol EGCG. *Nat. Struct. Mol. Biol.* 11: 380-381.

[68] Kumazoe M, Sugihara K, Tsukamoto S, Huang Y, Tsurudome Y, et al., (2013) 67-kDa laminin receptor increases cGMP to induce cancer-selective apoptosis. *J. Clin. Invest.* 123: 787-799.

[69] Ren X, Guo X, Chen L, Guo M, Peng N, et al., (2014) Attenuated migration by green tea extract (-)-epigallocatechin gallate (EGCG): involvement of 67 kDa laminin receptor internalization in macrophagic cells. *Food Funct.*

[70] Tsukamoto S, Hirotsu K, Kumazoe M, Goto Y, Sugihara K, et al., (2012) Green tea polyphenol EGCG induces lipid-raft clustering and apoptotic cell death by activating protein kinase Cdelta and acid sphingomyelinase through a 67 kDa laminin receptor in multiple myeloma cells. *Biochem. J.* 443: 525-534.

[71] Wang CT, Chang HH, Hsiao CH, Lee MJ, Ku HC, et al., (2009) The effects of green tea (-)-epigallocatechin-3-gallate on reactive oxygen species in 3T3-L1 preadipocytes and adipocytes depend on the glutathione and 67 kDa laminin receptor pathways. *Mol. Nutr. Food Res.* 53: 349-360.

[72] Shammas MA, Neri P, Koley H, Batchu RB, Bertheau RC, et al., (2006) Specific killing of multiple myeloma cells by (-)-epigallocatechin-3-gallate extracted from green tea: biologic activity and therapeutic implications. *Blood* 108: 2804-2810.

[73] Kumazoe M, Kim Y, Bae J, Takai M, Murata M, et al., (2013) Phosphodiesterase 5 inhibitor acts as a potent agent sensitizing acute myeloid leukemia cells to 67-kDa laminin receptor-dependent apoptosis. *FEBS Lett.* 587: 3052-3057.

[74] Adachi S, Nagao T, To S, Joe AK, Shimizu M, et al., (2008) (-)-Epigallocatechin gallate causes internalization of the epidermal growth factor receptor in human colon cancer cells. *Carcinogenesis* 29: 1986-1993.

[75] Hong J, Lu H, Meng X, Ryu JH, Hara Y, et al., (2002) Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (-)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. *Cancer Res.* 62: 7241-7246.

[76] Ravindranath MH, Saravanan TS, Monteclaro CC, Presser N, Ye X, et al., (2006) Epicatechins Purified from Green Tea (Camellia sinensis) Differentially Suppress Growth of Gender-Dependent Human Cancer Cell Lines. *Evid. Based Complement Alternat*. Med. 3: 237-247.

[77] Nihal M, Ahmad N, Mukhtar H, Wood GS (2005) Anti-proliferative and proapoptotic effects of (-)-epigallocatechin-3-gallate on human melanoma: possible implications for the chemoprevention of melanoma. *Int. J. Cancer.* 114: 513-521.

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Chapter 8

THE USE OF GREEN TEA IN TREATING OBESITY

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ABSTRACT

Obesity has been increasing at an alarming rate in the last several decades, both in developed and in developing countries, reaching epidemic proportions among young people and adults as well. Unfortunately, nowadays obesity has become a global health problem. It raises the risk of morbidity from a great number of diseases like: diabetes mellitus Type 2, dyslipidemia, arterial hypertension, coronary heart disease, stroke, cancer and respiratory problems including sleep apnea, etc. Both the direct and indirect costs of obesity and obesity-related morbidity have strong economic impact on the whole society. Therefore, the prevention and treatment of obesity remain and should be a priority worldwide. Besides other possible ways of treatment, phytotherapy has an important role in both scientific research and traditional medicine as well.

Green tea beverage made from the dried, non-fermented leaves of the plant *Camellia sinensis*, has been consumed by humans for thousands of years. It has attained high reputation as a health promoting herb. The increasing interest in the effects of green tea is directed towards its ingredients: catechins, caffeine and theanine, all of which possess various biologically and pharmacologically effects. Some of these compounds are highly attractive in drug discovery programs.

It is traditionally thought that green tea consumption decreases the risks for obesity, reduces body weight and helps in treating overweight patients. Consequently, health abnormalities related to obesity may be alleviated by green tea consumption. A number of extensive experimental research and epidemiological studies supported the anti-obesity effects of green tea and its various forms, and proposed very complicated mechanisms concerning its potential influence on the body weight and composition. At least, the

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modulation of lipid and carbohydrate metabolism, body energy balance and food intake could be obtain by consuming green tea. Its antioxidant effects in treating obesity are also exploited.

Green tea and its commercial forms (which generally contain ingredients like catechins and caffeine in a higher concentration than the typical green tea beverage) have proved to be highly successful in controlled experiments, but they did not demonstrate identical and unambiguous effects in randomised clinical trials (RCTs). That is the reason why its safety and efficacy could not be properly judged and claimed to be a complementary and alternative medicine used to aid weight loss and weight maintenance. To overcome the problem of the insufficient number of RCTs, a lot of systematic reviews and meta-analyses have been conducted. However, in spite of some benefits shown in decreasing body weight and weight maintenance, the obtained improvement, in general, did not reach statistical significance. That could be the result of great heterogeneity of these trials. Thus, well-characterised, randomised controlled clinical trials are needed in order to assess the promising effects of different forms of green tea on health promotion in overweight and obese humans. In order to avoid possible misleading and aggressive commercial practices conducted by the advertisers, such reliable information is extraordinary important for health-care workers, and for green tea consumers as well.

INTRODUCTION

Obesity, as a lifestyle-related disease, is a serious health problem worldwide in spite of its preventability. It has been increasing at an alarming rate in the recent years, reaching epidemic proportions, affecting both children and adults [1].

One of the major concerns related to the increasing obesity incidence is its association with metabolic complications and a large number of related (obesity caused and/or worsened) pathologies [2]. Obesity increased health-care costs, reduced the quality of life and increased the risk of premature death. Therefore, decreasing the incidence of obesity and associated chronic conditions/diseases, will have long-term beneficial consequences on humans and the health care system as a whole. Very serious requirements in terms of prevention/treatment of obesity have been set in the professional and scientific world. It is thereby not surprising that, in addition to drug development in this area, this market is actually very big, offering a variety of products that usually belong to the family of supplements (complex mixtures of different compounds). **S**ome of these products may or may not provide therapeutic activity, or could even be harmful. That market has expanded to become a multimillion dollar industry worldwide. Some of these products are fraudulent and could be extremely harmful to human health. However, potential users are often not properly informed and familiar with their potential negative effects and health threats.

Among various commercially available teas, green tea is ranked as the second most consumed beverage in the world, with water holding the first place. Green tea has gained a high reputation as a nature's gift in both health promotion and longevity. In spite of some controversies and inconsistencies, the mounting evidence of epidemiological and other kinds of studies, pinpoints the favorable effects of green tea in many chronic disorders. Furthermore, green tea was proved to decrease mortality [3].

The anti-inflammatory, anti-carcinogenic, anti-arteriosclerotic and anti-bacterial properties of green tea were registered, as well as its immune-boosting and antidiabetic effects in the diabetes mellitus Type 2 [4, 5, 6]. Green tea might also be applied in the

management of rheumatoid arthritis [7]. Some very significant positive effects of green tea on cognitive functions and memory impairment were registered in neurodegenerative diseases, thus increasing its usage in treating these disorders [8, 9].

Obesity is one of the diseases in which the effectiveness of green tea is expected. A great number of studies conducted *in vitro,* in animals and in humans, were directed to verify and clarify the potential effects of green tea in treating obesity and obesity-induced diseases [10, 11, 12, 13, 14, 15].

OBESITY

According to the World Health Organisation (WHO), "Overweight and obesity are abnormal or excessive fat accumulation that may impair health" [1]. Obesity arises when the energy intake exceeds the energy expenditure. Its etiopthogenesis is complex and depends on a great numbers of factors, like diet, age, physical activity, genetic predisposition, intake of some drugs, etc.

Based on the body mass index in the assessment of obesity (BMI: person's weight in kilograms divided by the square of his height in meters; kg/m^2), the WHO distinguishes between being overweight and obese:

- a BMI greater than or equal to 25 is overweight;
- a BMI greater than or equal to 30 is obesity.

The WHO estimated that, in 2014, more than 1.9 billion adults, aged 18 years and older, were overweight. More than 600 million of these people were obese. These results show that 39% of the world population was overweight, without significant differences between males and females. In 2013, 42 million children under the age of 5 were overweight or obese. The rate of increase of childhood overweight and obesity has been more than 30% higher in developing than in developed countries.

Obesity Comorbidities (Obesity Complications)

Obesity doesn't denote just being overweight. The disturbances are much more serious and they are represented as the metabolic disorder that leads to a dramatic increase in the incidence of comorbidities (secondary diseases to obesity) [2]. This happens because of the inability of the subcutaneous adipose tissue to store the extra fat so that the fat is accumulated and stored in other organs and tissues such as the liver, skeletal muscles, heart and pancreas. This ectopic fat deposition has a strong impact on the organ/tissue function and leads to many disorders. Their clinical manifestation involves diseases such as diabetes mellitus, cardiovascular disease metabolic syndrome, sleep apnea, neuropsychiatric diseases, malignances, rheumatoid arthritis, gastrointestinal, orthopedic, urinary, reproductive/sexual dysfunctions and many other diseases (Figure 1) [2, 16, 17, 18].

However, it became evident that the connection between the body mass and diseases was much more complicated than it was previously thought. It was recognized for the first time in

1947 that a great number of morbidities associated with obesity were also seen in people who were not obese [19]. Later on, the relationship between the body shape and the incidence of comorbidities was established to be of extreme importance. Increased attention was paid to the specific regional distribution of adipose tissue [20]. This distribution might be more important than the total amount of body fat, which has been traditionally been associated with obesity. The accumulated intra abdominal adipose (visceral) tissue is the most important health risk associated with overweight and obesity. People with such kind of obesity are more prone to developing complications [19]. Waist circumference became the measure for estimating the severity of obesity and predicting its complications.

Adipose tissue is not a passive, inert reservoir for lipid storage but a very dynamic endocrine organ [21]. It is the source of variety bioactive compounds known as adipokines or adipocytokines, which circulate in the blood throughout the body. They regulate appetite and food intake, energy balance, immunity, etc. Some adipokines can act pro- or anti-inflammatory. Leptin, adiponectin, resistin and ghrelin participate in the multiple mechanisms of adipokine actions of the systemic energy homeostasis regulation. The first three aforementioned compounds are produced by adipose tissue [22].

Inflammation and Oxidative Stress in Obesity

It has been found obese-associated low-grade, chronic and systemic inflammation. It is developed due to increased production of proinflammatory cytokines [23]. Obese individuals also exhibit an increase in circulating inflammatory mediators [24]. These disturbances are implicated as the underlying cause of obesity comorbidities, i.e., obesity associated diseases arise as a consequence of inflammation that affects different organs, together with obesity accompanied metabolic disorders [25, 24]. For example, neuroinflammation is associated with cognitive dysfunction and was also found in some psychiatric disorders, especially mood disorders that are common in depression and bipolar diseases [26, 27].

Figure 1. Obesity comorbidities.

Pro-inflammatory processes and oxidative stress are strongly related. When the balance of adipokines is shifted to the pro-inflammatory state, as it is found in obesity, generation of free radicals is permanently increased [28]. The consequently developed oxidative stress leads to irregular production of other adipokines and to the development of obesity-related complications [29]. That is in harmony with the findings of the elevated levels of systemic oxidative stress and decreased antioxidant defense in obese people [25, 30].

Obesity Treatment

Obesity and obesity related comorbidities together represent an enormous health and economic burden. Thus, in the treatment of obesity itself and obesity related diseases, the priority is to decrease obesity. Weight loss can improve obesity complication. Striking benefits of decreased body weight was found in obese persons with the Type 2 diabetes [31]. Recent findings about atrial fibrillation, where its severity was dose-dependent with long term weight loss, are also an excellent example [32].

The body weight regulation and energy homeostasis are controlled by many different neurological end endocrine pathways. Obesity treatment is directed towards the regulation of peripheral fat-borne signals that are sent to the brain, and brain neuronal circuits, both of which are involved in energy homeostasis [33]. Taking into account all the facts about obesity and its regulation, specific guidance are made on how to approach the obesity treatment [34].

Chronic weight management and weight loss are the goal in obesity treatment. However, obesity treatment is very difficult. That is because of the extremely complex mechanisms of regulation of energy balance. In line with this are the findings that, in spite of rapid weight loss, the body's tendency to return to the previous weight is more powerful than to maintain the obtained weight reduction.

Lifestyle changes, for example changes in eating habits and physical exercises, represent the mainstay of obesity treatment. Some drugs could reduce appetite and fat absorption. The people with a history of unsuccessful effects of diet plans or exercise alone can be given these drugs together with the reduced-calorie diet plan and increased physical activity [35]. The products derived from medicinal plants are an abundant source of biologically active components. A number of them are believed to be effective in preventing and treating obesity [36]. One of them is green tea which could affect several biological pathways in the regulation of energy homeostasis and complications of obesity [15, 37, 38].

GREEN TEA

Green tea, derived from dried, non-fermented leaves of the plant *Camellia sinensis*, is believed to be a classical tea in the Chinese tradition. It has a great number of components, some of which possess various biological and pharmacological effects. Among them are polyphenols - known as catechins, caffeine, theanine, carotene and vitamins B, C and E. The four main catechins are: (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin and (-)-epigallocatechin-3-gallate (EGCG). EGCG has been suggested to be the most pharmacologically active one and most effects of green tea are being attributed to it [39].

The pleiotropic and complex anti-obesity effects of green tea are being increasingly investigated nowadays. Their exact molecular basis, pharmacokinetics and pharmacodynamics remain only partially known, though. The adipose tissue, liver, intestines and skeletal muscles were noticed to be the target organs of green tea, through which it mediates anti-obesity effects.

Some findings of preclinical studies (*in vivo, ex vivo* and in animals) are of great importance for many properties of green tea, but they cannot adequately document a relationship between its ingredients and treating diseases in humans. Thus, to evaluate whether short- or long-term consumption of green tea in humans may decrease the incidence of obesity, or, even be useful in obesity treatment, these effects must be studied in humans. That makes such studies still very common and attractive.

GREEN TEA USAGE IN WEIGHT LOSS AND WEIGHT MAINTENANCE IN OVERWEIGHT OR OBESE ADULTS

The convincing experimental evidence shows that the balance between the energy intake and expenditure is tightly regulated, making the need for the multidimensional treatment of obesity entirely clear. The main clinical task in obesity treatment is to determine the impact of green tea on body weight (kg), BMI, fat mass (%), and waist and hip circumference (cm). In order to treat obesity, governments, families, and individuals would have to make a great effort. There are several goals in treating obesity, such as:

- The reduction of body weight;
- Changing the body composition;
- The reduction of complications (comorbidities) of obesity;
- The long-term weight maintenance that should follow weight loss;
- The clinical significance of weight reduction.

Animal Studies

A great number of studies in animals (genetic and dietary models of obesity) have been conducted to examine the effects of green tea on obesity and obesity related pathologies. In most studies the green tea extract or EGCG was used, applied in a range from a couple of hours up to 16 weeks in total. The green tea-treated animals showed lesser weight and body fat gain, in contrast with the green tea untreated animals, and showed reduced visceral fat [12, 40]. The green tea-treated animals also had reduced adipose tissue, including visceral fat and hepatic steatosis, and obese-associated biochemical parameters (such as total lipids, triglycerides and total cholesterol), and also had an improved glucose tolerance and increased fecal lipid concentrations [10, 11]. These effects might be at least partially mediated via the regulation of the intestinal fat absorption and the expression of multiple genes involved in adipogenesis, lipolysis, β-oxidation, thermogenesis and inflammation [12].

Human Studies

Apart from other kinds of clinical studies, an increasing number of randomized control trials (RCT) have been conducted during the recent years, aiming to evaluate/confirm the role of green tea in weight loss and weight maintenance. This role was visible in many studies, including both *in vivo* and *in vitro* as well.

Green tea extract and EGCG, with or without caffeine, were given to overweight and obese humans for up to 24 weeks in total. Such treatment was often combined with a low energy diet plan and/or exercising [41]. The success of the green tea treatment, evaluated on the basis of numerous clinical, antropometric and biochemical parameters of obesity, was usually registered, but mixed results were demonstrated as well. The cumulative results of these studies involve: decreased body weight, BMI, waist circumference, body fat (including visceral fat mass established as the adipose tissue storage dysfunction and ectopic triglyceride accumulation), and increased lean body mass (Figure 2) [13, 14]. All these effects lead to a better *body composition*. That could be very beneficial, because the impaired body shape developed in obesity is associated with the appearance of comorbidities. Further examination showed a dose-dependent impact of EGCG and caffeine on body weight and fat percentage reduction.

The improved clinical manifestation of obesity was associated, at the same time, with some considerably beneficial effects of green tea on lipid profiles, such as lowered plasma levels of total cholesterol, low density lipoprotein-cholesterol and triglyceride, and increased high density lipoprotein-cholesterol as well [42].

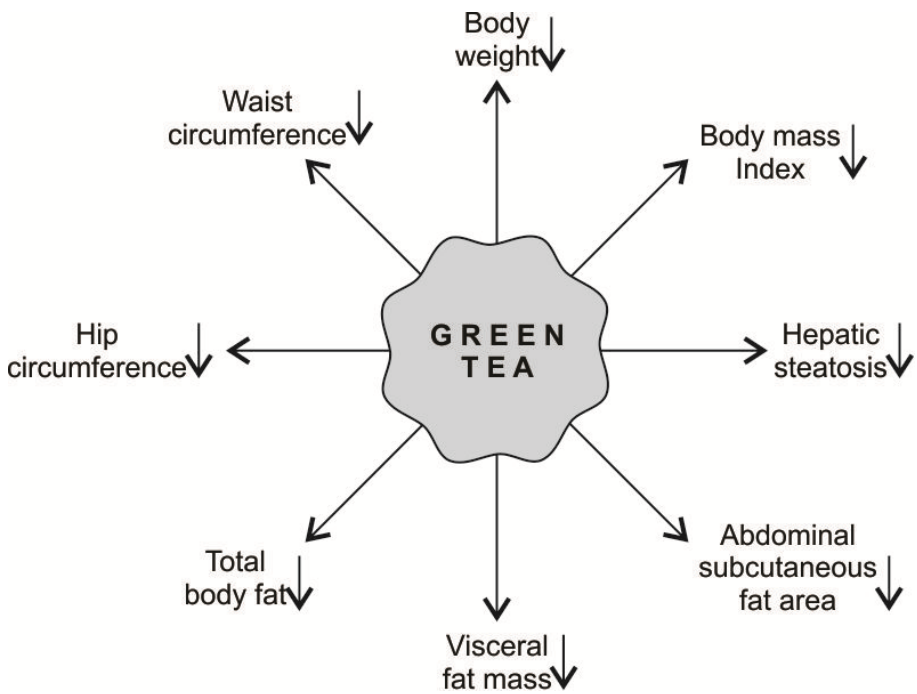

Figure 2. Proposed anti-obesity effects of green tea.

The obtained results considering *weight maintenance* after weight loss are inconsistent. Many of them documented that, together with successful weight loss, a prolonged intake of green tea and caffeine (up to three months) has significantly helped to maintain the weight reduction [15]. On the other hand, some RCT and meta-analytic studies showed contradictory findings to the above mentioned [43, 44].

The overall results do indicate that green tea leads to the reduction of body weight in overweight and obese patients, but some studies suggested that the obtained reduction was not significant [44]. Furthermore, the achieved weight loss did not appear to have any clinical significance.

Thus, green tea, although being highly successful in controlled experiments concerning obesity, did not demonstrate identical and unambiguous effects in the RCTs which could judge its efficacy and safety in overweight or obese humans properly. A number of reasons could lead to the discrepancy in the results, especially in humans. For example, the use of different green tea sources of pharmacologically active ingredients (brewed tea, extracts or supplements) results in totally different amounts of the active substances [45]. Also, if the design of the studies was not uniform (considering the nature of the study population: the extent of obesity, body composition, age, sex and race) and the tested products were not exactly the same (considering their chemical form, composition of pharmacologically active ingredients, their bioavailability, dosage, route, as well as the length of the test product administration), if the patients had different dietary habits, intensity of physical activity, and the genetic population background, the results of the aforementioned studies could not be compared.

Moreover, the optimal dose of green tea active ingredients with anti-obesity properties has not been established yet, and that could be the main reason for obtaining considerably different results from studies with a similar design.

Different and inconsistent findings of the aforementioned studies imposed a necessity to introduce well-characterized, randomized and controlled clinical trials, in order to assess the promising effects of different forms of green tea on the improvement of health in overweight and obese humans. Such reliable information is extremely important for both health-care workers and green tea consumers.

THE PROPOSED MECHANISMS OF ACTION OF GREEN TEA IN OBESITY

There are only two ways to treat obesity. One of them is to reduce the energy intake, while the other one is to increase the energy expenditure. According to their hypothesized mechanism of action, weight-loss substances could be divided into these categories:

1. The suppression of appetite (food intake);
2. The reduction of nutrient absorption;
3. The increment of energy expenditure (thermogenesis and fat oxidation);
4. The antioxidant effects of green tea;
5. Other antiobesity effects.

The aforementioned effects of green tea are proposed to be obtained through few target organs/tissues: the small intestine, the liver, the adipose tissue, and skeletal muscle.

1. The Suppression of Appetite (Food Intake)

A number of neuroendocrine signals participate in the body-weight homeostasis, including the appetite in which the sympathetic nervous system (SNS) holds the major place [46]. It affects both the energy expenditure and substrate utilization through thermogenesis, lipolysis and fat oxidation. In animal models of obesity, green tea reduced food intake, decreased obesity and visceral fats significantly [40]. When given concomitantly with soluble fiber, green tea reduced appetite and increased the feeling of satiety in overweight and obese humans [47].

2. The Reduction of Nutrient Absorption

Whether green tea could influence the absorption of nutrients in the gastrointestinal tract has been an issue examined systematically for many decades. In general, the results look promising considering obesity and obesity-concomitant diseases. A wide variety of studies demonstrated a modulation in dietary carbohydrate and lipid absorption as a result of the green tea treatment.

Carbohydrates. Starch and disaccharides like sucrose and lactose, are the main source of dietary glucose. They have to be fully converted into glucose, which is then rapidly absorbed in the small intestine by different glucose transporters.

The results of the oral glucose tolerance test, and the analysis of starch ingestion showed that green tea reduced glycaemia in humans and animals [48]. Some data indicate the importance of timing of the green tea intake for glucose absorption and metabolism. It seems that stronger effects could be obtained when green tea is co-administered with glucose [48, 49]. Polyphenols could also decrease the food glycemic index. The extent of postprandial glycaemia directly depends on this index. The glycemic effects of polyphenols could open a new field as far as diabetes mellitus is concerned.

The glycemic effects of green tea could be the result of two main mechanisms: the inhibition of transporters responsible for glucose absorption, and the inhibition of α-amylase activity that hydrolyses starch, as well as the enzymes which convert disaccharides into glucose.

Lipids. Dyslipidemia is the main risk factor for atherosclerosis. Because of that, multiple positive effects of green tea on fat metabolism are encouraging. The decreased amounts of different kinds of lipids in serum and liver and the increased amounts in feces**,** which usually indicate lower and delayed lipid intestinal absorption, were found. One of the major reasons for this is the inhibition of luminal lipid absorption as a result of the binding interactions of EGCG and gastric and pancreatic lipase. These effects were dose-dependent [50].

Furthermore, green tea changes the physiochemical properties of a lipid emulsion. It interrupts the lipid emulsification by increasing the lipid particle size and reducing the surface area, which consequently slows down the intestinal absorption of fats.

The lipid-lowering effects of green tea result from the disrupted fat digestion and lipid transport across enterocytes along with the consequently decreased postprandial plasma levels of dietary and biliary cholesterol and triglycerides that could reduce the risk for obesity and cardiovascular diseases.

3. The Increment of Energy Expenditure

Green tea has fat-burning properties. The increment of energy expenditure could be achieved either through upregulated thermogenesis, fat oxidation or fecal lipid excretion. Caffeine is the major metabolically active component in tea. Catechins and caffeine, are each believed to have a role in increasing the energy metabolism [51]. However, the interaction between green tea catechins and caffeine in the thermogenesis, seems to be more promising than when either compound is acting alone, as well as in the prolonging and augmenting of fat oxidation as well. They stimulate the body to burn calories and enhance the loss of fat, including belly fat.

The excess energy intake leads to the SNS (adrenergic) activation and the increased diet-induced thermogenesis and expenditure of energy. Catechol-O-methyl-transferase (COMPT), the major catalyst of polyphenol metabolism, degrades catecholamines like noradrenaline, which activates adipocyte receptors. Green tea catechins are capable of inhibiting COMPT [52]. However, COMPT inhibition is only one of the components in lipid metabolism, and it is not sufficient to fully explain the anti-obesity effects of green tea.

Short- and long-term consumption of caffeine promotes fat oxidation. Increased oxidation is also found in men treated with EGCG [53]. This catabolic reaction takes place in mitochondria. Mitochondrial fatty acid oxidation is involved in generation the chemical energy-adenosine triphosphate (ATP) and reductive equivalents which, beside other effects, hold the oxido-reductive balance.

Furthermore, caffeine could inhibit itracellular phosphodiesterases, the enzymes which break down cyclic adenosine monophosphate (cAMP) generated by noradrenaline. Thus, EGCG and caffeine increase the sympathetic activity that stimulates thermogenesis which is proposed to be one of mechanisms of green tea anti-obesity effects after a short or long intake [54, 15].

4. The Antioxidant Effects of Green Tea

There is a multitude of evidence regarding the obesity-induced oxidative stress that has been examined by different methods. Both, the positive correlation between obesity and different parameters of oxidative stress, and the inverse correlation between obesity and antioxidant defense, were found [57].

The hypertrophied adipocytes and chronic inflammation developed in obesity are significant sources of reactive oxidative and nitrosative species (ROS, RNS) [24, 29]. They may contribute to the development of obesity-associated complications, i.e. obesity-related comorbidities [25]. Therefore, antioxidant products could represent an important therapeutic strategy in the treatment of obesity. Consequently, most studies considering green tea usage

were directed to its anti-oxidative and anti-inflammatory properties, which might have the most powerful impact on human health, including obesity.

Green tea possesses strong antioxidant properties. There are too many mechanisms underlying these effects. They are partially attributed to the trapping of the reactive oxidative and nitrosative species, and the chelating of transitional metal ions which participate in free radical production. In the first case, the quenching of superoxide, hydroxyl and peroxyl radicals, singlet oxygen, as well as nitric oxide and peroxynitrite, reduces the damaging effects of free radicals on all kinds of biomolecules, including genetic material. In the second case, the generation of free radicals is prevented.

Green tea demonstrates antioxidant effects in healthy and obese animals, and obese people. They are based on the improved mitochondrial function, and the enzymatic and non-enzymatic antioxidant capacity [59, 10, 60, 61]. In line with the green tea antioxidant effects is also the increased plasma enzymatic antioxidant capacity (catalase and glutathione peroxidase) after eight weeks of consuming green tea by obese people. Furthermore, reduced glutathione, the major non-enzymatic antioxidant defense, was increased, as well. The decreased oxidative stress and pro-inflammatory signaling were also demonstrated in these persons.

The results of the consumption of green tea beverage for 12 weeks are in line with the previous findings. This treatment decreased the oxidative stress measured by the oxidized low density lipoproteine (LDL) in overweight men in whom the decrement was stronger than in non- obese ones [37]. That was the result of the daily dose of catechins approximately 200-400 mg, since the beverage was prepared by using 2 g of tea leaves. At the same time, clinical parameters of obesity were reduced: the body weight, BMI, waist circumference, body and fat mass and subcutaneous fat area. All this proves that the effects of green tea in the treatment of obesity are very complex.

5. Other Anti-Obesity Effects of Green Tea

The proliferation and differentiation of adipocytes

Adipocytes are not only a triglyceride-storage tissue, but they play the central role in the maintenance of lipid homeostasis, energy balance and weight control. The synthesis of fatty acids, the key step in the lipogenesis, is carried out in the adipose tissue and liver. Both the hypertrophy and hyperplasia of adipocytes are characteristic features of obesity, which has numerous harmful consequences.

Green tea reduces the differentiation of adipocytes and inhibits their proliferation. This happens through the down-regulated gene expression for adipocyte differentiation and adipogenesis, lipogenesis and lipid accumulation [55]. Inhibition of adipocyte differentiation has also been noticed through a cascade of reactions which are mediated *via* the activation of AMP-activated protein kinase, which is involved in the regulation of energy homeostasis [14, 56].

Lipogenesis

Apart from inhibiting fat absorption and increasing lipolysis, the modulation of lipid metabolism in liver and/or adipose tissue by green tea refers to the expression and/or activity

of enzymes related to the synthesis and oxidation of fatty acids [55, 51].It was found, for example, a reduced activity and/or gene expression of lipogenic enzymes essential for fatty acid biosynthesis, such as acetyl-CoA carboxilase and fatty acid synthase that is also present in cells of some malignant diseases (prostate and breast cancer), and some other lipogenic enzymes, Thus, the result of green tea intake could be the reduction of lipid content in the liver, fat cells, and blood [50].

Inflammation

An important feature of obesity is chronic and systemic low-grade inflammation [24]. The adipose tissue, the largest endocrine organ in the body, produces cytokines and chemokines and induces acute-phase inflammatory signaling [29]. Some adipokines promote inflammation and oxidative stress [57]. It is also believed that the infiltration of macrophages into the adipose tissue is involved in obesity-induced inflammation, since adipocytes recruit monocytes which are then transformed into macrophages [58]. Therefore, the suppression of chronic inflammation might be one of the strategies in the prevention and/or treating obesity. In this respect are the results of a number of studies that suggest positive effects of green tea in obesity *via* its ability to suppress chronic inflammation [42, 38].

THE POTENTIAL DANGERS OF GREEN TEA

Despite all the benefits, green tea consumption could also cause some unwanted effects. Those might be the result of:

1. Self-medication;
2. Safety problems;
3. Advertizing.

1. Self-Medication

Patients are often interested in herbal products because they usually perceive them as being natural and, therefore, harmless. Since not marketed as drugs, herbal products are not subjected to rigorous efficacy, safety and quality control testing. Green tea is one of them. Thus, patients usually have a misconception about the consumption of green tea and its safety.

Most herbal products, including all forms of green tea, are sold without prescription. Unfortunately, they are found to be the most widely used form of self-medication which patients take on their own, without informing their physicians [62]. However, that is very risky and could potentially have many harmful consequences due to:

- The incorrect diagnosis;
- The incorrect treatment;
- A delay in seeking medical advice;
- The potential masking and/or worsening of the self-treated disease;

- The complications caused by the applied products, including:
  - adverse reactions
  - interactions, etc.

Extremely aggressive herbal product advertising is one of the main reasons for the increased herbal self-medication and all the side effects that go with it.

2. The Safety of Green Tea

Nowadays there is no doubt that botanical products induce:

- Adverse reactions and
- Interactions with conventional drugs and herbal products.

The adverse effects caused by herbal products applied in therapeutic purposes, or taken as part of traditional habits, emphasize the problem of their safety aspect [63]. The incidence and prevalence of adverse effects and interactions haven't been clarified. That might be due to the fact that the trials are neither uniform nor sufficiently reliable, or to the fact that it is extremely difficult to provide a proper diagnosis of adverse effects, i.e. to prove the causative relation between the accused product (drug) and the obtained health disorder [64].

Adverse Effects

In general, green tea consumption is safe and well tolerated. To date, however, a number of observational cases of green tea adverse effects have been reported and it was suggested that they were the result of the consumption of green tea/green tea ingredients in high doses.

Among all pharmacologically active ingredients in green tea, the major dose-limiting adverse effects are thought to be caffeine related. The adverse effects are mild (minimally clinically significant) to modest (moderately clinically significant). Numerous disturbances have been reported:

- Gastrointestinal: flatulence, nausea, abdominal bloating;
- Neurological: insomnia, tremor, headache, paresthesia;
- Cardiovascular: tachycardia, palpitation.

Similar types of adverse reactions attributed to caffeine were also observed when the higher dose of EGCG was used individually, without caffeine (800 mg daily for 4 weeks) by healthy people [65]. Nausea, abdominal pain and headache were the most commonly reported disturbances. However, synergistic effects of catechins and caffeine were detected in many cases [66].

Green tea intake was also associated with severe adverse effects. For example, it is hepatotoxicity, which is manifested as an acute liver failure [67]. Most of these effects are self-limited and patients recover soon after they stop consuming green tea. Unfortunately, in the case of fulminant hepatitis, transplantation surgery is the only possible solution [68].

The amount of green tea causing hepatotoxicity, its prevalence and incidence have not been established yet. In some case reports of the established causality between green tea and acute liver toxicity, the dose of green tea ingredients was much higher than that conventionally used. Moreover, recent research suggested that EGCG may trigger hepatotoxicity by worsening the pre-existing mitochondria abnormalities [69]. The possibility of hepatotoxicity should be considered when applying other complex herbal products, not only green tea. The fact that a large number of them contain unlabeled green tea should be taken into consideration.

According to other available data, however, the side effects associated with green tea consumption are generally not causally connected to green tea itself [44].

Interactions of Green Tea with Other Substances

Many patients (around 45%) take conventional drugs and herbal products at the same time [70]. Such combinations are usually taken without their physician's knowledge (80%). That increases the potential for the herbal product-conventional drug interaction when concomitantly used, and such interaction was registered in almost 22% of these patients [71]. However, the results are inconsistent, including green tea-drug interactions, and they have to be estimated considering their clinical importance, especially the results for drugs with narrow therapeutic index and in patients currently taking multiple medications.

The potential interactions between biologically active compounds of green tea and conventional drugs have been demonstrated both *in vitro* and *in vivo* studies. They could be synergistic and antagonistic, and from mild to modest in magnitude [72]. In the pharmacokinetic interaction, which is more often than the pharmacodynamic one, green tea influence on CYP enzymes that are mainly present in liver and intestines, drug transporters and proteins for the drug efflux, holds the major place [11].

About 70% of drugs, whose activities show large interindividual variations in healthy people, are metabolized by CYP enzymes. In spite of green tea altered bioavailability of tamoxifen, closapine and midazolam in rats, these interactions were inconsistent in humans. By modulating cell membrane transporters, EGCG could influence the pharmacokinetics of verapamil, nicardipine, tamoxifen and doxorubicin in humans. EGCG could inhibit the biliary elimination of irinotecan and its metabolite, resulting in their prolonged half-life, and, thus, increasing their potential toxicity. Some interactions were also found with buspirone in humans [73].

Why bortezomib and sunitinib cannot be given concomitantly with green tea? Bearing in mind the potential of green tea interaction, the EGCG and other catechins can neutralize bortezomib in serum and the tumoricidal effects of this proteasome inhibitor have been reported to be reduced both *in vitro* (by 100%) and *in vivo*. Green tea also reduces the intestine absorption of sinutinib in humans. Both the interactions have clinical implications considering their efficacy and possible life-threatening consequences [74].

Green tea may interfere with the oral bioavailability or activity of cardiovascular drugs by various mechanisms, thus, potentially leading to the reduction of drug efficacy or increased drug toxicity. Literature data indicate antagonistic effects of green tea against warfarin, which is still controversial [75]. An explanation could take into account the effects of vitamin K from green tea. Additionally, two-fold higher concentration of simvastatine metabolite was found in the plasma of humans taking green tea. The pharmacokinetic and pharmacodynamic interactions with nadolol could diminish its antihypertensive responses. As

far as hydrochlorothiazide is concerned, green tea reduced its side-effects dose-dependently and exhibited myocardial protection [76].

If green tea is taken habitually, in small quantities, the aforementioned interactions with cardiovascular drugs are of little clinical relevance, except in a limited number of sensitive humans. However, understanding the relationship between diet-derived substances and conventional medicines in humans is still vague and information about it is scarce. Interactions between conventional drugs and botanical products have to be thoroughly investigated before recommending their concomitant use as safe.

3. Advertizing

Due to difficulties in curing obesity, weight-loss products, usually of herbal origin, are extremely aggressively advertised. The electronic media, but also printed, represent the most commonly used channel by the advertisers and sellers. Herbal advertizing could bring a lot of complications due to:

- The unqualified health claims and
- Hidden drugs.

Herbs and their products are classified as dietary supplements. There are tens of thousands of such products on the market. They are subject to the legislation concerning the registration of supplements. Consequently, manufacturers are not required to have evidence about their efficacy and safety before registration. On the other hand, in medicine registration these data are unconditionally required. Thus, health claims about the relationship between herbal products and a particular disease/health-related condition must never be advertised until they have been proven under the same regulations which are applied to drugs. Qualified health claims are characterized by a proven relationship between a substance and a disease or health-related condition. As far as green tea is concerned, we could, for example, cite the notification given by Food and Drug Administration (FDA), in which they issued a warning about the wrong heath claims considering green tea and its possibility to reduce the risk of cardiovascular diseases (CVD). FDA wrote a warning letter to the manufacturers: "…there is no credible scientific evidence to support qualified health claims about the consumption of green tea or green tea extract and a reduction of a number of risk factors associated with CVD" [77]. Each user of such products needs to know these facts, in order to avoid the pitfalls of their health omnipotence, which usually can be seen from a very aggressive advertising.

There is a plethora of products for treating obesity that are advertised to be ancient, unique, miraculous, quick and never seen before. It could be said that the world is being flooded with them. As a rule, they are all of natural origin. However, most of them are ineffective and/or fraudulent [78]. The latter ones contain one or more hidden active ingredients including drugs, which are not stated in the product label, for example: sibutramine, caffeine, ephedra, phenolphtaleine, etc. These compounds are most likely to produce harmful effects since consumers take them unknowingly, believing to consume herbal products. Consumers must be aware of such fraudulent claims.

CONCLUSION

The epidemic proportions of obesity have promoted the growing range of dietary products, supplements and so-called natural medicines, most of which are unproven or having little scientific evidence to support this indication. Scientific interest in green tea and its ingredients as therapeutic agents concerning obesity and weight reduction maintenance is rapidly increasing. There is a lot of evidence about its anti-obesity effects that is achieved by a number of actions of biologically and pharmacologically active ingredients of green tea, but there is a number of anti-obesity effects inconsistencies, as well. That could be the consequence of multifactorial reasons, such as the lack of uniformity in study designs, different forms and doses of green tea and its ingredients used in the studies, small study groups, short intervention periods, the concomitant application of an energy-restricted diet or increased energy expenditure, little or no follow-up, etc.

Nowadays consumers, medical doctors and pharmacists are being faced with aggressive advertizing and the extensive usage of herbal products, including green tea. Self-medication is becoming a tremendously widespread practice, and that is why both health professionals and laymen should be informed and become aware of possible harmful effects, adverse reactions and interactions between herbs/botanicals and drugs. Due to many limitations in previous studies, there is a need to conduct well-designed and controlled clinical studies to validate the existing and encouraging findings about green tea and its ingredients in the prevention and treatment of obesity.

REFERENCES

[1] World Health Organization. Obesity and overweight. Fact sheet N°311. Updated January 2015. Accessed 10 February 2015. Available: http://www.who.int /mediacentre/factsheets/fs311/en/#.

[2] Bray, GA. Medical consequences of obesity. *J Clin Endocrinol Metab.*, 2004, 89, 2583-9.

[3] Gardener, H; Rundek, T; Wright, CB; Elkind, MS; Sacco, RL. Coffee and tea consumption are inversely associated with mortality in a multiethnic urban population. *J Nutr.*, 2013, 143(8), 1299-308.

[4] Bashir, S; Khan, BM; Babar, M; Andleeb, S; Hafeez, M; Ali, S; et al., Assessment of bioautography and spot screening of TLC of green tea (Camellia) plant extracts as antibacterial and antioxidant agents. *Indian J Pharm Sci.*, 2014, 76(4), 364-70.

[5] Ognjenović, J; Stojadinović, M; Milčić, M; Apostolović, D; Vesić, J; Stambolić, I; et al., Interactions of epigallo-catechin 3-gallate and ovalbumin, the major allergen of egg white. *Food Chem.*, 2014, 164, 36-43.

[6] Lecumberri, E; Dupertuis, YM; Miralbell, R; Pichard, C. Green tea polyphenol epigallocatechin-3-gallate (EGCG) as adjuvant in cancer therapy. *Clin Nutr.*, 2013, 32(6), 894-903.

[7] Al-Okbi, SY. Nutraceuticals of anti-inflammatory activity as complementary therapy for rheumatoid arthritis. *Toxicol Ind Health*., 2014, 30(8), 738-49.

[8] Ide, K; Yamada, H; Takuma, N; Park, M; Wakamiya, N;, Nakase, J; Ukawa, Y; et al., Green tea consumption affects cognitive dysfunction in the elderly: a pilot study. *Nutrients.*, 2014, 6(10), 4032-42.

[9] Noguchi-Shinohara, M; Yuki, S; Dohmoto, C; Ikeda, Y; Samuraki, M; Iwasa, K; et al., Consumption of green tea, but not black tea or coffee, is associated with reduced risk of cognitive decline. *PLoS One.* 2014 May 14, 9(5), e96013. doi: 10.1371/journal.pone. 0096013. eCollection 2014.

[10] Chung, MY; Park, HJ; Manautou, JE; Koo, SI; Bruno, RS. Green tea extract protects against nonalcoholic steatohepatitis in ob/ob mice by decreasing oxidative and nitrative stress responses induced by proinflammatory enzymes. *J Nutr Biochem.*, 2012, 23(4), 361-7.

[11] Friedrich, M; Petzke, KJ; Raederstorff, D; Wolfram, S; Klaus, S. Acute effects of epigallocatechin gallate from green tea on oxidation and tissue incorporation of dietary lipids in mice fed a high-fat diet. *Int J Obes (Lond).*, 2012, 36(5), 735-43.

[12] Cunha, CA; Lira, FS; Rosa, Neto, JC; Pimentel, GD; Souza, GI; da Silva, CM; et al., Green tea extract supplementation induces the lipolytic pathway, attenuates obesity, and reduces low-grade inflammation in mice fed a high-fat diet. *Mediators Inflamm.* 2013, 635470. doi: 10.1155/2013/635470.

[13] Wang, H; Wen, Y; Du, Y; Yan, X; Guo, H; Rycroft, JA; et al., Effects of catechin enriched green tea on body composition. *Obesity (Silver Spring*)., 2010, 18(4), 773-9.

[14] Wang, S; Moustaid-Moussa, N; Chen, L; Mo, H; Shastri, A; Su, R; et al., Novel insights of dietary polyphenols and obesity. *J Nutr Biochem.*, 2014, 25(1), 1-18.

[15] Hursel, R; Westerterp-Plantenga, MS. Catechin- and caffeine-rich teas for control of body weight in humans. *Am J Clin Nutr.*, 2013, 98(6 Suppl), 1682S-1693S.

[16] Després, JP. Obesity and cardiovascular disease: weight loss is not the only target. *Can J Cardiol.*, 2015, 31(2), 216-22.

[17] Nguyen, NT; Magno, CP; Lane, KT; Hinojosa, MW; Lane, JS. Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: findings from the National Health and Nutrition Examination Survey, 1999 to 2004. *J Am Coll Surg.*, 2008, 207, 928-34.

[18] Renehan, AG; Tyson, M; Egger, M; Heller, RF; Zwahlen, M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet.*, 2008, 371, 569-78.

[19] Tchernof, A; Després, JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev.*, 2013, 93(1), 359-404.

[20] Min, KB; Min, JY. Android and gynoid fat percentages and serum lipid levels in United States adults. *Clin Endocrinol.*, 2015, 82(3), 377-87.

[21] Frühbeck, G. Overview of adipose tissue and its role in obesity and metabolic disorders. *Methods Mol Biol.*, 2008, 456, 1-22.

[22] Henry, BA; Clarke, IJ. Adipose tissue hormones and the regulation of food intake. *J Neuroendocrinol.*, 2008, 20(6), 842-9.

[23] Sumarac-Dumanovic, M; Stevanovic, D; Ljubic, A; Jorga, J; Simic, M; et al., Increased activity of interleukin-23/interleukin-17 proinflammatory axis in obese women. *Int J Obes (Lond).*, 2009, 33(1), 151-6.

[24] Fernández-Sánchez, A; Madrigal-Santillán, E; Bautista, M; Esquivel-Soto, J; Morales-González, A; Esquivel-Chirino, C; et al., Inflammation, oxidative stress, and obesity. *Int J Mol Sci.*, 2011, 12(5), 3117-32.

[25] Matsuda, M; Shimomura, I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obes Res Clin Pract.*, 2013, 7(5), e330-41.

[26] Miller, AA; Spencer, SJ Obesity and neuroinflammation: a pathway to cognitive impairment. *Brain Behav Immun.*, 2014, 42, 10-21.

[27] van Reedt Dortland, AK; Giltay, EJ; van Veen, T; Zitman, FG; Penninx, BW. Longitudinal relationship of depressive and anxiety symptoms with dyslipidemia and abdominal obesity. *Psychosom Med.*, 2013, 75(1), 83-9.

[28] Marseglia, L; Manti, S; D'Angelo, G; Nicotera, A; Parisi, E; Di Rosa, G; et al., Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci.*, 2014, 16(1), 378-400.

[29] Le Lay, S; Simard, G; Martinez, MC; Andriantsitohaina, R. Oxidative stress and metabolic pathologies: from an adipocentric point of view. *Oxid Med Cell Longev.*, 2014, 2014:908539. doi: 10.1155/2014/908539. 10.1155/2014/908539.

[30] Amirkhizi, F; Siassi, F; Djalali, M; Shahraki, SH. Impaired enzymatic antioxidant defense in erythrocytes of women with general and abdominal obesity. *Obes Res Clin Pract.*, 2014 Jan-Feb, 8(1), e26-34. doi: 10.1016/j.orcp.2012.07.004.

[31] Sattar, N; Gill, JM. Type 2 diabetes as a disease of ectopic fat? *BMC Med.* 2014 Aug 26, 12, 123. doi: 10.1186/s12916-014-0123-4.

[32] Pathak, R; Middeldorp, M; Meredith, M; Mehta, A; Mahajan, R; Wong, C; et al., Long-term effect of goal directed weight management in an atrial fibrillation cohort: A long-term follow-up study (LEGACY Study). *J Am Coll Cardiol*, 2015, DOI:101016/jacc.2015.03.002. Available at: http://content.onlinejacc.org/ journal. aspx.).

[33] Skolnik, NS; Ryan, DH. Pathophysiology, epidemiology, and assessment of obesity in adults. *J Fam Pract.*, 2014, 63(7), S3-S10.

[34] Jensen, MD; Ryan, DH; Apovian, CM; Ard, JD; Comuzzie, AG; Donato, KA; et al., 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation.*, 2014, 129(25 Suppl 2):S102-38.

[35] Apovian, CM; Aronne, LJ; Bessesen, DH; McDonnell, ME; Murad, MH; Pagotto, U; et al., Pharmacological management of obesity: an endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.*, 2015, 100(2), 342-62.

[36] Hasani-Ranjbar, S; Nayebi, N; Larijani, B; Abdollahi, M. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. *World J Gastroenterol.*, 2009, 15(25), 3073-85.

[37] Nagao, T; Komine, Y; Soga, S; Meguro, S; Hase, T; Tanaka, Y; Tokimitsu, I. Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am J Clin Nutr.*, 2005, 81(1), 122-9.

[38] Stote, KS; Clevidence, BA; Novotny, JA; Henderson, T; Radecki, SV; Baer, DJ. Effect of cocoa and green tea on biomarkers of glucose regulation, oxidative stress,

inflammation and hemostasis in obese adults at risk for insulin resistance. *Eur J Clin Nutr.*, 2012, 66(10),1153-9.

[39] Braicu, C; Ladomery, MR; Chedea, VS; Irimie, A; Berindan-Neagoe, I. The relationship between the structure and biological actions of green tea catechins. *Food Chem.*, 2013, 141(3), 3282-9.

[40] Heber, D; Zhang, Y; Yang, J; Ma, JE; Henning, SM; Li, Z. Green tea, black tea, and oolong tea polyphenols reduce visceral fat and inflammation in mice fed high-fat, high-sucrose obesogenic diets. *J Nutr.*, *2014*, 144(9), 1385-93.

[41] Roberts, JD; Roberts, MG; Tarpey, MD; Weekes, JC; Thomas, CH. The effect of a decaffeinated green tea extract formula on fat oxidation, body composition and exercise performance. *J Int Soc Sports Nutr.* 2015 Jan 21, 12(1), 1. doi: 10.1186/s12970-014-0062-7. eCollection 2015.

[42] Bogdanski, P; Suliburska, J; Szulinska, M; Stepien, M; Pupek-Musialik, D; Jablecka A. Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutr Res.*, 2012, 32(6), 421-7.

[43] Mielgo-Ayuso, J; Barrenechea, L; Alcorta, P; Larrarte, E; Margareto, J; Labayen, I. Effects of dietary supplementation with epigallocatechin-3-gallate on weight loss, energy homeostasis, cardiometabolic risk factors and liver function in obese women: randomised, double-blind, placebo-controlled clinical trial. *Br J Nutr.*, 2014 Apr 14, 111(7), 1263-71.

[44] Jurgens, TM; Whelan, AM; Killian, L; Doucette, S; Kirk, S; Foy, E. Green tea for weight loss and weight maintenance in overweight or obese adults. *Cochrane Database Syst Rev.*, 2012 Dec 12;12:CD008650. doi: 10.1002/14651858.CD008650.pub2.

[45] Janssens, PL; Hursel, R; Westerterp-Plantenga, MS. Long-term green tea extract supplementation does not affect fat absorption, resting energy expenditure, and body composition in adults. *J Nutr.*, 2015 Mar 4. pii: jn.114.207829. [Epub ahead of print].

[46] Westerterp-Plantenga, MS. Green tea catechins, caffeine and body-weight regulation. *Physiol Behav.*, 2010, 100(1), 42-6.

[47] Carter, BE; Drewnowski, A. Beverages containing soluble fiber, caffeine, and green tea catechins suppress hunger and lead to less energy consumption at the next meal. *Appetite.*, 2012, 59(3), 755-61. doi: 10.1016/j.appet.2012.08.015.

[48] Williamson, G. Possible effects of dietary polyphenols on sugar absorption and digestion. *Mol Nutr Food Res.*, 2013, 57(1), 48-57.

[49] Forester, SC; Gu, Y; Lambert, JD Inhibition of starch digestion by the green tea polyphenol, (-)-epigallocatechin-3-gallate. *Mol Nutr Food Res.*, 2012, 56(11), 1647-54.

[50] Thavanesan, N. The putative effects of green tea on body fat: an evaluation of the evidence and a review of the potential mechanisms. *Br J Nutr.*, 2011, 106(9), 1297-309.

[51] Lu, C; Zhu, W; Shen, CL; Gao, W. Green tea polyphenols reduce body weight in rats by modulating obesity-related genes. *PLoS One.* 2012, 7(6), e38332. doi: 10.1371/journal.pone.0038332.

[52] Shixian, Q; VanCrey, B; Shi, J; Kakuda, Y; Jiang, Y. Green tea extract thermogenesis-induced weight loss by epigallocatechin gallate inhibition of catechol-O-methyltransferase. *J Med Food.*, 2006, 9(4), 451-8.

[53] Venables, MC; Hulston, CJ; Cox, HR; Jeukendrup, AE. Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. *Am J Clin Nutr.*, 2008, 87(3), 778-84.

[54] Dulloo, AG; Duret, C; Rohrer, D; Girardier, L; Mensi, N; Fathi, M; Chantre, P; Vandermander, J. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr.*, 1999, 70(6), 1040-5.

[55] Lee, MS; Kim, CT; Kim, Y. Green tea (-)-epigallocatechin-3-gallate reduces body weight with regulation of multiple genes expression in adipose tissue of diet-induced obese mice. *Ann Nutr Metab*., 2009, 54(2), 151-7.

[56] Stevanovic, D; Starcevic, V; Vilimanovich, U; Nesic, D; Vucicevic, L; Sumarac-Dumanovic, M; et al., Immunomodulatory actions of central ghrelin in diet-induced energy imbalance. *Brain Behav Immun.* 2012; 26(1), 150-8.

[57] Karbownik-Lewinska, M; Szosland, J; Kokoszko-Bilska, A; Stępniak, J; Zasada, K; Gesing, A; et al., Direct contribution of obesity to oxidative damage to macromolecules. *Neuro Endocrinol Lett.*, 2012, 33(4), 453-61.

[58] Abdali, D; Samson, SE; Grover, AK. How effective are antioxidant supplements in obesity and diabetes? *Med Princ Pract.*, 2015 Mar 14. [Epub ahead of print].

[59] Jelenković A; Jovanović MD; Stevanović I; Petronijević N; Bokonjić D; Zivković J; et al., Influence of the green tea leaf extract on neurotoxicity of aluminium chloride in rats. *Phytother Res.*, 2014, 28(1), 82-7.

[60] Basu, A; Betts, NM; Mulugeta, A; Tong, C; Newman, E; Lyons, TJ. Green tea supplementation increases glutathione and plasma antioxidant capacity in adults with the metabolic syndrome. *Nutr Res.*, 2013, 33(3), 180-7.

[61] Ellinger, S; Müller, N; Stehle, P; Ulrich-Merzenich, G. Consumption of green tea or green tea products: is there an evidence for antioxidant effects from controlled interventional studies? *Phytomedicine*. 2011, 18(11), 903-15.

[62] Bent, S. Herbal medicine in the United States: review of efficacy, safety, and regulation: grand rounds at University of California, San Francisco Medical Center. *J Gen Intern Med.*, 2008, 23(6), 854-9.

[63] Singh, D; Gupta, R; Saraf, SA. Herbs-are they safe enough? An overview. *Crit Rev Food Sci Nutr.*, 2012, 52(10), 876-98.

[64] Jelenković, A; Panić, V; Popović, A; Mitić, S; Milić, M; Janošević-Nešić, Lj; et al., Clinical practice and knowledge of primary health care physicians toward adverse drug reactions: can we recognize adverse drug reactions and what should we do to maximize patient safety and to improve self-confidence of doctors when prescribing medicines? *Br J Med Medical Res.*, 2015, 7(6), 481-93.

[65] Chow, HH; Cai, Y; Hakim, IA; Crowell, JA; Shahi, F; Brooks, CA; et al., Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin Cancer Res.*, 2003, 9(9), 3312-9.

[66] Schönthal, AH. Adverse effects of concentrated green tea extracts. *Mol Nutr Food Res.*, 2011, 55(6), 874-85.

[67] Navarro, VJ; Bonkovsky, HL; Hwang, SI; Vega, M; Barnhart, H; Serrano, J. Catechins in dietary supplements and hepatotoxicity. *Dig Dis Sci.*, 2013, 58(9), 2682-90.

[68] Molinari, M; Watt, KD; Kruszyna, T; Nelson, R; Walsh, M; Huang, WY; et al., Acute liver failure induced by green tea extracts: case report and review of the literature. Liver *Transpl.*, 2006, 12(12), 1892-5.

[69] Weng, Z; Zhou, P; Salminen, WF; Yang, X; Harrill, AH; Cao, Z; et al., Green tea epigallocatechin gallate binds to and inhibits respiratory complexes in swelling but not normal rat hepatic mitochondria. *Biochem Biophys Res Commun.*, 2014, 443(3), 1097-104.

[70] Djuv, A; Nilsen, OG; Steinsbekk, A. The co-use of conventional drugs and herbs among patients in Norwegian general practice: a cross-sectional study. *BMC Complement Altern Med.*, 2013 Oct 30, 13, 295. doi: 10.1186/1472-6882-13-295.

[71] Blalock, SJ; Gregory, PJ; Patel, RA; Norton, LL; Callahan, LF; Jordan, JM. Factors associated with potential medication-herb/natural product interactions in a rural community. *Altern Ther Health Med.*, 2009, 15(5), 26-34.

[72] Yang, CS; Pan, E. The effects of green tea polyphenols on drug metabolism. *Expert Opin Drug Metab Toxicol.*, 2012, 8(6), 677-89.

[73] Chow, HH; Hakim, IA; Vining, DR; Crowell, JA; Cordova, CA; Chew, WM; et al., Effects of repeated green tea catechin administration on human cytochrome P450 activity. *Cancer Epidemiol Biomarkers Prev*., 2006, 15(12), 2473-6.

[74] Jia, L; Liu, FT. Why bortezomib cannot go with 'green'? *Cancer Biol Med*., 2013, 10(4), 206-13.

[75] Werba, JP; Misaka, S; Giroli, MG; Yamada, S; Cavalca, V; Kawabe, K; et al., Overview of green tea interaction with cardiovascular drugs. *Curr Pharm Des.*, 2015, 21(9), 1213-9.

[76] Chakraborty, M; Kamath, JV. Pharmacodynamic interaction of green tea extract with hydrochlorothiazide against ischemia-reperfusion injury-induced myocardial infarction. *J Adv Pharm Technol Res.*, 2014, 5(3), 134-9.

[77] Schneeman, BO. Qualified health claims: letter of denial - green tea and reduced risk of cardiovascular disease (Docket No. 2005Q-0297). Accessed 16 December 2014. Available: http://www.fda.gov/food/ ingredientspackaginglabeling/labelingnutrition/ ucm073207.htm.

[78] Jelenković, A. Counterfeit drugs and other products and substances: review of drugs for influenza prevention and treatment including pandemic influenza a (HIN1). *Vojnosanit Pregl.*, 2010, 67(6), 501-6. (Serbian).

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Chapter 9

AROMATIZED GREEN TEAS – THE CONTENT OF FLAVONOIDS AND METALS

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ABSTRACT

The aromatized green teas are popular due to their aroma and organoleptic properties. In this study the content of flavonoids in the infusions of five commercially available teas was determined by HPLC-MS in the negative electrospray ionization mode. All types of tea contained epigallocatechin-3-gallate in the greatest amounts, followed by epicatechin and catechin. Some teas with citrus aromas or fruits contain also the glycosides of other flavonoids such as naringin and hesperidin. The mineral contents of dry teas and the infusions produced from them were also determined by inductively coupled optical emission spectrometry. Aluminium and manganese were major constituents in dry teas, followed by iron, zinc, copper and nickel. The release of metals into tea infusions depends on whether they are strongly bond to the organic matrix or more soluble in the solution. Iron exhibited the lowest efficiency of extraction by hot water from all studied teas. Copper and nickel fractions transferred into tea brew exceeded 50% in some cases.

INTRODUCTION

Green tea, like black and oolong teas, is manufactured from dried leaves of *Camelia sinensis*. It differs from the other tea types by the processing method as well as organoleptic

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taste, flavor and chemical content. To produce green tea, freshly harvested leaves are steamed to prevent fermentation, yielding a dry, stable product. In addition, various kinds of flavored or fruit teas became popular in many European countries due to their fragrance, therapeutic application and lower content of caffeine, which could inhibit calcium absorption [1]. These teas contain natural aromas as well as dry fruits or herbs, which are added to tea leaves in the last stage of processing before packing.

In recent years, tea is extensively investigated mainly regarding its influence on human health [2, 3]. Several studies tested the qualities of green tea as an antioxidant, anti-mutagenic and anti-carcinogenic substances, and its role in hypertension prevention, cardiovascular risk modification, ultraviolet radiation protection, bodyweight management and oral health improvement [4-7].

Flavonoids and polyphenolic acids are the most abundant bioactive compounds in tea leaves and extracts. They are primarily responsible for the beneficial healthful properties of tea [8]. The main polyphenols in green tea are catechins (flavan-3-ols) and their content varies with climate, season, horticultural practices and leaf age. Similarly to fruits and vegetables, green tea contains additional antioxidants such as carotenoids, tocopherols (vitamin E derivatives) and vitamin C. In addition to organic compounds, different minerals and trace metals are present in tea leaves and their infusions. Some of these elements are components of enzymes and greatly influence the biochemical processes in cells. Generally, the metal content in tea leaves tea is influenced by the soil composition and local environmental factors. Owing to the importance of minerals present in tea, many studies were carried out to determine their levels in tea leaves and their infusions [9-12]. Several elements, such as K, Na, Ca, Mg, Al and Mn are present at mg/g level, whereas Cr, Co, Ni, Zn, Cu and Fe have been found at the level of μg/g. The presence of some toxic elements such as Pb, Cd and As has been also demonstrated.

The objective of this study was to evaluate and compare the phenolic composition and metal content of commercially available aromatized green tea infusions. The content of selected flavonoids was determined by high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS).

MATERIALS AND METHODS

Chemicals

The commercial standards of flavonoids as well as the other chemicals were purchased from Sigma (Steinheim, Germany). Acetonitrile was of HPLC gradient grade from Merck (Darmstadt, Germany). Ultra pure water from Milli-Q system (Millipore, Bedford, MA, USA) was used in all experiments. Stock solutions of flavonoids were prepared in methanol. Diluted mix standards were prepared with water or in methanol. All solutions were filtered through PTFE 0.45 μm membrane filters (Millipore) and degassed prior to use.

Teas and Preparation of Infusions

All bagged teas Lipton brand were purchased from a local market. The aromatized green teas contain, except tea leaves, dry fruit and herbs which are visible, as well as some natural aroma. List of teas under study with the ingredients shown in the labels is presented in Table 1. The tea bags were dipped into 200 mL of freshly boiled water for 5 min to represent the typical quantity consumed by tea drinkers. After the infusion time, the bags were removed and the partly turbid solutions were filtered paper after cooling to room temperature through the Whatman filter. In the prepared infusions the content of polyphenolic compounds and metal ions were determined.

Dried sample of 0.250 g of each tea was mineralized in the presence of concentrated HNO_3 (3 mL) and HF (0.2 mL) in the microwave oven. The power was gradually increased at 160 W for 3 min, at 350 W for 4 min, 750 W for 5 min and finally 1000 W for 6 min. After cooling the residue was transferred into a volumetric flask and made up to 25 mL with distilled water. Digestion was made in triplicate for each kind of tea.

Chromatographic Analysis

Chromatographic analysis was performed with a Shimadzu LC system consisted of binary pumps LC20-AD, degasser DGU-20A5, column oven CTO-20AC, autosampler SIL-20AC, detector UV SPD 20A connected to 3200 QTRAP Mass spectrometer (Applied Biosystem/MDS SCIEX). A MS system equipped with electrospray ionization source (ESI) operated in negative-ion mode and a quadrupole mass analyser in a scan mode from 50 to 1500 m/z. ESI conditions were following: capillary temperature 450 °C, curtain gas at 0.3 MPa, auxiliary gas at 0.3 MPa, negative ionisation mode source voltage – 4.5 kV. Nitrogen was used as curtain and auxiliary gas.

Compounds were separated on Kinetex™ (Phenomenex) C-18 column (100 x 2.1 mm, 2,6 μm) with precolumn at 30 °C. Formic acid (2 mmol/L, pH 2.8) as eluent A and acetonitrile as eluent B were used. The mobile phase was delivered at 0.2 mL/min in linear gradient mode: 0-5 min. 20% B, 10 – 15 min 25 % B, 20-25 min 30% B, 30-31 min 90% B, 32 min 20% B. Compounds were identified by comparing retention time and *m/z* values obtained by MS and MS^2 with the mass spectra from standards tested under the same conditions. Quantification of compounds was done from the calibration curves obtained in Multiple Reaction Mode (MRM) [13].

Determination of Metal Ions

A quadrupole spectrometer ICP-QMS (ELAN 6100 DRC, Perkin-Elmer Sciex, Boston, MA, USA) equipped with conventional Scott spray chamber and Meinhard nebulizer was used for the determination of metal ions. The working conditions of spectrometer were optimized daily in order to obtain the maximal sensitivity and stability. The operating conditions were as follows: plasma Ar gas flow rate 15 L/min, auxiliary Ar gas flow rate 1.2 L/min, nebulizer gas flow rate 0.74 L/min, solution uptake rate 1 mL/min, dwell time 100 ms, and the number of replicates 6. The isotopes ^{63}Cu, ^{57}Fe, ^{61}Ni and ^{66}Zn were used for

quantification. ^{103}Rh was used as internal standard. During measurements, care was taken to avoid memory effect and therefore wash-out time of 20 min was applied. All standard solutions used were prepared by appropriate dilution of 1 mg/mL single element standard solutions from Merck (Darmstadt, Germany).

Table 1. List of the analyzed green teas

Code	Commercial name	Ingredients (as per label)
S1	Green Indonesia	Green tea (98.8*), natural aroma (1.2)
S2	Asian Temple	Aroma (19.3), petals of rose (0.9), violet (0.8) and jasmine (0.8)
S3	Green Citrus	Natural aroma of grapefruit (6), peels of lemon, lime, orange and grapefruit (2.1)
S4	Green Mint	Natural mint aroma (7.9)
S5	Green mandarin	Tangerine and orange aroma (15.5), peels of orange (1.2) and tangerine (1.2)

*g per 100 g tea

RESULTS AND DISCUSSION

Flavonoid Composition

Epigallocatechin 3-gallate (EGCG) is the major flavonoid constituent of studied green teas. It levels ranged from 67.8 to 152.5 mg per 100g of dry tea. It. The high antioxidant activity of these compound has been widely described [8]. Sing et al., [14] reported that EGCG acts as an angiogenesis inhibitor by modulating protease activity during endothelial morphogenesis. Angiogenesis is a crucial step in the growth and metastasis of cancer. EGCG decreased in amount with increasing fermentation process from green to oolong and black tea as did total catechins [15]. The level of catechin and epicatechin in studied tea infusions were in the ranges of 26.8-64.4 and 31.7-75.6 mg per 100 g of dry matter. Table 2 summarises these results. Generally, the highest level of studied catechins was found in sample S1, which contains only 0.7 g of the additives per 100 g of dry *Camelia sinensis* leaves (Table 1). Example of the chromatogram of tea infusions by LC/MS/MS are presented in Figure 1.

Except catechins, the most important green tea flavonols are kaempferol, quercetin and myricetin, which mainly occurring as glycosides (Table 2). Only quercetin in the form free aglycone was found at trace levels (0.31 – 0.78 mg per 100 g of dry tea) in the studied infusions. Samples S1, S3 and S4 contained the highest content of rutin (quercetin-3-rutinoside), while sample G2 was characterised by the lowest content of rutin, nicotoflorin (kaempferol-3-rutinoside) and myricetrin (myricetin-3-glucoside).

Table 2. Content of selected flavonoids in studied tea infusions (expressed in mg per 100g dry tea)

	ECGC	Epicatechin	Catechin	Rutin	Nicotoflorin	Myricetrin	Hesperidin	Naringin
S1	129 ± 5.40	60.6 ± 5.97	50.9 ± 3.71	92.3 ± 5.8	99.6 ± 6.52	67.8 ± 6.40	1.0 ± 0.09	0.5 ± 0.04
S2	13.2 ± 6.91	65.4 ± 6.30	55.7 ± 4.22	72.4 ± 4.75	95.8 ± 6.13	28.3 ± 1.84	nd	0.04 ± 0.01
S3	125 ± 6.13	74.6 ± 8.11	63.6 ± 6.99	92.4 ± 6.02	93.1 ± 6.42	32.9 ± 2.09	4.3 ± 0.32	13.2 ± 1.14
S4	94.2 ± 5.90	54.2 ± 5.98	44.9 ± 3.65	95.8 ± 6.73	77.5 ± 8.23	53.2 ± 5.21	10.1 ± 0.98	nd
S5	66.9 ± 7.80	32.5 ± 1.51	26.9 ± 1.81	67.9 ± 5.99	84.4 ± 5.63	27.0 ± 2.11	8.2 ± 0.61	2.8 ± 0.17

*Mean ± SD (n=3), nd < limit of detection

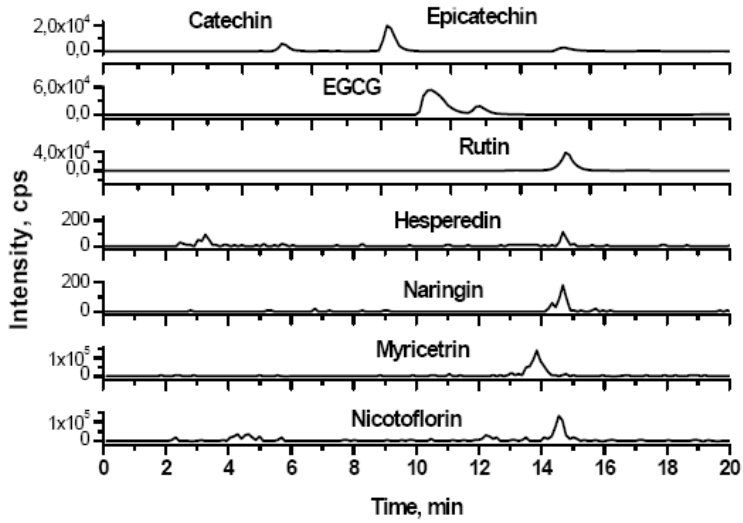


Figure 1. Extracted ion chromatograms of flavonoids in the extract of *Green Mint* (S4).

HPLC analysis of S3 and S5 samples (teas with citrus aromas or part of dry fruits) showed the significant level of naringin (naringenin-7-rutinoside) and hesperidin (hesperetin-7-rutinoside). These compounds are the predominant flavonones in citrus fruits [16]. Jung et al., [17] found that supplementation with hesperidin and naringin significantly reduced blood glucose, while the bone and lipid benefits of hesperidin make it an attractive dietary agent for the management of the health of postmenopausal women [18]. Sample S4 (*Green Mint*) also contained significant level of hesperidin, which could be found in mint leaves [19]. Thus, these flavored green teas would support the human diet with a satisfactory source of naringin and hesperidin.

Metals in Teas and Their Infusions

The content of six metals (Al, Cu, Fe, Mn, Ni and Zn) were determined in dry leaves of aromatized green teas after their mineralization.. The results of the analysis by ICP OES method are presented in Table 3. All studied samples contained significant values of microelements but their contents presented a wide variability.

Table 3. Contents of metals in the studied dry teas (expressed in μg/g)

Tea	Al	Cu	Fe	Mn	Ni	Zn
S1	1771 ± 92	19.5 ± 1.6	248 ± 21	626 ± 38.1	2.21 ± 0.13	42.5 ± 2.57
S2	1672 ± 84	12.9 ± 1.1	363 ± 18	743 ± 45	3.10 ± 0.14	36.0 ± 1.84
S3	2129 ± 127	18.5 ± 0.97	159 ± 10	593 ± 31	2.15 ± 0.22	28.5 ± 1.15
S4	807 ± 48	14.0 ± 0.76	98.1 ± 4.9	708 ± 35	2.64 ± 0.13	23.5 ± 1.09
S5	1382 ± 69	12.0 ± 1.0	140 ± 9.0	1230 ± 62	4.32 ± 0.23	24.5 ± 1.53

*Mean ± SD (n=3).

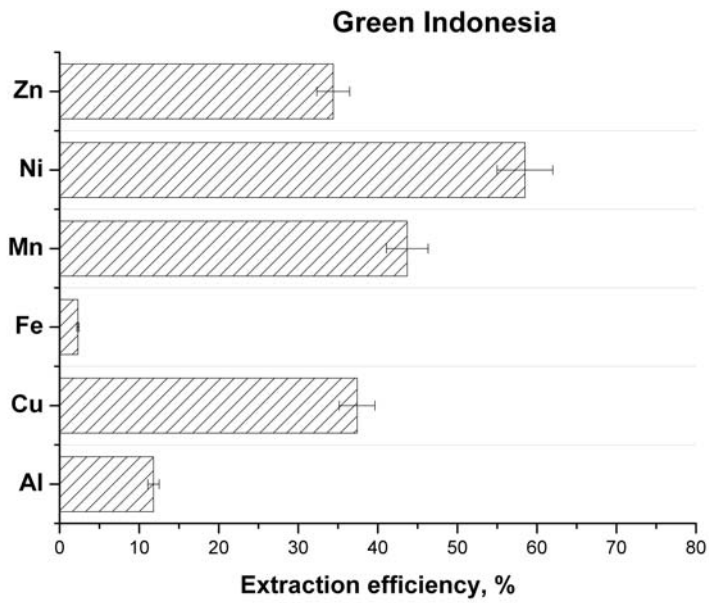
The highest content (2129 μg/g) was found for Al, particularly for *Green Citrus* tea (S3 - 2129 μg/g). Dry *Green Mint* tea contains the lowest content of this metal (807 μg/g). Due to

favorable conditions and easy aluminium absorption by the root system of a tea bush, Al is one of main elements which occur in tea leaves. The content of aluminium in several black teas in two price groups (>1€ and <1€) occurred at similar level (825-1050 μg/g) independently on the price group [20].

The studied aromatized green teas also contained contents of manganese, 593 μg/g (sample S3) to 1230 μg/g (sample S5). The comparison performed by Kumar et al., [21]. revealed that most of tea leaves from various countries have Mn content in the range of 300-900 μg/g, except for those from Turkey and Japan (1100 - 2678 μg/g).

The contents of other determined metals decrease in the order: iron > zinc > copper > nickel (Table 2). Han et al., [22] reported concentration of Cu in 811 tea samples (from the main tea producing provinces of China) in the range of 2.04-447.5 μg/g, with the mean value of 18.33 μg/g. Among the different types of tea, black tea had the highest copper level, probably due to the use of Cu-bearing-Bordeaux mixture to protect plants against diseases. The mean nickel content in black teas from South India was in the range of 1.1-5.3 μg/g [23], while the range of 10.1-55.4 μg/g was reported for Zn content in *Camellia sinensis* plants by Nookabkaew et al., [24].

The level of metal concentration in the studied tea infusions may be affected by a number of parameters such as organic matrix of corresponding tea, original mineral content as well as extraction conditions (amount of material relative to water, infusion time and temperature). The content of metals in tea infusions may be also affected by different properties or structures of plants as well as level of polyphenolic compounds which could bind metals [25].



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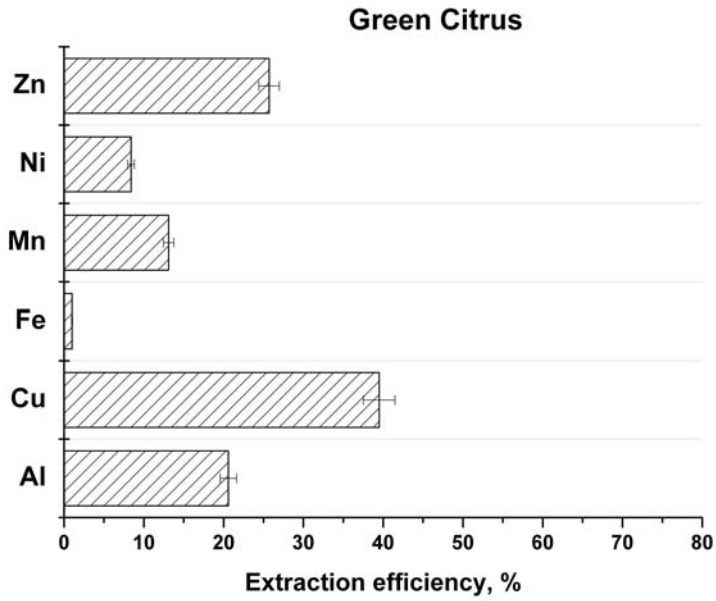

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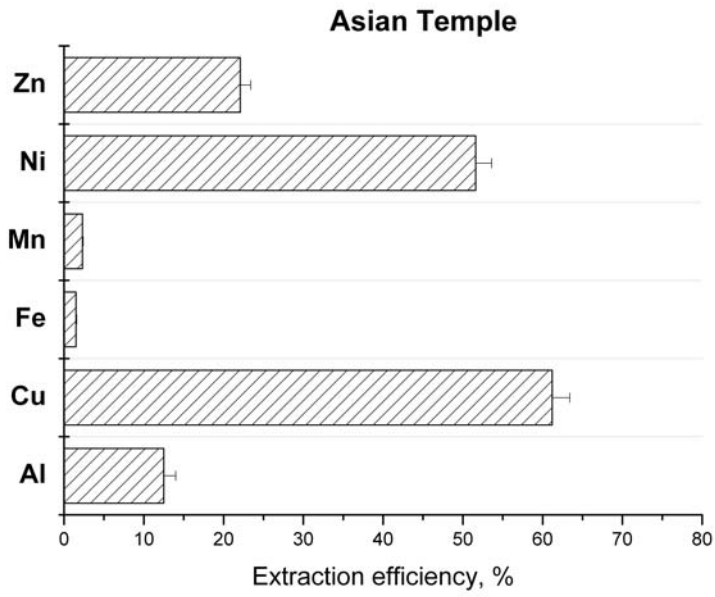

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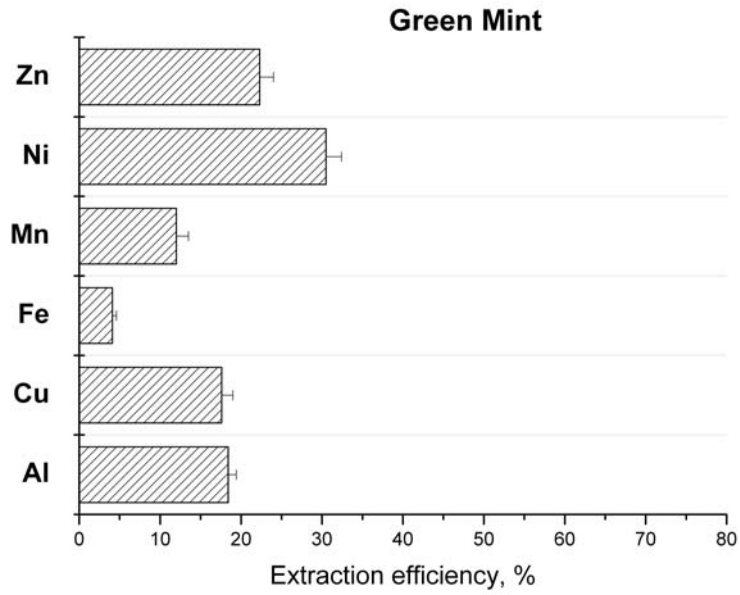

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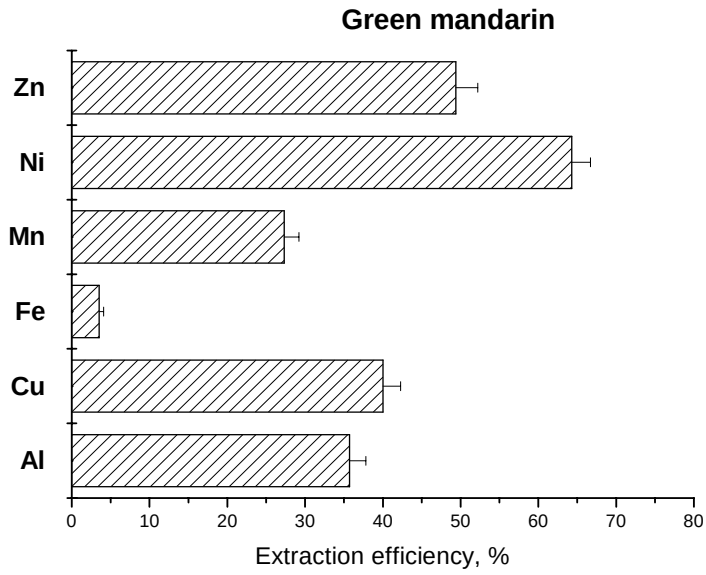

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Figure 2. The efficiency of metal extraction from the studied teas.

The effectiveness of metal transfer from dry tea leaves to infusion are shown in Figure 2. As can be seen, the percentage transfer of each metal varied widely for the different tea samples. Generally, Fe had the lowest percent release in all infusions. The low extractability of iron was also reported and dominating metal fraction in the infusions was the cationic form, which is considered as the most bioavailable [26]. The high efficiency of extraction from tea leaves was observed for nickel (except sample S2), copper and zinc. The studied tea

infusions, particularly *Green Indonesia* and *Green mandarin*, could be a good source of manganese, which is in agreement with literature data [21]. Manganese is an essential element and is bound to a number of essential enzymes; for example the activity of superoxide dismutase is suppressed by low Mn status. Although the content of manganese in tea sample S1 was two ones smaller than in sample S5 (Table 2), the extraction efficiency of Mn was higher for the first tea.

However, the actual bioavailability and toxicity of trace elements to the human organisms depend not only on the total element amount of the metals but also on their existing physicochemical forms [27-29]. Apparently, the trace and major metals can be present in the infusions as simple ions but also as the complexes formed with the endogenous bioligands.. Pohl and Prusisz [26] reported that zinc was present mainly in tea infusion in the cationic forms which account for 58-71% and 84-86%, respectively, in the black and the green teas. Mn can be present in brews not only in the form of the predominant simple cations or other small cationic species (up to 80%) but also may be bound with the polyphenolic species or other macromolecular compounds [30]. 10–19% of total aluminum in black tea infusion was present as cationic species, while about 28–33% of as hydrolyzable polyphenolic compounds [31].

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REFERENCES

[1] H.Y. Chen, Y.C. Lin, C. Hsieh, Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. *Food Chem.* 10, 1418 (2007).

[2] A.B. Sharangi, Medicinal and therapeutic potentialities of tea (*Camelia sinensis* L.) – A review. *Food Res. Inter.* 42, 529 (2009).

[3] B. Narotzki, A.Z. Reznick, D. Aizenbud, Y. Levy, Green tea: A promising natural product in oral health. *Arc. Oral Biol.* 57, 429 (2012).

[4] E.G. de Mejla, M.V. Ramirez-Mares, S. Puangpraphant, Bioactive components of tea: Cancer, inflammation and behaviour. *Brain Behav. Immun.* 23, 721 (2009).

[5] J.D. Lambert, R.J. Elias, The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Arch. Biochem. Biophys.* 501, 65 (2010).

[6] M. Bancirova, Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. *Food Res. Inter.* 43, 1379 (2010).

[7] X.Y. Qin, Y. Cheng L.C. Yu, Potential protection of green tea polyphenols against intracellular amyloid beta-induced toxicity on primary cultured prefrontal cortical neurons of rats. *Neuroscien. Lett.* 57, 170 (2012).

[8] N. Khan, H. Mukhar, Tea polyphenols for health promotion. *Life Science* 81,519 (2007).
[9] T. Karak, R.M. Bhagat, Trace elements in tea leaves, made tea and tea infusion: A review. *Food Res. Inter.* 43, 2234 (2010).
[10] A. Szymczycha-Madeja, M. Welna, P. Pohl, Elemental analysis of teas and their infusions by spectrometric methods. *Trends Anal. Chem*. 35,165 (2012).
[11] A. Pękal, M. Biesaga, K. Pyrzynska, Trace metals and flavonoids in different types of tea. *Food Sci. Biotechnol.* 22, 925 (2013).
[12] E.M. Derun, Determination of essential mineral concentrations in some Turkish teas and the effect of lemon addition. *Food Sci.Biotechnol.* 23, 671 (2014).
[13] M. Biesaga, K. Pyrzynska, Liquid chromatography/tandem mass spectrometry studies of the phenolic compounds in honey. *J. Chromatogr.* A, 1216, 6620 (2009).
[14] A.K. Sing, A.P. Seth, M.M. Husain, S. Madhavan, H. Mukhtar, R.K. Maheshwari, Green tea constituent epigallocatechin-3-gallate inhibits angiogenic differentiation of human endothelial cells. *Arch. Biochem. Biophys.* 40, 29 (2002).
[15] J. Peterson, J. Dwyer, S. Bhagwat, D. Haytowitz, J. Holden, A.L. Eldridge, G. Beecher, J. Aladesanmi, Major flavonoids in dry tea. *J. Food Comp. Anal.* 18, 487 (2005).
[16] G. Gattuso, D.D. Barreca, C. Gargiulli, U. Leuzzzi, C. Caristi. Flavonoid composition of *Citrus* juices. *Molecules* 12, 1641 (2007).
[17] U.J. Jung, M.K. Lee, K.S. Jeong, KS M.S. Choi, The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. *J. Nutrit.* 134, 2499 (2004).
[18] M.N. Horcajada, V. Habauzit, A. Trzeciakiewicz, C. Morand, A. Gil-Izquierdo, A. Mardon, P. Lebecque, M.J. Davicco, W.S. Chee, V. Coxam, E. Offord, Hesperidin inhibits ovariectomized-induced osteopenia and shows differential effects on bone mass and strength in young and adult intact rats. *J. Appl. Physiol.* 104, 648 (2008).
[19] H. Aoshima, S. Hirata, S. Ayabe, Antioxidative and anti-hydrogen peroxide activities of various herbal teas. *Food Chem.* 1003, 617 (2007).
[20] M. Frankowski, Aluminium and its complexes in teas and fruity brew samples, speciation and ions determination by ion chromatography and high-performance liquid chromatography-fluorescence analytical methods. *Food Anal. Meth.* 7, 1190 (2014).
[21] A. Kumar, A.G.C. Nair, A.V.R. Reddy, A.N. Garg, Availability of essential elements in Indian and US tea brands. *Food Chem.* 89, 441 (2005).
[22] W.Y. Han, Y.Z. Shi, L.F. Ma, J.Y. Ruan, Arsenic, cadmium, chromium, cobalt and copper in different types of Chinese tea. *Bull.* Environ. Contam.Toxicol. 75, 272 (2005).
[23] S. Seenivasan, N. Manikandan, N. Muraleedharan, R. Ivasundaram (2008). Heavy metal content of black teas from south India. *Food Control* 19, 746 (2008).
[24] S. Nookabkaev, N. Rangkadilok, I. Satayavivad, Determination of trace elements in herbal tea products and their infusions consumed in Thailand. *J. Agric. Food Chem.* 54, 6939 (2006).
[25] A. Pękal, M. Biesaga, K. Pyrzynska, Interaction of quercetin with copper ions: complexation, oxidation and reactivity towards radicals. *Biometal*s 24, 41 (2010).
[26] P. Pohl, B. Prusisz, Simple and versatile operational fractionation of Fe and Zn in dietary products by solid phase extraction on ion exchange resins. *Talanta* 71,411 (2007).

[27] T.P. Flaten, Aluminum in tea – concentrations, speciation and bioavailability. *Coord. Chem. Rev.* 228, 385 (2002).
[28] J.J. Powell, T.J. Burden, R.P. Thompson, In vitro mineral availability from digested tea: a rich dietary source of manganese. *Analyst* 123, 1721 (1998).
[29] A. Pękal, K. Pyrzynska (2013). Availability of some elements from different types of teas. *Natur. Prod. J.* 3, 292-295.
[30] P. Pohl, B. Prusisz (2007). Fractionation analysis of manganese and zinc in tea infusions by two-column solid phase extraction and flame atomic absorption spectrometry. *Food Chem.* 102, 1415-1424.
[31] S.B. Erdemoğlu, K. Pyrzynska, S. Gucer (2000). Speciation of aluminum in tea infusion by ion-exchange resins and flame AAS detection. Anal. Chim. Acta 411, 81–89.

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Chapter 10

APPLICATION OF HILIC COLUMN FOR THE DETERMINATION OF CATECHINS

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ABSTRACT

The goal of this study was to investigate chromatographic behavior of catechins in hydrophilic interaction liquid chromatography (HILIC). Two different HILIC columns were used: cross-linked DIOL (Luna HILIC) and zwitterionic sulfoalkylbetaine (SeQuant ZIC-HILIC). Separation parameters such as content of acetonitrile (ACN) and pH of the aqueous fraction of an eluent were studied. On the ZIC column, the retention factor of catechins increased with decreasing water content in the mobile phase and the increase in pH of the aqueous component mainly affects the polarity of the analytes. DIOL stationary phase showed more or less apparent dual retention mechanism; HILIC at ACN content $\geq$ 75% (v/v) and reversed-phase (RP) with lower content of organic modifier. Retention times for catechins are longer for zwitterionic stationary phase. In the presence of ammonium acetate in the mobile phase, retention of catechins increases slightly for both columns without change in the selectivity of separation. Significant higher sensitivity was observed under HILIC conditions due to much higher content of ACN in the mobile phase than in RP mode. The elution order increases in the order:: catechin < epicatechin < epigallocatechin gallate.

INTRODUCTION

Green tea infusions not only gives specific taste and flavor, but also contain many essential dietary compounds for human health, such as proteins (considerable parts of them are enzymes) carbohydrates and lipid components [1]. Besides macronutrients, green tea also includes vitamins (B, C, E), xanthic bases such as caffeine and theophylline, pigments (chlorophyll and carotenoids) and several trace elements [2,3]. Another important green tea

components are polyphenols, amongst them catechins (flavan-3-ols) are the main compounds. There is a growing evidence that these compounds have strong antioxidant activity and have numerous potentially beneficial effects on living organisms [4,5].

The current interest in the health effects of green tea has necessitated the development of new analytical methods for determination of catechins in natural products and HPLC is the most widely used separation techniques for these purposes [6-8]. The HPLC conditions mainly include the use of C_{18} reverse-phased (RP) columns, a binary solvent gradient and diode array detector or tandem mass spectrometry. The mobile phase usually consists of an aqueous solution of acid and an organic solvent (acetonitrile or methanol). HPLC-MS combines the separation of LC with the selectivity and sensitivity of the MS detector to permit the identification of individual compounds from the complex matrices.

Hydrophilic interaction chromatography (HILIC), which involves polar stationary phases and predominantly organic eluents, is a fast-growing sub-technique of HPLC aimed mainly at separating polar compounds that show inadequate retention and thus poor resolution in RP LC [9,10]. The mechanism of retention in HILIC appears to be complex. The primary retention is supposed to be partition of the analyte between the organic-rich mobile phase and the immobilized aqueous layer at the surface of the stationary phase. Interaction between the solute and the functional groups of the stationary phase (dipole-dipole, hydrogen bonding and electrostatic interactions) also occurs as evidenced by the different separations observed when the stationary phase is varied [11,12]. Both partition and adsorption mechanism are thus believed to contribute to the overall retention of analytes in HILIC conditions. The extent to which each mechanism dominates is dependent on the type of stationary phase used and eluent conditions. An important feature of HILIC is the improved sensitivity with electrospray mass spectrometry [13]. This is significant for the analysis of components existing in small concentration in multicomponent mixtures.

Critical parameters for the retention mechanism in HILIC are the structure of the solute, the composition of the mobile phase and the polar functional moieties on the stationary phases. Polar stationary phases commonly used in HILIC separations consist of bare silica and silica phases modified with neutral, ionic or zwitterionic functional groups [14]. In a typical HILIC mobile phase, acetonitrile is used as a week eluent and water or aqueous buffer as a strong eluent.

In this work, HILIC mode was used to investigate chromatographic behavior of several catechins – catechin, epicatechin and epigallocatechin-3-gallate (EGCG). For this purpose two HILIC stationary phases: diol-bonded phase and with zwitterionic sulfoalkylbetaine groups on silica surface (ZIC) were used. HILIC columns have proven to be very useful for the separation of a variety of polar compounds [15-17].

MATERIALS AND METHODS

Chemicals

The commercial standards of catechins as well as the other chemicals were purchased from Sigma (Steinheim, Germany). Acetonitrile was of HPLC gradient grade from Merck (Darmstadt, Germany). Ultra pure water from Milli-Q system (Millipore, Bedford, MA, USA)

was used in all experiments. Stock solutions of the analytes were prepared in methanol. All solutions were filtered through PTFE 0.45 μm membrane filters (Millipore) and degassed prior to use.

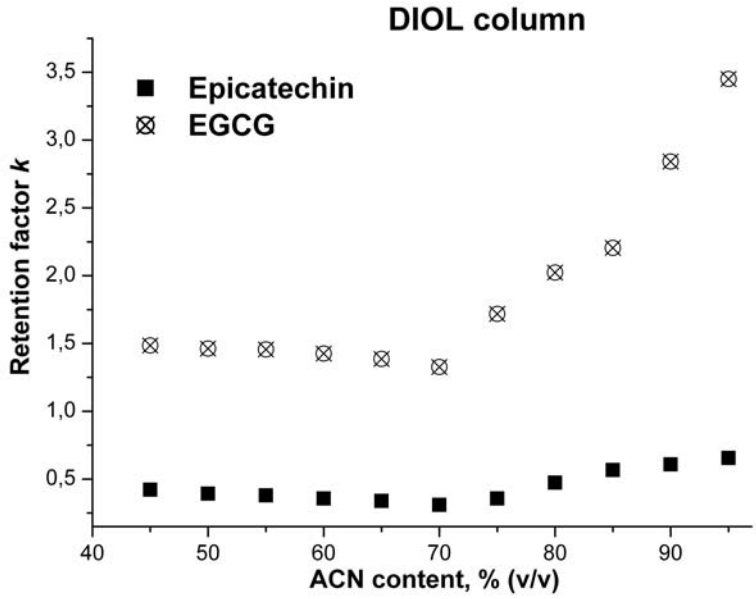
Chromatographic Analysis

Chromatographic analysis was performed with the liquid chromatography system (Shimazu, Duisburg, Germany) consisted of binary pumps LC20-AD, degasser DGU-20A5, column oven CTO-20AC, autosampler SIL-20AC and 3200 QTRAP Mass spectrometer (Applied Biosystem/MDS SCIEX, Foster City, California, USA). The mass spectrometry system was equipped with electrospray ionization source (ESI). ESI in negative mode conditions were following: capillary temperature 450 °C, curtain gas at 0.3 MPa, auxiliary gas at 0.3 MPa, the ionisation mode source voltage 4.5 kV. Nitrogen was used as curtain and auxiliary gas. For each compound the optimum conditions of Selected Reaction Mode (SRM) were determined in infusion mode. Standard solutions were infused into the electrospray source via a 50 μm *i.d*. polyether ether ketone capillary employing the pump at 10 μL min^{-1}. (Harvard Apparatus, Holliston, Massachusetts, USA).

Chromatographic measurements were performed on two different polar stationary phases: diol-bonded phase Luna HILIC (100x2.0 mm, 3.5 μm) from Phenomenex and zwitterionic-bonded phase ZIC-HILIC (100x2.1 mm, 3.0 μm) with bare silica based material from Phenomenex. The mobile phases were prepared by mixing appropriate volumes of ACN and acetic acid, water or ammonium acetate solution. Toluene was applied as the void time marker. The mobile phase was delivered at 0.2 mL/min in the isocratic mode. For the analysis of green tea extracts 15 mM of ammonium acetate or 15 mM HCOOH as eluent A and acetonitrile as eluent B were applied. The mobile phase was delivered in the gradient mode: 0-4 min 98%B, 6-7 min 90% B, 8-8.4 min 80% B, 8.4-12 min 50% B and 13-20 min 98% B. Compounds were identified by comparing retention time and *m/z* values obtained by MS and MS^2 with the mass spectra from standards tested under the same conditions. Quantification of compounds was done from the calibration curves obtained in Multiple Reaction Mode (MRM) [18].

RESULTS AND DISCUSSION

The effect of the content of organic modifier (acetonitrile) in the mobile phase on the retention behavior of catechin, epicatechin and epigallocatechin-3-gallate (EGCG). was investigated at neutral pH. It should be noted that the pH value of the mobile phases refer to their aqueous portion. Thus, at pH 7.0 the analytes are less or more dissociated and exist in the equilibrium between the charged and the neutral forms. ZIC phase contains basic quaternary ammonium groups and acidic sulfonic groups which exist in a molar ratio of 1:1. The small negative charge arising from the distal sulfonic groups is pH independent [19]. The obtained retention factors were plotted against the ACN content in the range of 45-95% (v/v) as shown in Figure 1.



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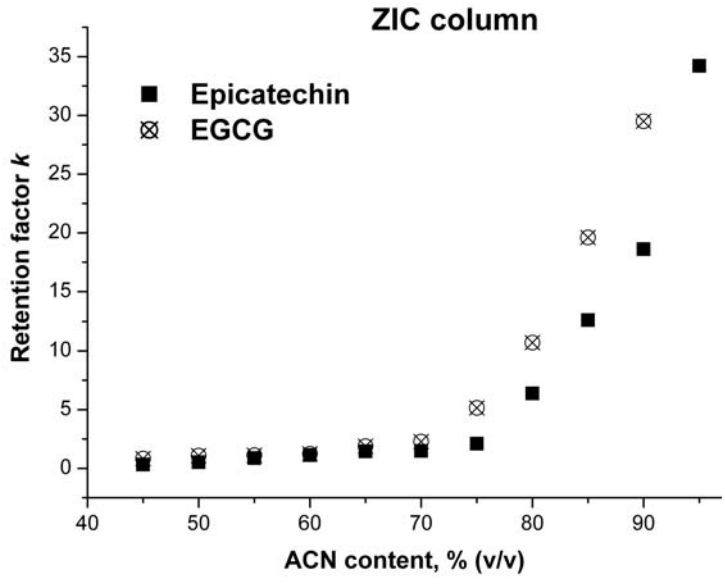

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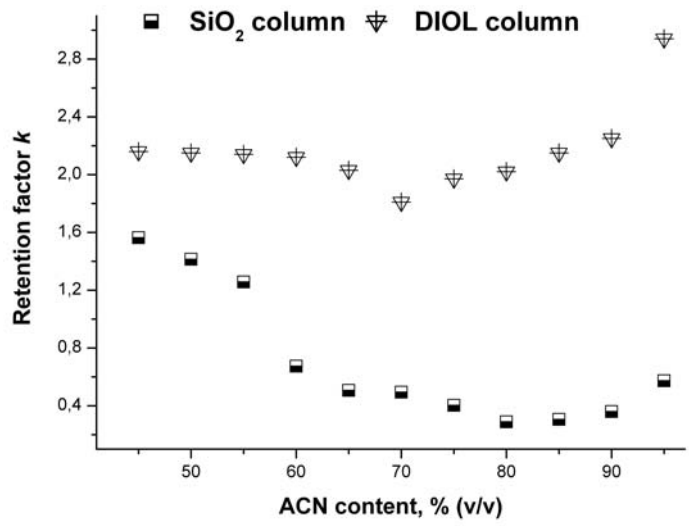
Figure 1. Influence of acetonitrile content on the retention factors of epicatechin and EGCG on different HILIC columns at pH 7.0.



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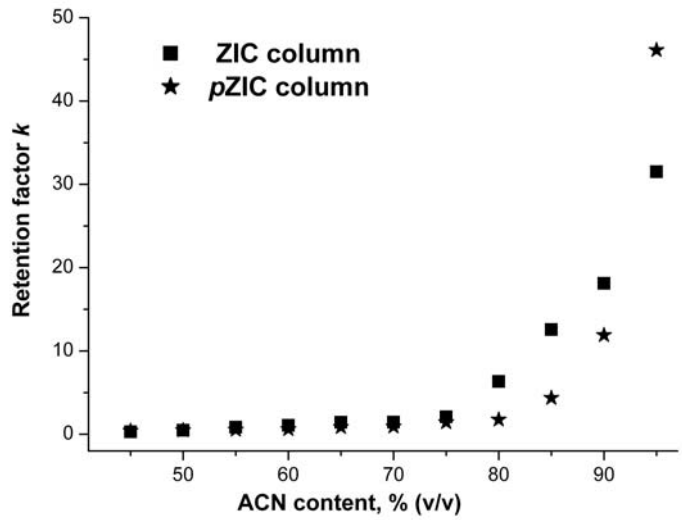

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Figure 2. Influence of acetonitrile content on the retention factors of catechin on different HILIC columns at pH 7.0.

Increasing content of ACN in the mobile phase enhances the retention of studied catechins on ZIC column, which is in line of perception of a HILIC-type retention mechanism [10,20]. At increasing concentration of acetonitrile, water is adsorbed more strongly on the surface of the polar stationary phase. The more hydrophilic are the analytes, the more the partitioning equilibrium is shifted towards the adsorbed water layer on the stationary phase, and the more the analytes are retained. On the DIOL column, U-shaped curves are obtained when plotting solute retention factors versus the ACN content (Figure 1). The chemical bonded diol phase demonstrates high polarity and does not contain ionisable groups, other

than non-reacted residual silanols [14]. This stationary phase shows more or less apparent dual retention mechanism, HILIC at the concentration of ACN higher than 70%, and RP in mobile phases with lower content of organic modifier. Similar U-shape retention curves were also found on polyethylene glycol and diol bonded stationary phases [21].

Retention characteristics for catechin on ZIC and DIOL columns were similar to those obtained for epicatechin. The influence of ACN content on retention of this compound were also investigated on additional HILIC columns - bare silica phase Altantis HLIC (100x2.1 mm, 3.0 μm) and zwitterionic-bonded phase ZIC-*p*HILIC (150 x 2.1 mm, 5.0 μm) covalently attached to porous polymer beads (Figure 2). The bare silica packing consistently delivered the lowest retention factor values for catechin which was a clear indication that among the examined columns, had the lowest polarity. Both stationary phases – DIOL and bare silica - exhibit dual retention mechanism. Significantly higher values of retention factors were observed for the zwitterionic-bonded phases under the same elution conditions. ZIC columns have probably additional electrostatic contributions from the sulfonic groups, presumably because it is a distal charge moiety in the zwitterionic functional group. Salts are usually added to the mobile phase to control electrostatic interactions between charged analytes and stationary phase [20].

As a further parameter, the effect of column temperature was investigated at isocratic conditions using constant concentration of ACN (95%, *v/v*, pH 7). The temperature was varied over the range of 20-35 ^{o}C (higher temperatures were not use due to instability of the column according to the manufacturer`s data). The obtained ln *k* values for catechin were plotted versus 1/T according to van`t Hoff equation. As can be seen from Figure 3 column temperature has a minimal impact on catechin retention. In HILIC mode, the retention is probably controlled by entropic contributions, possibly originating in different levels of sample salvation in the stationary nad bulk mobile phase [2]. Calculated enthalpy changes (ΔH) showed values between -0.25 and -7.70 kJ/mol. Chirita et al., [22] postulated that linear van`t Hoff plots can be an indicator that partitioning is the predominant mechanism under the experimental conditions. Regarding separation, variations in temperature did not seem to be critical. Neither the efficiency nor the selectivity of the separation was significantly affected.

The potential of HILIC separation was demonstrated for the analysis of bioactive compounds in the aqueous extracts of green tea. Infusion was carried out by pouring 1.7 g of dried plant (a typical bag) in 50 mL of freshly boiled water and allowed to steep for 5 min. DIOL column and gradient elution of increasing part of water content in the mobile phase was used to reduce the retention times of catechins. Extracted ion chromatograms of catechins on DIOL column in Lipton Green Mint tea infusion using different eluents were presented in Figure 4. That stationary phase was chosen as on ZIC column retention times were very long and the peaks of studied catechins were unresolved. Generally, the elution increases in the order:: catechin < epicatechin < epigallocatechin gallate, similar like in RP mode [6,23]. The addition of formic acid in the mobile phase has the significant effect on the resolution of catechin/epicatechin peaks as well as reduction of retention time for EGCG (Figure 4A). In the presence of ammonium acetate these peaks were unresolved and retention time for EGCG was very long.

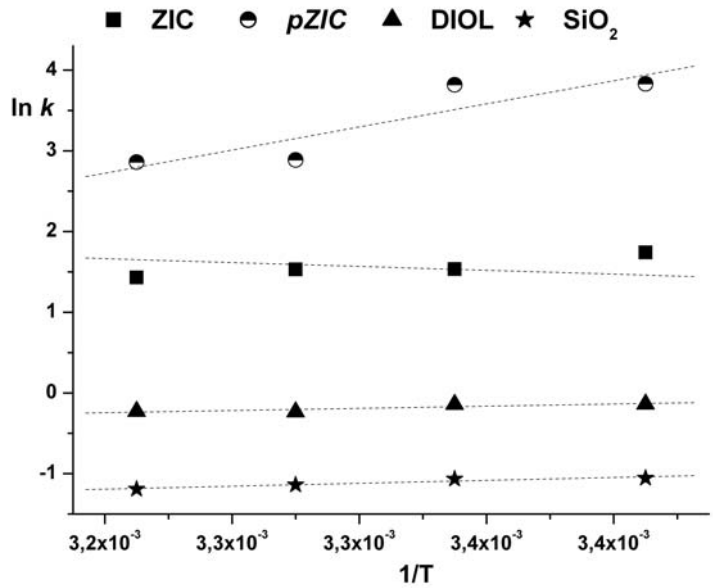
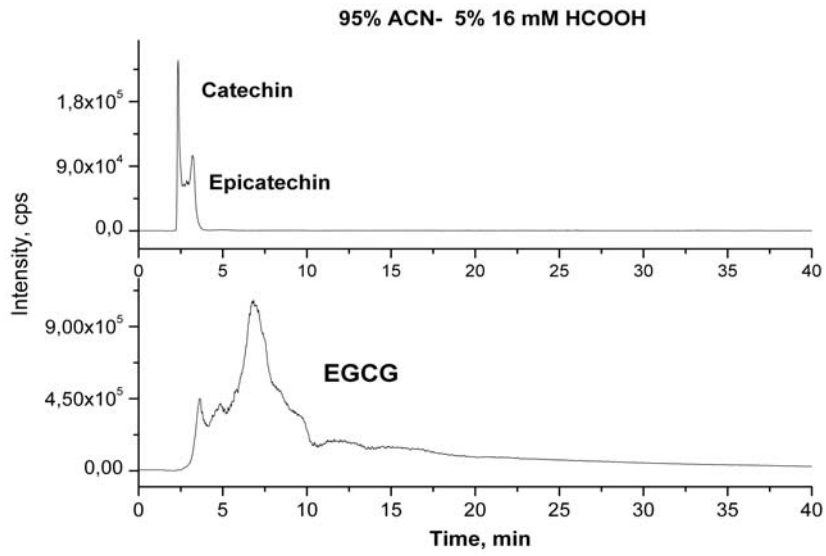
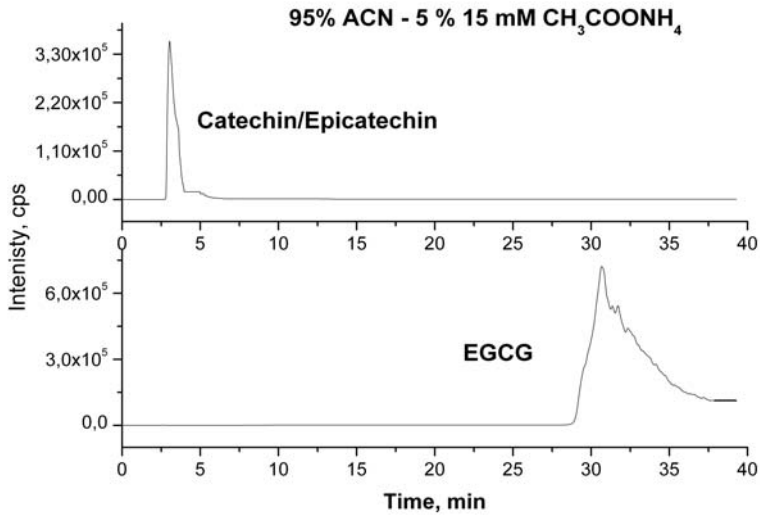


Figure 3. Temperature effects on the retention factors of catechin on different HILIC columns. Eluent: 95%ACN/H_2O.



a

Figure 4. (Continued).



b

Figure 4. Extracted ion chromatograms of catechins in the aqueous extract of Lipton Green Mint tea using different eluents. DIOL column (100x2.1 mm, 3.0 μm).

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REFERENCES

[1] B. Narotzki, A.Z. Reznick, D. Aizenbud, Y. Levy, Green tea: A promising natural product in oral health. *Arc. Oral Biol.* 57, 429 (2012).

[2] E.G. de Mejla, M.V. Ramirez-Mares, S. Puangpraphant, Bioactive components of tea: Cancer, inflammation and behaviour. *Brain Behav. Immun.* 23, 721 (2009).

[3] T. Karak, R.M. Bhagat, Trace elements in tea leaves, made tea and tea infusion: A review. *Food Res. Inter.* 43, 2234 (2010).

[4] N. Khan, H. Mukhar, Tea polyphenols for health promotion. *Life Science* 81,519 (2007).

[5] J.D. Lambert, R.J. Elias, The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Arch. Biochem. Biophys.* 501, 65 (2010).

[6] M.S. Al-Shawi, A. Hamza, S.O. Bahaffi, Al-Sibaai, T.N. Abduljabbar, Analysis of some selected catechins and caffeine in green tea by high performance liquid chromatography. *Food Chem*. 134, 2268 (2012).

[7] K. Pyrzynska, A. Sentkowska, Recent developments in the HPLC separation of phenolic food compounds. *Crit. Rev. Anal. Chem*. 45, 41(2015).

[8] Z. Spàčil, L. Novàkova, P. Solich, Comparison of positive and negative ion detection of tea catechins using tandem mass spectrometry and ultra high performance liquid chromatography. *Food Chem*. 123, 535 (2010).

[9] Y. Guo, S. Gaiki, Retention and selectivity of stationary phases for hydrophilic interaction liquid chromatography. *J. Chromatogr*. A 1218: 5920 (2010).

[10] J. Bernal, A.M. Ares, J. Pol, S.K. Wiedmer, Hydrophilic interaction liquid chromatography in food analysis, *J. Chromatogr*. A 1218, 7438 (2011).

[11] Y. Kawachi, T. Ikegami, H. Takubo, Y. Ikegami, M. Miyamoto, N. Tanaka, Chromatographic characterization of hydrophilic interaction liquid chromatography stationary phase: Hydrophilicity, charge effects, structural selectivity and separation efficiency. *J. Chromatogr*. A 1218, 5903 (2011).

[12] A.E. Karatapanis, Y.C. Fiamegos, C.D. Staikas, A revisit to the retention mechanism of hydrophilic interaction liquid chromatography using model organic compounds. *J. Chromatogr*. A 1218, 2871 (2011).

[13] Y. Hsieh, Potential of HILIC-MS in quantitative bioanalysis of drugs and drug metabolites. *J. Sep. Sci.* 31, 1481 (2008).

[14] P. Jandera, Stationary and mobile phases in hydrophilic interaction chromatography: a review. *Ana.l Chim. Acta* 692, 1 (2011).

[15] A. Sentkowska, M. Biesaga, K. Pyrzynska, Effects of the operation parameters on HILIC separation of flavonoids on zwitterionic column. *Talanta* 115, 284 (2013).

[16] T. Tetaz, S. Detzner, A. Friedlen, B. Molitor, J.L. Mary, Hydrophilic interaction chromatography of intact, soluble proteins. *J. Chromatogr*. A 1218, 5892 (2011).

[17] S. Noga, P. Jandera, B. Buszewski, Retention mechanism studies of selected amino acids and vitamin B6 on HILIC columns with evaporative light scattering detection. *Chromatographia* 76, 929 (2013).

[18] M. Biesaga, K. Pyrzynska, Liquid chromatography/tandem mass spectrometry studies of the phenolic compounds in honey. *J. Chromatogr.* A, 1216, 6620 (2009).

[19] Y. Guo , S. Gaiki , Retention of small polar compounds on polar stationary phases in hydrophilic interaction chromatography. *J. Chromatogr*. A 1074, 71 (2005).

[20] B. Buszewski, S. Noga, Hydrophilic interaction liquid chromatography (HILIC) - a powerful separation techniques. *Anal. Bioanal. Chem.* 402, 31 (2012).

[21] P. Jandera, T. Hajek, Utilization of dual retention mechanism on columns with bonded PEG and diol stationary phases for adjusting the separation selectivity of phenolic and flavones natural antioxidants; *J. Sep. Sci*. 32: 3903 (209).

[22] R.I. Chirita, C. West, S. Zubrzycki, A.L. Finaru, C. Elfakiri, Investigations on the chromatographic behavior of zwitterionic stationary phases used in hydrophilic interaction chromatography. *J. Chromatogr*. A 1288, 5939 (2011).

[23] M./ Naidi, J. Fiori, R. Gotti, A. Périat, J.L. Veuthey, G. Guillarne, V. Andrisanop, UHPLC determination of catechins for the quality control of green teas. *J. Pharm. Biomed. Anal*. 88, 307 (2014).

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