Review and Perspective on the Composition and Safety of Green Tea Extracts

Jeffrey B. Blumberg1*, Bradley W. Bolling2, C. Y. Oliver Chen1 and Hang Xiao3

1Friedman School of Nutrition Science and Policy, Tufts University, 711 Washington Street, Boston, MA 02111, USA.
2Department of Food Science, University of Wisconsin-Madison, 1605 Linden Drive, Madison, WI 53706, USA.
3Department of Food Science, University of Massachusetts Amherst, 338 Chenoweth Laboratory, 100 Holdsworth Way, Amherst, MA 01003, USA.

Authors’ contributions

This work was carried out equally in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2015/12712

ABSTRACT

The growing body of evidence regarding the putative health benefits of green tea (Camellia sinensis), including reduced risk of cancer and cardiovascular disease, has led to an increase in the consumption of brewed green tea and the formulation of green tea extracts (GTE) into a variety of food and beverage products and food supplements. The principal bioactive ingredients in green tea beverages and GTE are polyphenols, particularly the flavan-3-ols, which have been shown to act on antioxidant, anti-inflammatory, glucoregulatory, and cell signaling pathways. Some experimental evidence and case reports suggest the use of green tea and GTE is associated with the potential for inducing liver injury. The ability to extrapolate findings from in vitro and animal model studies is always limited and the available results on green tea- and GTE-induced liver injury in humans have presented clinical and regulatory challenges due to the difficulty of demonstrating a causal relationship between intake and harm. Attention to the risk for hepatotoxicity has largely been focused on GTE. Existing data are insufficient to identify the causative agent in the preparation or composition of GTE or its dose or duration of use as well as nutrigenetic, medical, and other factors that may contribute to the risk of hepatotoxicity. Responses by different government regulatory agencies regarding the safety of GTE are inconsistent with one another, including the dosage and derivation of its bioactives from aqueous versus hydro-alcoholic extracts. Restrictions on the production of GTE limit the application of innovative extraction...
1. INTRODUCTION

Based on archeological evidence, the consumption of tea appears to have first occurred about 5000 years ago during the Paleolithic period, predating the legend of its discovery by the mythical Chinese emperor, Shen Nung. By the fourth century, tea was an important part of Chinese life because of its perceived value as a medicine for the treatment of a variety of ailments [1]. Today, after water, tea is the widely consumed beverage in the world. Tea is made from leaves of Camellia sinensis (L.) O. Kuntze and is cultivated in more than 30 countries [2,3]. The usual amount of dry green tea leaves used to brew a cup (237 mL) is 1.8-2.5 g. The global average consumption of tea is about 0.5 kg per capita, though amounts (in kg per capita) are greater where tea drinking is common such as India (0.73), China (0.95), Japan (0.96), Ireland (1.90), United Kingdom (1.97), Turkey (2.04), and Libya (1.90) [4]. Tea is especially rich in flavan-3-ols (also referred to as catechins) but also serves as an important source of flavonols contributing over 90 and 40% of these flavonoids, respectively, to the American diet [5]. Data from the USDA Continuing Survey of Food Intake by Individuals reveals that at the 90th percentile of intake, American men and women consume 3.5 and 3.0 cups (or 830 and 711 mL) daily providing 454 and 393 mg of flavan-3-ols, respectively [6]. These data, together with reports of green tea drinkers in other countries suggest a range of intake of 500-2360 mL daily providing up to 931 mg of flavan-3-ols [7-12].

Numerous research studies suggest that regular consumption of tea is associated with an array of bioactivities, including antibacterial, antioxidant, anti-inflammatory, antiviral, glucoregulatory, and immune-stimulant actions, and putative health benefits, including a reduced risk of chronic conditions like arthritis, some forms of cancer, cardiovascular disease, dental caries, type 2 diabetes mellitus, dyslipidemia, and neurodegenerative diseases [2,13-17]. This diversity of actions appears largely related to the flavonoid constituents of tea via mechanisms partly overlapping with those of other dietary polyphenols, including the modulation of protein kinases, growth factors and transcription factors as well as the modification of intracellular signaling by activation of membrane receptors or molecular targets within cells [15,18,19]. These actions may result directly or indirectly from the antioxidant capacity of green tea in vivo to reduce oxidative stress [20-22] and associated DNA damage and lipid peroxidation [23-25].

Given the wide use of tea at 4,624,625 tonnes or 2.3 trillion cups per year, even modest benefits on health could have significant implications for the promotion of public health [4]. An effort to further broaden these benefits beyond drinking tea infusions has led to the extraction of green tea polyphenols for use in functional foods and food supplements. A growing body of research on dried green tea extracts (GTE) suggests similar benefits can be derived from the use of food products containing GTE [26]. Specifications for GTE apply to its constituent polyphenols (especially (-)-epigallocatechin gallate or EGCG) as well as caffeine and L-theanine, other major bioactive components. Green tea consumption has a long, safe history of use [27-30] and green tea preparations such as essential oils, solvent-free oleoresins, and natural extractives (including distillates) are deemed Generally Recognized As Safe (GRAS) [31] for use in foods. GTE are currently formulated into foods, beverages, candy and gums, chocolates, and dietary or food supplements as well as toothpaste. However, concerns have been expressed about the potential for harm of GTE preparations consumed at high doses in supplement form, particularly an idiosyncratic hepatotoxicity [32-38]. The objective of this review is to characterize the phytochemical profile of green tea and GTE, provide an updated description of the methods used to extract green tea constituents, and

**Keywords:** Green tea extract; flavan-3-ols; hepatotoxicity; cancer; cardiovascular disease; extraction; regulations.
discuss recent considerations on the potential for harm from consumption of GTE and products containing GTE.

2. COMPOSITION OF PHYTOCHEMICALS AND HEAVY METALS IN GREEN TEA LEAVES AND GREEN TEA

On a dry weight basis, the main constituents of tea leaves are polyphenols [39], particularly flavan-3-ols (Table 1). Other constituents of the tea leaf include fiber, carbohydrates (cellulose, pectin, glucose, fructose, sucrose), proteins (enzymes constituting an important fraction), amino acids (L-theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine), alkaloids (principal coffee and smaller amounts of theophylline and theobromine), minerals and trace elements, pigments (chlorophyll and carotenoids), volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons), lipids (linoleic and α-linolenic acids), sterols (stigmasterol), and vitamins (vitamins A, C, E, and K as well as several B vitamins)[40-43]. The composition of tea leaves varies widely, being substantially dependent on agricultural, botanical and genetic factors — such as climate, season, growing location, horticultural practice, cultivar, age of the leaves and the plant — and post-harvest factors like the conditions and duration of storage.

The flavan-3-ols in tea leaves, green tea infusions and extracts include EGCG, (-)epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (+)-catechin (C), and (+)-gallocatechin (GC) [39] (Table 2). The chemical structures of these compounds are presented in (Fig. 1). Other flavonoids and phenolic acids are present at <1% of dry weight. The major flavonols in tea are glucose or rhamnose conjugates of quercetin and kaempferol [44]. Phenolic acids in tea leaves include quinic acid esters of gallic acid and caffeic acid [44]. The polyphenols are not evenly distributed in the tea plant with the leaf bud and first leaves generally containing the highest concentrations [45]. Flavan-3-ols are concentrated in mesophyll cells of the leaf structure in close proximity to epidermal cells [46].

EGCG is the most abundant flavan-3-ol in tea leaves and green tea infusions, but with its concentration varying about 8-fold between teas and tea preparations [47-54] (Table 2). Though showing less variation in concentration, the next most abundant tea flavan-3-ols are EGC and ECG. In green tea infusions, only EGCG and EC are present consistently. While the content of EGC and ECG in green tea is lower than EGCG, their values in the infusion appear disproportionally larger. The range of caffeine concentrations in green tea infusions is comparable to that of EGCG, suggesting that flavanols and alkaloids leach out the tea matrix in different degrees during steeping or brewing. These variations can be attributed not only to intrinsic factors but also to the methods employed in extraction of flavonoids from tea leaves and green tea for quantification.

Tea trees accumulate significant amounts of trace metals in their leaves [55]. While some of these minerals are essential for human health, some might be associated with toxicity when consumed in large amounts or for long durations if the leaves are consumed directly or dissolved in the infusion. Among the metals and elements listed in the (Table 3) [55], aluminum, arsenic, cadmium, fluoride, and lead can induce acute or chronic toxicity if sufficient exposure is achieved. However, while the amount of these metals in fresh tea leaves and green tea can exceed recommended safe levels for these elements, they are not fully extracted by aqueous or alcoholic solvent, and are present in much lower amounts in infusions [55]. The level of arsenic in green tea infusions is 1000-fold larger than the level generally found in U.S. drinking water (2.0 µg/L, although levels of 0.138 to 1,700 µg/L have been measured in surface water in the U.S.) [56]. As the minimal risk level (MRL) for acute-duration oral exposure (≤14 d) to inorganic arsenic is 5 µg/kg bw/d, daily consumption of 230 mL green tea infusion containing 1.53 µg/L arsenic (the highest identified arsenic content [55]) for 14 d would not attain the MRL for a 70 kg adult. The U.S. Environmental Protection Agency (EPA) requires water suppliers to limit the cadmium concentration in water to <5 µg/L, which is 7- to 47-fold larger than cadmium concentrations reported in green tea infusions. The content of lead in green tea infusions is comparable to levels in surface water and groundwater in the US at 0.005 and 0.03 µg/mL, respectively [57]. Most drinking water supplies in the U.S. contain <5 µg/L of chromium [58]. Green tea infusions may become a significant source of chromium among those who drink a substantial volume daily; the Adequate Intake for chromium established by the Institute of Medicine is 25 and 35 µg/d for women and men, respectively. The
The majority of chromium in tea is the less-toxic trivalent form, whereas water-soluble hexavalent chromium ranges from 0-10% of total chromium from green tea leaves, depending on its origin; the highest amount of hexavalent chromium found in a cup of green is ~70-fold lower than the maximum acceptable concentration [59]. Importantly, the level of metals and elements in GTE can be readily identified and controlled by parameters in its specification.

Table 1. Approximate composition of tea leaves

| Constituents             | % dry weight |
|--------------------------|--------------|
| Polyphenols              | 30           |
| Fiber                    | 26           |
| Protein                  | 15-20        |
| Carbohydrate             | 5-7          |
| Minerals                 | 4-9          |
| Alkaloids (caffeine)     | 2-4          |
| Amino acids              | 1-4          |
| Pigments                 | Trace        |
| Lipids and sterols       | Trace        |
| Volatile compounds       | Trace        |
| Vitamin                  | Trace        |

Table 2. Range of flavonoids and alkaloid contents in tea leaves, green tea, and green tea infusion

| Constituents                      | Fresh tea leaves | Green tea leaves | Green tea in infusions |
|-----------------------------------|------------------|------------------|------------------------|
|                                   | mg/g dry weight  | mg/L             |                        |
| Flavonoids                         |                  |                  |                        |
| (-)-EGCG                           | 68 - 134         | 13 - 113         | 95 - 712               |
| (-)-EGC                            | 22 - 53          | ND - 45          | ND - 471               |
| (-)-EC                             | 5.9 - 18         | 5.7 - 51         | ND - 1508              |
| (+)-GCG                            | 1.9 - 11         | 1.9 - 21.1       | 25 - 216               |
| (+)-CG                             | ND               | ND - 3.7         | ND - 9.3               |
| (+)-C                              | ND               | ND - 1.2         | ND - 688               |
| EGCMG                              | NR               | 0.3 - 1.2        | NR                     |
| Quercetin-rhamnoside-galactoside   | trace - 1.0      | NR               | NR                     |
| Quercetin-rhamnoside-glucoside     | 0.5 - 1.9        | NR               | NR                     |
| Rutin                              | 0.6 - 2.6        | NR               | NR                     |
| Kaempferol                         | NR               | NR               | 3.6 - 6.4              |
| Myricetin                          | NR               | NR               | 4.8 - 7.3              |
| Quercetin                          | NR               | NR               | 13.4 - 20.7            |
| Thearubigins                       | NR               | NR               | ND - 221               |
| Alkaloids                          |                  |                  |                        |
| Caffeine                           | 16 - 28          | 26 - 39          | 99 - 338               |
| Theobromine                        | 0.2 - 1.6        | NR               | 7.6 - 86               |

1Chaturvedula and Prakash [39]

Table 2. Range of flavonoids and alkaloid contents in tea leaves, green tea, and green tea infusion

| Constituents                      | Fresh tea leaves | Green tea leaves | Green tea in infusions |
|-----------------------------------|------------------|------------------|------------------------|
|                                   | mg/g dry weight  | mg/L             |                        |
|                                    |                  |                  |                        |
| Flavonoids                         |                  |                  |                        |
| (-)-EGCG                           | 68 - 134         | 13 - 113         | 95 - 712               |
| (-)-EGC                            | 22 - 53          | ND - 45          | ND - 471               |
| (-)-EC                             | 5.9 - 18         | 5.7 - 51         | ND - 1508              |
| (+)-GCG                            | 1.9 - 11         | 1.9 - 21.1       | 25 - 216               |
| (+)-CG                             | ND               | ND - 3.7         | ND - 9.3               |
| (+)-C                              | ND               | ND - 1.2         | ND - 688               |
| EGCMG                              | NR               | 0.3 - 1.2        | NR                     |
| Quercetin-rhamnoside-galactoside   | trace - 1.0      | NR               | NR                     |
| Quercetin-rhamnoside-glucoside     | 0.5 - 1.9        | NR               | NR                     |
| Rutin                              | 0.6 - 2.6        | NR               | NR                     |
| Kaempferol                         | NR               | NR               | 3.6 - 6.4              |
| Myricetin                          | NR               | NR               | 4.8 - 7.3              |
| Quercetin                          | NR               | NR               | 13.4 - 20.7            |
| Thearubigins                       | NR               | NR               | ND - 221               |
| Alkaloids                          |                  |                  |                        |
| Caffeine                           | 16 - 28          | 26 - 39          | 99 - 338               |
| Theobromine                        | 0.2 - 1.6        | NR               | 7.6 - 86               |

1Abbreviations: C, catechin; CG, catechin gallate; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; EGCMG, epigallocatechin 3-O-methylgallate; GCG, gallocatechin gallate; ND, not detected; NR, not reported

2Compiled from Bhagwat et al. [47]; Cabrera et al. [48]; Friedman et al. [49]; Komes et al. [50]; Nishitani and Sagesaka [51]; Peterson et al. [52]; Wang et al. [53]; Xu et al. [54]

3Includes data from loose tea and tea bags

4Tea infusions made by brewing 2 g tea in 200 mL at 80°C for 3 min
Table 3. Range of trace metal and elemental content in tea leaves, green tea, and green tea infusion

| Trace metals | Fresh tea leaves | Green tea | Green tea infusion |
|--------------|------------------|-----------|-------------------|
|              | µg/g dry weight  | µg/mL    | µg/mL             |
| Aluminum     | 13 - 11,981 (177) | 211 - 4074 (41) | 0.7 - 6 (24) |
| Arsenic      | 0.021 - 0.073 (13) | trace - 1.66 (536) | 0.02×10⁻³ - 1.53×10⁻³ (17) |
| Cadmium      | ND³ - 1.27 (63)   | 0.013 - 0.114 (141) | 0.04×10⁻³ - 0.24×10⁻³ (17) |
| Chromium     | trace - 3.41 (103) | 0.45 - 0.99 (2) | ND - 6.91×10⁻³ (17) |
| Copper       | 9.4 - 127 (59)    | 4.7 - 36.5 (169) | 0.033 - 0.191 (50) |
| Fluoride     | 49 - 808 (33)     | 49 - 344 (14)   | 0.59 - 2.52 (20) |
| Lead         | ND - 14.5 (181)   | 0.11 - 1.93 (141) | 0.004 - 0.032 (17) |
| Manganese    | ND - 3154 (56)    | 211 - 2081 (33) | 0.52 - 10.09 (54) |
| Nickel       | 6.6 - 14 (43)     | ND - 9.35 (28)  | 0.04 - 0.269 (21) |

³Summarized from Karak and Bhagat [55]; The range of trace metal and other element contents in green tea infusion is due partly to the different brewing methods, including tea:water ratio, water temperature, and brewing duration; Values in parentheses are the number of samples tested; ND, not detected.

3. ADVANCED EXTRACTION TECHNOLOGIES FOR PLANTS/BOTANICALS

To obtain natural products from plant materials, effective extraction techniques are required to remove unwanted constituents and concentrate bioactive compounds. Traditional techniques, such as Soxhlet extraction, have been used for many decades, while novel techniques have been developed more recently to improve extraction efficiency and the cost-effectiveness of the process. It is worthwhile noting that pretreatment of the raw plant material, e.g., breaking, crushing, and milling, can significantly affect the kinetics of solvent extraction [60]. Extraction efficiency is related to the solvent to leaf ratio and depends substantially on polarity of the constituent compounds and the type of solvent. For example, acetone, ethyl acetate, acetonitrile, isopropanol, ethanol, methanol, and...
water are preferred for extraction of the more amphipathic compounds, such as polyphenols and their glycosides and hexane, petroleum ether, benzene, and chloroform are utilized to maximize extraction of lipophilic constituents, such as fats and essential oils [61]. Extraction solvents can be chosen based on solvent selectivity, capacity, reactivity, stability, solubility, regeneration capability, and toxicity [62]. Several additional novel extraction technologies have emerged that may be useful for the extraction of green tea constituents. For example, ionic liquids have been used for extraction of the stilbene resveratrol from the dried rhizome of Polygonum cuspidatum [63]. A surfactant and micelle approach has been used to extract flavonoids from licorice root [64]. Supercritical CO₂ or dimethyl ether with solvent modifiers can be used to decaffeinate tea and extract flavan-3-ols [65]. This section provides a brief overview of novel extraction technologies suitable for consideration of plant polyphenols.

3.1 Accelerated Solvent Extraction (ASE)

ASE is also known as pressurized solvent extraction, subcritical solvent extraction or pressurized liquid extraction [66]. ASE is an automated process and widely used in the extraction of functional components from botanicals and plants [67-71]. The high temperature and pressure applied in this protocol not only facilitate extraction efficiency but also reduce solvent use and extraction time by increasing the solubility of target compounds and the rate of solvent diffusion into the plant matrix as well as decreasing solvent viscosity and surface tension [69,72]. Extraction efficiency for thermally labile components can decrease when temperatures are set too high, resulting in a lower quality extract [71]. Since ASE is a relatively fast process, prolonged extraction time does not raise the yield [70-74]. Importantly, ASE often allows for the use of environmentally safe solvents, such as water and aqueous ethanol to achieve more exhaustive extractions of plant materials relative to use of these same solvents under normal extraction conditions. In general, smaller particle sizes of the plant material combined with larger volumes of solvent can increase the amount of functional components dissolved, resulting in greater extraction efficiency [73,75,76].

3.2 Supercritical Fluid Extraction (SFE)

Supercritical fluid is a phase between gas and liquid, and its temperature and pressure are above the critical point. Its density is similar to liquid but its viscosity is similar to gas, resulting in a higher diffusion coefficient than liquid. Due to its high dissolving capacity for many compounds, including phytochemicals, supercritical fluid can be used as an efficient extraction solvent. The principal SFE solvent is CO₂ due to its relatively low critical conditions (30.9°C and 73.8 bar), profile of safe use, and relatively low cost. Thus, SFE with CO₂ presents a good method for the extraction of thermally labile phytochemicals [77,78]. Supercritical CO₂ has a very high diffusion coefficient, leading to a much faster extraction rate than conventional solvent extraction [79]. Due to its non-polarity, CO₂ may not be an efficient solvent for extraction of polar bioactives such as phenolic compounds. However, the efficiency of SFE for polar compounds is significantly increased when combined with organic solvent modifiers like ethanol [80,81]. While extracted phytochemicals can be easily recovered by decreasing the pressure of the supercritical CO₂ [78], the high cost and sophisticated operation conditions limit the broad utilization of SFE. However, it is noteworthy that SFE is the method of choice for the commercial production of quality decaffeinated tea.

3.3 Ultrasound-Assisted Extraction (UAE)

Ultrasound waves can change the physical and chemical properties of plant material and induce cavitations causing an increase in pressure and temperature close to the plant surface, disrupting cell walls and enhancing the release of intracellular compounds into the extraction solvent [82,83]. UAE can also cause swelling of the plant material that can facilitate the extraction of phytochemicals. UAE is a simple and cost-effective method which has been shown to improve the extraction of polyphenols, though extraction efficiency is dependent on the nature of the plant matrix [66,72]. UAE is also suitable for thermally labile compounds because satisfactory extraction can be achieved at relatively low temperature [72].

3.4 Enzyme-Assisted Extraction (EAE)

The principle of EAE is use of enzymes, such as cellulases, pectinases and hemicellulase, to break the plant cell wall to facilitate the extraction of targeted constituents from plantmatrices [84]. EAE has been used for extraction of proteins, phenolics, oils, and carotenoids [85-89]. Although EAE may offer better extraction efficiency in
comparison with conventional extraction methods, this method extends considerably the time for completion of conventional extraction protocols and may not be better than other novel techniques using pre-extraction treatment, e.g., UAE [89,90]. During EAE, specific enzymes are needed for different raw materials, which require the information on the exact composition of the cell walls. In one study, UAE pretreatment led to a better and faster extraction of phenolic compounds from acerola than an EAE-added protocol [90]. Additional factors that limit the application of EAE to green tea include the relatively high cost of enzymes and environmental sensitivity regarding the use of these enzymes.

3.5 Pulsed Electric Field (PEF) Assisted Extraction

PEF assisted extraction uses an electrical field to induce electroporation of cell membranes which results in an increased permeability of extraction solvent into the plant matrix and extraction efficiency [66,91,92]. PEF treatment improves the extraction of phytochemicals, including polyphenols like anthocyanins and tannins [91]. Compared to other pre-treatments such as UAE, PEF assisted extraction requires less time and lower energy costs[93]. PEF has been used as a pre-treatment before pressing to enhance the yield and quality (turbidity and odor intensity) of juices from different fruits and vegetables [94,95]. PEF may yield comparable or better extraction efficiency than UAE and requires less time (seconds vs. minutes) and energy than UAE [91,96].

3.6 Microwave Assisted Extraction (MAE)

As electromagnetic radiation, microwaves can penetrate plant materials and interact with polar molecules such as water to generate heat [72]. The heat generated internally in the plant materials disrupts cell structure and facilitates the dissolution of phytochemicals from the plant matrices [95]. Moreover, homogenously increased temperature of extraction solvent and plant materials by MAE generally leads to enhanced extraction efficacy. MAE also reduces extraction time in comparison to conventional extraction methods [97-99]. However, in certain cases, unwanted products can be generated during MAE, especially when the temperature is too high [100]. The major advantage of MAE is that it offers significant reductions in extraction time and solvent usage with a similar or even better extraction efficacy in comparison to the conventional extraction methods [66].

4. TECHNOLOGIES FOR GREEN TEA EXTRACTION

On a commercial scale, extractions of green tea for beverages or food supplements are typically conducted using aqueous, ethanolic or supercritical fluid solvents with either batch or continuous processes [101]. A variety of laboratory-scale methods for GTE production have been reported and demonstrate that various solvents, temperatures, and other process variables contribute to a wide range of constituents in the final product. Market studies of commercial GTE have reported a marked variation in the content of EGCG (11.9-fold), polyphenols (2.6-fold), and caffeine (6.5-fold) [102-104] (Table 4). It is noteworthy that the variation in the phytochemical content of GTE in these studies is equivalent or less than that reported in surveys of commercial green teas used for infusions.

4.1 Continuous or Batch Aqueous Extraction

A single aqueous batch extraction for GTE most closely mimics the typical home preparation of green tea. Phytochemical yields have been reported for both batch and continuous extraction processes, including reverse flow continuous extraction. For batch processes, time, temperature, and tea:water ratios have been manipulated in laboratory studies. Total flavan-3-ol content plateaus from 30 to 120 min during aqueous batch extractions of green tea [50,105]. Vuong et al. [105] reported that EC content plateaued at 30 min, but catechin content continued to increase to 120 min in a batch aqueous process. Similarly, L-theanine aqueous batch extraction was maximized at 80°C for 30 min [106]. Aqueous shaking extraction of flavan-3-ols from dry green tea leaves at 25°C peaked at 2 h, but had only minor differences from 0.5 to 24 h with a maximum 8% difference between 2 and 6 h [107]. Reflux extraction of green tea leaves beyond 30 min up to 3 h at 80°C did not yield significantly more flavan-3-ols or caffeine [107]. Longer aqueous extraction resulted in lower caffeine:flavan-3-ol ratios [108].
4.2 Extraction Temperature

Extraction temperature plays an important role in the yields of flavan-3-ols from green tea with efficiency varying dependent on the individual compound [113]. Perva-Uzunalić [110] obtained maximum aqueous extraction efficiency of flavan-3-ols after 20 min at 80°C and 10 min at 95°C, suggesting an interactive effect of temperature and time on extraction efficiency. Response surface methodology, which is generally employed to generate an optimal response by conducting a sequence of designated experiments, has been used characterize the yield of bioactive components of GTE. Zhang et al. [114] reported that steeping 1 g tea powder in 16 mL water for 40 min at 96°C extracted the most EGCG, theanine, and total phenols. A potential degradation of flavan-3-ols may occur when a protocol includes both high temperature and extended extraction. For example, aqueous extraction of green tea for 7 h at 98°C reduced the flavan-3-ol content of GTE by 20% [115]. Increasing temperature from 80 to 95°C augmented epimerization of EGCG to (-) gallocatechin gallate (GCG) by 52% from 0.25 g/100 g green tea leaves [115]. The caffeine content of aqueous extracts reaches a plateau at 80°C from 20-40 min extraction, and then decreases slightly at 90°C [113]. Thus, temperature employed during extraction not only affects extraction efficiency of flavan-3-ols but can also contribute to an altered flavonoid profile through epimerization.
4.3 Extraction Solvent

Laboratory-scale preparations of GTE have utilized a variety of solvents with various effects on extract yields [107,110,116] (Table 5). Friedman et al. [104] compared an 80% ethanol extraction to an aqueous extraction of 24 green teas and found that aqueous alcohol extraction yielded 36% more flavan-3-ols, 12% more caffeine, ~50% more theobromine, and 30-200% more theophylline than water alone. As shown in (Table 5), the percentage of ethanol in the extraction solvent is crucial to the yield of flavan-3-ols, but not necessarily caffeine, with 40% ethanol in water being the best as compared to all other aqueous ethanol solvents and methanol, acetonitrile, and acetone [107,110,116]. Similarly, Rusak et al. [117] noted the yield of flavan-3-ols from loose green tea extracted for 30 min was highest with 40% ethanol compared to 10 and 70% ethanol, and water. The 40% ethanol extract recovered 18% EGC, 74% ECG, 40% GCG, and 97% ECG more than water extraction. Thus, aqueous ethanol provides a larger ratio of flavan-3-ols to caffeine than other extraction solvents.

In contrast, the flavonol and proanthocyanin yield from green tea leaves appear only marginally affected by ethanol or aqueous ethanol extraction [110]. However, acetonitrile and acetone extraction provides 33 to 83% flavonols and proanthocyanidins compared to water extraction. Recovery of caffeine and theobromine was greatest with 70 and 15% methanol in water, respectively, following three 10-min sequential extractions of green tea leaves [118]. Theophylline was only recovered in the 70% methanol extract, but not in methanol alone, water, acetonitrile or acetone [118].

Less information is available about how solvents affect other green tea constituents. Mossion et al. [119] reported that mineral content of water decreased extraction of aluminum, total organic carbon, and total polyphenols. Kanrar et al. [120] demonstrated an ethyl acetate-cyclohexane mixture (9:1, v/v) was effective at extracting pesticide residues from green tea. Pollution associated with industrialization near tea cultivation areas can increase the presence of heavy metals, particularly lead, in raw tea leaves. Different solvent extractions vary in their capacity for extracting lead from tea plants [121,122]. Sullivan et al. [123] have noted that while hydroethanolic solvents are not completely efficient in the extraction of heavy metal accumulations, they can be an effective decontamination step in herbal product processing.

4.4 pH

The pH of aqueous extraction solvents varies from ~5.5 to 7.2 based on temperature and the quantity of green tea leaves utilized for extraction [124]. Yoshida et al. [124] reported that the aqueous pH was nearly constant at ~6.5 when the ratio of green tea leaves to water was between 0.5 and 3%. Stability of tea flavan-3-ols decreases with increasing pH above pH 3, at which they are quite stable [107]. Thus, the pH of extraction solvent strongly determines extraction efficiency of these flavonoids from green tea leaves. The effect of pH varies during extraction, with a pH of 4.5 providing a maximum recovery of gallic acid, EGC, and ECG, whereas ECG and EC are stable up to pH 6.5 [102]. Extraction at pH 7 results in greater proportions of EC and ECG to other flavan-3-ols [102]. GTE extraction held at 80°C for 20 min at pH 8, led to twice the epimerization of EGC to GC compared to pH 6 [124]. The mineral content of GTE is also affected by the acidity of extraction solvents. Water extraction with 0.12 µmol/L HCl provides a yield with 44% aluminum, 19% calcium, and 44% magnesium of the original amount in the green tea leaves [112].

**Table 5. Effect of extraction solvent on green tea extract phytochemical yield, as fold-difference of water extract**

| Phytochemical     | Aqueous ethanol | Methanol | Acetonitrile | Acetone |
|-------------------|-----------------|----------|--------------|---------|
|                   | 20% 40% 50% 80%|          |              |         |
| EGCG              | 2.76 3.32 3.26 1.92 | 1.3-3.2 | -            | -       |
| Catechins         | 1.46 1.61 1.46 0.79 - 1.15 | 1.17 | 1.24 | 0.63 |
| Flavonols         | - - 0.91 1.02 | 0.97 | 0.39 | 0.33 |
| Proanthocyanidins | - - 0.97 0.96 | 0.91 | 0.83 | 0.83 |
| Caffeine          | 1.03 1.03 0.97 | - | - | - |

*Compiled results from Perva-Uzunalić et al. [110]; Meterc et al. [116]; Choung and Lee [107].

*not determined*
4.5 Other Extraction Technologies

Other extraction technologies for preparing GTE may modulate the yield of flavan-3-ols. In a semi-continuous SFE using CO₂, Sosa et al. [125] tested whether encapsulation with a poly-ε-caprolactone, a biodegradable particle, applied during the solvent removal step would affect the constituent profile of the final GTE. They found that total phenols varied 60-100% of the maximum theoretical composition, but caffeine content was reduced to 13%. Ultrasonic extraction of green tea leaves for 1 h had 5% less flavan-3-ols, mainly as EGC and ECG, and the same caffeine content relative to a 2 h aqueous extraction [107]. Conventional hot-water steeping of green tea yielded 55% magnesium, 260% calcium, and a similar percentage of aluminum compared to focused microwave oven heating [112]. Ionic liquids have also been used to extract caffeine and theophylline from commercial tea drinks [126].

4.6 Procedures for Solvent Removal

GTE is dried prior to distribution. Spray drying, drum drying, or lyophilization can be used to process GTE. Excessive heat during a high pressure spray drying process of 145°C pre-expansion temperature and 79°C resulted in 88% less polyphenols relative to optimized conditions at ≤130°C [116]. Temperatures during spray drying between 33 and 65°C did not affect the amount of total polyphenols but the residual water content increased with lower temperatures, a condition which is unacceptable for commercial applications.

5. POSTPRODUCTION HANDLING OF GREEN TEA

5.1 Grinding

The size of the dry tea leaf particles also affects the yield of extracted compounds as smaller particle sizes increase surface area available for extraction. Aqueous extraction of green tea leaves with a particle size ≤1 mm have ~10% greater yields of flavan-3-ols [105]. Gains in fluoride yields with smaller particle sizes relative to flavonoids maybe exploited to limit fluoride in GTE [111]. Caffeine and total flavonoids, but not EGCG, are dependent on particle size for supercritical CO₂ decaffeination [65].

5.2 Storage

The duration that green tea leaves are stored before use may affect GTE composition. Friedman et al. [104] found that after 6 mo storage at 20°C, EGCG concentration in green tea leaves decreased by 28% and total flavan-3-ols decreased by 32%. Storing tea leaves under vacuum or nitrogen and/or low humidity and temperature can minimize flavonoid oxidation.

5.3 Decaffeination

Green tea may be decaffeinated prior to extraction. At optimal conditions for supercritical CO₂ decaffeination of ground green tea leaves, 99.6% caffeine, 40.5% flavan-3-ols, and 43.1% chlorophyll are extracted using ethanol as a co-solvent [127]. Ethanol is superior to water in this regard, and an 50% aqueous ethanol modifier for supercritical CO₂ decaffeination resulted in 70% caffeine and 6% flavan-3-ols being removed from green tea powder [65]. Perva-Uzunalic et al. [110] noted that supercritical CO₂ reduces flavan-3-ols by 17% and flavonols by 10% from the starting material. Up to 43% of chlorophylls are removed after 120 min of supercritical CO₂ decaffeination with an ethanol co-solvent [128]. While the loss of polyphenols can be minimized during the decaffeination process, commercial decaffeinated green teas generally have a lower content of flavan-3-ols than non-decaffeinated green tea [102].

6. POTENTIAL HEALTH BENEFITS OF GREEN TEA INFUSIONS AND EXTRACTS

Before discussing the reported potential for adverse effects of GTE, it is worth briefly noting the potential health benefits associated with green tea and GTE. A wealth of basic research as well as evidence from observational studies and randomized clinical trials indicate the consumption of green tea infusions or GTE to be associated with an improvement in intermediary biomarkers of chronic disease and/or a reduced risk of some forms of cancer, cardiovascular disease, type 2 diabetes mellitus, neurodegenerative conditions like Alzheimer’s disease and Parkinson’s disease, and other age-associated diseases. These putative benefits appear due principally to the array of bioactivity of the flavonoid constituents of green tea, particularly the flavan-3-ols, including antioxidation, anti-inflammation, glucose regulation, anti-proliferation, energy regulation, and modulation of signal transduction pathways [16,129-135]. A brief summary of selected systematic reviews and meta-analyses of the association between green tea and health outcomes are presented in (Tables 6 and 7).
addition, green tea infusions and GTE have a modest effect on weight loss and/or weight management, though this benefit appears due principally to an interaction between the constituent flavonoids and caffeine. Randomized clinical trials indicate green tea flavonoids have a positive impact on blood glucose, suggesting a potential benefit to people with impaired glucoregulation and an increased risk of type 2 diabetes. Evidence from clinical studies also indicates a modest effect of green tea in decreasing serum total cholesterol and LDL-cholesterol [155]. Data from observational studies showing green tea is associated with a reduced risk of stroke is consistent with results from clinical trials. While rodent model studies reveal a marked chemopreventive effect of green tea on several forms of cancer, results from observational studies and from clinical trials examining intermediary biomarkers are mixed and dependent on cancer type, sex, and study design.

7. SAFETY PROFILE OF GREEN TEA EXTRACTS

Green tea consumption has a long, safe history of use as a beverage and green tea preparations are deemed GRAS for use in foods; however, concerns about the potential for hepatic toxicity of GTE prepared by aqueous solvent extractions, particularly hydro-alcoholic extracts, have been raised. As discussed above, the polyphenol profile of alcohol extractions of green tea leaves appears to affect overall GTE composition, and the extraction solvent causes a wider effect on more than processing steps or raw materials. Based on laboratory analysis of 17 to 54 samples of quality grade commercial green tea infusions (Table 3), aqueous and hydro-alcoholic extracts of green tea are expected meet guidelines for the suggested safe levels of metals in herbsals, food ingredients, and dietary supplements (Table 8). For example, the aluminum content reported in green tea infusions (0.7 - 6 mg/mL) is ~33-fold less than CODEX standards. Depending on the location that a tea is grown, the content of arsenic, cadmium, copper, and lead in dry tea may exceed recommendations for exposure to these elements, but are not fully extracted by aqueous or alcoholic extraction, and are present in much lower amounts in infusions [55]. Reports on the heavy metal content of commercial or laboratory-prepared GTE are lacking in the literature, however, commercial products are commonly controlled for contaminants inclusive of heavy meals through established quality specifications in accordance with applicable current Good Manufacturing Practices (cGMP). Upon drying, residual solvents are not expected to be at concentrations above toxic threshold due to the drying process (Table 9). While tea leaves and infusions are unlikely to have residue solvents as their processing typically does not include water or organic solvents, data from these sources and GTE are not readily available from literature. While decaffeinating tea with methylene chloride may leave residual traces, it is excluded from import into the United States and some other countries. In contrast, ethanol is regarded as a solvent with low toxic potential. There is no evidence that hydro-alcoholic processes extract significantly different proportions of bioactive or toxic compounds from green tea that would lead to toxicity. However, components of GTE other than polyphenols and caffeine are rarely reported in animal experiments or case studies of toxicity, so data on these constituents of GTE are quite limited.

Green tea flavonoids consumed in a phospholipid complex are better absorbed than in a free form but do not appear to be associated with toxicity [160]. Nanoparticles and other technologies for enhancing the bioavailability and/or distribution of tea flavonoids have been tested in animal models without evidence of harm but remain untested in humans. In humans, green tea flavan-3-ols appear similarly bioavailable from infusions and GTE. Importantly, foods co-consumed with tea may impair maximum plasma flavonoid status and the time required to achieve maximal concentrations [161]; such actions would likely reduce any potential toxicity associated with tea polyphenols [162]. Indeed, consumption of GTE in a fed state resulted in lower and less variable toxicity than found under fasted conditions in dogs fed GTE at 50 to 1000 mg/kg body weight (bw)/d [163,164]. Further, clinical toxicokinetic studies by Ullmann et al. [165,166] confirmed that GTE under fasting conditions or following a single bolus led to more marked increases in bioavailability and plasma concentrations of EGCG than administration with split doses or with food. Rarely, moderate consumption of aqueous green tea infusions, aqueous GTE, and hydro-ethanolic GTE have been associated with liver injury in humans but confounding factors and effect modifiers make it impossible to assign a direct causal relationship to individual or combined tea flavonoids or related polyphenols [167]. Evidence regarding toxicity from tea and tea polyphenols is described below.
Table 6. Meta-analyses and systematic reviews of randomized clinical trials on the health benefits of green tea or green tea catechins in anthropometry, glucoregulation, lipid profile, blood pressure, and coronary heart disease risk

| Authors                | Number of study (n) | Subject (n) | Duration | Dosage in RCT | Objectives | Findings |
|------------------------|--------------------|-------------|----------|---------------|------------|----------|
| **Anthropometry**      |                    |             |          |               |            |          |
| Jurgens et al. [132]   | RCT (14)           | 1562        | 12-13 wk | 140.85-1206.9 mg GTC/d | BW in overweight or obese adults | BW: ↔ outside Japan; ↓ in Japan. WC: ↔ |
| Hursel et al. [136]    | RCT (11)           | 1188        | 12-13 wk | 270-1207 mg GTC/d | BW         | BW: ↓ Effect modification by caffeine and ethnicity |
| Phung et al. [137]     | RCT (15)           | 1243        | 8-24 wk  | 141-1207 mg GTC/d | BMI, BW, WC, WHR | GTC with caffeine BMI: ↓ BW: ↓ WC: ↓ WHR: ↔ |
| **Glucoregulation**    |                    |             |          |               |            |          |
| Zheng et al. [138]     | RCT (22)           | 1548        | 3-24 wk  | 236-1207 mg GTC/d | Glucoregulation | FG: ↓ Fl: ↔ HbA1C: ↔ HOMA: ↔ |
| Liu et al. [139]       | RCT (17)           | 1133        | 4-14 wk  | 208-1207 mg GTC/d | Glucoregulation | FG: ↓ Fl: ↓ HbA1C: ↓ |
| **Lipid profile**      |                    |             |          |               |            |          |
| Kim et al. [133]       | RCT (20)           | 1415        | 3-24 wk  | 145-3000 mg GTC/d | Lipid profile | TC: ↓ LDL-C: ↓ HDL-C: ↔ TG: ↔ |
| Zheng et al. [140]     | RCT (14)           | 1136        | 3-24 wk  | 150-2500 GTC mg/d | Lipid profile | TC: ↓ LDL-C: ↓ HDL-C: ↔ TG: ↔ |
| **Coronary heart disease** |                |             |          |               |            |          |
| Hooper et al. [141]    | RCT (170)          | 6557 (515 green tea) | 0-52 wk (0-12 wk for green tea) | Not available | CHD risk | LDL-C: ↓ BP: ↔ |
| Taubert et al. [142]   | RCT (10)           | 516 (343 green tea) | 2 mo     | 1 tea bag/d, 544 mg phenols | BP | BP: ↔ |

Abbreviations: BMI, body mass index; BP, blood pressure; BW, body weight; CHD, coronary heart disease; FG, fasting blood glucose; Fl, fasting insulin; GTC, green tea catechins; HbA1C, glycated hemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low density lipoprotein cholesterol; RCT, randomized clinical trial; TC, total cholesterol; TG, triglyceride; WC, waist circumference; WHR, waist and hip ratio; ↓, decrease; ↔, no change

7.1 Evidence from Cell Studies

Primary hepatocytes from adult male Wistar rats were used to investigate the potential toxicity of hydro-ethanolic GTE constituents [168]. Hepatocellular necrosis was observed at doses of 1 to 3 mg GTE/mL medium. However, individual flavan-3-ols, caffeine, and theanine were not cytotoxic up to 3 mg/mL medium except for the observation that EGCG reduced cell viability at the 1 mg/mL dose only. The lipidsoluble fraction did not appear to account for increased cytotoxicity. With an LD₅₀ of 200 μM, Galati et al. [169] reported that EGCG was the
most potent of green tea phenolics in inducing mitochondrial membrane potential collapse in isolated rat hepatocytes. In contrast, Nishikawa et al. [170] found a benefit of EGCG at 50-100 μg/mL in stimulating apoptosis of human hepatocellular carcinoma cell lines. However, the direct relevance and ability to extrapolate these findings is limited as only sub-micromolar concentrations are observed in vivo following purified flavan-3-ol and flavonol administration to rodents and humans [171].

Table 7. Meta-analyses and systematic reviews of observational studies on the health benefits of green tea or green tea catechins in coronary heart disease, stroke, and cancer risk

| Authors          | Number of study (n) | Subject (n) | Duration | Objectives  | Findings          |
|------------------|---------------------|-------------|----------|-------------|-------------------|
| **Coronary heart disease** |                     |             |          |             |                   |
| Wang et al. [143] | Cohort (12), Case-control (6) [5 green tea] | 416,676 (13,978 cases) | 5-20 y follow up | CAD (CHD and MI) | RR=0.72 (0.58-0.89) 1 cup/d: ↓ 10% CAD risk |
| **Stroke** |                     |             |          |             |                   |
| Arab et al. [144] | Cohort (8), Case-control (2), Cross-sectional (1) | 213,897 (5,537 cases) | 4-15 y | Total stroke | RR=0.79 (0.73-0.85, ≥3 vs.<1 cup/d) |
| **Cancer** |                     |             |          |             |                   |
| Zheng et al. [145] | Case-control (20), Cohort (4) | 7,376 cases, 487,894 control in case-control; 8,874,734 person-y in cohort | NA³ | Esophageal cancer | Cohort: RR=0.77 (0.57-1.04, highest vs. non/lowest) Case-control: RR=0.70 (0.51-0.96) |
| Zheng et al. [146] | Case-control (8), Cohort (2) | 3,731 (3,557 cases) | 0-9 y | Esophageal cancer | Overall: ↔ Case control: RR=0.32 (0.10-0.54, high), 0.43 (0.21-0.66, medium), 0.45 (0.10-0.79, low) vs. non-drinker in female only |
| Kang et al. [147] | Cohort (7), Case-control (11) | 223,044 (6814 cases) | NA | Stomach cancer | Overall: RR=0.86 (0.74-1.00) subgroup analysis (6 studies): RR=0.68 (0.53-0.87, ≥5 cup/d vs. lowest) |
| Myung et al. [148] | Case-control (8), Cohort (5) | 254,869 (6,636 cases) | NA | Stomach cancer | RR=1.10 (0.92-1.32, highest vs. lowest consumption) |
| Wang et al. [149] | Cohort (6) | 352,275 (1,675 cases) | NA | Colorectal cancer | Overall RR=0.90 (0.72-1.08) Shanghai: RR=0.70 (0.55-0.85, highest vs. lowest) Singapore: RR=1.36 (1.06-1.74) |
| Tang et al. [150] | Cohort (5), Case-control (7) | 106,069 (5,495 cases) | NA | Lung cancer | RR=0.78 (0.61-1.00) in highest intake |
Table 7 Continued...............

| Study | Design | Case-control | Cases | RR (95% CI) |
|-------|--------|--------------|-------|-------------|
| Ogunleye et al. [151] | (9) | 164,943 (5,617 cases) | NA Breast cancer | RR=0.81, (0.75-0.88, >4 vs. ≤1 cup/d) |
| Tang et al. [152] | Cohort (2), Case-control (5) | 107,764 (3,487 cases) | NA Endometrial cancer | Overall: RR=0.85 (0.77-0.94) Cohort: RR=1.05 (0.85-1.28) Case control: RR=0.81 (0.71-0.93) |
| Zheng et al. [153] | Cohort (6), Case-control (7) | 111,499 (3,608 cases) | NA Prostate cancer | Overall: RR=0.72 (0.45-1.15) Case control: RR=0.43 (0.25-0.73) Cohort: RR=1.00 (0.66-1.53) |
| Nagle et al. [154] | Case-control (3) | 6,092 (2,567 cases) | NA Ovarian cancer | RR=0.58 (0.33-1.01), ≥1 cup/d vs. never/seldom |

*Abbreviations: CAD; coronary artery disease; CHD, coronary heart disease; MI, myocardial infarction; RR, relative risk; Values in parenthesis is the 95% confidence interval; NA: not available

Table 8. Established safe levels of metals in herbals, foods, and food ingredients, and as provided by dietary supplements

| Metal | Herbal products (w/w) | Foods and ingredients (w/w) | Dietary supplements (dose) |
|-------|-----------------------|-----------------------------|-----------------------------|
| Aluminum | 10.2 mg/mL | - | - |
| Arsenic | 0.1-0.5 mg/kg | 0.3-400 µg/kg | 5 µg/d |
| Cadmium | 0.3 mg/kg | 0.1-0.4 mg/kg | - |
| Copper | - | 0.1-2 mg/kg | - |
| Lead | 10 mg/kg | 1-100 µg/kg | 10 µg/d |
| Mercury | - | - | 15 µg/d |

*Abbreviations: -, no established guidelines; WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007 [156]; EPA 40 CFR part 143 [157]; Joint FAO/WHO Food Standards Programme, CODEX Committee on Contaminants in Foods, Sixth Session, Maastricht, The Netherlands. Working document for information and use in discussions related to contaminants and toxins in the GSCFTT (General Standard for Contaminants and Toxins in Foods and Feeds), March 26-30, 2012 [158]; United States Pharmacopeial Convention Chapter 2232 Elemental Contaminants in Dietary Supplements, USP 36-NF31 S1 [159]; Hg in total as methylmercury, 2 µg/d

Table 9. Recommended limits for residual solvents in U.S. dietary supplements with relevance to GTE

| Solvent category | Residual solvent | Recommended limits† (mg/d) |
|------------------|------------------|-----------------------------|
| Class 2 (solvents to be limited) | acetonitrile | 4.1 |
| | chloroform | 0.6 |
| | dichloromethane | 6 |
| | hexane | 2.9 |
| | methanol | 30. |
| | acetic acid‡ | 50 |
| | acetone | 50 |
| | ethanol‡ | 50 |
| | ethyl acetate | 50 |

*American Herbal Product Association Guidance Policies [159]; †Based on a 10 g dose; ‡ Higher levels permitted when intended as part of the product formulation

7.2 Evidence from Animal Models

In rodents, the No Observed Adverse Effect Level (NOAEL) with oral ingestion ranges from 65.5 to 2500 mg GTE/kg body weight (bw), providing intakes of flavan-3-ols of 120 to 3542 mg/kg bw/d and EGCG of 50 to 1500 mg/kg bw/d over periods ranging from 4 to 26 weeks.
An increase in plasma alkaline phosphatase and 5% (w/w) of the diet for 90 d and observed only Takami et al. [178] fed male and female F344 greater sensitivity of mice to flavan adverse effect. An amount in humans would be typically consumed from 10.5 cups of green tea, and noted this equivalency to this Lambert et al. [181] calculated the minimum administration. Based on allometric scaling, liver. Plasma biomarkers of oxidative stress in plasma and apoptosis was associa...
found under fasting conditions. Importantly, Chow et al. [162] reported the same relationship in humans wherein volunteers consuming an acute dose of 400 to 12000 mg of GTE showed a >3.5-fold increase in the average maximum plasma concentration of EGCG when taken in a fasting condition than when taken with food.

In contrast to evidence from animal models of hepatotoxicity, several reports have shown a hepatoprotective action of green tea preparations. For example, Chen et al. [184] found a protective effect of intraperitoneal EGCG for 3 d at 50 and 75 mg/kg bw against acute carbon tetrachloride hepatotoxicity in mice and Bruno et al. [185] found GTE reduced serum alanine transaminase and aspartate transaminase activity as well as hepatic steatosis in obese mice. Fiorini et al. [186] found EGCG at 85 mg/kg bw administered via intraperitoneal injection or drinking water protected against hepatic ischemia/reperfusion injury in steatotic mice. Dobrzynska et al. [187] and Zhang et al. [188] both reported that feeding green tea polyphenols to rats reduced the adverse effects of ethanol toxicity in the liver.

7.3 Evidence from Human Studies

As it is unethical to attempt to induce harm in human studies, there is no direct evidence of toxicity from green tea or GTE. Clinical toxicity from GTE or purified tea polyphenols can only be deduced from animal model data and/or observational evidence. Similar to rodents, humans extensively metabolize flavonoids to glucuronidated, methylated, and sulfated forms for relatively rapid excretion through bile, urine or feces and do not appear to accumulate these compounds in plasma or tissues for long periods [189]. In general, EGCG, EGC, and EC reach their maximum plasma concentrations within 1.5-2.5 h after consumption and return to the baseline values within 24 h [190]. Flavan-3-ol bioavailability is also dose-dependent in acute oral administrations, e.g., at 50, 100, 200, 400, 800 or 1600 mg EGCG [165]. Plasma and urine concentrations of flavan-3-ols are absent 24 h after an acute dose and their accumulation is essentially undetectable even after repeated doses of 200, 400, or 800 mg EGCG daily for ≥10 d [166,191]. However, these data do not imply an absence of bioactivity, as repeated provision of 800 mg/d green tea polyphenols resulted in an inhibition in the capacity of phase II enzymes in plasma and a consequent increase in the maximum of free EGCG concentration in plasma, but the kinetics of circulating EGCG were not altered.

Only a few, minor adverse side effects have been reported from randomized clinical trials of GTE, though most of these studies have had durations of less than 6 mo. Reports of minor to moderate side effects of GTE consumption include gastric irritation, diarrhea, nausea, and vomiting [192]. These untoward reactions appear unrelated to the chemical composition of GTE [28,130,132].

In 2008, up to 34 cases of liver damage associated with GTE had been reported worldwide and were summarized by Sarma et al. [28]. For most case reports, it is usually unclear whether the toxicity was due to GTE per se or related to other chemicals introduced during the extraction process, to concomitant medications, to other intentional or adulterant ingredients in the product or other factors. Though of variable quality, 27 of these reports were categorized according to the Naranjo causality algorithm scale as possible causality and 7 as probable causality. These cases were associated with the use of 6 different products varying markedly in product composition, solvent extraction, dose, and duration of use. The reported hepatotoxicity was coincident with increases in the activity of serum alanine transaminase and/or alkaline phosphatase or acute hepatitis-like syndrome [193]. However, the Naranjo scale has been found to lack validity and reproducibility when evaluating hepatotoxicity and is no longer recommended for such assessments [194]. The NIH Liver Tox database includes case reports of GTE extract and indicates no dose-response relationship between GTE intake and hepatotoxicity [193]. Many of the cases of liver toxicity associated with GTE were identified in Europe and linked to the use of alcohol extracts; however, hepatotoxicity has been reported for both aqueous and alcoholic GTE [28]. Liver injury has also been reported in at least 3 individuals drinking green tea infusions: an adult consuming 4-6 cups/d for 6 mo; an individual drinking an unspecified amount of green tea daily, and an individual consuming 6 cups/d of a commercial green tea infusion for 4 mo [33,194-196]. More recently, Teschke et al. [197] analyzed 185 case reports, spontaneous reports, review articles, and comments regarding liver injury associated with the use of 60 herbs, herbal drugs, and herbal dietary supplements, including GTE, and concluded that convincing causality assessment was rarely provided. The most robust current
approach for structured, quantitative, and validated hepatotoxicity cases is the Council for International Organizations of Medical Sciences (CIOMS) scale for initial causality assessment [198]. While not without limitations, the CIOMS scale compiles liver specific criteria for challenge, de-challenge, risk factors, exclusion of unrelated diseases, and co-medication [199,200].

In contrast to case reports, randomized clinical trials of GTE do not provide evidence of hepatotoxicity. For example, in a 3-wk randomized clinical trial testing a GTE daily containing ~670 mg flavan-3-ols in healthy men, Frank et al. [201] found no untoward effects on \(\gamma\)-glutamyl-transpeptidase, alanine transaminase or aspartate transaminase. In a 12-wk phase II randomized clinical trial of patients with oral premalignant lesions, Tsao et al. [202] found no adverse effects, including on hematological or blood chemistry outcomes, from GTE supplementation at 500 to 1000 mg/m\(^2\) (~14.7 to 29.4 mg/kg bw or ~1034 to 2068 mg/d). Similarly, Bettuzzi et al. [203] reported no adverse effects following the administration of green tea flavanols at 600 mg/d for 1 y in a safety assessment study among men with high-grade prostate intraepithelial neoplasia. In a randomized clinical trial of 40 overweight and obese Japanese children, 6 to 16 y, Matsuyama et al. [204] tested the effect of 575.9 vs 75.4 mg daily of green tea flavanols and found no significant effects on \(\gamma\)-glutamyl-transpeptidase, alanine transaminase, aspartate transaminase or albumin. In contrast, supplementation with a GTE at 1300 mg/d for a mean of 34.5 d in men with prostate cancer and scheduled for a radical prostatectomy was associated with statistically significant decreases in aspartate transaminase and alkaline phosphatase; non-significant decreases in alanine transaminase, \(\gamma\)-glutamyl-transpeptidase, and lipase were also reported [205]. While these results indicate no adverse effect on liver function and suggest a favorable trend, it is important to note that all these values were within normal limits throughout the study.

Hepatotoxicity from herbal products and pharmaceuticals is not uncommon and has often raised concerns about product quality [206-208]. Navarro et al. [38] assayed 97 herbal dietary supplements and found that 29 of 73 products that did not identify GTE or any of its constituents did contain flavan-3-ols, suggesting that packaging and labels of such products seem to be unreliable as regards GTE content. In their study matching clinical histories with the analysis of tea flavonoids, among patients with confirmed hepatotoxicity, no statistically significant association was observed between the presence of flavan-3-ols or the dose consumed and liver injury causality score, severity, or pattern of liver injury [38]. Liver injury associated with green tea infusions and GTE is consistent with the definition of idiosyncratic drug-induced hepatotoxicity based on its rare occurrence and lack of dose-dependency [38,209]. While green tea infusions and GTE appear well tolerated in most all individuals, they have been associated with liver injury in rare circumstances, possibly as a result of specific environmental, genetic, and/or medical conditions.

8. AUTHORITATIVE REVIEWS OF GREEN TEA EXTRACTS

In 2003, the French Health Product Safety Agency (AFSSAPS) and the Spanish Food Safety and Nutrition Agency suspended the marketing authorization of an 80% ethanolic GTE (Exolise®) due to reports of hepatic disorders [210]. This product was indicated at 375 mg/d of GTE, standardized to 25% EGCG, and obtained using 80% ethanol as the solvent. More recent cases were reported to AFSSAPS in 2006 with aqueous GTE and AFSSA, the French Food Safety Agency, in 2009 [32,211,212]. In 2007, the U.S. Pharmacopeia (USP) determined GTE was a Class 2 substance and issued a press release suggesting specific cautionary wording be added to any dietary supplements containing GTE as an ingredient: “Take with food. Discontinue use and consult a healthcare practitioner if you have a liver disorder or develop symptoms of liver trouble such as abdominal pain, dark urine, or jaundice.” [213]. However, two years later, the USP undertook a follow-up comprehensive review and, based on updated information, reclassified GTE as an article falling within Class A (Admitted into the Compendia – does not require a caution/warning statement in labeling) and canceled the published proposal for a cautionary labeling statement [214]. Additional expert reviews based on case reports, observational studies, and clinical trials confirm the general safety of green tea and GTE consumption [28,30,130,132,215-217] (Table 10).

In 2009, the European Food Safety Authority Scientific (EFSA) Cooperation Group (ESCO) published guidance for the safety assessment of botanicals in which green tea was considered based upon knowledge of the risk of
hepatotoxicity and the worldwide, long-term consumption of traditional tea infusions; intakes of brewed green tea from the mean to 95th percentile of intake at 362 to 1097 g/d, respectively, were noted in China and Japan and were associated with mean and 95th percentile intakes of EGCG at 95 to 289 and 148 to 448 mg/d and of total flavan-3-ols at 186 to 565 and 307 to 931 mg/d, respectively [216]. The ESCO concluded that traditional green tea infusion is assumed to be safe on level A (safety presumed based upon available knowledge derived from long-term use). Dried aqueous GTE which have a similar composition and do not exceed the concentration of polyphenols compared to a traditional green tea infusion were also classified to be on level A when consistent to EFSA guidance on the safety assessment of botanicals intended for use in the preparation of beverages [216,218]. In marked contrast, this EFSA report [216] questions the safety of use of GTE for use in food supplements and recommends application of a margin of safety (MOS) – the ratio between the NOAEL (determined from animal studies) and daily intake for EGCG – of 100. Though absent specific information, the Norwegian Food Safety Authority recently issued a warning about using highly concentrated GTE supplements based upon some cases of hepatotoxicity reported by the Norwegian Medicines Agency [219].

Similar to EFSA guidelines, France has published restrictions on the use of preparations of green tea in food supplements limiting them to traditional extracts (i.e., aqueous extracts at a maximal amount equivalent to 30 mg/d of EGCG).

### Table 10. Summary of systematic reviews that include safety assessment of green tea or GTE

| Review | Date | Conclusions |
|--------|------|-------------|
| US Pharmacopeia, Safety of Green Tea Extract<sup>1</sup> | 2008 | Assigned Class 2 safety rating, suggested labeling: “Take with food. Discontinue use and consult a healthcare practitioner if you have a liver disorder or develop symptoms of liver trouble such as abdominal pain, dark urine, or jaundice” |
| Green tea consumption and liver disease; interventional and observational studies with liver cancer, cirrhosis, and fatty liver disease as outcomes<sup>2</sup> | 2008 | Increased green tea consumption may reduce the risk of chronic liver diseases. |
| US Pharmacopeia, Green Tea Extract Monograph<sup>3</sup> | 2009 | Class A safety rating, no cautionary label statement |
| Green tea for the prevention of cancer<sup>4</sup> | 2009 | Green tea consumption appears safe at moderate, regular, and habitual use (3-5 cups/d, providing ~250 mg catechins/d) |
| EFSA ESCO advise on the EFSA guidance for the safety assessment of botanicals<sup>5</sup> | 2009 | Aqueous GTE for food supplement use given A except for weight reduction purposes which received grade B, other GTE (e.g. hydro-alcoholic preparations) given grade B |
| Green tea for weight loss and weight maintenance in overweight or obese adults<sup>6</sup> | 2012 | Assessed randomized control trials of at least 12 wk duration of green tea preparations. Most adverse effects reported appeared unrelated to green tea or control intervention. |
| French Agency for Food, Environmental and Occupational Health Safety (ANSES)<sup>7</sup> | 2012 | Based on case reports, hepatitis severity following GTE consumption could not be conclusively associated with GTE consumption, dose response could not be achieved. |

<sup>1</sup>Sarma et al. [28]; <sup>2</sup>Jin et al. [215]; <sup>3</sup>Sarma. [30]; <sup>4</sup>Boehm et al. [130]; <sup>5</sup>EFSA J. [215]; <sup>6</sup>Jurgens et al. [132]; <sup>7</sup>ANSES, 21011-SA-0108 [217]
for a person weighing 60 kg) and excludes hydro-alcoholic extracts and powders [220]. In some contrast, the French Agency for Food, Environmental and Occupational Health Safety recently evaluated 17 case reports of hepatitis associated with GTE consumption and found no conclusive evidence for a causal relationship because the data on quantity of green tea and GTE consumed were lacking so that no dose-response relationship could be established between intake and severity of hepatitis [217]. Belgium allows a maximum dose of EGCG at 1600 mg/d and a maximum amount of 25% ethanol permitted in the extraction process [221]. Currently, the Plant LIBRA project, co-financed in the context of the 7th European Union Framework Program, is evaluating the safety of GTE using several assessment approaches, including the Observed Safe Level [222], and Minimum Anticipated Biological Effect Level [223] and Estimation of the Optimal Range [224,225].

More recently, Cochrane database reviews have confirmed the general safety of consuming green tea infusions at 3-5 cups/d [130] and the safety of infusions and GTE associated with weight loss studies [132]. The safety of GTE was reviewed by the US Pharmacopeia and assigned as a Class A substance that does not require a caution or warning statement [28,30]. GTE prepared as essential oils, solvent-free oleoresins, and other natural extractives are currently classified as GRAS for food and ingredient use by the U.S. Food and Drug Administration [226]. Further, purified tea flavan-3-ols, an aqueous GTE, and L-theanine from green tea have self-determined GRAS status (Table 11).

| Product                                      | Manufacturer | Date  |
|----------------------------------------------|--------------|-------|
| Teavigo, green tea extract standardized to 94% EGCG¹ | DSM          | 2005  |
| Purified green tea catechins¹                | Kao, Inc.    | 2007  |
| L-theanine extracted from tea leaves³        | Blue California | 2010 |
| AssuriTEA aqueous green tea extract¹         | Kemin        | 2012  |

¹AIBMR Life Sciences, GRAS Self-determination Inventory Database [227] ²Assuming maximum use of 540 mg catechins/350 mL, with up to 1 to 1.5 g green tea catechins presumed safe; FDA GRN No. 225 & 259 [228] ³FDA GRN No. 338 [229]

9. SUMMARY AND PERSPECTIVE

The interest in the putative health benefits of green tea has led to an increase in the consumption of brewed green tea and the application of GTE to a variety of food and beverage products and food supplements. With some experimental evidence of toxicity from green tea and GTE and a number of case reports of hepatotoxicity, the concept of a safety threshold for green tea and GTE has been raised. While there are several approaches to determining safety thresholds [222,224,225, 230,231,232], some suggest a conservative threshold for nutrients and food supplements should be equivalent to at least 10-fold less than the lowest dose associated with toxicity observed in humans. In the case reports of GTE-associated hepatotoxicity summarized by Bonkovsky [34], the reported range of intake was 10-29 mg GTE/kg bw/d, using the lower 10 mg/kg bw dose, an estimated acceptable intake would be 1.0 mg/kg bw/d or 70 mg EGCG daily for a reference 70 kg person. However, such a calculation is in marked contrast to usual intakes and the GRAS status of green tea and GTE and the expert and systematic reviews on the safety of consuming moderate levels of GTE and green tea infusions [28,30,130,132,215]. For example, the 90th percentile of EGCG intake associated with green tea consumption in the U.S. is 189 and 218 mg/d for women and men, respectively; the 95th percentile of EGCG intake associated with green tea consumption in China and Japan is 285 and 447 mg/d [4,8]. These values are about 3- to 6-times higher than a 10-fold safety threshold of 1.0 mg/kg bw/d and suggest an inappropriate application of the precautionary principle, particularly in the absence of established causality. Although most individual studies have been relatively short-term, recent Cochrane database reviews confirm the general safety of green tea and GTE [130,132]. Nonetheless, it is noteworthy that Bettuzzi et al. [203] found no untoward responses in a year-long randomized, placebo-controlled trial of a GTE providing 311 mg/d EGCG and 142 mg/d of other flavan-3-ols. The noted gap could be attributed to the factors such as dosing frequencies and conditions, as well as the medical conditions, concomitant use of other products and sensitivities of individuals.

Responses by government regulatory agencies to the experimental data and case reports of hepatotoxicity associated with use of GTE are contradictory, e.g., with EFSA stating that GTE
for food supplement use cannot be considered safe and suggesting a MOS (calculated with data from animals) of at least 100 [216] and the USP admitting GTE into its Compendia without a requirement for any caution/warning label (though USP’s Dietary Supplement Information Expert Committee continues to monitor clinical case reports) [213]. After an accountability assessment of 17 cases of hepatitis linked to green tea and GTE reported between 2009 and 2011, the French Agency for Food, Environmental and Occupational Health Safety concluded that these cases could not be clearly associated with green tea due to confounding by other likely causes and an inability to obtain adequate information on intake [217]. This contrast between approaches and recommendations arises in part from the rare and idiosyncratic nature of the injury and the apparent absence of a dose-response relationship between GTE and hepatotoxicity [36, 37, 193]. The sensitivity and extremely low occurrence of hepatotoxicity with GTE may have a nutrigenetic basis, e.g., polymorphism(s) in pathways for flavonoid metabolism [35, 233]. This relationship is highlighted by the recent report of Navarro et al. [38] which indicated no statistically significant association between GTE dose or content of flavan-3-ols and liver injury causality score, severity, or pattern of liver injury. Despite concern raised in some reports about a great risk for harm induced by GTE derived from hydro-alcoholic versus aqueous extractions, specifically hepatotoxicity, there is no evidence that the former provides significantly different proportions of bioactive or toxic compounds than the latter. This work is essentially confirmed by the observation that hepatotoxicity associated with GTE have been reported from cases following use of variety of products varying markedly in composition, solvent extraction, dose, and duration of use. Although the pathogenesis of hepatotoxicity associated with GTE is unknown, it is important to note that EGCG, the component of GTE most often identified as the cause of hepatotoxicity, is readily soluble in both water and alcohol-water mixtures. Regulations ranking the potential hepatotoxicity of GTE via its extraction with water versus hydro-alcoholic solvents are not based on the available evidence. Rather than an extraction process, it appears that the greater number of cases linked to a limited number of GTE products may be largely due to their greater sales and indication for chronic use, such as for weight loss.

The evidence derived from observational studies and clinical trials over the last three decades suggest that flavan-3-ols from green tea may contribute importantly to reducing the risk of prevalent age-related chronic diseases. The biological plausibility underlying this association is derived from a large body of experimental studies revealing several relevant molecular mechanisms of action of these flavonoids. However, the application of this information, e.g., through the development of innovative dietary products containing GTE, requires careful consideration of the safety profile of its constituent phytochemicals. Clinical case reports of hepatotoxicity associated with use of GTE and products containing GTE have raised concern and generated responses from regulatory agencies. In addition to the usual limitations associated with the extrapolation of evidence from in vitro and animal model experiments, data on liver injury induced by GTE and its specific flavan-3-ols constituents revealed by these approaches are mixed in that some demonstrate hepatotoxicity while others show hepatoprotective actions. Case reports are important but also limited, especially with rare and idiosyncratic reactions, in their ability to reveal causality due to confounding medical and lifestyle factors of the patients.

10. CONCLUSION

The scientific evidence regarding the potential benefits and risks of green tea polyphenols and GTE continues to emerge. Existing data are insufficient to identify in the preparation or composition of GTE the causative agent, its dose or duration of use as well as nutrigenetic, medical, and other factors contributing to the risk of hepatotoxicity. While reference to quantitative equivalents associated with traditional dietary intakes of green tea, its constituent flavonoids, and related dietary polyphenols is important in evaluating GTE products, opportunities should be allowed for innovation to improve the efficiency, quality, and cost-effectiveness of their production through extraction technologies. This approach can also prove useful in minimizing the presence of endogenous or contaminant heavy metals as well as mycotoxins, pesticides, and other unwanted constituents.

ACKNOWLEDGEMENTS

This work was supported by an educational grant from the International Alliance of Dietary/Food Supplement Associations (IADSA) to the
Friedman School of Nutrition Science and Policy at Tufts University. IADSA had no role in the preparation or review of the manuscript. We appreciate the assistance of Jo Obelsky and Jennifer O’Leary-Chen in formatting the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. MacFarlane A, MacFarlane I, editors. The empire of tea: The remarkable history of the plant that took over the world. Woodstock, NY: The Overlook Press; 2003.

2. Hodgson JM, Croft KD. Tea flavonoids and cardiovascular health. Mol Aspects Med. 2010;31:495-502.

3. Mukhtar H, Ahmad N. Tea polyphenols: Prevention of cancer and optimizing health. Am J Clin Nutr. 2000;71:1698-702S.

4. International Tea Committee. Annual Bulletin of Statistics. 2013. Accessed 18 October 2013. Available: http://www.inttea.com/publications.asp

5. Chun OK, Chung SJ, Song WO. Estimated dietary flavonoid intake and major food sources in U.S. adults. J Nutr. 2007;137:1244-52.

6. CFSII. Continuing food survey of intake by individuals. 2003. Accessed 8 January 2014. Available: http://www.ars.usda.gov/Services/docs.htm?docid=14392.

7. Tomata Y, Kakizaki M, Nakaya N, Tsuboya T, Sone T, Kuriyama S, et al. Green tea consumption and the risk of incident functional disability in elderly Japanese: The Ohsaki Cohort 2006 Study. Am J Clin Nutr. 2012;95:732-9.

8. Robert Koch Institute. Verzehr von grüntee. Bundes-Gesundheitssurvey. 1998. Accessed 30 October 2013. Available: http://www.rki.de/EN/Content/Health-Monitoring/Health-Reporting/GBEDownloadsB/was_essen_wir_heute.pdf?blob=publicationFile.

9. Khokhar S, Magnusdottir SG. Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. J Agric Food Chem. 2002;50:565-70.

10. Kaegi E. Unconventional therapies for cancer: Green tea. Canadian Med Assoc J. 1998;158:1033-5.

11. Henderson L, Gregory J, Swan G. National Dietary and Nutrition Survey: Adults aged 19 to 64 years. Food Standards Agency. 2002. Accessed 8 October 2013. Available: http://food.gov.uk/multimedia/pdfs/ndsprintedreport.pdf.

12. Smith A. Effects of caffeine on human behavior. Food Chem Toxicol. 2002;40:1243-55.

13. Blumberg JB. Introduction to the proceedings of the fifth international scientific symposium on tea and human health. Am J Clin Nutr. 2013;98:1607-10S.

14. Bogdanski P, Suliburska J, Szulinska M, Stepien M, Pepek-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;32:421-7.

15. Del Rio D, Rodriguez-Mateos A, Spencer JP, Tognolini M, Borges G, Crozier A. Dietary (poly) phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. Antiox Redox Signal. 2013;18:1818-92.

16. Liu K, Zhou R, Wang B, Chen K, Shi LY, Zhu JD, et al. Effect of green tea on glucose control and insulin sensitivity: A meta-analysis of 17 randomized controlled trials. Am J Clin Nutr. 2013;98:340-8.

17. Mak JC. Potential role of green tea catechins in various disease therapies: progress and promise. Clin Exp Pharmacol Physiol. 2012;39:265-73.

18. Kim H-S, Quon MJ, Kim J-A. New insights into the mechanisms of polyphenols beyond antioxidant properties: Lessons from the green tea polyphenol, epigallocatechin-3-gallate. Redox Biol. 2014;2:187-95.

19. Lorenz M. Cellular targets for the beneficial actions of tea polyphenols. Am J Clin Nutr. 2013;90:1642-50S.

20. Katiyar SK, Afao F, Perez A, Mukhtar H. Green ptea polyphenol (-)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. Carcinogenesis. 2001;22:287-94.

21. Meng Q, Velalar CN, Ruan R. Regulating the age-related oxidative damage, mitochondrial integrity, and antioxidative...
22. Yan J, Zhao Y, Suo S, Liu Y, Zhao B. Green tea catechins ameliorate adipose insulin resistance by improving oxidative stress. Free Radic Biol Med. 2012;52:1648-57.

23. Basu A, Sanchez K, Leyva MJ, Wu M, Betts NM, Aston CE, Lyons TJ. Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. J Am Coll Nutr. 2010;29:31-40.

24. Nakagawa K, Ninomiya M, Okubo T, Aoi N, Juneja LR, Kim M, et al. Tea catechin supplementation increase antioxidant capacity and prevents phospholipid hydroperoxidation in plasma of humans. J Agric Food Chem. 1999;47:3967-73.

25. Orsolic N, Sirovina D, Gajski G, Garaj-Vrhovac V, Jembrek MJ, Kosalec I. Assessment of DNA damage and lipid peroxidation in diabetic mice: effects of propolis and epigallocatechin gallate (EGCG). Mutat Res. 2013;757:36-44.

26. Namal Senanayake SPJ. Green tea extract: Chemistry, antioxidant properties and food applications – a review. J Funct Food. 2013;5:1529-41.

27. Blumenthal M. editor. The ABC clinical guide to herbs. (1st ed.) American Botanical Council. Austin, TX. Thieme New York. New York. 2003;335-50.

28. Sarma DN, Barrett ML, Chavez ML, Gardiner P, Ko R, Mahady GB, et al. Safety of green tea extracts: A systematic review by the US Pharmacopeia. Drug Saf. 2008;31:469-84.

29. Shivanand P. Tea: effects on cardiovascular system, blood pressure, EEG and many other system of human being. Int J Pharm Sci Res. 2010;2:31-4

30. Sarma DN. Update on the USP green tea extract monograph. U.S. Pharmacopeia. 2009. Accessed 26 October 2013. Available: http://www.usp.org/usp-nf/notices/retired-compendial-notices/update-usp-green-tea-extract-monograph

31. GRAS. 21 CFR Ch. I (4-1-12 Edition) § 182.20. 2010. Substances generally recognized as safe, Subpart A – General Provisions Sec. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates). Food and Drug Administration, Dept. Health and Human Services. Washington, DC. Accessed 8 October 2013. Available: http://www.gpo.gov/fdsys/pkg/CFR-2012-title21-vol3_pdf/CFR-2012-title21-vol3-sec182-20.pdf

32. Gloro R, Hourmand-Ollivier I, Mosquet B, Mosquel L. Rousselot P, Salamé E, et al. Fulminant hepatitis during self-medication with hydroalcoholic extract of green tea. Eur J Gastroenterol Hepatol. 2005;17:1135-7.

33. Jimenez-Saenz M, Martinez-Sanchez MdC. Acute hepatitis associated with the use of green tea infusions. J Hepatol. 2006;44:616-7.

34. Bonkovsky HL. Hepatotoxicity associated with supplements containing Chinese green tea (Camellia sinensis). Ann Intern Med. 2006;144:68-71.

35. Javaid A, Bonkovsky HL. Hepatotoxicity due to extracts of Chinese green tea (Camellia sinensis): A growing concern. J Hepatol. 2006;45:334-5.

36. Mazzanti G, Menniti-Ippolito FM, Moro PA, Cassetti F, Raschetti R, Santuccio C, et al. Hepatotoxicity form green tea: A review of the literature and two unpublished cases. Eur J Clin Pharmacol. 2009;65:331-4.

37. Fong TL, Klontz KC, Canas-Coto A, Casper SJ, Durazo FA, Davern TJ, et al. Hepatotoxicity due to Hydroxycut: A case series. Am J Gastroenterol. 2010;105:1561-6.

38. Navarro VJ, Bonkovsky HL, Hwang S-I, Vega M, Barmhart H, Serrano J. Catechins in dietary supplements and hepatotoxicity. Dig Dis Sci. 2013;58:2682-90.

39. Chaturvedula VSP, Prakash I. The aroma, taste, color and bioactive constituents of tea. J Med Plants Res. 2011;5:2110-20.

40. Chacko SM, Thambi PT, Kuttan R, Nishigaki I. Beneficial effects of green tea: a literature review. Chin Med. 2010;5:13. DOI: 10.1186/1749-8546-7-13.

41. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea – a review. J Am Coll Nutr. 2006;25:79-99.

42. Wang Y, Ho CT. Polyphenolic chemistry of tea and coffee: A century of progress. J Agric Food Chem. 2009;57:8109-14.

43. Butt MS, Sultan MT. Green tea: Nature’s defense against malignancies. Crit Rev Food Sci Nutr. 2009;49:463-73.

44. Zhao Y, Chen P, Lin L, Harnly JM, Yu L, Li Z. Tentative identification, quantification, and
Karak T, Bhagat RM. Trace elements in tea by chemical analysis of the production season of Chinese green tea. J Food Compos Anal. 2005;18:487-91.

Suzuki T, Yamazaki N, Sada Y, Oguni I, Moriyasu Y. Tissue distribution and intracellular localization of catechins in tea leaves. Biosci Biotechnol Biochem. 2003;67:2683-6.

Bhagwat S, Haytowitz DB, Holden JM. USDA database for the flavonoid content of selected foods, Release 3.1. U.S. Department of Agriculture, Agricultural Research Service. Accessed 14 March 2014. Available: http://www.ars.usda.gov/nutrientdata/flavonoid.

Cabrera C, Giménez R, López MC. Determination of tea components with antioxidant activity. J Agric Food Chem. 2003;51:4427-35.

Friedman M, Levin CE, Lee S-U, Kozukue N. Stability of green tea catechins in commercial tea leaves during storage for 6 months. J Food Sci. 2009;74:H47-51.

Komes D, Horžič D, Belščak A, Ganič KK, Vulič I. Green tea preparation and its influence on the content of bioactive compounds. Food Res Int. 2010;43:167-76.

Nishitani E, Sagesaka YM. Simultaneous determination of catechins, caffeine and other phenolic compounds in tea using new HPLC method. J Food Compos Anal. 2004;17:675-85.

Peterson J, Dwyera J, Bhagwat S, Haytowitz D, Holden J, Eldridge AL, Beecher G, et al. Major flavonoids in dry tea. J Food Compos Anal. 2005;18:487-501.

Wang D, Lu J, Miao A, Xie Z, Yang D. HPLC-DAD-ESI-MS/MS analysis of polyphenols and purine alkaloids in leaves of 22 tea cultivars in China. J Food Compos Anal. 2008;21:361-9.

Xu W, Song Q, Li D, Wan X. Discrimination of the production season of Chinese green tea by chemical analysis in combination with supervised pattern recognition. J Agric Food Chem. 2012;60:7064-70.

Karak T, Bhagat RM. Trace elements in tea leaves, made tea and tea infusion: A review. Food Res Int. 2010;43:2234-52.

ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological profile for arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services. 2007a. Accessed 14 July 2014. Available: http://www.atsdr.cdc.gov/toxguides/toxguide-2.pdf.

ATSDR (Agency for Toxic Substances and Disease). Toxicological profile for lead. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services. 2007b. Accessed 1 June 2013. Available: http://www.atsdr.cdc.gov/toxguides/toxguide-13.pdf.

ATSDR (Agency for Toxic Substances and Disease). Toxicological profile for chromium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services. 2012. Accessed 4 July 2013. Available: http://www.atsdr.cdc.gov/toxguides/toxguide-7.pdf.

Mandiwana KL, Panichev N, Panicheva S. Determination of chromium (VI) in black, green and herbal teas. Food Chem. 2011;129:1839-43.

Eggers P, Pilz S. High pressure processing. In: Bart H-J, Pilz S, editors. Industrial Scale Natural Products Extraction. Weinheim, Germany: Wiley-VCH. 2011;87-122.

Kassing M, Jenelten U, Schenk J, Strube J. A new approach for process development of plant-based extraction processes. Chem Eng Tech. 2010;33:377-87.

Strube J, Backer W, Schulte M. Process engineering and mini-plant technology. In: Bart H-J, Pilz S, editors. Industrial scale natural products extraction. Weinheim, Germany: Wiley-VCH. 2011;123-80.

Du FY, Xiao XY, Li GK. Application of ionic liquids in the microwave-assisted extraction of trans-resveratrol from Rhizma Polygoni Cuspidati. J Chrom A. 2007;1140:56-62.

Jordan V, Muller U. Alternative solvents in plant extraction. In: Bart H-J, Pilz S, editors. Industrial scale natural products extraction. Weinheim, Germany: Wiley-VCH. 2011;55-86.

Sun QL, Hua S, Ye JH, Lu JL, Zhen XQ, Liang YR. Decaffeination of green tea by supercritical carbon dioxide. J Med Plant Res. 2010;4:1161-8.
66. Wijngaard H, Hussain MB, Rai DK, Brunton N. Techniques to extract bioactive compounds from food-by-products of plant origin. Food Res Int. 2012;46:505-13.

67. Klein AP, Beach ES, Emerson JW, Zimmerman JB. Accelerated solvent extraction of lignin from Aleurites moluccana (Candlenut) nutshell. J Agr Food Chem. 2010;58:10045-8.

68. Ibanez E, Herrero M, Martin-Alvarez PJ, Senorans FJ, Reglero G, Cifuentes A. Accelerated solvent extraction: A new procedure to obtain functional ingredients from natural sources. ACS Sym Ser. 2006;926:65-78.

69. Hussain MB, Barry-Ryan C, Martin-Diana AB, Brunton NP. Optimisation of accelerated solvent extraction of antioxidant compounds from rosemary (Rosmarinus officinalis L.), marjoram (Origanum majorana L.) and oregano (Origanum vulgare L.) using response surface methodology. Food Chem. 2011;126:339-46.

70. Herrero M, Martin-Alvarez PJ, Senorans FJ, Cifuentes A, Ibanez E. Optimization of accelerated solvent extraction of antioxidants from Spirulina platensis microalgae. Food Chem. 2005;93:417-23.

71. Wijngaard HH, Ballay M, Brunton N. The optimisation of extraction of antioxidants from potato peel by pressurised liquids. Food Chem. 2012;136:1123-30.

72. Wang LJ, Weller CL. Recent advances in extraction of nutraceuticals from plants. Trends in Food Sci Tech. 2006;17:300-12.

73. Luthria DL. Influence of experimental conditions on the extraction of phenolic compounds from parsley (Petroselinum crispum) flakes using a pressurized liquid extractor. Food Chem. 2008;107:475-52.

74. Rostagno MA, Palma M, Barroso CG. Pressurized liquid extraction of isoflavones from soybeans. Anal Chim Acta. 2004;522:169-77.

75. Denery JR, Dragull K, Tang CS, Li QX. Pressurized fluid extraction of carotenoids from Haematococcus pluvialis and Dunaliella salina and kavalactones from Piper methysticum. Anal Chim Acta. 2004;501:175-81.

76. Pompeu DR, Silva EM, Rogez H. Optimisation of the solvent extraction of phenolic antioxidants from fruits of Euterpe oleracea using response surface methodology. Bioresource Technol. 2009;100:8076-82.

77. Herrero M, Mendiola JA, Cifuentes A, Ibanez E. Supercritical fluid extraction: Recent advances and applications. J Chromatogr A. 2010;1217:2495-511.

78. Poiana M, Sicari V, Mincione B. Supercritical carbon dioxide (SC-CO2) extraction of grape fruit flavedo. Flavour Frag J. 1998;13:125-30.

79. Roy BC, Goto M, Kodama A, Hirose T. Supercritical CO2 extraction of essential oils and cuticular waxes from peppermint leaves. J Chem Tech Biotech. 1996;67:21-6.

80. Santos SAO, Villaverde JJ, Silva CM, Neto CP, Silvestre AJD. Supercritical fluid extraction of phenolic compounds from Eucalyptus globulus Labill bark. J Supercrit Fluid. 2012;71:71-9.

81. Casas L, Mantell C, Rodríguez M, Ossa EJMDL, Roldán A, Ory ID, et al. Extraction of resveratrol from the pomace of Palomino fino grapes by supercritical carbon dioxide. J Food Eng. 2010;96:304-8.

82. Chen BY, Kuo CH, Liu YC, Ye LY, Chen JH, Shieh CJ. Ultrasonic-assisted extraction of the botanical dietary supplement resveratrol and other constituents of Polygonum cuspidatum. J Nat Prod. 2012;75:1810-3.

83. Chemat F, Zill-e-Huma, Khan MK. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrason Sonochem. 2011;18:813-35.

84. Puri M, Sharma D, Barrow CJ. Enzyme-assisted extraction of bioactives from plants. Trends Biotechnol. 2012;30:37-44.

85. Chandran J, Amma KPP, Menon N, Purushothaman J, Nisha P. Effect of enzyme assisted extraction on quality and yield of volatile oil from black pepper and cardamom. Food Sci Biotech. 2012;21:1611-7.

86. Choudhari SM, Ananthanarayan L. Enzyme aided extraction of lycopene from tomato tissues. Food Chem. 2007;102:77-81.

87. Gibbins RD, Aksoy HA, Ustun G. Enzyme-assisted aqueous extraction of safflower oil: Optimisation by response surface methodology. Int J Food Sci Tech. 2012;47:1055-62.

88. Li BB, Smith AB, Hossain MM. Extraction of phenolics from citrus peels II. Enzyme-assisted extraction method. Sep Purif Technol. 2006;48:189-96.
89. Sari YW, Bruins ME, Sanders JPM. Enzyme assisted protein extraction from rapeseed, soybean, and microalgae meals. Industr Crops Products. 2013;43:78-83.

90. Le HV, Van VML. Comparison of enzyme-assisted and ultrasound-assisted extraction of vitamin C and phenolic compounds from acerola (Malpighia emarginata DC.) fruit. Int J Food Sci Tech. 2012;47:1206-14.

91. El Darra N, Grimi N, Maroun RG, Louka N, Vorobiev E. Pulsed electric field, ultrasound, and thermal pretreatments for better phenolic extraction during red fermentation. Eur Food Res Technol. 2013;236:47-56.

92. Puertolas E, Cregenzan O, Luengo E, Alvarez I, Raso J. Pulsed-electric-field-assisted extraction of anthocyanins from purple-fleshed potato. Food Chem. 2013;136:1330-6.

93. Huang W, Wue A, Niu H, Jia Z, Wang J. Optimised ultrasonic-assisted extraction of flavonoids from Folium eucummae and evaluation of antioxidant activity in multilist systems in vitro. Food Chem. 2009;114:1147-54.

94. Chittapalo T, Noomhorm A. Ultrasonic assisted alkali extraction of protein from defatted rice bran and properties of the protein concentrates. Int J Food Sci Tech. 2009;44:1843-9.

95. Turk MF, Vorobiev E, Baron A. Improving apple juice expression and quality by pulsed electric field on an industrial scale. Lwt-Food Sci Technol. 2012;49:245-50.

96. Corrales M, Toepfl S, Butz P, Knorr D, Tauscher B. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. Innovat Food Sci Emerg Tech. 2008;9:85-91.

97. Kaufmann B, Christen P, Veuthey JL. Parameters affecting microwave-assisted extraction of with anolides. Phytochem Anal. 2001;12:327-31.

98. Liazid A, Guerrero RF, Cantos E, Palma M, Barroso CG. Microwave assisted extraction of anthocyanins from grape skins. Food Chem. 2011;124:1238-43.

99. Perez-Serradilla JA, de Castro MDL. Microwave-assisted extraction of phenolic compounds from wine lees and spray-drying of the extract. Food Chem. 2011;124:1652-9.

100. Tsubaki S, Sakamoto M, Azuma J. Microwave-assisted extraction of phenolic compounds from tea residues under autohydrolytic conditions. Food Chem. 2010;123:1255-8.

101. Pfennig A, Delinski D, Johannisbauer W, Josten H. Extraction technology. In: Bart H-J, Pilz S, editors. Industrial Scale Natural Products Extraction. Weinheim: Wiley-VCH. 2011;181-218.

102. Henning SM, Fajardo-Lira C, Lee HW, Youssefian AA, Go VL, Heber D. Catechin content of 18 teas and a green tea extract supplement correlates with the antioxidant capacity. Nutr Cancer. 2003;45:226-35.

103. Seeram NP, Henning SM, Niu Y, Lee R, Scheuiller HS, Heber D. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. J Agric Food Chem. 2006;54:1599-603.

104. Friedman M, Levin CE, Choi S-H, Kozukue E, Kozukue N. HPLC Analysis of catechins, theaflavins, and alkaloids in commercial teas and green tea dietary supplements: Comparison of water and 80% ethanol/water extracts. J Food Sci. 2006;71:C328-37.

105. Vuong QV, Golding JB, Stathopoulos CE, Nguyen MH, Roach PD. Optimizing conditions for the extraction of catechins from green tea using hot water. J Sep Sci. 2011;34:3099-106.

106. Vuong QV, Stathopoulos CE, Golding JB, Nguyen MH, Roach PD. Optimum conditions for the water extraction of L-theanine from green tea. J Sep Sci. 2011;34:2468-74.

107. Choung M-G, Lee M-S. Optimal extraction conditions for simultaneous determination of catechins and caffeine in green tea leaves. Food Sci Biotech. 2011;20:327-33.

108. Astill C, Birch MR, Dacombe C, Humphrey PG, Martin PT. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J Agric Food Chem. 2001;49:5340-7.

109. Hicks MB, Hsieh YHP, Bell LN. Tea preparation and its influence on methylxanthine concentration. Food Res Int. 1996;29:325-30.

110. Perva-Uzunalič A, Škerget M, Knez Ž, Weinreich B, Otto F, Grüner S. Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. Food Chem. 2006;96:597-605.

111. Ma SC, Wang XL, Liang YR. Kinetic study
112. Costa LM, Gouveia ST, Nobrega JA. Comparison of heating extraction procedures for Al, Ca, Mg, and Mn in tea samples. Anal Sci. 2002;18:313-8.

113. Ziaedini A, Jafari A, Zakeri A. Extraction of antioxidants and caffeine from green tea (Camellia sinensis) leaves: kinetics and modeling. Food Sci Technol Int. 2010;16:505-10.

114. Zhang X, Xu F, Gao Y, Wu J, Sun Y, Zeng X. Optimising the extraction of tea polyphenols, (-)-epigallocatechin gallate and theaene from summer green tea by using response surface methodology. Int J Food Sci Tech. 2012;47:2151-7.

115. Chen Z-Y, Zhu QY, Tsang JH, Huang Y. Degradation of green tea catechins in tea drinks. J Agric Food Chem. 2000;49:477-82.

116. Meterc D, Petermann M, Weidner E. Extraction of green tea and drying with a high pressure spray process. Hem Ind. 2007;61:222-8.

117. 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 Chem. 2008;110:852-8.

118. Sharma V, Gulati A, Ravindranath SD, Kumar V. A simple and convenient method for analysis of tea bioactive components by reverse phase HPLC. J Food Comp Anal. 2005;18:583-75.

119. Mossier A, Potin-Gautier M, Delerue S, Le Hécho I, Behra P. Effect of water composition on aluminum, calcium and organic carbon extraction in tea infusions. Food Chem. 2008;106:1467-75.

120. Kanrar B, Mandal S, Bhattacharyya A. Validation and uncertainty analysis of a multiresidue method for 67 pesticides in made tea, tea infusion, and spent leaves using ethyl acetate extraction and gas chromatography/mass spectrometry. J AOAC Int. 2010;93:411-24.

121. Wan CX. Primary speciation analysis of lead in tea. J Jiamusi Univ. 2012;30:798-800.

122. Xu J, Yu MG, Chen YX, Fu XP, Guan DC. Characteristic of distribution and chemical forms of Pb in tea plant varieties. Chinese J Appl Ecol. 2011;22:891-6.

123. Sullivan J, Greenfield J, Cumberford G, Grant J, Stewart J. Extraction efficiencies of heavy metals in hydroethanolic solvent from herbs of commerce. J AOAC Intl. 2010;2:496-8.

124. Yoshida Y, Kiso M, Goto T. Efficiency of the extraction of catechins from green tea. Food Chem. 1999;67:429-33.

125. Sosa MV, Rodríguez-Rojo S, Mattea F, Cismondi M, Cocero MJ. Green tea encapsulation by means of high pressure antisolvent coprecipitation. J Supercrit Fluid. 2011;56:304-11.

126. Tian M, Yan H, Row KH. Solid-phase extraction of caffeine and theophylline from green tea by a new ionic liquid-modified functional polymer sorbent. Anal Lett. 2009:43:110-8.

127. Park HS, Im NG, Kim KH. Response surface analysis of caffeine and chlorophyll extraction in supercritical decaffeination of green tea. J Biotechnol. 2008;136:477-85.

128. Park HS, Im NG, Kim KH. Extraction behaviors of caffeine and chlorophylls in supercritical decaffeination of green tea leaves. LWT - Food Sci Technol. 2012;45:73-82.

129. Arab L, Khan F, Lam H. Tea consumption and cardiovascular disease risk. Am J Clin Nutr. 2013;98:1651-59S.

130. Boehm K, Borrelli F, Ernst E, Habacher G, Hung SK, Milazzo S, et al. Green tea (Camellia sinensis) for the prevention of cancer. Cochrane Database Syst Rev. 2009;3:CD005004.

131. Grassi D, Desideri G, Di Giosia P, De Feo M, Fallini E, Cheli P, Ferri L, Ferri C, et al. Tea, flavonoids, and cardiovascular health; Endothelial protection. Am J Clin Nutr. 2013;98:1660-66S.

132. 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;12:CD008650.

133. Kim A, Chiu A, Barone MK, Avino D, Wang F, Coleman CI, et al. Green tea catechins decrease total and low-density lipoprotein cholesterol: A systematic review and meta-analysis. J Am Diet Assoc. 2011;111:1720-9.

134. Shen C-L, Chyu M-C, Wang J-S. Tea and bone health: Steps forward in translational nutrition. Am J Clin Nutr. 2013;98:1694-9S.

135. Yuan J-M. Cancer prevention by green tea: Evidence from epidemiologic studies. Am J Clin Nutr. 2013;98:1676-81S.

136. Hursel R, Viechtbauer W, Westerterp-Plantenga MS. The effects of green tea on...
weight loss and weight maintenance: a meta-analysis. Int J Obes (Lond). 2009;33:956-61.

137. Phung OJ, Baker WL, Matthews LJ, Lanosa M, Thorne A, Coleman CI. Effect of green tea catechins with or without caffeine on anthropometric measures: A systematic review and meta-analysis. Am J Clin Nutr. 2010;91:73-81.

138. Zheng XX, Xu YL, Li SH, Hui R, Wu YJ, Huang XH. Effects of green tea catechins with or without caffeine on glycemic control in adults: A meta-analysis of randomized controlled trials. Am J Clin Nutr. 2013;97:750-62.

139. Liu K, Zhou R, Wang B, Chen K, Shi LY, Zhu JD, Mi MT. Effect of green tea on glucose control and insulin sensitivity: A meta-analysis of 17 randomized controlled trials. Am J Clin Nutr. 2013;98:340-8.

140. Zheng XX, Xu YL, Li SH, Liu XX, Hui R, Huang XH. Green tea intake lowers fasting serum total and LDL cholesterol in adults: A meta-analysis of 14 randomized controlled trials. Am J Clin Nutr. 2011;94:601-10.

141. Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, et al. Flavonoids, flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. Am J Clin Nutr. 2008;88:38-50.

142. Taubert D, Roesen R, Schömig E. Effect of cocoa and tea intake on blood pressure: A meta-analysis. Arch Intern Med. 2007;167:626-34.

143. Wang ZM, Zhou B, Wang YS, Gong QY, Wang QM, Yan JJ, et al. Black and green tea consumption and the risk of coronary artery disease: A meta-analysis. Am J Clin Nutr. 2011;93:506-15.

144. Arab L, Liu W, Elashoff D. Green and black tea consumption and risk of stroke: A meta-analysis. Stroke. 2009;40:1786-92.

145. Zheng JS, Yang J, Fu YQ, Huang T, Huang YJ, Li D. Effects of green tea, black tea, and coffee consumption on the risk of esophageal cancer: A systematic review and meta-analysis of observational studies. Nutr Cancer. 2013;65:1-16.

146. Zheng P, Zheng HM, Deng XM, Zhang YD. Green tea consumption and risk of esophageal cancer: a meta-analysis of epidemiologic studies. BMC Gastroenterol. 2012;12:165.

147. Kang H, Rha SY, Oh KW, Nam CM. Green tea consumption and stomach cancer risk: A meta-analysis. Epidemiol Health. 2010;32:e2010001.

148. Myung SK, Bae WK, Oh SM, Kim Y, Ju W, Sung J, et al. Green tea consumption and risk of stomach cancer: A meta-analysis of epidemiologic studies. Int J Cancer. 2009;124:670-7.

149. Wang ZH, Gao QY, Fang JY. Green tea and incidence of colorectal cancer: Evidence from prospective cohort studies. Nutr Cancer. 2012;64:1143-52.

150. Tang N, Wu Y, Zhou B, Wang B, Yu R. Green tea, black tea consumption and risk of lung cancer: A meta-analysis. Lung Cancer. 2009;65:274-83.

151. Ogunleye AA, Xue F, Michels KB. Green tea consumption and breast cancer risk or recurrence: A meta-analysis. Breast Cancer Res Treat. 2010;119:477-84.

152. Tang NP, Li H, Qiu YL, Zhou GM, Ma J. Tea consumption and risk of endometrial cancer: a meta-analysis. Am J Obstet Gynecol. 2009;201:605.e1-8.

153. Zheng J, Yang B, Huang T, Yu Y, Yang J, Li D. Green tea and black tea consumption and prostate cancer risk: An exploratory meta-analysis of observational studies. Nutr Cancer. 2011;63:663-72.

154. Nagle CM, Olsen CM, Bain CJ, Whiteman DC, Green AC, Webb PM. Tea consumption and risk of ovarian cancer. Cancer Causes Control. 2010;21:1485-91.

155. Onakpoya I, Spencer E, Heneghan C, Thompson M. The effect of green tea on blood pressure and lipid profile: A systematic review and meta-analysis of randomized clinical trials. Nutr Metabol Cardiovasc Dis; 2014. DOI:10.1016/j.numecd.2014.01.016.

156. World Health Organization (WHO) guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2007. Accessed 1 March 2014. Available: http://www.who.int/medicines/docs/index/assoc/s14878e.pdf.

157. EPA 40 CFR part 143. Accessed 1 March 2014. Available: http://www.ecfr.gov/cgi-bin/text-idx?tpl=/ecfrbrowse/Title40/40cfr143_main_02.tpl.

158. Joint FAO/WHO Food Standards Programme, CODEX Committee on Contaminants in Foods, Sixth Session, Maastricht, The Netherlands. Working document for information and use in discussions related to contaminants and
toxins in the GSCFTT (General Standard for Contaminants and Toxins in Foods and Feeds), March 26-30, 2012. Accessed 1 March 2014. Available:ftp://ftp.fao.org/codex/meetings/cf/cf06_INFe.pdf.

159. United States Pharmacopeial Convention Chapter 2232 Elemental Contaminants in Dietary Supplements, USP 36-NF31 S1.

160. American Herbal Product Association Guidance Policies. Accessed 2 December 2013. Available:http://www.ahpa.org/default.aspx?tabid=223.

161. Pietta P, Simonetti P, Gardana C, Brusamolino A, Morazzoni P, Bombardelli E. Relationship between rate and extent of catechin absorption and plasma antioxidant status. Biochem Mol Biol Int. 1998;46:895-903.

162. Chow HH, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM, et al. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenol E in healthy individuals. Clin Cancer Res. 2005;11:4627-33.

163. Kapetanovic IM, Crowell JA, Krishnaraj R, Lindeblad M, Lyubimov A. Exposure and toxicity of green tea polyphenols in fasted and nonfasted dogs. Toxicol. 2009;260;28-36.

164. Wu KM, Yao J, Boring D. Green tea extract-induced lethal toxicity in fasted but not in nonfasted dogs. Int J Toxicol. 2011;30:19-20.

165. Ullman U, Haller J, Decourt JP, Girault N, Girault J, Richard-Caudron AS, et al. A single ascending dose study of epigallocatechin gallate in healthy volunteers. J Int Med Res. 2003;31:88-101.

166. Ullmann U, Haller J, Decourt JD, Girault J, Spitzer V, Weber P. Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. Int J Vitam Nutr Res. 2004;74:269-78.

167. Yarnell E, Abascal K. Hepatotoxicity of botanicals. Alt Comp Ther. 2014;20:136-44.

168. Schmidt M, Schmitz HJ, Baumgart A, Guedon D, Netsch MI, Kreuter MH, et al. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. Food Chem Toxicol. 2005;43:307-14.

169. Galati G, Lin A, Sultan AM, O’Brian PJ. Cellular and In vivo hepatotoxicity caused by green tea phenolic acids and catechins. Free Radic Biol Med. 2006;40:570-80.

170. Nishikawa T, Nakajima T, Moriguchi M, Jo M, Sekoguchi S, Ishii M, et al. A green tea polyphenol, epigallocatechin-3-gallate, induces apoptosis of human hepatocellular carcinoma, possibly through inhibition of Bcl-2 family proteins. J Hepatol. 2006;44:1074-82.

171. Lee MJ, Mallakal P, Chen L, Meng X, Bondoc FY, Prabhu S, et al. Pharmacokinetics of tea catechins after ingestion of green tea and (-) -epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev. 2002;11:1025-32.

172. Chan PC, Ramot Y, Malarkey DE, Blackshear P, Kissling GE, Travlos G, et al. Fourteen-week toxicity study of green tea extract in rats and mice. Toxicol Pathol. 2010;38:1070-84.

173. Hsu YW, Tsai CF, Chen WK, Huang CF, Yen CC. A subacute toxicity evaluation of green tea (Camellia sinensis) extract in mice. Food Chem Toxicol. 2011;49:2624-30.

174. Isbrucker RA, Edwards JA, Wolz E, Davidovich A, Bausch J. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: Dermal, acute, and short-term toxicity studies. Food Chem Toxicol. 2006;44:636-50.

175. Isbrucker RA, Edwards JA, Wolz E, Davidovich A, Bausch J. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 3: Teratogenicity and reproductive toxicity studies in rats. Food Chem Toxicol. 2006;44:651-61.

176. Morita O, Knapp JF, Tamaki Y, Stump DG, Moore JS, Memec MD. Effects of green tea catechin on embryo-fetal development in rats. Food Chem Toxicol. 2009;47:1296-1303.

177. Monta O, Kirkpatrick JB, Tamaki Y, Chengelis CP, Beck MJ, Bruner RH. Safety assessment of heat-sterilized green tea catechin preparation: A 6-month repeat-dose study in rats. Food Chem Toxicol. 2009;47:1760-70.

178. Takami S, Imai T, Hasumura M, Cho YM, Onose J, Hirose M. Evaluation of toxicity of green tea catechins with 90-day dietary administration to F344 rats. Food Chem
Pancreat Dis Int. 2006;5:268-9.

179. Chengelis CP, Kirkpatrick JB, Regan KS, Radovsky AE, Beck MJ, Morita O, et al. 28-Day oral (gavage) toxicity studies of green tea catechins prepared for beverages in rats. Food Chem Toxicol. 2008;46:978-89.

180. Hurst R, Westerterp-Plantenga. Catechin- and caffeine-rich teas for control of body weight in humans. Am J Clin Nutr. 2013;98:1682-93S.

181. Lambert JD, Kennett MJ, Sang S, Reuhl KR, Ju J, Yang CS. Hepatotoxicity of high oral dose (-)-epigallocatechin-3-gallate in mice. Food Chem Toxicol. 2010;48:409-16.

182. Bun SS, Bun H, Guédon D, Rosier C, Ollivier E. Effect of green tea extracts on liver functions in Wistar rats. Food Chem Toxicol. 2006;44:1108-13.

183. NTP Technical Report on the Toxicology of Green Tea Extract in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Green Tea Extract in Wistar HAN[Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies). NTP TR 585. NIH Publication No. 14-5927; 2014.

184. Chen JH, Tipone GL, Liong EC, So HS, Leung KM, Tom WM, et al. Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. Am J Clin Nutr. 2004;80:742-51.

185. Bruno RS, Dugan CE, Smyth JA, DiNatale DA, Koo SI. Green tea extract protects leptin-deficient, spontaneously obese mice from hepatic steatosis and injury. J Nutr. 2008;138:323-31.

186. Fiorini RN, Donovan JL, Rodwell D, Evans Z, Cheng G, May HD, Mil liken et al. Short-term administration of (-)-epigallocatechin gallate reduces hepatic steatosis and protects against warm hepatic ischemia/reperfusion injury in steatotic mice. Liver Transpl. 2005;11:298-308.

187. Dobrzenska I, Sniecinska A, Skrzydlew ska E, Figaszewski Z. Green tea modulation of the biochemical and electric properties of rat liver cells that were affected by ethanol and aging. Cell Mol Biol Lett. 2004;9:709-21.

188. Zhang XG, Xu P, Liu Q, Yu CH, Zhang Y, Chen SH, et al. Effect of tea polyphenols on cytokine gene expression in rats with alcoholic liver disease. Hepatobiliary Pancreat Dis Int. 2006;5:268-72.

189. Lee MJ, Wang ZY, 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;4:393-9.

190. Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol Biomarkers Prev. 1998;7:351-4.

191. 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;9:3312-9.

192. Gruenwald J, Brendler T, Jaenike C, editors. PDR for herbal medicines. 1st ed. Medical Economics Co. Inc. Montvale, NJ. 1998.

193. NIH. Green Tea (Camellia sinensis). LiverTox. 2013a. Accessed 26 October 2013. Available: http://livertox.nlm.nih.gov/GreenTea.htm.

194. García-Cortés M, Stephens C, Lucena MI, Fernández-Castañer A, Andrade RJ. Causality assessment methods in drug induced liver injury: Strengths and weaknesses. J Hepatol. 2011;55:583-91.

195. Federico A, Tiso A, Loguercio C. A case of hepatotoxicity caused by green tea. Free Radic Biol Med. 2007;43:474.

196. Rohde J, Jacobsen C, Kromann-Andersen H. Toxic hepatitis triggered by green tea. Ugeskr Laeger. 2011;173:205-6.

197. Teschke R, Wolff A, Frenzel C, Schulze J, Eickhoff A. Herbal hepatotoxicity: A tabular compilation of reported cases. Liver Int. 2012;32:1543-56.

198. Teschke R, Schwarzenboeck A, Hennermann KH. Causality assessment in hepatotoxicity by drugs and dietary supplements. Br J Clin Pharmacol. 2008;66:758-66.

199. NIH. Drug record. Herbs and dietary supplements. 2013b. Accessed 26 October 2013. Available: http://livertox.nlm.nih.gov/Herbals and Dietary_Supplements.htm.

200. Teschke R, Frenzel C, Schulze J, Eickhoff A. Herbal hepatotoxicity: challenges and pitfalls of causality assessment methods. World J Gastroenterol. 2013;19:2864-82.

201. Frank J, George TW, Lodge JK, Rodriguez-Mateos AM, Spencer JPE.
Minihane AM, et al. Daily consumption of an aqueous green tea extract supplement does not impair liver function or alter cardiovascular disease risk biomarkers in healthy men. J Nutr. 2009;139:58-62.

202. Tsao AS, Martin J, TangX-M, Lee JJ, El-Naggar AK, Wistuba I, Culotta et al. Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions. Cancer Prev Res. 2009;3:1099-117.

203. Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res. 2006;66:1234-40.

204. Matsuyma T, Tanaka Y, Kaminmaki I, Nagao T, Tokimitsu I. Catechin safely improved higher levels of fatness, blood pressure, and cholesterol in children. Obesity. 2008;16:1338-48.

205. McLarty J, Bigelow RLH, Smith M, Elmajian D, Ankem M, Cardili JA. Tea polyphenols decrease serum levels of prostate-specific antigen, hepatocyte growth factor, and vascular endothelial growth factor in prostate cancer patients and inhibit production of hepatocyte growth factor and vascular endothelial growth factor. In vitro. Cancer Prev Res. 2009;2:673-82.

206. Fontana RJ, Seeff LB, Andrade RJ, Björnsson E, Day CP, Serrano J, et al. Standardization of nomenclature and causality assessment in drug-induced liver injury: summary of a clinical research workshop. Hepatol. 2010;52:730-42.

207. Larrey D, Faure S. Herbal medicine hepatotoxicity: A new step with development of specific biomarkers. J Hepatol. 2011;54:599-601.

208. Appelhans K, Frankos V. Herbal medicine hepatotoxicity revisited. J Hepatol. 2012;56:504-5.

209. Corsini A, Ganey P, Ju C, Kaplowitz N, Pessayre D, Roth R, Watkins PB, et al. Current challenges and controversies in drug-induced liver injury. Drug Saf. 2012;35:1099-117.

210. AFSSAPS. French Agency for Sanitary Safety of Health Products. Press release. Suspension of the authorization for placing on the market of the medicinal product EXOLISE® (epigallocatechin gallate); 2003. Accessed 14 July 2014. Available: http://www.ansm.sante.fr/S-informer/Presse-Communiques-Points-presse/Suspension-de-l-autorisation-de-mise-sur-le-marche-de-la-specialite-pharmaceutique-EXOLISE-R-gallate-d-epigallocatechol

211. García-Cortés M, Borraz Y, Lucena MI, Peláez G, Salmerón J, Diago M, et al. Liver injury induced by “natural remedies”: An analysis of cases submitted to the Spanish Liver Toxicity Registry. Rev Esp Enferm Dig. 2008;100:688-95.

212. AFSSA. French Agency for Food Safety. Communication to the Working Group; 2009.

213. USP (U.S. Pharmacopeia). Powdered decaffeinated green tea extract: In-process revision. Pharmacopeial Forum. 2007;33:1220-3.

214. USP (U.S. Pharmacopeia). Update on the USP green tea extract monograph. 10 April 2009. Accessed 26 December 2013. Available: http://www.usp.org/uspnf/notices/retired-compendial-notices/update-usp-green-tea-extract-monograph.

215. Jin X, Zheng RH, Li YM. Green tea consumption and liver disease: A systematic review. Liver Int. 2008;28:990-6.

216. EFSA. EFSA Scientific Cooperation (ESCO) Report. Advice on the EFSA guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements, based on real case studies. EFSA J. 2009;7:280.

217. ANSES. French Agency for Food, Environmental and Occupational Health Safety. [The evaluation of risk of hepatotoxicity related to consumption of foods containing green tea.] 21011-SA-0108. 2012. Accessed 14 July 2014. Available:http://www.anses.fr/Documents/NUT2011sa_0108.pdf

218. EFSA Guidance. Guidance on the safety assessment of botanicals and botanical preparations intended for us as ingredients in food supplements. EFSA J. 2009;7:1249.

219. NFSA. 2014. Accessed 26 October 2013. Available: http://www.matsportalen.no/verktøy/advarsler/gronn_te_ekstrakt_kan Gi lever bivirkninger.

220. DGCCRF. Directorate General for
Competition and Consumer del Fraud. Re: Restriction on the use of green tea preparations in food supplements. Note of information no. 2011-2060; 2011.

221. Belgisch Staatsblad. [Royal Order for botanicals.] 2012. Accessed 14 July 2014. Available: 15 August 2013. Available: http://www.ejustice.just.fgov.be/mopdf/2012/04/04_1.pdf.

222. Hathcock JN, Shao A. Expanded approach to Tolerable Upper Intake guidelines for nutrients and bioactive substances. J Nutr. 2008;138:1992-5S.

223. EMA. European Medicines Agency, Committee for Medicinal Products for Human Use. 22 March 2007. Accessed 18 January 2014. Available: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC50002989.pdf.

224. Renwick AG. Risk characterization of chemicals in food. Toxicol Lett. 2004;149:163-76.

225. PlantLIBRA. EC Project No. 245199. 2013. Accessed 26 October 2013. Available: http://www.plantlibra.eu/web/node/44.

226. FDA. 21 CFR 182.20 Part 182 -- Substances generally recognized as safe, Subpart A--General Provisions. Sec. 182.20 Essential oils, oleoresins (solvent-free), and natural extractives (including distillates). In: Services DoHaH, editor. Washington, DC: United States Food and Drug Administration; 2012. Accessed 8 November 2013. Available: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCfr/CFRSearch.cfm?fpr=182.20.

227. AIBMR Life Sciences, GRAS Self-determination Inventory Database. Accessed 1 March 2014. Available: http://www.aibmrm.com/resources/GRAS-database.

228. Assuming maximum use of 540 mg catechins/350 mL, with up to 1 to 1.5 g green tea catechins presumed safe; FDA GRN No. 225 & 259. Accessed 1 March 2014. Available: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn000225.pdf.

229. FDA GRN No. 338. Accessed 1 March 2014. Available: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/GRN000338.pdf.

230. FAO/WHO. A model for establishing upper levels of intake for nutrients and related substances. FAO/WHO Technical Workshop on Nutrient Assessment. Geneva. 2005. Accessed 22 April 2014. Available: http://www.who.int/ ipcshighlights/full_report.pdf.

231. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes: A risk assessment model for establishing upper intake levels for nutrients. National Academy Press. Washington, DC; 1998.

232. Taylor CL, Tetley EA. Nutrient risk assessment as a tool for providing scientific assessments to regulators. J Nutr. 2008;138:1996-2002.

233. Lambert JD, Sang S, Yang CS. Possible controversy over dietary polyphenols: Benefits vs. risks. Chem Res Toxicol. 2007;20:583-5.