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The Role of Green Tea Polyphenols in the Protection from Hexavalent Chromium-Induced Genotoxic Damage

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Additional information is available at the end of the chapter

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Abstract

In this chapter, the proposal that green tea polyphenols can be used effectively to protect against genotoxic effects associated with hexavalent chromium (Cr(VI)) exposure is analyzed. After explaining the chemical mechanisms involved in oxidative stress associated with the reduction of Cr(VI) compounds, the relationship between green tea polyphenols and oxidative stress is analyzed. Particular emphasis is given in elucidating how these proposals fit with our own experimental results with green tea polyphenols and Cr(VI) compounds, which show an increase of apoptotic cells and a decrease in micronucleus frequency. Finally, the gaps in our understanding of the role of green tea and its polyphenols, as well as their key importance to human health, are highlighted.

Keywords: green tea polyphenols, genotoxic damage, hexavalent chromium, antioxidants, oxidative stress

1. Introduction

Recently, the food industry and the consumer sector have shown a growing interest in the research, development and commercialization of beverages with high nutritional content and particular properties relevant to human health. In this context, green tea infusions (*Camellia sinensis*) are rich in bioactive compounds, particularly in phenolic compounds with antioxidant activity. It is therefore not surprising that green tea has attracted significant attention for

its positive effects on health-related issues of oxidative stress such as cancer, cardiovascular and neurodegenerative diseases [1, 2].

The tea manufacturing process is designed to either preclude or permit tea polyphenolic compounds to be oxidized by naturally occurring polyphenol oxidase in the tea leaves during fermentation (white and green tea are unfermented; oolong tea is semi-fermented; and black tea is fully fermented). Green tea is produced by inactivating the heat-labile enzyme polyphenol oxidase in fresh leaves by either applying heat or steam, which prevents the enzymatic oxidation of polyphenolic compounds. Although the components of green tea include proteins, carbohydrates, lipids, alkaloids, vitamins and minerals, its health-beneficial properties are attributed mainly to its high content of catechins (flavan-3-ols, or flavanols), such as (–)-epicatechin (EC), (–)-epigallocatechin (EGC) and their gallate forms (+)gallocatechin (GC), (–)epicatechin-3-gallate (ECG) and (–)epigallocatechin-3-gallate (EGCG). Recent studies have also identified biological functionality of other phenolic compounds found at lower concentrations, particularly flavonols and phenolic acids [1, 3–5].

A cup of green tea contains approximately 300 mg of catechins. It is considered that EGCG intake in the form of green tea infusions should be safe up to a maximum consumption of 734 mg EGCG/person/day and even a regular or high dose of green tea (8–16 cups a day) has positive effects in general health [4–7]. The catechin content of green tea also depends on a number of factors including the growing conditions of the plant, age of leaves harvested and the method used to prepare the infusion [4, 8].

In the last part of the twentieth century, interest in food polyphenols has increased due to activities such as free radical scavenging, modulation of signal transduction and metal chelation, as well as anti-inflammatory, anti-microbial and anti-proliferation activities [9–11]. In addition, polyphenols may exert an indirect antioxidant effect by protecting endogenous antioxidant enzymes in the human body [12]. Thus, substances with antioxidant properties such as polyphenols emerge as putative preventives and coadjuvants in the treatment of chronic degenerative diseases related to oxidative stress and DNA damage [13].

2. Oxidative stress, antioxidants and green tea flavonoids

“Oxidative stress” is a term used mainly in the fields of biology and medicine since 1985. Initially, it was defined as the lack of balance between the formation of reactive oxygen species (ROS) and molecules capable of counteracting their action (antioxidant defense system). Naturally, as our understanding has increased over the past years, this concept has been accordingly redefined and more elements like the interruption of signaling and redox control have been added [14]. Nevertheless, oxidative stress has always had a negative connotation because it has been linked to various potentially severe human diseases, including neurological diseases such as Alzheimer’s and Parkinson’s, and metabolic diseases, like diabetes and atherosclerosis, in addition to being involved in the development of some types of cancers, inflammatory processes and cardiomyopathies, among others [15].

The terms “ROS” and “free radicals” are often used interchangeably. However, it is important to note that even though both terms might fulfill operational and practical purposes in some contexts, they are not always fully interchangeable. The term “free radicals” refers to a reactive chemical species that has an unpaired electron in its last orbital, identified in the nomenclature as a dot “•,” which makes them highly reactive species. However, ROS includes oxygenated free radicals, such as the superoxide radical ($\bullet\text{O}_2^-$) and the hydroxyl radical ($\bullet\text{OH}$), as well as the oxygenated molecule precursors of free radicals, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$) [15, 16]. In short, all oxygenated free radicals are ROS, but not all ROS are free radicals.

The generation and elimination of ROS are closely related processes. Living organisms possess regulatory systems to maintain ROS at safe levels, that is, their production and elimination are well balanced. However, under certain circumstances this balance can be disturbed. These include (i) increased level of endogenous and exogenous compounds entering autoxidation coupled with ROS production; (ii) depletion of reserves of low molecular mass antioxidants; (iii) inactivation of antioxidant enzymes; (iv) decrease in the production of antioxidant enzymes and low molecular mass antioxidants; and, finally, (v) certain combinations of two or more of the listed above factors [16]. When ROS levels increase, aerobic organisms employ defense mechanisms such as “antioxidants” which remove reactive species or transform them into stable molecules. The maintenance of tissue redox homeostasis is only possible through a balance between the generation and elimination of ROS. Therefore, an antioxidant can be defined as a molecule capable of delaying or preventing the oxidation of the substrate when it is at a lower concentration than the oxidizable substrate. In biological terms, a good antioxidant should be characterized by high effectiveness, versatility and operational variability to prevent formation, inhibit propagation and enhance the elimination of ROS and stimulate cell repair processes. In addition, they may act as chelating agents, inhibitors of oxidizing enzymes or cofactors of antioxidant enzymes [17].

Antioxidants can be classified as enzymatic or non-enzymatic based on their reactivity to ROS. Enzymatic antioxidants metabolize and stabilize ROS, while non-enzymatic antioxidants sequester metals that participate in the formation of ROS [17]. Therefore, the “first line of defense” is identified as the enzymatic antioxidant system, whose main function is to reduce the production of ROS by preventing interaction between reactive species or with transition metals that could give rise to species of greater reactivity. Since an imbalance or interference in the equilibrium of these enzymes could favor the increase of ROS and therefore cause cellular damage, the cellular maintenance of this system is essential for homeostasis.

The enzymatic antioxidant system is based on the joint action of three systems: (i) superoxide dismutase (SOD) catalyzes a dismutation reaction where one molecule of $\bullet\text{O}_2^-$ is oxidized to O_2 , while the other is reduced to H_2O_2 ; (ii) catalase (CAT) catalyzes the reduction of H_2O_2 into H_2O and O_2 and (iii) glutathione peroxidases (GPx) catalyze the reduction of a large variety of peroxides (including H_2O_2) with the aid of a hydrogen acceptor substrate, in this case glutathione (GSH), which is oxidized (GSSG) and then returned to its original state by the enzyme glutathione reductase [17, 18]. In addition to the endogenous enzymatic system, the intervention of other non-enzymatic compounds, the “second line of defense,” is essential

to ensure redox cell homeostasis. Reduced thiols and low molecular weight antioxidants like coenzyme Q, urate, lipoic acid and GSH are some examples of these non-enzymatic antioxidant compounds.

On the other hand, some exogenous dietary antioxidants can interfere with oxidative cycles to inhibit or retard oxidative damage to biomolecules. The major classes of compounds with antioxidant activity are ascorbate (AsCH⁻), tocopherol, carotenoids and polyphenols. These compounds show significant antioxidant power in the organism and can reach up specific sites of the cell with oxidative damage. Furthermore, it has been shown that these compounds also contribute to the endogenous antioxidant defense. It is suggested that the total amount and position of OH groups in the structure of these compounds may play a role in their anti-ROS activity (12, 17). **Figure 1** shows the main dietary sources of these compounds.

AsCH⁻ is the water-soluble bioactive form of vitamin C and is present in all body fluids. At physiological pH, 99% of vitamin C is present as AsCH⁻, 0.05% in the form AsCH₂ and 0.004% as dianion ascorbate (Asc₂⁻). AsCH⁻ is the chemical form that confers its main antioxidant effects. The antioxidant activity of vitamin C is either direct, through the purification of ROS, or indirect, through the regeneration of other antioxidant systems. Its antioxidant effects have been observed both in vitro and in vivo [19, 20].

Tocopherols and tocotrienols make up vitamin E. In humans, α-tocopherol is particularly important because it is found in cell membranes and plasma lipoproteins. The reactivity of

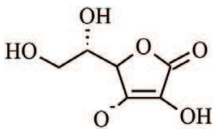
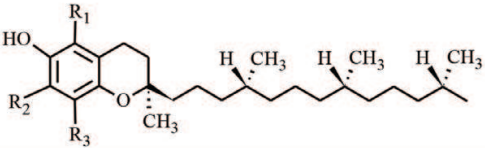
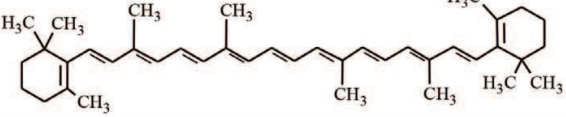
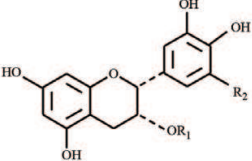
Structure of Antioxidants	Main Source in the Diet
Ascorbates 	Kiwi, strawberries, oranges, melon, grapefruit, red or green pepper, mango, broccoli, brussels sprouts, tomato.
Tocopherols 	Almonds, hazelnuts, peanuts, walnuts, sesame, rice bran, wheat germ, coconut, soy, olives, vegetable oils (palm, sunflower, olive).
Carotenes 	Carrots, papaya, tomatoes.
Polyphenols 	Green tea, wine (mainly red), grapes, cranberry, cherry tomato, soy, cherry, pomegranate, cabbage and red onions.

Figure 1. Main sources of diet with high in the antioxidant compounds ascorbate, tocopherol, carotenoids and polyphenols.

tocopherols with the organic peroxy radicals is associated with the redox properties of the chroman ring, which is responsible for its antioxidant capacity. Peroxy radicals formed during lipoperoxidation have a higher affinity for α -tocopherol OH, which makes it a less active

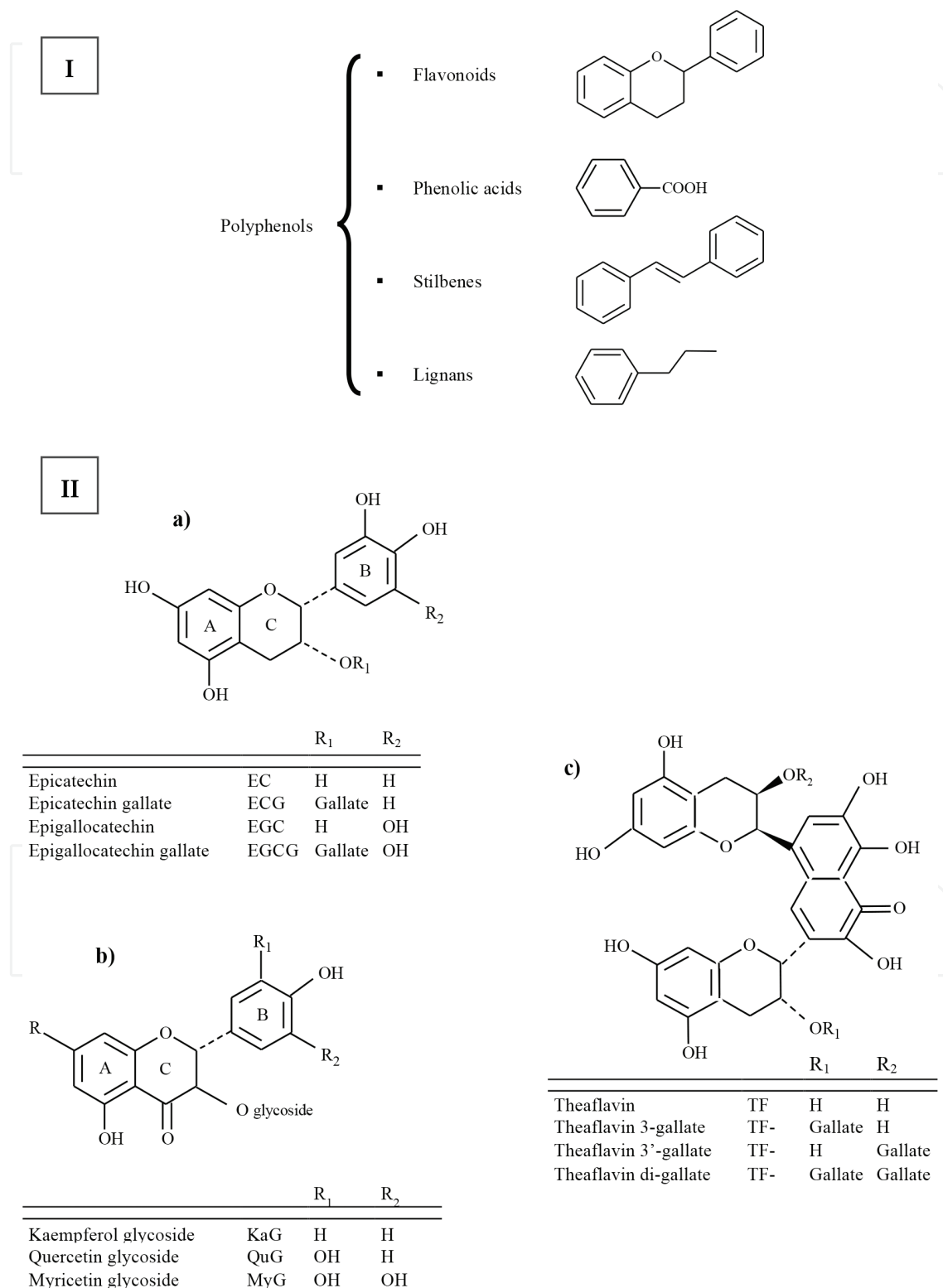


Figure 2. (I) The classification of the phenolic structures of polyphenols. (II) Structure of flavonoids; the core structure contains a diphenylpropane skeleton. The main flavonoids found in fresh green tealeaves are: (a) flavanols, (b) flavonols and (c) theaflavins.

radical and unable to react with other fatty acids, thus stopping the chain of lipoperoxidation reactions. At this point AsCH^- plays an important role as it regenerates the antioxidant form of vitamin E [21].

Carotenoids are lipid-soluble antioxidants and can react with ROS by three possible mechanisms: electron transfer, hydrogen abstraction and radical addition. The antioxidant activity of carotenoids is mainly due to their double-bond conjugate structure that can delocalize unpaired electrons, hence the excellent ability of carotene to neutralize $^1\text{O}_2$, $\bullet\text{O}_2^-$, $\bullet\text{OH}$ and peroxy radicals ($\text{ROO}\bullet$). Nevertheless, it has to be highlighted that although carotenoids are considered antioxidants, there is not enough evidence yet to support the notion that carotenoids actually function as antioxidants *in vivo*, except for their well-documented role as photoprotectors in the inhibition of $^1\text{O}_2$ generated by UV light in the skin and in the eyes [17, 22].

Polyphenols are compounds with variable phenolic structures that are generally classified as flavonoids, phenolic acids, stilbenes and lignans (**Figure 2 I**). The flavonoid compounds have a central structure containing a diphenylpropane skeleton. The primary flavonoids found in fresh green tea leaves are flavanols, flavonols and theaflavins (**Figure 2 II**). It has been observed that polyphenols act as inhibitors of lipoperoxidation and are capable of interacting directly with ROS, as well as acting as chelating agents, and they have indirect effects through their ability to modulate the levels of transcription factors and enzymes [23]. Furthermore, in the context of prophylaxis and cancer therapy polyphenols have manifested beneficial effects through the cytoprotective antioxidant response and proapoptotic action [24]. It has been observed that the anticarcinogenic activity of polyphenols is attributed to their pro-oxidant properties, which occur under certain conditions (i.e., low or very high concentration and presence of metal ions) increasing oxidant DNA damage [25, 26].

The flavonoids are the most powerful and effective antioxidants among the known plant phenols. For instance, EGCG is 20 times more active than vitamin C and 30 times more active than vitamin E. Just like other molecules, the chemical structures of catechins contribute to their antioxidant properties. Some catechins, including EGCG, possess an esterified gallate moiety at the third position of the C ring, the catechol group on the B ring and the OH groups at the fifth and seventh positions on the A ring (**Figure 2 II**). The potential free radical scavenging activity of EGCG has been attributed to the presence of the gallate group [10, 27].

3. Genotoxic roles of chromium and oxidative stress

The initial stages of the biological processes of mutagenesis, carcinogenesis and aging show permanent alterations of the genetic material. In fact, it has been well documented that in various cancer tissues, free radical-mediated DNA damage has occurred. Of all the ROS (half-life <1 ns), $\bullet\text{OH}$ is the most reactive and interacts with all components of the DNA molecule, inducing single- or double- stranded DNA breaks, DNA cross-links and purine, pyrimidine or deoxyribose modifications [22, 28, 29]. Most of the hydroxyl radicals ($\bullet\text{OH}$) generated

in vivo are derived from the metal-catalyzed breakdown of hydrogen peroxide (H_2O_2) via the Fenton and Haber-Weiss reactions [30, 31]:



Exposure to transition metal ions(n^+) such as chromium (Cr) represent a real in vivo production of ROS and free radicals due to intra-cellular reduction, since it has been established that redox-active metals participate closely in the generation of different free radicals [32]. The main genotoxic mechanism of Cr(VI) compounds has been linked to the intracellular reduction and generation of $\bullet\text{OH}$ [33, 34]. Furthermore, the way Cr(VI) produces ROS is a sophisticated step-wise process that starts by entering the cells through the mechanism of pinocytosis and endocytosis using channels for the transfer of isoelectric and isostructural anions, such as those for SO_4^{2-} and HPO_4^{2-} [35]. Inside the cell, Cr(VI) immediately binds with GSH-forming complexes, which causes it to reduce to Cr(V) and Cr(IV) intermediates (**Figure 3 R-I**). Alternatively, nicotinamide adenine dinucleotide phosphate (NAD(P)H) can reduce Cr(VI) to Cr(V), mediated by Asch⁻ (**Figure 3 R-II**). The generated Cr(V) and Cr(IV) intermediates can react with H_2O_2 forming $\bullet\text{OH}$ and $^1\text{O}_2$ [33, 36] via the Fenton reaction (**Figure 3 R-III**).

Nevertheless, the genotoxic mechanism of Cr(VI) can be neutralized or altered. Antioxidants such as Asch⁻ could react with $\bullet\text{OH}$, quenching and converting it into a poorly reactive semi-hydroascorbate radical, which is harmless to the DNA molecule. The C8-OH-adduct radical of deoxyguanosine is formed during catalysis of $\bullet\text{OH}$ in the reaction of 2-deoxyguanosine with molecular oxygen, (**Figure 3 R-IV**); since it induces DNA strand breaks, it is considered a form of oxidative DNA damage [37, 38]. Therefore, by activating repair mechanisms, this adduct can be removed through 8-hydroxydeoxyguanosine (8-OHdG, 7,8-dihydro-8-oxodeoxyguanosine), which is a marker repairer of oxidative stress in biological systems that can be measured in fluids such as blood, urine and saliva (**Figure 3 R-VII**). 8-OHdG undergoes keto-enol tautomerism, which favors the oxidized 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxo-dG) product. In the scientific literature both 8-OHdG and 8-oxo-dG are equivalent and refer to the same compound. The formation of the 8-OHdG adduct is of particular importance as it indicates the interaction between $\bullet\text{OH}$ and guanine [22, 39].

Although the direct relationship between DNA damage and $\bullet\text{OH}$ is not completely clear, it has been suggested that the ROS have a role in Cr(VI)-induced genotoxicity and cytotoxicity by showing Cr(VI)-induced genomic DNA damage through the formation of 8-OHdG [40]. Furthermore, it has also been observed that Cr(VI) produces oxidative stress by inducing time- and concentration-dependent cytotoxicity through suppression of antioxidant systems and by activation of p53-dependent apoptosis [41]. Other studies have called into question the genotoxic/mutagenic effect of $\bullet\text{OH}$ by Cr exposure, suggesting that reduction of Cr(VI) by physiological concentrations of Asch⁻ generates ascorbate-Cr(III)-DNA cross-links and binary Cr(III)-DNA adducts. Therefore, Cr-DNA adducts are responsible for both the mutagenicity and genotoxicity of Cr(VI) [42].

increase risk in human populations [36, 43–46]. There are three ways in which Cr(VI) could induce effects on human health: first, by the generation of $\bullet\text{OH}$ (oxidative stress); second, by the modification of antioxidant enzymes like SOD, CAT and peroxidase (POX) [10, 36, 46]; and finally, by the intervention of non-oxidative mechanisms of Cr(VI) [47].

It has been demonstrated that Cr(VI) compounds induce DNA damage, gene mutation, sister chromatid exchange, chromosomal aberrations, micronuclei and cell transformation. Dominant lethal mutations have also been observed in a variety of test systems in cultured human and animal cells and in experimental animals. These effects are related to multiple mechanisms of DNA damages including DNA adducts, DNA modification caused by the covalent attachment of a chemical, cross-links such as DNA protein cross-links and DNA–DNA cross-links, abasic sites and oxidized DNA bases. Cr(VI) also plays a critical role in altering gene expression [10, 36].

4. Protection against chromium(VI)-induced DNA damage by green tea polyphenols

Green tea and its polyphenols have shown the ability to quench free radicals generated by oxidative environmental toxicants and, consequently, to reduce genotoxic damage and cancer [48]. Particularly, it has been observed that the administration of green tea to mice CD-1 protects against genotoxic damage induced by metal compounds with carcinogenic potential such as Cr(VI), suggesting that its antioxidant compounds such as polyphenols have an antigenotoxic effect on the oxidative stress generated during reduction of Cr(VI) to Cr(III) [49]. However, the protection is only partial, and this may be related to different factors such as the origin of the tea, because the amount of polyphenols in plants is influenced by environmental factors (i.e., weather, light, nutrients, preparation process, storage, horticulture leaf age, etc.) [50]. In order to eliminate this source of variation, the effects of polyphenols (polyphenon60®, extracted from green tea) have been evaluated directly. The results showed that these polyphenolic extracts reduce almost 100% of the genotoxic damage induced by Cr(VI) compounds [13].

In other studies in which specific polyphenols of green tea have been tested individually, it has been observed that protection from the genotoxic damage induced by Cr(VI) compounds has the following order: rutin (82%) > EGCG (71%) > quercetin (64%) > quercetin-rutin (59%) (**Figure 4**) [10, 25]. Due to their phenolic structure, it is possible that these polyphenols may act as hydrogen donors to suppress the formation of lipid radicals and free radicals, including the $\bullet\text{O}_2^-$ and $\bullet\text{OH}$ generated during reduction of Cr(VI) to Cr(III), in addition to being able to chelate metals [51, 52]. The decrease in genotoxic damage by these extracted polyphenols was greater than those observed when administering green tea or red wine (**Figure 4**) [49, 53].

Apparently, the sugar of rutin makes it more efficient by protecting against the genotoxic damage induced by Cr(VI) by increasing its bioavailability and absorption. Rutin is hydrolyzed to its aglycone forms (quercetin) by β -glycosidase and is thus metabolized more slowly, which leads to increased activity [54]. The route of administration of polyphenols plays an important

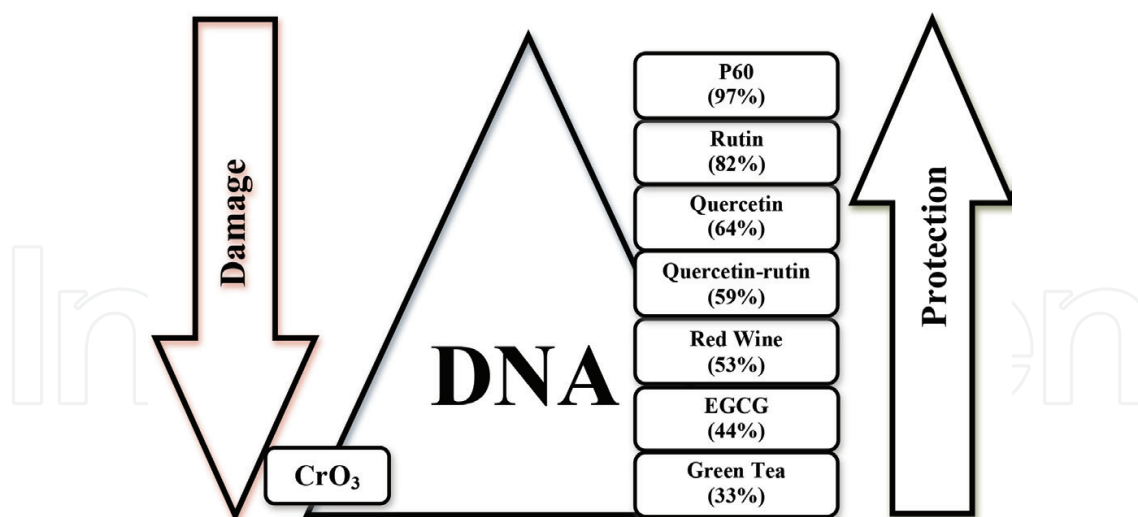


Figure 4. Levels of protection of different compounds from DNA damage caused by CrO_3 .

role in how efficiently they protect against genotoxic damage. For example, EGCG protected cells against Cr(VI)-induced genetic damage more effectively when administered orally than when administration following the ip route was done [10, 25]. While the ip route is more sensitive and direct [55] and therefore useful for detecting inducibility of micronuclei in polychromatic erythrocytes in short-term protocols (peripheral blood); when testing compounds with potential clastogenic properties, it is an artificial exposure route, and the route for human exposure to EGCG is oral. This is important because it has been observed that polyphenols might be biotransformed into more bioavailable forms in the gut [56] sometimes by intestinal bacteria [57]. Therefore, it is considered that the effects of polyphenols may be affected by (i) the kinetics of their absorption and elimination, (ii) the nature and the extent of their metabolism (e.g., conjugation and methylation) and (iii) the activity of each circulating compound [25].

There are two ways in which polyphenols can protect from DNA oxidative damage induced by Cr(VI) compounds. First, polyphenols can react with $\bullet\text{OH}$, generating an unreactive radical and therefore preventing damage to DNA (**Figure 3 R-V**). Second, polyphenols can activate repair mechanisms to remove adducts through 8-OHdG (**Figure 3 R-VI**) which is subsequently eliminated (**Figure 3 R-VII**). If the oxidative damage to DNA is not repaired, breaks can lead to formation of micronuclei [13]. The administration of green tea polyphenol extracts and EGCG led to an increase in the average number of apoptotic cells. Even when green tea polyphenol extracts and EGCG was administered prior to Cr(VI), the frequency of apoptotic cells was higher than with Cr(VI) treatment alone. The enhanced induction of apoptosis following polyphenols and Cr(VI) treatments suggests that this process may contribute to elimination of the cells with Cr(VI)-induced DNA damage (micronuclei) [10, 13]. Also, it has been observed that in vivo dietary polyphenols in combination with other antioxidants such as ascorbic acid enhance inhibition of micronuclei formation induced by endogenous nitrosation in mice [58]. This proposal is consistent with the observed protection against genetic damage by antioxidants, since the frequencies of apoptotic cells increase with the administration of

antioxidants. Hence, it is suggested that the combined treatments of antioxidants contribute positively to the elimination of cells with DNA damage through apoptosis [10, 59].

Apoptosis plays a crucial role in a number of physiological and pathological processes and is accompanied by characteristic morphological changes that include cytoplasmic shrinkage, plasma membrane blebbing, condensation or fragmentation of nuclei and extensive degradation of chromosomal DNA. Polyphenols are capable of regulating cell signaling pathways related to proliferation and apoptosis [60, 61]. It has been observed that polyphenols such as EGCG not only protect normal cells against genotoxic alterations induced by N-methyl-N'-nitro-N-nitrosoguanidine but that they are able to remove cancer cells by apoptosis in vitro [62]. In addition to other mechanisms, at a human achievable dose, EGCG is known to activate cell death signals and to induce apoptosis in precancerous or cancer cells, resulting in inhibition of tumor development and/or progression [63]. Therefore, it is plausible that substances able to induce apoptosis in cancer cells could be used as new anticancer agents. In fact, these findings suggest and strongly encourage more investigation into the potential of polyphenols in the treatment of cancer. Currently, few clinical trials are being carried out, and further studies are urgently needed to assess the anticancer activity of polyphenols in vivo.

Figure 5 summarizes the proposed interaction between polyphenols and Cr(VI) compounds; polyphenols can: (i) scavenge ROS such as $\bullet\text{OH}$ generated by Cr(VI) during its reduction to Cr(III), inhibiting their genotoxic effects; (ii) reactivate the repair mechanisms inactivated by Cr(VI), contributing to the elimination of 8-OHdG; (iii) regulate cell signaling pathways to eliminate the cells with DNA damage (micronuclei) via apoptosis.

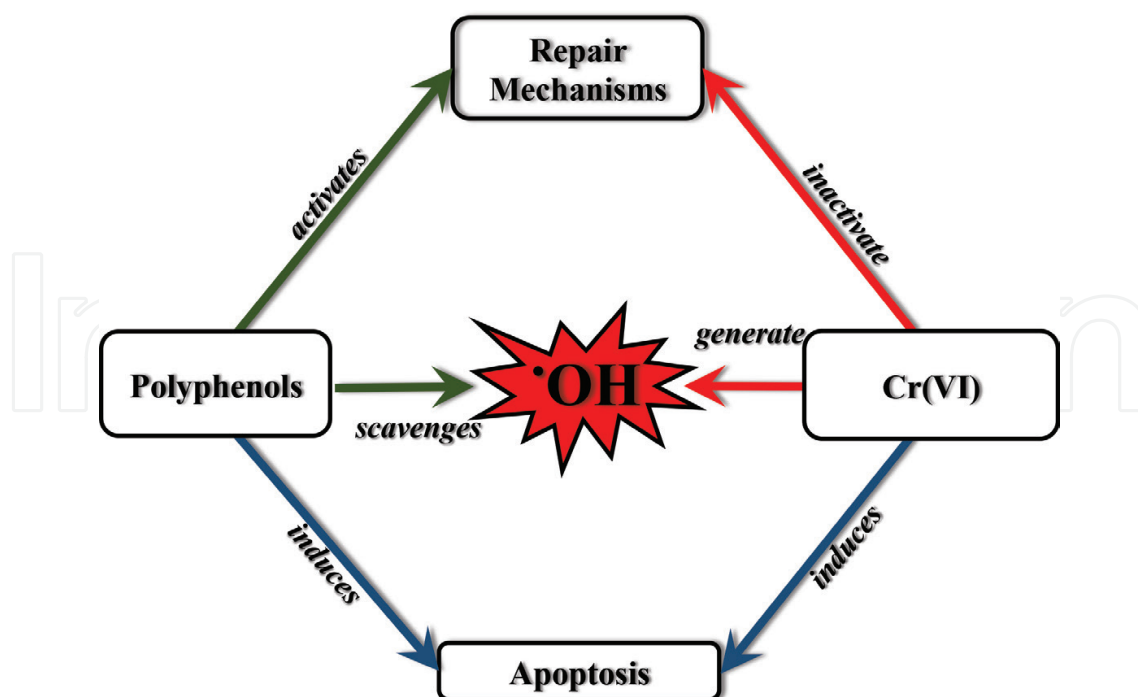


Figure 5. Summary of the interaction between polyphenols and heavy metals.

5. Conclusions

The relationship between diet and health has aroused great scientific interest. The consumption of antioxidants naturally present in the diet is of particular interest due to their action against the harmful effects of oxidative stress. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommend the intake of a minimum of 400 g of fruit and vegetables a day (excluding potatoes and other starchy tubers) to prevent chronic diseases such as cancer, especially in less developed countries, on the basis that at least one-third of all cancers can be prevented [1].

A healthy diet with a sufficient daily intake of fruits and vegetables with a high content of antioxidants can contribute to the prevention of diseases caused by exposure to pollutants with carcinogenic potential, such as heavy metals associated with oxidative stress. Antioxidants found in fresh fruits and vegetables can be easily absorbed and distributed at a physiologically relevant level in tissues and biofluids where they can play an essential role in capturing ROS, chelating redox metals and regenerating other antioxidants within the “antioxidant network.” Dietary antioxidants such as polyphenols are able to protect against genotoxic damage caused by Cr(VI) metal compounds, which could be related to the prevention of carcinogenic processes associated with these metals. Although the main mechanism described for antioxidants is the clearance of ROS, DNA repair and apoptosis are possible additional pathways involved in the protection and modulation of damage to genetic material.

Although compelling new evidence shows promising protective effects of the polyphenols in green tea against genotoxic damage induced by Cr(VI) compounds, there is a lack of clinical evidence that needs to be addressed in future studies. Some suggestions include the development of predictive biomarkers for green tea polyphenols consumption in the human population. These markers will greatly improve our current understanding of the relationship between polyphenols and the endogenous and exogenous factors that affect its bioavailability, which will in turn help establish safe and effective doses for human consumption.

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Conflict of interest

The authors declare that they do not have any competing interests.

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