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Polyphenols

Edited by Janica Wong

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Meet the editor

Dr. Janica Wong is an accomplished research scientist with 17 years of extensive experience in stem cell biology, metabolism, and oncology. She received her BSc degree in biochemistry and MSc degree in physiology from the Hong Kong University, China. Later, she went to the United States and completed her PhD degree at UNLV, with a research focus on the roles of nitric oxide signaling in cancer and stem cells. She conducted her doctoral research under the mentorship of Dr. Fiscus, whose work in nitric oxide with Dr. Murad led to the Nobel Prize in Physiology or Medicine in 1998. Dr. Wong then continued her post-doctoral training at Stanford University to study adult stem cell fate. Her research resulted in patents, book chapters, and many high-impact journal articles. She is currently leading organoids/cancer stem cell research at Merck Research Labs.

Contents

Preface

Polyphenols, which mostly contain phenol structures, are phytochemicals found naturally in many types of food such as nuts, cocoa, berries, tea, and red wine. Emerging studies have shown that polyphenols can have multiple beneficial effects on the human body, such as antioxidative, anticancer, and antidiabetic effects. For example, resveratrol, a polyphenol found in grapes and red wine, has been shown to exert anticancer effects by regulating the cell cycle, cell proliferation, apoptosis, invasion, and metastasis of cancer cells as well as by inhibiting tumor angiogenesis (*i.e.,* inhibiting the formation of new blood vessels that provide extra blood flow to the growing tumor). Many polyphenolic extracts from grapes or olives, like many other natural products, are commercially available as dietary supplements. Although increasing evidence supports the theory that moderate intake of these supplements may act as an antioxidant to help lower risk factors of many diseases, more conclusive research is needed to investigate these mechanisms specifically, for example, how polyphenols help protect cells from free radical damage.

The present book encompasses many key aspects of polyphenols-from classification, dietary sources, health benefits, to possible mechanisms of action. The book chapters were written based on systematic reviews and referencing experimental findings from many published studies.

I would like to thank all the authors who contributed to very interesting topics on polyphenols, including polyphenols from olive oil, green tea, red wine, flavonoids, their bioavailability, and the use of polyphenols in technology, e.g., bone implant devices. This book is aimed at a wide range of readers and it is hoped that this book can help readers gain scientific knowledge and a more in-depth understanding of why and how polyphenols can exert multiple health benefits.

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Section 1

Polyphenols in Diet: The Benefits and Possible Mechanisms

Chapter 1

A Systematic Review: Polyphenol Contents in Stressed-Olive Trees and Its Fruit Oil

Muhittin Kulak and Hakan Cetinkaya

Additional information is available at the end of the chapter

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Abstract

Olive oil includes high amounts of phenols and polyphenols. Through health benefits to humans, the antioxidant role of polyphenols that contain more than two phenolic hydroxyl groups has been well proven. Of those polyphenols, oleuropein, hydroxytyrosol, catechin, chlorogenic acids, hesperidin, nobiletin, and isoflavones are major compounds. Along with the present study, (1) the uses and biological roles of polyphenols have not been limited to their physiological roles to human health; their physiological roles for plant and aromatic values for plant are also evaluated; (2) possible roles of major components in response to environmental stressors are discussed; (3) bibliometric analysis of studies concerned with polyphenols in olive fruit oil has been done to evaluate the research trends concerned with polyphenol in olive fruit oil, considering the main theme of the studies. The study is concluded with highlights, limitation, and future outlooks.

Keywords: olive fruit oil, olive leaves, polyphenol, Turkey, *Olea europaea* L., plant stress physiology

1. Introduction

Stress is a difficult concept to be described in biology. Briefly described in various forms, it is defined as the power (potential) that brings damage to living things. This damage is the result of the degradation of metabolism. As a result, it can cause a reduction in the growth and productivity of a plant or plant organ or even death. Stress severity is within very wide limits ranging from stress-free (optimal conditions favoring for the sustainability of the plant) to moderate and severe stress. But in nature, a completely stress-free environment is not possible for any living organisms, not only for plants. In addition to stress severity, duration of the stress is also essential. In a short period of stress, the reaction of the plant may not emerge. What we mean here is that the visible reaction for growth or development of the plant may not be measured or observed. We should not ignore the endogenous invisible changes in metabolites. Stress degree depends on the plant species. That is, a stress factor that causes severe stress in a plant species may cause moderate stress or zero stress in another species. The degree of stress also depends on the amount of energy that affects the change in metabolic events in living systems. While whole or some parts of a plant (seeds, dormant seeds and dormant cells) may be tolerant to stress, some parts (meristem tissues, succulent organs, young seedlings) are susceptible to stress [1].

In the present chapter, we have mainly focused on the two subtopics for phenolic content. For the first subtopic, we have discussed about the stress physiology, mainly considering abiotic stress and, in the second subtopic, a bibliometric analysis associated with phenolic contents in fruit, which are edible and consumed parts of the plant. So, the first subtopic can be considered as the contents used for plant health itself; the second one can be considered as the contents used for people health.

1.1. A brief outlook on Turkey and Mediterranean region with emphasis on olive orchards

Olive belonging to the Oleaceae family is represented with many genera, and some of the genera have been devoted to produce oil. They are related to *Olea* genus and are usually found in subtropical and tropical climate regions, on the world's middle belt and where Mediterranean climate dominates. *Olea europaea* L. distributes in Upper Mesopotamia including the Southeastern Anatolian Region and South Asia [2]. Approximately 97% of world olive tree and olive production belongs to Mediterranean countries [3].

According to the data extracted from the Food and Agriculture Organization of the United Nations [4], with respect to the world area harvested for olives, world olive production, ranking for top producer countries and Turkey, was documented from 1961 to 2018. In 1961, area harvested for olive was reported to be 2,608,804 ha. The highest value was 10,650,068 ha in 2016, and the lowest value was recorded in 1966 with 2,578,098 ha (**Figure 1**).

For the data about olive production, the lowest quantity was documented in 1962 with a value of 5,410,901 tonnes, and highest quantity was about 22,025,129 tonnes in 2013 (**Figure 2**). In **Figure 3**, top olive-producing countries were represented. Spain was top producer with a remarkable quantity as 9,276,100 according to the data extracted for 2013. **Figure 4** illustrates the yield and production of olives for Turkey, indicating an increasing trend in parallel with the worldwide values.

1.2. Polyphenols versus phenols

Phenolic compounds are the most common secondary metabolites in the plant kingdom. Phenolic compounds are rarely found in bacteria, algae and fungi. Bryophytes are regular producers of polyphenols, including flavonoids, and all of the polyphenols are found in vascular plants. It is estimated that approximately 2% of all carbons photosynthesized by plants are transformed into flavonoids or closely related compounds [5].

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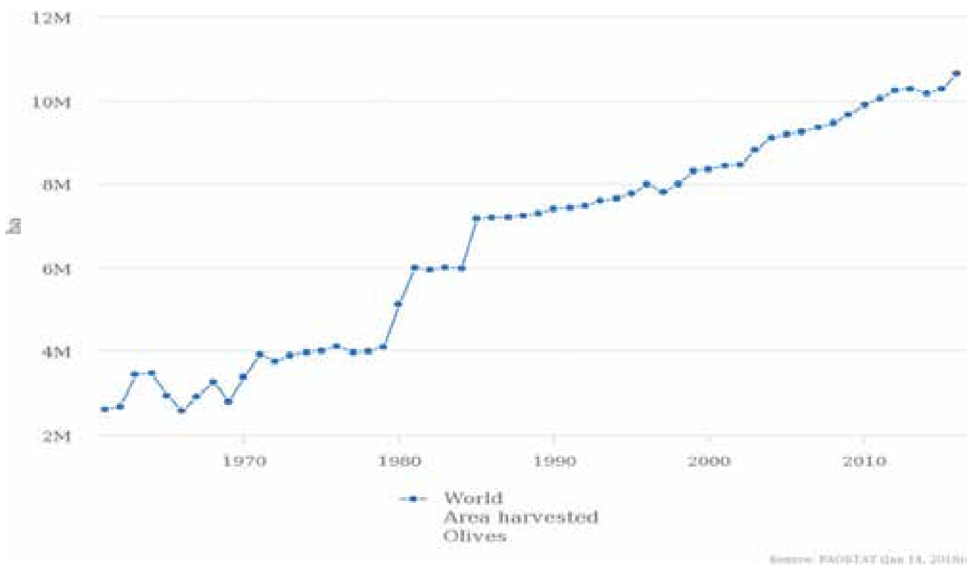

Figure 1. World area harvested for olives (ha).

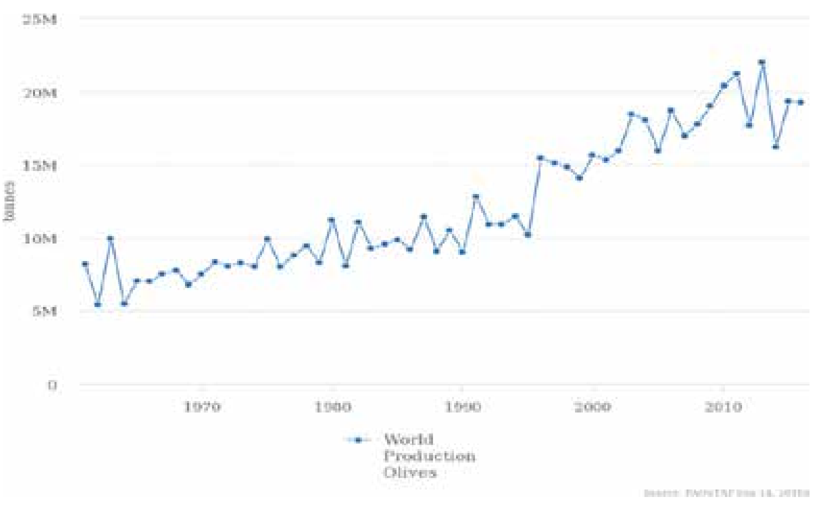

Figure 2. World olive production (tonnes).

The structure of the secondary metabolite of a certain plant is quite complex: (I) the contents of secondary metabolites specifically vary according to the tissue and organ; (II) may also differ between the developmental stages of the plant, for example, the organs important for survival and reproduction have the highest and strongest secondary metabolites; and (III) may also differ between plant individuals and populations. These secondary metabolites are divided into various groups according to biosynthesis pathways and structural properties [6].

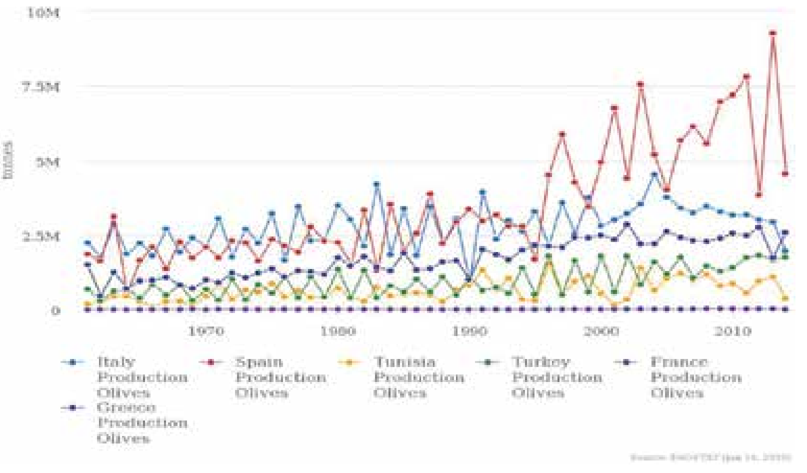

Figure 3. Ranking for top producer countries.

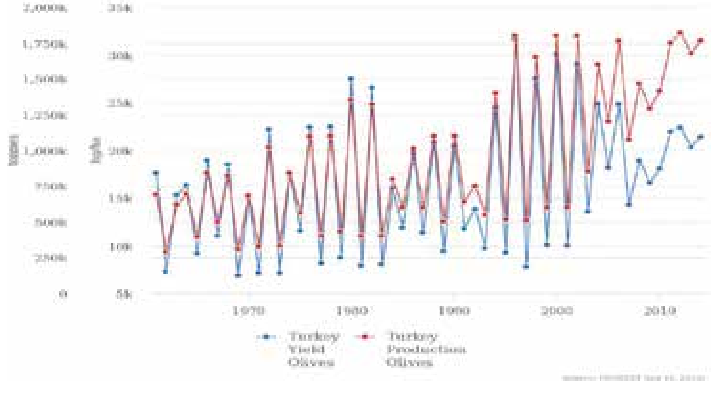

Figure 4. Yield and production values for Turkey.

Before going further, we should simply describe the difference between "phenol" and "polyphenol" terms. Can the terms phenol and polyphenol be used for the same purpose or meaning? In general, with respect to the definition of plant phenolic, phenol is a chemical term that defines a phenyl ring bearing one or more hydroxyl substituents. Polyphenol can be used to describe natural products having at least two phenyl rings carrying one or more hydroxyl substituents including functional derivatives (e.g., esters and glycosides), but such a definition in the context of plant phenolic is unsatisfactory because phenolic carotenoid 3-hydroxyisorenetin, which is a terpenoid, and female sex hormone estrone are also defined under this definition [7].

1.3. What about possible potential roles of phenolic compounds against environmental stress conditions?

Plants face adverse growth conditions during their lifetime such as drought, salinity, cold, low temperature and high temperature. Environmental stress factors can disrupt cellular structures and weaken important physiological functions of the plant. Drought, salinity and low temperature stress can cause osmotic stress, resulting in loss of turgor in plants. Disruption in the stability of the cell membrane, loss of activity or denaturation of proteins and excessive accumulation of the reactive oxygen species rather than optimal content for the essential physiological plants are of the consequences of stress conditions. As a result, photosynthesis inhibition, metabolic dysfunction, damage to cellular structures, growth retardation, loss of yield and early senescence can occur [8]. These undesirable conditions can delay the growth and development of the plant, decrease the productivity and cause the plant to die depending on the severity and duration of the stress. The response of the plant to stress conditions is dynamic, and as a response against unfavorable conditions, plants exhibit mechanisms such as regulation of metabolism and gene expression for the physiological and morphological adaptation of the plant [8, 9].

Phenolic compounds are secondary metabolites which are derivatives of pentose phosphate, shikimate and phenylpropanoids pathways in plants [10]. These compounds, which are one of the most common groups of phytochemical groups in plants, have important physiological and morphological importance in plants. These compounds play an important role in growth and proliferation by providing protection against pathogens and predators [11].

The interaction of plants with their own and biotic and abiotic surroundings has been a driving force in the emergence of certain natural products. In this context, plants are thought to accumulate phenolic material in the tissues of plants as a common response to adverse environmental conditions in the course of their spread and adaptation to the earth. Plant phenolics are thought to play an important role as defense compounds in situations where environmental stresses such as high light, low temperature, pathogenic infections, herbivores and nutrient deficiencies may lead to increased production of free radicals and other oxidative species. Increasing scientific studies and evidence showed that plants respond to biotic and abiotic stress factors by increasing the sweeping capacity of reactive oxygen species. The induction of gene expression of the secondary metabolism with biotic and abiotic stress is mediated by the integration of signaling molecules such as salicylic acid, jasmonic acid and their derivatives [12–14].

1.4. Phenolic profile of olive leaves, fruit and fruit oil

Olive leaves are rich in bioactive compounds, especially polyphenols. The reason of high concentration of polyphenols has been attributed to the long periods of sunlight and attack of many pathogens and insects to olive leaves in Mediterranean region. As a defense mechanism to cope with those stressors, a large quantity accumulation or storage of polyphenols has been reported in the canopy leaves. However, the production, accumulation or secretion of the fatty acids and polyphenols are not only consequences of exogenous factors but also endogenous factors of the plant itself such as cultivar and age of plant [15–19]. Of those compounds, oleuropein, hydroxytyrosol, tyrosol, chlorogenic acid, caffeic acid, p-coumaric acid, verbascoside, gallic acid, ellagic acid, epicatechin, quercitrin, kaempferol, luteolin, luteolin-7-o-glucoside, luteolin-4′-o-glucoside, quercetin, quercetin-7-o-rhamnoside, rutin and apigenin-7-o-glucoside are of the phenolic alcohols, phenolic acids, flavonoids, and secoiridoids analyzed in olive leaves [15, 17, 20–24].

In the Mediterranean region, olive fruit is the prevalent crop as a source of oil. The phenolics in oil composition are considered to contribute to the antioxidant properties [25, 26]. The olive fruits were reported to possess phenolic alcohols, phenolic acids, flavonoids and secoiridoids including oleuropein, ligstroside, demethyloleuropein, hydroxytyrosol, tyrosol, chlorogenic acid, verbascoside, luteolin-7-o-glucoside, luteolin, quercetin-3-o-rhamnoside, cyanidin-3-o-glucoside, cyanidin-3-o-rutinoside and apigenin-7-o-glucoside [27, 28].

There is a negligible number of some secondary metabolites in olive oil, and most of the secondary metabolites are composed of phenolic compounds. Of those compounds, α-tocopherol, oleuropein, hydroxytyrosol, tyrosol, caffeic acid, ferulic acid, p-coumaric, vanillic acid, apigenin, luteolin, pinoresinol, 1-acetoxypinoresinol, oleocanthal and oleacein have been reported in olive oils. As expected, the quantity and quality of the compounds are not stable corresponding to the developmental stage or environmental fluctuations. In short, multivariate interactions of endogenous and exogenous factors are predictive factors on the phenolics. For example, increases in number of phenolic compounds have been recorded from the green to the spotted stages of maturation, and then it decreases until maturity [29]. In this context, Guo et al. [26] suggested that the optimal harvest time for each olive variety may contribute to obtain the highest phenolic content.

1.5. Do polyphenol contents vary with the plant age, cultivation and storage conditions, harvesting times or biotic and abiotic stress factors?

This section can be considered as core of the present chapter. Not all but some studies concerned with polyphenol profile under different conditions or in different developmental stages are briefly summarized. Initially, we should emphasize that nothing stays as its former form. As a simple law of physics, when there is an action, there is a reaction. Any simple change deviating from the present situation of the plant itself or any deviation from optimal growth conditions of the plant—note that plants differ according to the their optimal growth conditions—cause changes in the biochemistry and physiology of the plant. There will always be change even sometimes statistical analysis states no differences between the experimental and control groups of the study. We should note that the change may not be in the measured parameters of the complex-structured whole plant. Plants are open system—not localized in a vacuumed media—and are continuously interacted with their surrounding conditions. This interaction is about the abiotic and biotic environmental conditions. It should not be ignored that the changes in metabolites of the plant might be consequences of the ontogenetic stages of plant itself. In this section, we will briefly represent some studies regarding with external or exogenous applications on the phenolic profile of the olive leaves and fruit oil.

On the hypothesis that the olive trees are exposed to water stress during summer, Petridis et al. [30] conducted a study to determine the effects of water deficit stress for 2 months on in total phenol content, oleuropein and hydroxytyrosol in the leaves of four olive cultivars. As expected and documented in previous studies, water stress triggered the biosynthesis of phenolic compounds, suggesting the possible antioxidant roles of phenolics, but the hydroxytyrosol content declined with the progression of water stress. We should note that the term "phenolic content" means total of thousands of components acting together. The general idea on protective role is that an increase is an indicator of possible antioxidant activity but any decline in any compounds under stress conditions may also be considered as antioxidant agent since synergistic or antagonistic interaction of compounds decides the protective roles. Unless individual activity of isolated pure compounds themselves is not tested, measurement of any individual in total phenolics pool using mass spectrometric analytical techniques may not be certain explanation for protective role of the compound.

Cetinkaya [31] examined the effects of tree age, cultivar and irrigation on flavanol content of two olive cultivar leaves (Kilis Yağlık and Gemlik). The study was designed as a 12-month observation under two irrigation conditions (irrigated and nonirrigated) using two cultivars differing in age. What achieved in the study was cultivar "Kilis Yağlık" contained more flavanols than cultivar "Gemlik". The age effect was not found to be significant, but nonirrigated conditions lead to higher content of flavanols, which were the possible stress defensive strategies of the plant. Considering the variations of flavanol by months, the content increased from flowering stages to harvest time of olive. Based on the study, we can conclude that flavanols are not only important for their preventive roles versus stress conditions but also important for developmental stages of olive.

Çetinkaya and Kulak [32] proposed the relationship between total phenolics, total flavonoid and oleuropein in different aged (9 and 65 years old) olive (*Olea europaea* L.) cultivars (Kilis Yağlık). Young trees exhibited higher phenolics and oleuropein content, while the higher total amounts of flavonoids were obtained in old trees.

Cetinkaya et al. [33] conducted a 12-month field manipulation irrigation experiment in two olive cultivars. The cultivars were exposed to irrigation and nonirrigation (April–September) and natural climate conditions of the region (October–March). The highest total phenolic contents were produced in cultivar "Kilis Yaglik" in February under natural conditions of the region, but irrigation regimes did not cause any statistical significance changes. The lowest phenolic content was found in cultivar "Kilis Yaglik" in August under irrigated conditions, while nonirrigation treatment increased the content. This decline may be explained with the defense-related functions of phenolic compounds. Moreover, the lowest content of flavonoids was determined in spring for both cultivars (in April for cultivar "Gemlik" and in May for cultivar "Kilis Yaglık"), whereas the highest content was observed in summer. The contents were remained at high levels for both cultivars during autumn. Those results propose the hypothesis that the increase in flavonoid content in summer could be induction of their biosynthesis in leaves to ensure a protection against stress induced by ultraviolet irradiations since the flavonoid roles against ultraviolet irradiation stress have been well documented [14, 34, 35].

Changes in phenolic compounds of *Olea europaea* cultivars contrasting in salt tolerance was reported by Rossi et al. [36]. The accumulation of phenolics was correlated with the content of sodium. Salt-sensitive cultivar (Leccino) had higher concentration of phenolic in their root, stem and old leaves, but salt-tolerant cultivar (Frantoio) exhibited higher concentration in their new leaves. The concentration of phenolics doubled under high salt stress in new leaves of salt-tolerant cultivar. On the other hand, phenolic content in new leaves of salt-sensitive remained stable by increasing salt stress. Of the phenolic compounds, kaempferol concentration was found to be higher in new leaves of salt-sensitive cultivar but lower in old leaves of the cultivar. Quercetin concentration was reported to be so low that it was below the detection threshold, and it increased in salt-tolerant cultivar with increases in salt stress. Salt-tolerant cultivars exhibited more abundant phenolic compounds, which were subsequently increased with the increase in salt stress in new and old leaves. The phenolic compounds in salt-sensitive cultivars, in which leaves accumulated more sodium, were reported to be stable or depleted with salt stress for new leaves. In the same study, genes responsive to phenylpropanoid metabolic pathway were correlated with biosynthesis of phenolic compounds. As a result, upregulation of the pathway was observed for the salt-sensitive cultivars and vice versa.

In one more study by Petridis et al. [37], salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) were monitored. Accordingly, severe salt stress triggered accumulation of total phenolic compounds (TPC) in leaves, but moderate salt stress level induced formation of TPC in roots. Of the quantified phenolic compounds, oleuropein was reported to be major compound in both leaves and roots, suggesting the possible protective roles against salt stress.

As an exogenous factor, Stanković et al. [38] examined the ecological variability of the phenolic compounds of *Olea europaea* L. leaves from natural habitats and cultivated conditions. In this context, samples from wild-spread regions (Tunisia, Malta and Montenegro) and from cultivated conditions (France and Serbia) were scanned for their total phenolic content, flavonoid content and antioxidant properties. As expected result, polyphenol content and antioxidant properties were found to be habitat dependent. Interesting output of the research was that samples from cultivated plants exhibited higher total quantity of phenols and flavonoids, but the values associated to antioxidant properties were higher in samples from natural habitats. The researchers deduced that natural habitats contained metabolites with high antioxidant activity, proposing that those substances may be active players for adaptation of the plants against stress conditions. Using mass spectrometric technologies for profiling the individual compounds with their quantities may contribute more to increase our understanding of important roles of polyphenolic compounds as a response to the changing environmental conditions.

Higher total quantity of phenols and flavonoids in young plants of four Tunisian olive cultivars (Chetoui, Ouslati, Jarboui and Meski) grown under water deficit conditions for two months were determined but the quantity differed according to the cultivars. Furthermore, cultivar with high content of phenolic and flavonoid exhibited important antiradical activity under water deficit [39].

Aparicio et al. [40] reported the effects of saline condition (0 and 200 mM NaCl for 12 weeks) on physiological properties of six olive genotypes. Out of the tested cultivars, total quantity of phenolics of four cultivars increased with increase in salt stress. The preventive roles of phenolic compounds as compatible organic solutes and as molecular antioxidants through their free radical scavenging abilities have been re-reported [41].

1.6. How do phenolic compounds exhibit antioxidative properties in plants? Which parts of phenolics are used to combat with free radicals?

Reactive oxygen species (ROS) production, which damages the cellular structure, is a common result of stress at the cellular level [42]. The prominence of the phenol compounds is that the hydroxyl group has an inhibitory potential for free radicals. Therefore, an increase in this compound means an increase in an antioxidative activity. ROS production is normally detoxified by a number of antioxidants including phenolics and antioxidant enzymes [43]. In addition to the free radical scavenger roles, phenolics also act as a radiation-absorbing filter limiting chlorophyll excitation for photosynthesis under adverse conditions [44]. Depending on the decrease of water content in leaf tissues, protective mechanisms involving the synthesis of phenolic compounds are triggered and then neutralized by absorption of radiation and conversion to blue fluorescence [45].

In the presence of suitable functional groups (hydroxyl and carboxyl), phenolic compounds have the ability to chelate iron and copper ions. Plants with a content of tannins were documented to possess capability to tolerate the high concentrations of manganese in a soil through chelation of these ions [46, 47]. Posmyk et al. [48] suggested the roles of peroxidases and phenolic compounds in defense reaction in response to the oxidative stress mediated by copper ions.

1.7. The remarkable changes of phenolic compounds during alternation: a prominent compound, "chlorogenic acid"

Alternate bearing or irregular bearing is of the major unfavorable attribute decreasing crop production and consequently results in significant economic loss in some years. The phenomenon of alternate bearing is described as the processes associated with the plants (both deciduous and evergreen trees) bearing an irregular crop year after year, usually heavy yields which are followed by light ones [49], proposing a biennial cycle (sorted as "on" and "off" year) of fruit production. High yield in "on" year and little or no yield in "off" year are representatives of the alternation. In order to understand and reveal the underlying mechanisms for alternate bearing, a number of physiological, biochemical and molecular studies have been carried out [50–54]. The quality and subsequently the aromatic value or bio-efficacy of the oil or table olives have been well documented to be related to the content and pattern of phenolic compounds of the fruit. The phenolic contents are not only strongly influenced by cultivar and stage of the maturation but also by plant genetic structure and alternate bearing phenomenon [52]. Profiling the polyphenolic content and composition is also essential physiological basis for enlightening this phenomenon. In this context, Mert et al. [21] monitored the quantitative seasonal changes in the leaf phenolic content related to the alternate-bearing patterns of olive (*Olea europaea* L. cv. Gemlik). In this context, oleuropein, chlorogenic acid, caffeic acid, 3-hydroxycinnamic acid, scopolin and p-coumaric acid concentration in leaves were investigated. Significant differences in concentration of the measured phenolic compounds in 2008 (off year) and 2009 (on year) were reported. The higher concentration of chlorogenic and p-coumaric acids during "on" year was determined, and lower levels of other phenolic compounds were reported and vice versa for the "off" year samples. In general, higher levels of phenolic compounds were measured during the dormant season. Also, the central role of chlorogenic acid in alternation has been identified, exhibiting 3–4 times greater concentration in fruit-bearing years once compared to the non-fruit-bearing period [55–57]. The chlorogenic acid concentration in old season leaves exhibited dramatically variations between seasons [58].

2. A bibliometric analysis of olive fruit oil

The research questions of this section are as follows. Along with the present study, (i) what are the research trends concerned with polyphenol in olive fruit oil, considering the main theme of the studies? (ii) What is the spatial distribution of the researches? Do the Mediterranean countries mostly conduct studies or not, considering the attributes influential on the performing the studies? (iii) How far Turkey is away from worldwide studies regarding with polyphenol contents in olive fruit oil? To answer these questions, we performed a bibliometric analysis using VOSviewer v.1.61 (Centre for Science and Technology Studies) program.

2.1. Methods

2.1.1. Data source

The data were retrieved from online version of Scopus database on December 7, 2017, which indexed 37.956 major journals across many different scientific disciplines. The Scopus is considered as most comprehensive and reliable bibliographic source. Thus, the Scopus database was used to identify research articles on the topic of polyphenols. All records with the term "polyphenols" in the "article title, abstract and keywords" were retrieved. Accordingly, 47,821 documents containing the word "polyphenols" were recorded. The search was then restricted for publications that contain the words "olive or *Olea europaea* and fruit oil" in the title and abstracts. The inclusion criteria were all relevant available scientific publications in field of polyphenols in olive fruit oil. The search was conducted on December 7, 2017. The documents published during the period of 1995–2017 were included. After assessment literature in the title and abstract of all documents, duplicated articles, errata and undefined documents were excluded. No language limitation was considered. Finally 434 documents were analyzed. Workflow for the present study was illustrated in **Figure 5**.

Furthermore, VOSviewer v.1.61 (Centre for Science and Technology Studies) was used to construct bibliometric diagrams for visualization of co-citation, co-occurrences extracted from the title and abstract fields of the articles. Co-citation is described as any two items (authors) that have been jointly cited by another item (author). Hence, the more co-citations two items received correspond to the more likely they are related.

2.2. Results

2.2.1. Co-occurrence network of terms and country rankings

Figure 6 shows the co-occurrence of terms that occurred in the title and abstract fields. After inclusion of some limitations, the most relevant terms were displayed in **Figure 6**. Three main clusters were identified as the first cluster (red), the second cluster (blue) and the third cluster (green). To conclude, the clusters are found to be associated with (i) the first cluster (red), biochemistry of olive fruit oil; (ii) the second cluster (blue), genetics concerned with diversity of olive; and (iii) the third cluster (green), agricultural applications concerned with irrigation treatments for yield. In **Figure 7**, the co-occurrence terms that appeared in Turkish-originated

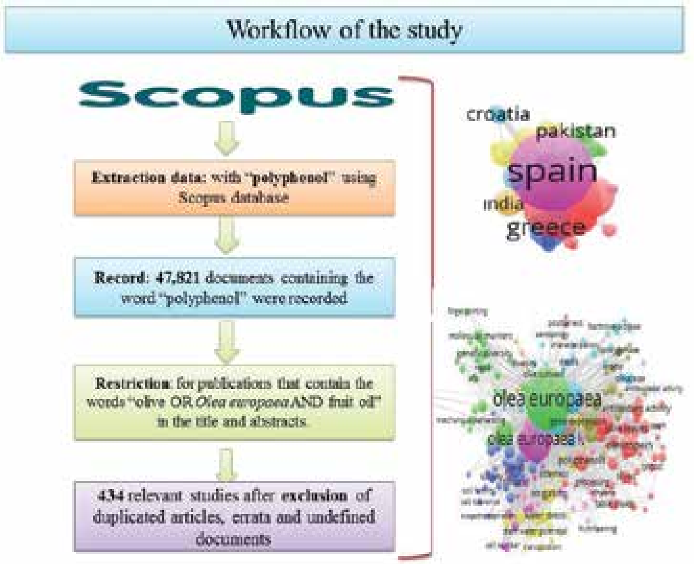

Figure 5. Workflow for the present study.

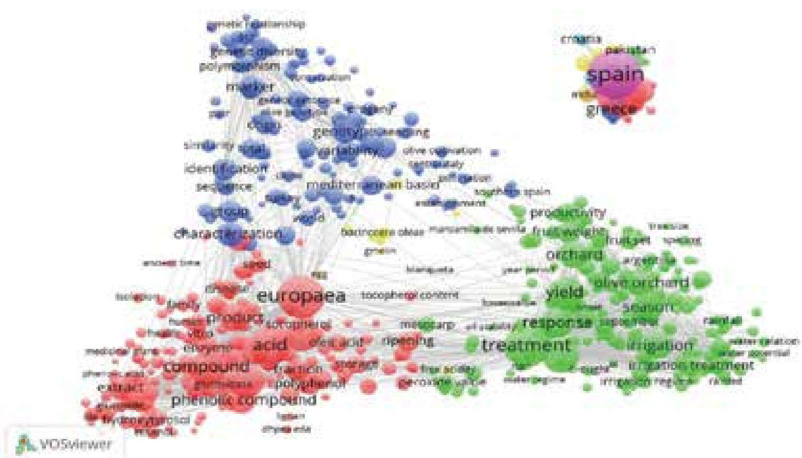

Figure 6. Co-occurrence network of terms occurred in the title and abstract of the documents on polyphenol contents and olive fruit oils between 1995 and 2017. Four clusters were identified and displayed in different colors.

studies are shown. Three clusters were also identified. The first cluster (green) and second cluster (blue) were related to biochemistry of olive fruit oil including phenolics and fatty acid composition, and the third cluster (red) was associated with genetics concerned with diversity of olive and its cultivars. To conclude that the worldwide trend for olive studies is not only limited to profile, the biochemical or nutritional composition of olives but also exogenous agricultural practices for yielding higher oil content. For Turkish studies, the works are mainly concentrated on monitoring or profiling the phenolic and fatty acid composition

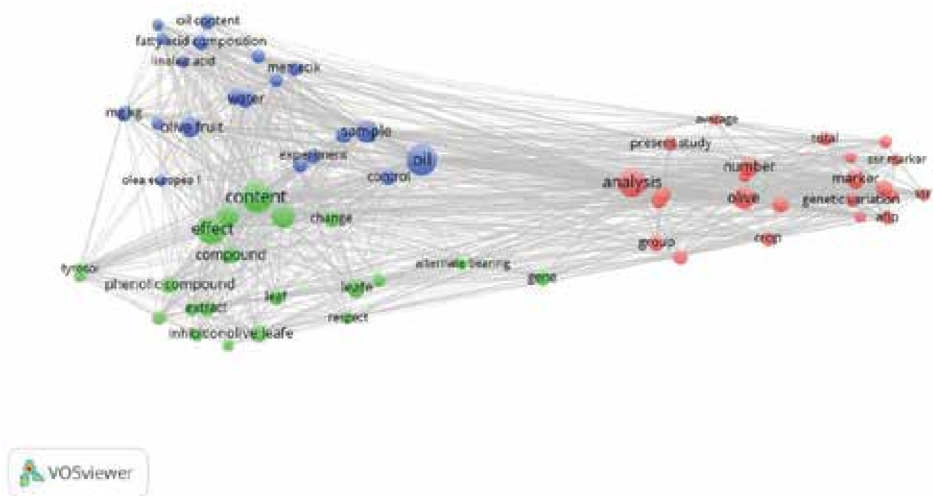

Figure 7. Co-occurrence network of terms occurred in the title and abstract of the documents on polyphenol contents and olive fruit oils between 2001 and 2017. Three clusters were identified and displayed in different colors. The terms were retrieved from Turkish studies.

with their total yield. We should also note that the extracted documents were mostly limited to the agricultural and biological sciences. Furthermore, the genetic studies in Turkey are concomitant with the worldwide studies. Of the publishing countries, as expected Mediterranean countries—Spain, Italy, Tunisia, Greece, Turkey and France—were predominant countries. Olive tree is a typical Mediterranean plant grown in Marmara, Aegean, Mediterranean and South East Anatolian regions of Turkey and important oil sources for Mediterranean countries, fulfilling 90% of the world olive oil production. Spain, Italy, Greece, Tunisia and Turkey are of the important producer and stakeholders of olive oils [59].

2.2.2. Contributions of keyword by authors

The simple keyword extraction provides raw information about the research topics, but they are assigned to documents to represent the core content of their papers. In this regard, keyword analysis can be used to determine the progress the research frontiers associated with a knowledge [60], proposing the gap of keyword analysis in polyphenol studies in olive fruit oils. Herewith the core results, this section should be considered as the most important contribution of the manuscript [61]. Co-occurrence and author keywords might be considered as one of the factors to provide information on polyphenol studies in olive fruit oils. **Figures 8** and **9** represent the core content of the studies for worldwide and Turkey-centered studies, respectively. For the worldwide studies (**Figure 8**), the researches on molecular approaches (molecular markers, genetic diversity, RAPD, AFLP methods), biochemical approaches (polyphenol content, olive leaves, table olive, processing for quality and antioxidant activity), stress physiology (irrigation, water stress, salinity, salt tolerance, water potential and transpiration) were performed. For Turkey-centred studies (**Figure 9**), the studies are mostly concentrated on polyphenol content and fatty acid composition. For molecular studies, some cultivars have been compared with wild cultivars using molecular markers.

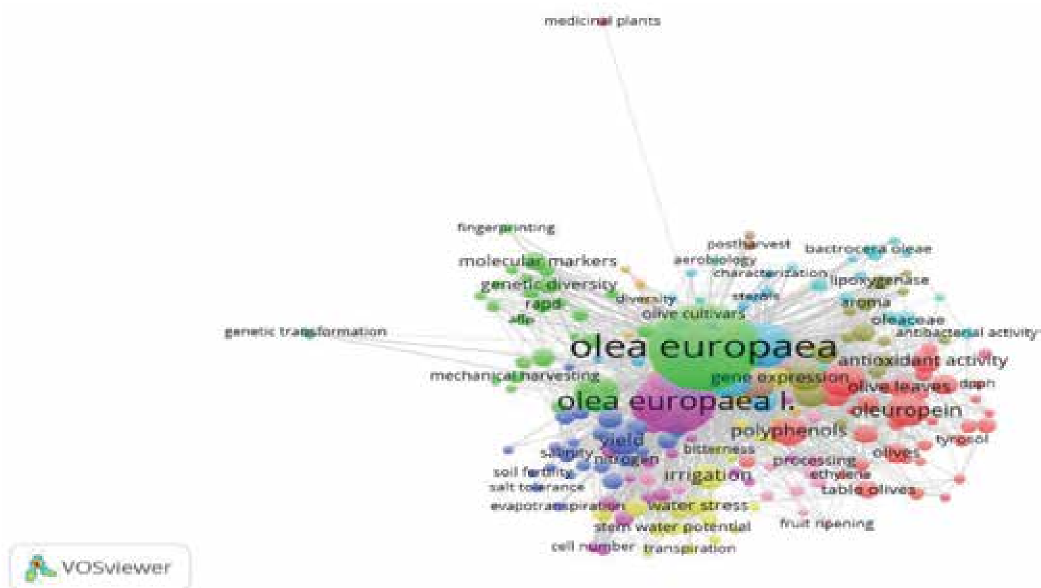

Figure 8. Co-occurrence network of keywords occurred in the title and abstract of the documents on polyphenol contents and olive fruit oils between 1995 and 2017. Thirteen clusters were identified and displayed in different colors.

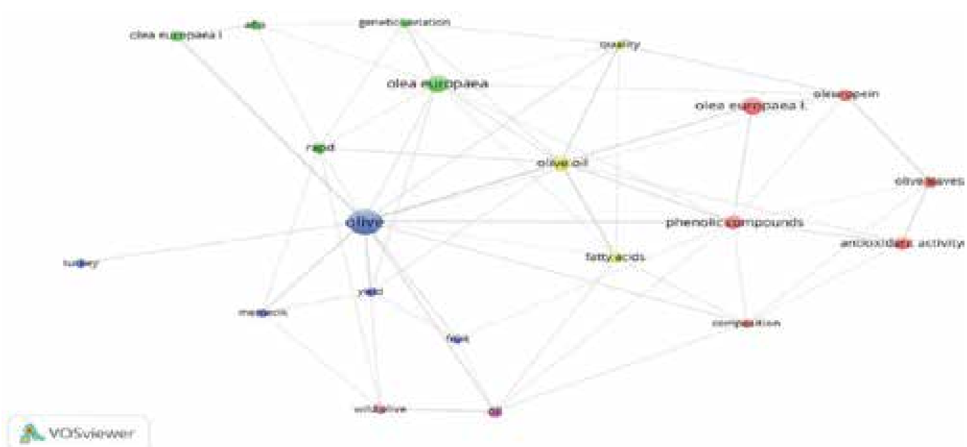

Figure 9. Co-occurrence network of keywords occurred in the title and abstract of the documents on polyphenol contents and olive fruit oils between 2001 and 2017. Five clusters were identified and displayed in different colors. The keywords were retrieved from Turkish studies.

2.3. Future outlook

In this section, we focused on the gaps and gave suggestions for future studies associated polyphenols for Turkish olive cultivars:

1. Polyphenol content and patterns of the olive cultivars and wild ones might be documented in order to point out the aromatic value.
2. Biotic and abiotic stress-tolerant cultivars might be monitored in response to the probable sharp and extreme environmental conditions.
3. Exogenous stressors (water stress, salt stress, etc.) might be applied to enhance the stress tolerance and increase the essential secondary metabolites.
4. Stressed olive cultivar extracts might be tested for their plausible biological activities.

2.4. Highlights of the study

Along with the present study, we have illustrated a schema as studies regarding to (i) the current state knowledge of polyphenol studies or profiling the changes in polyphenolic components in Turkish olive cultivars, (ii) pointing out the stages of development of the studies, (iii) presenting assessments for the significance of the studies performed and (iv) giving suggestions for the key areas for further work.

2.5. Limitations of the study

Although this is the first study—up to our best survey—to present the most comprehensive and specific view of available research on for polyphenol content of olive fruit oil from the largest existing database using VOSviewer program, we have several limitations in this study. (1) We only extracted data from SCOPUS, and so documents in nonindexed plant journals have not been considered. (2) The search was then restricted for publications that contain the words *olive or Olea europaea and fruit oil* in the title and abstracts. (3) Hence some publications might not contain polyphenol and olive fruit oil-related terms in the publication title and abstracts, so it is possible that not all publications for polyphenol content of olive fruit oil were identified.

3. Conclusions

We can list the main conclusions of the review as follows:

1. The content and pattern of the polyphenolic compounds are dynamic, not constant. They exhibit variations with the genetic structure, alternate bearing or irregular bearing, plant age, cultivation and storage conditions, harvesting times or biotic and abiotic stress factors.
2. Upregulation of the phenolic biosynthesis pathway was observed for the salt-sensitive cultivars and vice versa, deciphering the plausible defense roles of phenolics under stress conditions.
3. The roles of phenolic compounds in defense mechanism have been well documented. Neutralizing the effects of oxidative stress and chelating heavy metals are the major mechanisms to combat stress factors but in the presence of suitable functional groups.

4. Chlorogenic acid was of the outstanding compounds in response to the alternation phenomenon.

5. According to the co-occurrence network of terms and country rankings, the worldwide trend for olive studies is not only limited to profile the biochemical or nutritional composition of olives but also exogenous agricultural practices for yielding higher oil content. For Turkish studies, the works are mainly concentrated on monitoring or profiling the phenolics and fatty acid composition with their total yield.

6. Of the publishing countries, as expected Mediterranean countries—Spain, Italy, Tunisia, Greece, Turkey and France—were predominant countries.

7. Even though there are some limitations of the present study, we pointed the stages of the development of the olive studies but only for phenolics, giving suggestions for the key areas for further works.

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Section 2

Protection Against Oxidative Stress by Tea Polyphenols

Chapter 2

Oxidative Stress Diminishing Perspectives of Green and Black Tea Polyphenols: A Mechanistic Approach

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Additional information is available at the end of the chapter

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Abstract

Polyphenols have credentials to tackle the oxidative stress. Oxidative stress is the imbalance between free radicals production and antioxidant enzymes ability to tackle these radicals resulting the onset various metabolic related disorders. Polyphenols based foods have credential as a shield against these glitches mainly owing to their antioxidant potential. In this context, tea polyphenols have gained paramount attention of scientific community as therapeutic agents for the prevention and treatment of various oxidative stress induce maladies owing to their structural diversity, strong antioxidant ability and capacity to modulate various expression involved in the pathogenesis of these maladies. The notable polyphenols are catechins which are mainly present in green tea and further subdivided into various compounds like ECG, EGC, EGCG which has their unique therapeutic potential. The catechins undergo various structural changes and transformed into theaflavins and thearubigins in the process of black tea formation. These are high molecular weight polyphenols and promising candidates in obesity, diabetes and cancer treatment. Mechanistically, these polyphenols ameliorate oxidative stress by trapping the noxious radicals like superoxide and peroxyl, promote the activity of glutathione, suppressing the malondialdehyde (MDA) activity. The current chapter is an attempt to highlight the therapeutic potential of tea polyphenols.

Keywords: polyphenols, diabetes, oxidative stress, cardiovascular complications, catechins

1. Introduction

A fundamental relationship between nutrition, health and disease is diverting the attention of consumers from a medicine towards plant based natural products. Science is progressing with leaps and bounces and the modern era of food technology makes it easy to produce plant based natural products to combat life threatening diseases. In diet based regimen, functional and nutraceutical foods are getting popular especially among scientific community because of their therapeutic potential [1]. Likewise, tea polyphenols have gained paramount importance in modern era of dietary regimen and proved to have potential against several chronic maladies. In addition, tea either green or black acts as functional food and is easily available, cheap and safe to use [2]. Tea; a wonderful beverage that has strong historical background and it is being consumed in various part of the world from last 5000 years [3]. Tea has three basic types: black tea, green tea, and oolong that vary due in biological, chemical profile, and in processing methods. However, processing of green and black tea is quite different from each other. Firstly, fresh harvested leaves gone through steaming process in order to avoid fermentation, as a result stable dry final product is obtained. Enzymes which are responsible for the catalysis of color pigments in the leaves is eventually destroy by steaming [4]. Consequently, with the help of this process green color and natural polyphenols remain stable and provide health promoting properties to tea. Secondly, green tea is fermented to Oolong, and then subsequently, into the black tea. Leaves of black tea are crushed and subjected towards enzymatic oxidation process called fermentation. Green tea and black tea both have different biological properties because the polyphenols of green tea dimerized into several the aflavins as well as higher molecular weight polymers i.e., thearubigins. In other words, these two compounds impart specific flavor and color to black tea [4].

Globally, tea is the most famous beverage but now recent evidences show that it is more than a beverage because it has characteristics of functional food. In addition, it contains unique aroma, desirable taste, caffeine and potential therapeutic property. Tea is produce from plant named "*Camellia sinensis*" family "Theaceae", which is consumed worldwide as green or black tea because of its nutraceutical properties [4]. According to an estimate, tea leaves production in the world is about 3.6 million tons annually, whilst consumption as120 mL/capita/day [5]. China, India, Sri Lanka, Vietnam and Indonesia are the major producers of tea but among them china is the dominant in production line. Green tea contributes about 20% of total tea production, and famous beverage among East and South-East Asia. On other hand, black tea covers up about 78% of the world share and primarily, consumed in North America, Europe and North Africa.

1.1. Bioactive components of tea

Green tea has complicated chemical composition: proteins including some amino acids like theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates including pectins, glucose and fructose; minerals including calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel,

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potassium, fluorine, and aluminum; trace amount of lipids, sterols, vitamins, pigments, caffeine and volatile compounds [6]. Epigallocatechin gallate is the major component of green tea, make green tea extract more stable due to the presence of some antioxidant as compared to pure form of epigallocatechin gallate [7]. In contrast, major portion of black tea leaves consist of polyphenols. Whereas, along with some macronutrients also contains 6.5% lignin, 5% ash, 1.5% organic acid, 6–9% flavonols, 10–12% phenolic acids, 8–12% methylxanthines, 0.5% chlorophyll, 0.1% carotenoids, and 0.1% volatile substances. Naturally, tea contains phenolic acid, theophylline and linalool as flavoring agents. Furthermore, leaves of black tea have alkaloids including caffeine and theobromine [6]. Like other plant based food products, tea also contains a wide variety of bioactive components, which provide therapeutic potential, including antioxidant, anti-inflammatory, anti-hypertensive, anti-cancerous and immune-modulatory effects [8]. Especially, polyphenols in tea help to prevent from cardiovascular diseases, hyperlipidemia, cancer, diabetes and scavenge free radical species [9]. For instance, kaempferol has potential against metabolic disorders; myricetin modify white blood cell ability to scavenge free radical; quercetin act as antioxidant: so, prevent from cancer; theaflavin work against oxidative stress and cell toxicity thus prevent from DNA cleavage [8, 10, 11] (**Table 1**).

1.2. Classification of polyphenols and its properties

Tea polyphenols has a complex acidic structure which consists of 2-phenylbenzpyran skeleton with several hydroxy groups and aromatic ring. So, electron density of their structure is decrease which leads to weak O—H bond strength and promote proton loss [14]. Presence of asymmetric carbons at the 2 and 3 positions of the pyran ring of the 2-phenybenzopyran nucleus give it therapeutic and functional potential. Therefore, activation of numerous enzyme systems indicates structural and functional alteration of tea polyphenols [15]. Eventually, black tea holds array of polyphenols approximately 36%; which is in form of oxidized and un-oxidized polyphenols. About 5% are in un-oxidized form like catechins and remaining is in oxidized form including theaflavin and thearubigins which is also produced from catechins

Nutrients	Concentration (%)	
	Black tea	Green tea
Carbohydrates	10–15	5–7
Proteins	12–14	15–20
Lipids	2	Trace amount
Minerals	5	55
Vitamins	Trace amount	Trace amount
Fibers	15–20	Trace amount
Alkaloids	2–5	3–4

Source: [6, 12, 13].

Table 1. Percentages of nutrients in black and green tea.

oxidation. Enzyme mediated fermentation: a step in black tea processing converts 3–6% of catechins into its oxidized form [16]. Simultaneously, 12–19% thearubigins are produce when catechins is further oxidized. Significantly, only 5–10% catechins are able to maintain their structure in form of epigallocatechin gallate and provide anti-oxidative potential [12]. Catechins are present in various form like epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) and gallocatechin, epigallocatechin digallate, 3-methylepicatechin gallate, catechin gallate, gallocatechin gallate in smaller amount [17]. Certainly, catechins content: present more in black tea as compare to green tea and amount depends upon the age of leaves. During tea production, approximately 75% of tea catechins undergone oxidation and partial polymerization with the help of enzyme called tea leaf polyphenols oxidase. However, black tea composition depends upon technological process, so there is no definitive composition [18]. In addition, it also has a wide range of flavonoids especially flavanols and flavonols. The promising candidates of aforementioned categories of tea flavonoids and other polyphenols of both types of tea are illustrated in **Figure 1** [19]. Major class of polyphenols in tea is flavonoids which is synthesized when carbohydrates act as precursor and adopt metabolic pathways including shikimic and *p*-coumaric acid. Phenylalanine ammonia lyase triggered flavonoids biosynthesis: a photosensitive mechanism [21].

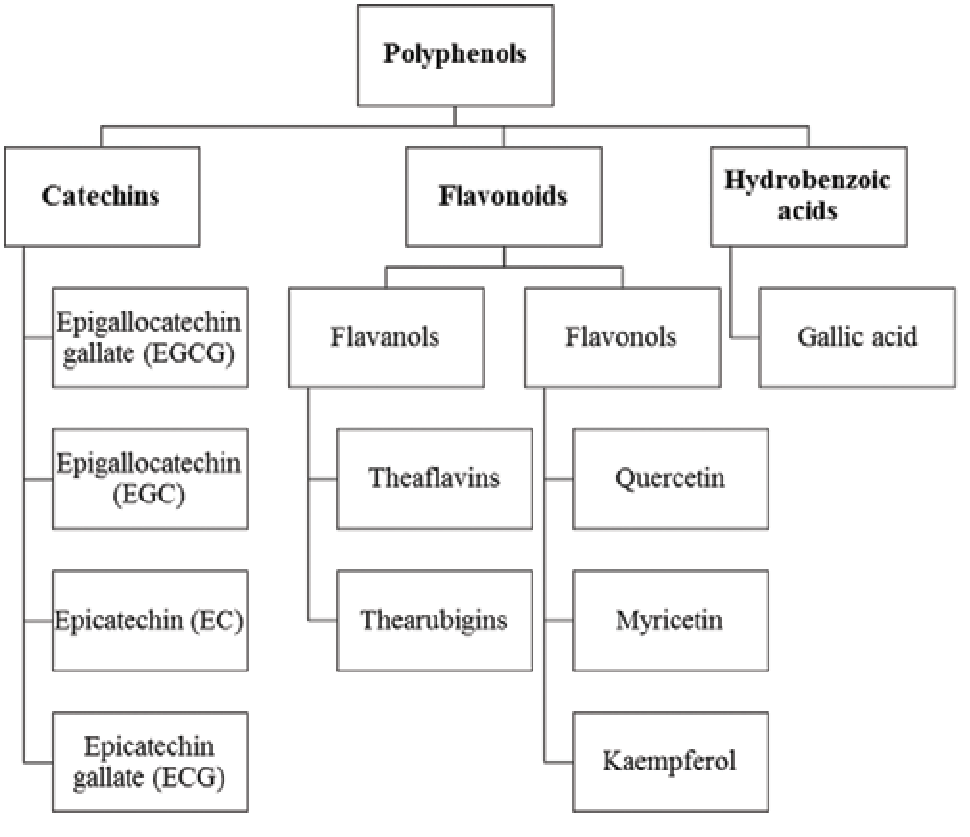


Figure 1. Classification of polyphenols in tea. *Source*: [19, 20].

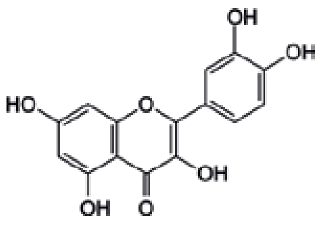
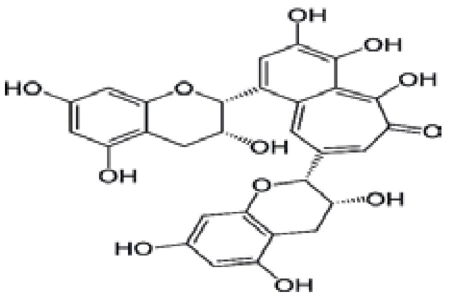
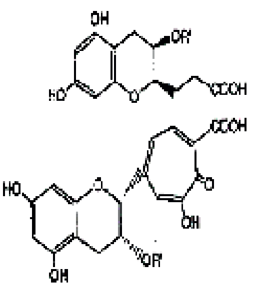
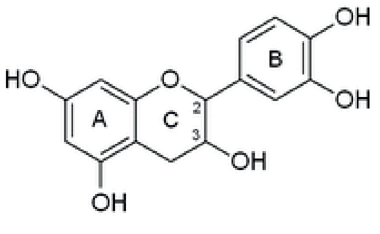
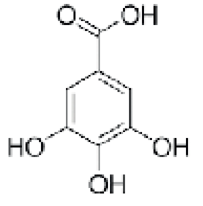
Polyphenol	Structure	Functions	References
Catechins		Act as antioxidant. So, inhibit LDL oxidation. Consequently, prevent heart disease, diabetes and obesity	[12, 16]
Theaflavins		Help to reduce glucose level in the body. However, effective against cancer and neurological disorders	[3, 9]
Thearubigins		Assist in excretion of toxins, especially, in inflammatory diseases. Moreover, reduce oxidative stress and risk of cardiovascular diseases	[23]
Flavonols		Has property to scavenge free radicals and metal chelation. Hence, act as antioxidant, anti-oncogenic, anti-inflammatory and helps in reduction of foam cell formation	[10, 11]
Gallic acid		Protect against oxidative damage	[10]

Table 2. Structure and functions of polyphenols.

Alternatively, Green tea also contains 30% polyphenols: flavanols, flavandiols, flavonoids and phenolic acids. Among them flavonols is most common which is also known as catechins; green tea hold catechins in greater proportion as compared to the black or oolong tea [22]. In green tea, four different kinds of catechins are present: epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin gallate. Whereas, proportion of polyphenols especially catechins vary both qualitatively and quantitatively, depends upon the preparation methods [20] (**Table 2**).

2. Tea polyphenols and its health claims

2.1. Free radical scavenging activity and its anti-oxidative potential

Reactive oxygen species (ROS) are formed due to mitochondrial oxidative metabolism and some cellular response against xenobiotics, cytokines, and bacterial invasion. Although, excessive ROS leads to imbalance in oxidative stress, they may also cause damage at macromolecular level including lipids, proteins, carbohydrates, DNA; and results in various diseases: atherosclerosis, diabetes, aging, cancer, neurological damage and so forth [24, 25]. Moreover, some other outcomes of oxidative stress are DNA damage, production of mutated tumor suppressor genes and may induce cell death [26, 27].

Antioxidants provide protection against free radicals: by neutralizing and donating electron to free radicals [28]. Improvement in antioxidant defense system helps to reduce free radicals production. Admittedly, glutathione is natural anti-oxidant of the body that maintains intracellular redox status and it acts as co-factor in metabolic reactions. All polyphenols in tea has property of antioxidant, which lessen oxidative stress in the body [29]. Ref. [30] demonstrated antioxidant activities of water extracts of different tea including reducing power, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potential and the inhibition of hemolysis caused by 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH)-induced lipid oxidation in erythrocyte membranes and they supported the use of green tea as significant antioxidant. To illustrates, green tea polyphenols: an antioxidant against iodophenol-derived phenoxyl radicals, superoxide anion radicals and lipid peroxidation was observed in rat liver microsomes [31, 32]. Furthermore, several factors are involved in the production of reactive oxygen species; which disrupt the overall intrinsic environment of the body. However, tea polyphenols prevent from DNA damage, reduce oxidation of low density lipoprotein and some other factors shown in **Figure 2**. Thereby, prevent from various life-threatening diseases like cancer, heart disease, inflammatory disorders [29].

2.2. Immunomodulatory and anti-inflammatory effect of polyphenols

Immunity is defined as an ability to fight against infection, any abnormal function within the body and prevent from diseases [35]. Certain organs are involved to build up defense mechanism in body including thymus gland, spleen, lymph nodes and bone marrow [36]. Generally, humoral and cell-mediated immunity is enhanced by green tea consumption and reduce the risk of cancers and cardiovascular disease [37]. For instance, tea polyphenols act as antioxidant

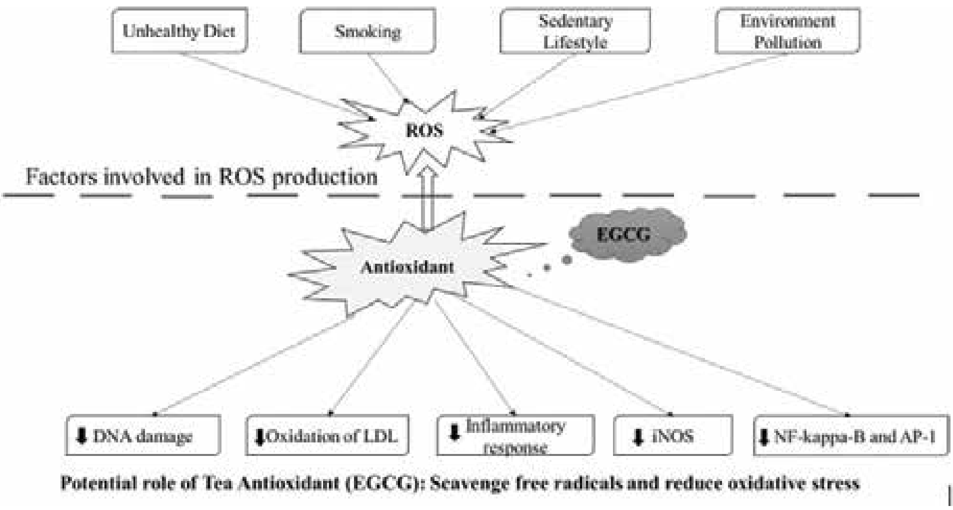


Figure 2. Free radical scavenging activity of tea antioxidants. *Source*: [29, 33, 34].

and anti-inflammatory agent [38, 39]. Although, inflammation is fundamental to immune system but in some complicated disease, it become causative agent [40]. Dona et al. [41] demonstrated molecular and cellular insight properties of green tea and found that epigallocatechin-3-gallate (EGCG) assist to reduce inflammation. It was observed that mice injected with lipopolysaccharide (LPS), fed them with green tea polyphenols showed a significant reduction in production of tumor necrosis factor- (TNF) and prevented from death even after administration of lethal dose of LPS [42]. Moreover, some studies showed a reduction in joint diseases like arthritis due to consumption of tea polyphenols [43, 44].

Many chemicals are used in order to induce inflammatory response like topical application of phorbol esters: TPA, to check the effect of anti-inflammatory agents. Several studies proved a beneficial effect of green tea polyphenols, by inducing tea polyphenols in mice skin that inhibits TPA-mediated induction of epidermal ornithine decarboxylase (ODC) activity in a dose-dependent manner [45]. Polyphenols prevents TPA-induced oxygen radical-induced cytotoxicity, inhibits intercellular communication in normal human epidermal keratinocytes and also inhibits TPA-induced protein kinase-C activity [46, 47]. Further, tea polyphenols inhibits 12-0-tetradecanoylphorbol-13-acetate and other skin tumor -promoter-caused induction of protein and mRNA expression of the pro-inflammatory cytokines interleukin (1L)-1 α and TNF-α. Skin applications of green tea polyphenols inhibits UV-radiation-induced local and systemic suppression of contact hypersensitivity and edema responses in C3H/HeN mice. In various *in-vitro* studies, green tea polyphenols/crude extracts of green tea have shown preventive effects in system considered essential in inflammatory processes [48]. Flavonoids in tea i.e., epigallocatechin-3gallate inhibits the activation of NF-kappa-B; helps in modulation of MyD88- and TRIF-dependent signaling pathways of TLRs and subsequent inflammatory target gene expression [49]. Nuclear factor-kappa-B is a transcriptional factor in oxidative stress that regulates numerous gene expression crucial in cellular responses, including inflammation, innate immunity, and growth. In a similar study [50] researchers illustrated that EGCG inhibits LPS-induced inducible nitric-oxide synthase gene expression in mouse peritoneal macrophages by subsequently, lowering NF-κB. To put all aforementioned mechanism in nut shell, tea has certain elements like polyphenols that proved to have anti-inflammatory response.

3. Regulation of several physiological process and disease prevention by tea polyphenols

3.1. Therapeutic properties of polyphenols in hyperlipidemia and hypercholesteremia

Hyperlipidemia is characterized as high lipid level in the body which may leads to certain metabolic dysfunctions: cardiovascular diseases, high blood pressure and stroke. Black tea is proved to be beneficial against hypercholesterolemia and platelet aggregation because it contains oxidized and un-oxidized catechins. In addition, black tea contains antioxidants which can combat with oxidative stress, endothelium dysfunction and arterial complications [51, 52]. Tea polyphenols: prevent LDL oxidation; balance HDL level and limits intestinal cholesterol absorption [53]. A scientist [54] mentioned in their study that polyphenols administration about 7 g/L for a course of 35 days attenuating lipid profile and hepatic oxidative abnormality in both normal and hypercholesterolemic male Wistar rats. It was observed that, black and green tea increases fecal excretion of fatty acids and sterols, thus provides protection against serum and hepatic abnormalities in hypercholesterolemic phase [55]. Moreover, Black tea polyphenols in amount: 500 and 1000 mg/kg body weight provision to male Wistar rats for 8 weeks caused a significant reduction in total cholesterol, increase LDL and triglycerides along with better HDL profile [56].

Theaflavin is helpful against lipid related abnormalities because it stimulates cellular energy expenditure at mitochondrial level. However, it also suppresses FAS expression by down regulating EGF-receptor/PI3K/Akt/Sp-1 signal transduction pathway, hence, inhibits the cellular lipogenesis and tissue growth. Furthermore, theaflavin especially TF-3, ultimately suppresses biosynthesis of cholesterol, triglycerides and fatty acids by inhibiting growth factor EGF; binding to receptor EGFR, restricts PI3K/Akt signal pathway activation and decreases DNA-binding capacity of nuclear transcription factor Sp-1; consequently, down regulates FAS gene and modulates LDL receptors that facilitate in cholesterol and triglycerides reduction [57, 58]. Likewise, theaflavin a potent antioxidant, stimulates lipid metabolism by regulating pancreatic and gastric secretion; thereby, cause reduction in lipid level and fatty acid synthase enzyme shown in **Figure 3**. Another possible route, cholesterol micelle solubilization interferes with lipid lowering [60]. Theaflavin causes change in structure of micelles and leads to reduction in cholesterol re-synthesis and eventually, alters its metabolism [57, 61]. Development of atherosclerosis takes place due to LDL oxidation: abnormal changes in macrophages and join with macrophage scavenger receptor; results in foam cell formation that deposit in the walls of arteries. Tea polyphenols has an ability to scavenge free radicals and inhibit foam cell formation and deposition [56, 62]. Tea polyphenols also scavenge H_2O_2, so, a natural antioxidant including glutathione, glutathione peroxidase and glutathione reductase activity is increased. Moreover, it enhances LDL particle size and improve the adiponectin metabolism

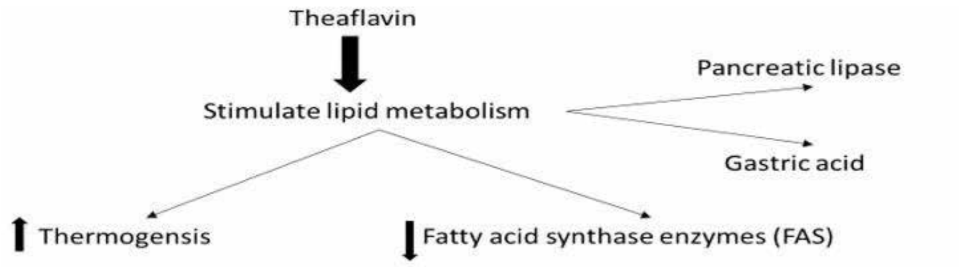


Figure 3. Role of theaflavin in regulating lipid mechanism. *Source*: [14, 56, 59].

[63, 64]. It is observed that there is a direct relationship between high fat diet or high sucrose diet and triglycerides production which triggers lipogenesis [32]. Whereas, tea polyphenols possess potential to normalize lipid abnormalities: inhibit intestinal lipid absorption, increasing fecal excretion of fat through bile acid, suppressing the activity of fat synthesis enzymes and thereby, prevent lipogenesis [65]. Furthermore, high sucrose diet cause hypertriglyceridemia because production of acetyl CoA elevated which in turn raise triglycerides. Polyphenols activate activation of LKB1-AMPK pathway; AMPK (protein) involve in glucose homeostasis that consequently, involve in the synthesis of glucose-6-phosphate and cause fatty acid metabolism. Hence, in short tea provide a helping hand towards lipid metabolism; reduce lipid oxidation and lipid absorption; thus reducing risk of hyperlipidemia [58].

3.2. Effect on carbohydrates metabolism-diabetes mellitus

Diabetes has been increasing at an alarming rate, due to poor dietary habits and sedentary lifestyle. According to an estimate, 300 million people will be affected by diabetes until 2025. In general, type 2 diabetes is defined as malfunctioning of pancreatic β-cell but dietary intervention and healthy life style is the best way to manage diabetes. Moreover, evidence showed that tea act as anti-diabetic agent: attenuate hyperglycemic state; thus, modify glucose metabolism and insulin secretion [66]. Tea polyphenols improve glycogen syntheses system by re-activating the glycogen synthesis and lower liver glucose-6-phosphatase activity [67]. Similarly, in one study, 16 subjects were provided 75 g of glucose and water per day with simultaneous provision of 3 g instant black tea. It was found that tea polyphenols help to stimulate the pancreatic enzymes that enhance β-cell ability towards insulin [68]. Mechanistically, tea: a hypoglycemic agent, modulate glucose transporter (GLUTs) that helps to maintain glucose homeostasis and requires IRβ and AMPKR proteins for their translocations. However, in hypercholesterolemia, diet high in fats leads to reduce GLUT4 and other associated proteins. Subsequently, there is a disturbance in glucose incorporation into the cells, thus, cause progression of diabetes and insulin resistance [69, 70]. Surprisingly, tea polyphenols inhibit α-glucosidase and α-amylase activity which leads to reduction in intestinal glucose absorption and insulinomimetic action [71]. Further, diet rich in fructose and sucrose trigger abnormal glucose production, and effect hormones in plasma including adiponectin and intestine GLUT1 [72]. Studies proved that tea has an ability to improve insulin resistance in both hypercholesterolemic and hyperglycemic models [65]. It has been estimated that about 15-fold increase in insulin activity due to black tea extract consumption was observed in *in-vitro* study.

The identified bioactive constituents theaflavin, thearubigins, EGCG and catechins in tea enhanced activity of GIP and GLP-1 factors in order to improve the insulin secretion. Besides, theaflavin, its derivatives and thearubigins regulate insulin signaling process including growth factors insulin/IGF-1 in FOXO1a, PEPCK of mammalian cells thus, improved dysregulation of hepatic gluconeogenesis [73]. Glycemic response can be managed through tea polyphenols intake, that in turn lower risk of diabetes and increase insulin sensitivity; a mechanism described in **Figure 4**. Thereby, tea polyphenols proved to reduce the risk of diabetes and diabetic complications by improving glucose uptake in adipocytes and decreasing leptin production [71, 74].

3.3. Effect on obesity

Obesity and the existence of other diseases associated with obesity persist a global health problem. Current estimates in the USA demanded a fear full situation that almost one-third of the adult peoples obese. Obesity defined as body mass index ≥30kg/m^2 is a universal lifestyle-related illness increasing at an upsetting rate. Among related factors, dietary habits are measured one of reasons for its postponement [47]. During the last limited years, enlarged consumption of carbohydrate and animal fat has backed to obesity thereby increased occurrence of hyperlipidemia and diabetes mellitus. For the cause, functional ingredients are getting devotion to improve lipid metabolism and resistor obesity. In this background, black tea is a promising tool to improve thermogenesis and fat oxidation. Provision of black tea remove (0.2%) rich in polyphenols caused significant decline in the markers related with obesity (body weight) of obese CF-1 mice [39]. Black tea antioxidants like theaflavin and thearubigins have probable to prevent fat oxidation and decrease the fascination of nutrients in gastrointestinal track. Furthermore, they manage the energy feeding thus prevent LDL testimony and obesity. In an animal trial model, rats were fed on high fat diet with immediate drink delivery containing 5% black tea polyphenols cutting (BTPE) and seen 44.2% decrease in weight [16]. Mechanically, the aflavin constrains the pancreatic lipase activity together with intestinal lipid absorption thus lessens the gain in weight. Previous research inquiries have painted that the

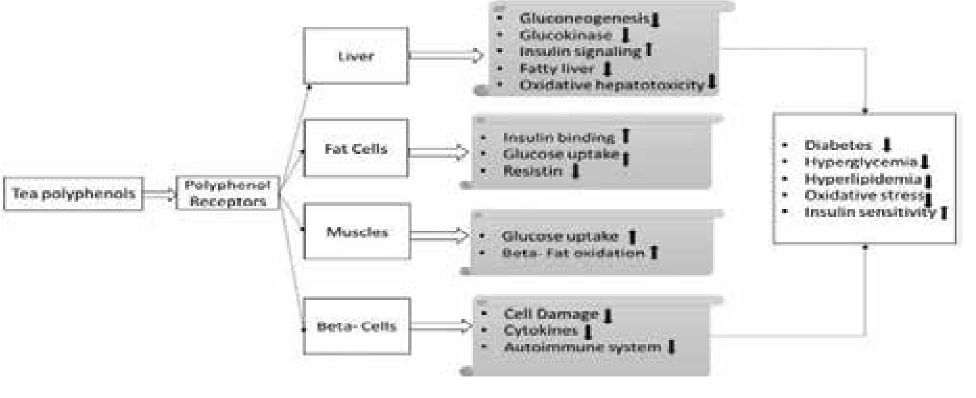


Figure 4. Glycemic response management: mechanistic illustration of black tea polyphenols. *Source*: [74].

compounds containing galloyl moiety blocked the post prandial hypertriacylglycerolemia by decelerating down the triacylglycerol absorption through the hang-up of pancreatic lipase [9]. The aflavin comprises two digallate groups thereby have more probable for weight managing than thearubigins. On molecular level, dissimilar enzymes played a key part to regulate lipid metabolism yet, fatty acid synthase (FAS) is a thoughtful factor. Its Imbalance activates the cascade of certain sicknesses like obesity, cardiovascular problems and cancer uprising [28]. The FAS inhibitors may assistance in weight management and in this background, black tea theaflavin is a gifted ingredient. It blocks FAS over the deactivation of PI-3 K/AKT/Sp-1 trail owing to galloyl moiety. Unlike scientific opinions are explained that the black tea gallate polyphenols (theaflavin) achieve the body weight by moderating the cholesterol metabolism, constrain the reabsorption of bile acid and delay the synthesis of fatty acid enzymes via impersonating the AMP-activated protein kinase trail in HepG2 cells [22]. Among the other likely anti-obesity ways are modulating the action of superoxide dismutase and catalase that hunk the start of oxidative stress, by up regulating the GLUT1 and GLUT4 genes appearance, different genes expression and defend the hepatic tissue through black tea polyphenols therefore helpful in the weight management package [25]. Many observational studies have interweaved the ingesting of black and green tea with reduced low-density lipoprotein oxidation and improved insulin action in animals and humans. In a community based test, tea polyphenols administration resulted helpful impact on the aging diabetic subjects by controlling insulin and glucose metabolism [13] (**Figure 5**).

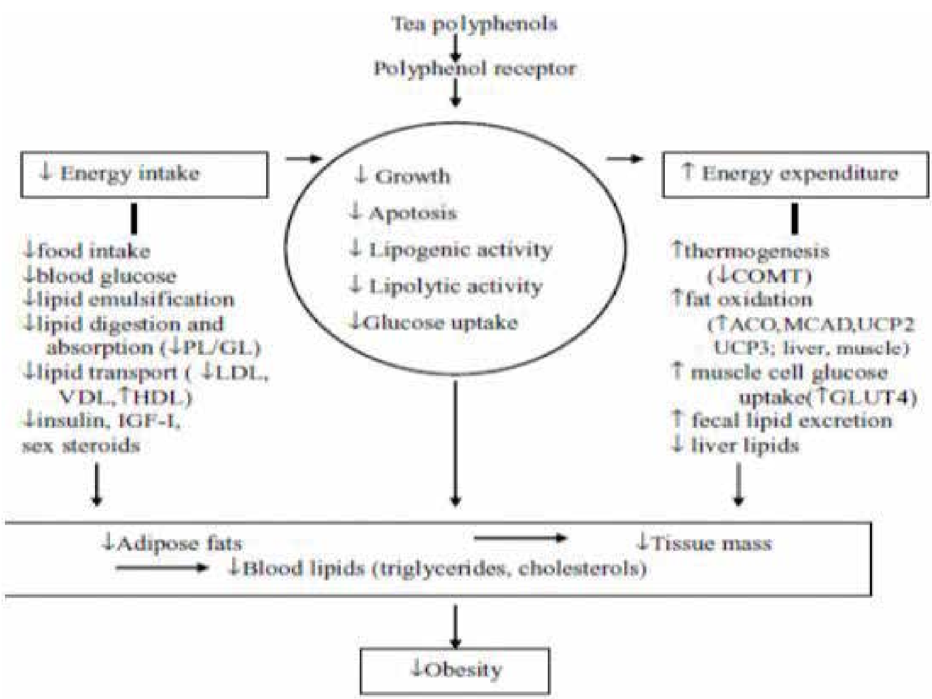


Figure 5. Mechanism of tea polyphenols on obesity reduction. *Source*: [64].

High cholesterol diet i.e., 1.5% of cholesterol beside with high sucrose diet 40% was assumed to the normal rats to persuade hypercholesterolemia as well as obesity. Periodic examination of rats was approved out to assess the orientation of obesity. The functional drinks were providing to the rats alongside to harmonize their effect on the own group [17]. Obesity is related with systemic oxidative stress, adipokine inequity and condensed antioxidant defenses, top to dyslipidemia, vascular disease and hepatic steatosis. Gastrointestinal lipase inhibitors delay fat digestion and fascination. Phenolic lipase inhibitors such as epigallocatechin-3-gallate, grape seed, kaempferol, quercetin, ellagitannin, tannins and proanthocyanidins are existing in green and black tea, berries (lingonberry, bearberry, arctic bramble, cloudberry, strawberry, raspberry and blueberry); garden pea (*Pisum sativum*), Norway spruce (*Picea abies*), large-leaved lime (*Tilia platyphyllos*) [23].

Obesity is related with bigger health-care costs, compact quality of life, and rise risk for premature death, Cholesterol is one of the vibrant compounds, lipophilic in nature that does numerous metabolic functions in the body. It is essential four lipoproteins; chylomicron (CM), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) for its carriage. In hypercholesterolemic state, numerous metabolic dysfunctions like coronary complications, high blood pressure and stroke are the allied syndromes [66]. Black tea polyphenols deliver protection in contradiction of hypercholesterolemia and platelet combination owing to the occurrence of oxidized and un-oxidized catechins. Moreover, black tea antioxidants have possible to challenge oxidative stress, endothelium dysfunction and arterial complications [2].

3.4. Cardiovascular diseases and hypertension

Cardiovascular diseases are leading reason of morbidity and mortality all over the world. High cholesterol and oxidation of LDL activate the cascade of events leading to start of atherosclerosis. Defective immune system results in onset of numerous health disparities branded as autoimmune disorders and immune dysfunction [7]. In the light of some previous epidemiological studies it was assumed that the higher flavonoids consumption through natural commodities measured allied with decrease risk for cardiovascular disease by improving endothelial role. Inhibit low-density lipoprotein and recover dyslipidemia. In the routine of diet based therapy, polyphenols reached core care as a coronary cover agent [14]. Among the polyphenols, tea bioactive moieties catechins, theaflavin and thearubigins are in attention for curtailing the menace. Many scientific explorations exposed an opposite suggestion between tea intake and lipid abnormalities as it opposites LDL oxidation and rises HDL level in obese and diabetic models. The slogan "good cholesterol" is credited to HDL due to its skill to reverse the cholesterol transport (RCT), eliminates the extra cholesterol from the tissues and arteries back to liver. It chiefly acts on sub-endothelial planetary in medium caliber artery that is the place where actual cholesterol testimony in the form of atheroma happens [26]. In contrary, LDL called as bad cholesterol because it transports the cholesterol from liver to the body. Now, great attention is being paid to the decrease of LDL by different therapeutic devices but, various epidemiological studies also specified the role of HDL for the administration of cardiovascular health [43].

Decreasing the atherogenic index and reverse the oxidation of fat are the possible mechanisms by which theaflavin may improve the plasma HDL level. Atherogenic index is the proportion between LDL and HDL and theaflavin significantly modifies the cholesterol metabolism, overturns the activity of lipid synthesis enzymes and protects the LDL in contradiction of oxidation thus improve the HDL. Cholesterol testimony in arterial wall needs a receptor called as prostacyclin [38]. Frequent studies floodlit that the high HDL may stop this process. In fresh lipid talking therapies, HDL enhancement advances importance and in this context prostacyclin inhibitors are in attention. Black tea polyphenols have skill to act as prostacyclin inhibitors thus progresses HDL that finally protect cardiovascular health [51].

Flavonoids contain of a large group of 6000 familiar phytochemicals originate in vegetables and fruits. Fresh scientific exploration point outs upon their antioxidant and anti-inflammatory actions. Such flavonoids typically don't deliver any nutrition, because they display very important useful act in the cardiovascular, gastrointestinal, cancer, and neurodegenerative like life risking maladies prevention. Metabolic syndrome is a disorder of at least three of the cardiovascular risk factors: obesity, unnecessary visceral fat storage, dyslipidemia, hypertension and hyperglycemia or Type 2 diabetes. It is a state of insulin fight, oxidative stress and chronic inflammation. Cardiovascular disease is the uppermost cause of death globally. Convinced dietary components and over 800 plants help stop or moderate metabolic syndrome by secondary the body homeostasis mechanisms [1].

Hypertension, also known as high blood pressure, is extra condition related to metabolic syndrome. Tea polyphenols has been shown to decrease blood pressure and recover endothelial function in animal studies. Endothelial dysfunction is a change of endothelial cells, resultant from oxidative stress and reduced vasodilator reply. Both hypertension and disturbed homeostasis of the ratio of HDL-cholesterol and low-density lipoprotein-associated (LDL)-cholesterol are danger factors for cardiovascular disease. The effect of green tea cutting on arterial hypertension in Sprague-Dawley rats was inspected [73]. The animals were preserved with angiotensin (Ang) II to encourage to the progress of hypertension. At the end of 13 days experiment, Ang II treated rats had amplified blood pressure and left ventricle mass. Co-treatment with 0.6% green tea quotation as the sole basis of drinking fluid rounded these rises. Green tea treatment also condensed Ang II-induced rises in plasma hydroperoxides and aortic endothelial appearance of hemeoxygenase I and SOD, representative a decrease in vascular oxidative stress. A second study by the similar group originate that 0.6% green tea quotation as the sole source of drinking fluid abridged final systolic and diastolic blood pressure by 20 and 24%, correspondingly in Ang II treated rats after 14 days. Gene expression trainings in the hearts of treated rats displayed that green tea extract treatment abridged Ang II persuaded expression of NAD(P)H oxidase appearance and activity linked to Ang II-treated controls [19]. This protein plays a key role in the initiation of endothelial oxidative stress by Ang II. Alike decreases in the appearance of Akt and extracellular responsive kinase (Erk) 1/2 were observed. Both enzymes are downstream effectors of NAD(P)H oxidase. He examined the effect of EGCG on spontaneously hypertensive rats (SHR), a sketch of hypertension, insulin resistance and obesity. The absence of effect on blood pressure may be due to change in this model from the SHR model, alterations in dose, or some other factor [36]. Treatment of a type 2 diabetes rat model, the Otsuka Long-Evans Tokushima Fatty rat, with 30 mg/kg/day tea catechins for 12 weeks was revealed to advance endothelial function. Systolic blood pressure was abridged by 10% associated to saline-treated regulator rats. Catechin-treated rats also showed increased vasodilation in answer to sodium nitroprusside treatment. These effects look to associate with decreased NADH oxidase appearance and activity. Green tea provisions have been exposed to encourage vasodilation *in vitro*. Using rat aortic rings, have exposed that EGCG can facilitate dose-dependent vasodilation. Green tea and green tea polyphenols have been revealed to modulate plasma and tissue levels of both HDL- and LDL-cholesterol. Many investigators have reported that tea polyphenols can stop the oxidation of LDL cholesterol *in vitro*. For example, 1–10 μg/mL green tea extract was shown to dose powerlessly reduce LDL oxidation persuaded by umbilical vascular endothelial cells. A 61% decrease in LDL oxidation was experiential following treatment with 10 μg/mL green tea excerpt [74].

3.5. Hepatic disorder and oxidative stress

Liver has a pivotal role in modification of numerous physiological processes in the body such as metabolism, secretion and storage. It has great size to detoxicate toxic matters and synthesize useful principles. Therefore, harm on the liver imposed by hepatotoxic agents is of grave significances [35]. Indications developed over the last years have optional that numerous forms of liver damages may be caused by free radical creation and subsequent oxidative stress. It is supposed that reactive oxygen species (ROS), such as hydroxyl radical, superoxide radical anion and nitric oxide may hurt cell membranes done lipid peroxidation [40]. Deceptively ROS adjust or damage biomolecules, i.e., proteins, lipids, carbohydrates and DNA [7].

3.6. Prevention of obesity-related fatty liver disease by green tea polyphenols

Hepatic steatosis (fatty liver) is a disorder that is defined by fat addition within hepatocytes that surpasses 5% of the liver by weight [39]. Firstly, it was supposed that this condition was mostly attributable to additional alcohol ingesting, but studies in the last numerous decades have also related obesity and diabetes to the attendance of fatty liver [60]. Characteristically, fatty liver syndrome related to etiological factors other than alcohol is mentioned to as non-alcoholic fatty liver illness (NAFLD). Now, non-alcoholic fatty liver disease (NAFLD) is the most mutual form of liver disease [8]. It is now extensively putative that NAFLD is the hepatic constituent of the metabolic syndrome; risk factors for the disease comprise obesity, insulin confrontation, and hypertriglyceridemia [30]. Dysfunction in lipoprotein metabolism may also play a part in the growth of hepatic steatosis. Treatments with tea in animal models have exposed to modulate numerous of these conditions. More studies essential to be lead on the specific mechanisms of green tea that arbitrate its benefits on liver function, and the fundamental mechanisms of action. For example, EGCG and other catechins decrease fatty acid synthase in cells and cell-free studies but effects by exact tea catechins need to be proved *in vivo* [49].

3.7. Oxidative stress and safety concerns

Oxidative stress is an inequity between the reactive oxygen species and endogenous antioxidants that disturbs normal detoxification of free radicals, made in a biological system. This situation troubled the body redox potential and compensations the cell mechanisms including protein and lipid thereby changes the cellular motioning [44]. Reactive oxygen spices (ROS) are produced continuously within the body though; some factors like unhealthy diet, smoking, deskbound lifestyle, environmental pollutant etc. may improvement their production. Improvement in the antioxidant defense system is of primary anxiety to mitigate free radicals manufacture. In this milieu, intake of polyphenols rich diet is inevitable to maintain the body antioxidant potential [14, 68].

3.8. Renal complication

Kidney does numerous life supporting functions counting body homeostatic, directive of electrolyte stability, blood pressure and exclusion of poisons in the form of urine. Throughout the recent era, in the emerging countries there is a fast rise in the chronic kidney disease (CKD) due to chemical contact, environmental toxins and poor dietary ways. In CKD, nephrons lost their physical and functional integrity that clues to reduce glomerulus filtration, rise blood urea and creatinine [3]. Chronic kidney disease (CKD) is a quiet killer branded by the liberal loss in renal function at a gentler pace. It comprises blood vessel disorders foremost to nephrons dysfunction that eventually reduces the glomerulus filtration. Renal dysfunctionality is more predominant in patients with high blood pressure, diabetes and cardiovascular problems [10]. Raised creatinine and blood urea levels were seen in the chronic renal letdown due to damage in glomerular filtration rate thus reduce urinary excretion. Recent studies supported the competence of black tea polyphenols to trigger antioxidant enzymes thus recover kidney detoxifying ability. Similarly, plasma creatinine and blood urea nitrogen levels were weakened in the diabetic rats after tea polyphenols treatment [47]. Black polyphenols decrease creatinine level by their anti-platelet exploit and allow kidneys to recover their normal function. It has been experiential that black tea polyphenols exhibit diuretic result thereby enhance the general kidney functioning like renal blood flow, capillary expansion and glomerular filtration. The *in vivo* renal functioning parameters like blood urea nitrogen and creatinine were augmented during the oxygen lacking state. However, black tea abridged urea and creatinine by 11.74 and 14.62%, respectively. The oxidative stress persuaded some morphological abnormalities in glomerulus, capillaries and tubules structures [59]. Furthermore, inflammation, sore lesion and distortion in tubules were also experimental. Provision of black tea elevates the renal functioning by mitigating the abnormal signs of kidney and inflammation. In a study, improvement in urea and creatinine stages in rats fed on high arginine diet was experiential due to the manufacture of uremic acid toxins and conquest of certain key hormones. However, black tea polyphenols supplementation caused discount in these abnormal indicators. The effective role of tea polyphenols in the arachidonic acid metabolism pathway may be one of the likely route by which they regularize the kidney malfunctioning. Numerous scientific evidences are in errand that tea polyphenols abridged kidney inflammation by overpowering the prostaglandin (PG), thromboxane $A_2$and cyclooxygenase words of arachidonic acid in microsomes and glomeruli. Impaired glomerulus filtration that is a first sign of CKD and black tea is lime lighted for handling the irregularities of glomerulus filtration by dipping toxic impact of sensitive oxygen species and refining the overall antioxidant status. Black tea polyphenols resulted noticeable decline in the creatinine level by their act on platelets thus allows kidneys to recover their normal functioning [46]. Furthermore, the diuretic effect of black tea improves renal blood flow, capillary expansion and glomerular filtration. The black tea decreases the formation of toxins, quenches free radicals and counteracts reactive oxygen species together with diuretic effect. Furthermore, improvement in inflammation, sore lesion and deformation in tubules are the foremost routes for renal modulating action. In an initial attempt, renal dysfunctionality was persuaded in Sprague Dawley rats by sub chronic administration of 3-methyl-2-quinoxalin benzenevinylketo-1,4-dioxide (QCT). The rats were providing black tea polyphenols blood urea, creatinine and urinary 8-OHdG levels were the board outcomes. The QCT persuaded higher urea, creatinine and 8-OHdG that were weakened significantly by black tea polyphenols [64].

4. Dosage and adverse effect of excessive use of black tea

4.1. Black tea side effects

4.1.1. Diarrhea

As caffeine boost up digestive secretions so if you drink black tea in large quantities, then it might have an adverse effect on your health. As a basic ingredient of black tea, it also results frequent and watery stool.

4.1.2. Constipation

As minor agent's black tea contains lot of tannins which might cause constipation and also makes the stool hard to pass out due to more water absorption capacity of tannins.

4.1.3. Disturbed stomach

Your stomach may feel irritation and discomfort due to excessive caffeine in black tea. The major side effect associated with heavy black tea consumption is increased gastric abnormalities.

4.1.4. Heart diseases

For patients recovering from heart attacks or acute cardiovascular disorders black tea is highly limited due to its unfavorable effects on heart muscles.

4.1.5. Other health hazards

Women of reproductive age should not consume more than 2 cups a day of black tea due to increased chances of miscarriage. The high caffeine content is also associated with a negative effect on people eyes health, high blood pressure and anxiety related disorders [33].

4.2. The side effects of green tea

4.2.1. Stomach maladies

Caffeine could be the most widely recognized offender. Although it has a lower measure of caffeine than different sorts of tea, still it can cause issues. This is because caffeine expands the measure of corrosive engaged with the stomach related process. This can cause torment or sickness [26]. Likewise, however green tea has been touted to anticipate growth, particularly gastric tumor, contemplates say that there is deficient data in such manner.

4.2.2. Iron deficiency and anemia

Green tea contains tannins that square the assimilation of iron from sustenance and nourishment supplements. Certain sources say that adding lemon to green tea or savoring it between suppers can counter this issue. Additionally, expending tea can diminish the assimilation of iron from plant-based sources (as much as by 64%). For alleviating the impact, one can drink tea no less than 1 h earlier or after dinners; and furthermore, incorporate more sustenance rich in vitamin C (as vitamin C helps in press assimilation). This happens when the polyphenols tie to the iron in the intestinal cells and keep it from entering the circulation system. This polyphenol-press complex is in the long run discharged from the body [65].

4.2.3. Irregular heartbeat

Once more, due to the caffeine, green tea may make mellow extreme cerebral pains. What's more, cerebral pains can likewise be caused by press insufficiency, which, as we have seen as of now, could happen through an over the top admission of green tea. Aside from migraines, green tea can likewise cause discombobulation. What's more, according to contemplates, the most extreme endured dosage of green tea in people is 9.9 g for every day—which is generally comparable to some the refreshment in a day. One vital point to note is that however the green tea extricate is recorded in more than 100 over-the-counter natural supplements and arrangements, its utilization as a treatment for any sickness isn't entirely managed by the FDA. Likewise, the security of the long-haul utilization of green tea separates is not unmistakably characterized. Green tea can make one feel unsteady and temperamental, which may not be the situation with decaffeinated green tea items [69].

4.2.4. Vomiting and diarrhea

According to one Indian investigation, green tea polyphenols can, truth be told, cause oxidative pressure. What's more, exorbitant admission of caffeine, including that from green tea, can trigger sickness and regurgitating. Direct measures of caffeine are noted to be 300–400 mg for each day. In the event that the sum surpasses, it can bring about certain symptoms, including retching. Loose bowels could happen on the off chance that you are new to green tea. Free stools could be one of the gentle symptoms (because of the caffeine content), which can in the long run die down as you get used to the drink. The runs can likewise occur with over the top admission of green tea. One approach to stop this is to decrease the utilization [55].

4.2.5. Muscle tremors and contractions

Over the top caffeine utilization has likewise been connected to muscle fits and jerking. Also, people with a strange sinus musicality must utmost caffeine admission. Caffeine has likewise been connected to anxious leg disorder. In the event that you are a person with mellow to direct seriousness of this condition, it is better you check your green tea (or caffeine) consumption. One basic normal for fretful leg disorder is the indications happening when the individual is dormant. This could occur in the night while going to bed, or an irritated agony at night, or jerking of the legs in the evening time [53]. Caffeine could irritate any of these side effects, including muscle fits. 240 ml of green tea contains around 25 mg of caffeine, and as indicated by one report, this caffeine can likewise cause tremors.

4.2.6. Heartburn

Green tea is acidic, and thus can aggravate the esophageal coating, causing indigestion or indigestion. The condition could deteriorate if an individual is now experiencing indigestion (or heartburn). Despite the fact that ordinary prepared green tea could not be so intense, the packaged green tea that you so frequently find in the business sectors could be the genuine risk. This is on account of the greater part of the green teas that come in bottles are strengthened with an acidic additive like ascorbic corrosive. This additive can relax the lower esophageal sphincter, which generally shields the stomach corrosive from ascending the throat [52].

4.2.7. Hepatic disorders

These days, green tea removes are enthusiastically showcased as weight reduction supplements. In spite of the fact that there is little proof supporting the adequacy of green tea in this viewpoint, certain genuine reactions, including intense liver disappointment, are being accounted for further research. The catechins in green tea convey tremendous advantages like brought down cholesterol and lessened danger of tumor and cardiovascular infection. In any case, if taken in high measurements, particularly like the dose in weight reduction supplements, these catechins cause liver poisonous quality. Green tea supplements contain tremendous measures of polyphenols, the most surely understood of them being EGCG (likewise called epigallocatechin gallate). According to certain case reports, utilization of 700–2000 mg of EGCG every day prompted genuine liver issues [18]. Consequently, on the off chance that you are at a danger of building up a liver infection, confine your green tea admission.

4.2.8. Osteoporosis

Caffeine has been found to restrain calcium ingestion. It can likewise build the rate of calcium discharge in the body. According to one investigation by the University of Connecticut, utilization of green tea extricates brought about lower femur length. It likewise prompted bring down volume, mineral substance, cortical volume and thickness of the bone. This recommends utilization of substantial amounts of green tea can prompt a lessened rate of bone collection amid the developmental years of a person.

4.2.9. Kidney issues

An audit of studies has demonstrated that the very polyphenols that are credited with averting tumor and coronary illness can likewise cause kidney harm if taken in over the top amounts. According to specialists, individuals devouring green tea supplements must exercise alert, specifically.

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5. Safe dose level of green and black tea

A perfect measurement of green tea is 3–5 glasses for every day, which could be equivalent to 1200 ml (or 250 mg of catechins). Never take green tea on an unfilled stomach as it may cause liver poisonous quality. It is recommended to take 2 or less than 4 cups of black tea per day for a normal adult to be active due to high concentration of caffeine in black tea and its associated side effects prevention [61].

6. Summary

Tea is an important commodity with significant health benefits. The tea polyphenols are of sufficient capabilities to ameliorate numerous lifestyle related maladies. A wide range of functional ingredients from tea phytoceutics provide a great tool to be utilized as a dietary remedy to avoid health issues like diabetes, cardiovascular disorders, hepatic and renal stress. Alongside, antioxidant potential of black and green tea is promising to prevent oxidative stress induced metabolic malfunctions in the body. Tea polyphenols also exhibit anti-obesity and anti-inflammatory effects. These further assuage lipid metabolism and show positive effects on lipid profile of an individual. Despite various health promoting perspectives, overconsumption of black and green tea may give rise to certain uncomfortable side effects. However, these side effects can be managed by utilizing recommended amounts of tea. In a nutshell, tea polyphenols are promising dietary components with significant disease preventing perspectives and easy to be utilized hence, providing a low cost strategy in prophylaxis of oxidative stress mediated malfunctions.

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Acronyms and abbreviations

EC	Epicatechins
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
PPO	Polyphenol oxidase
ROS	Reactive oxygen spices
DPPH	1,1-diphenyl-2-picrylhydrazyl

AAPH	2,2′-azo-bis(2-amidinopropane) dihydrochloride
LPS	Lipopolysaccharide
TNF	Tumor necrosis factor
TPA	Tropical phorbol esters
ODC	Ornithine decarboxylase
1L-1 α	cytokines interleukin
TC	Total cholesterol
TFs	Theaflavins
TG	Triglycerides
TRBs	Thearubigins
NF-kappa-B	Nuclear factor-kappa-B
TRIF	TIR-domain-containing adapter-inducing interferon-β
TLRs	Toll-like receptors
FAS	Fatty acid synthase enzyme
EGFR	The epidermal growth factor receptor
SP1	Specificity protein 1
H_2O_2	Hydrogen peroxide
AMPK	Activation of activated protein kinase
CoA	Co-enzyme A
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
GLTU	Glucose transporters
PEPCK	Phosphoenolpyruvate carboxykinase
RCT	Reverse the cholesterol transport
PG	Prostaglandin
SHR	Spontaneously hypertensive rats
NAFLD	Non-alcoholic fatty liver disease
OHdG	8-hydroxy-2′-deoxyguanosine
CKD	Chronic kidney disease
QCT	3-methyl-2-quinoxalin benzenevinylketo-1,4-dioxide

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Chapter 3

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 ($\bullet O_2^-$) and the hydroxyl radical ($\bullet OH$), as well as the oxygenated molecule precursors of free radicals, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) [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 $\bullet O_2^-$ 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, lopoic 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 $AscH_2$ and 0.004% as dianion ascorbate (Asc_2^-). $AscH^-$ 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

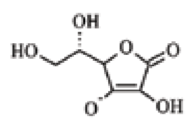
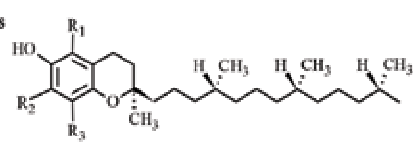
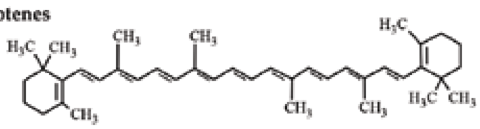
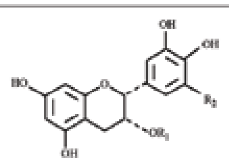
Structure of Antioxidants	Main Source in the Diet
Ascorbates 	Kiwi, strawberries, oranges, melon, grapefruit, red or green pepper, mango, broccoli, brussels sprouts, tomato.
Tocopherols 	Almonds, hazelnuts, peanuts, walnuts, sesame, rice bran, wheat germ, coconut, soy, olives, vegetable oils (palm, sunflower, 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.

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

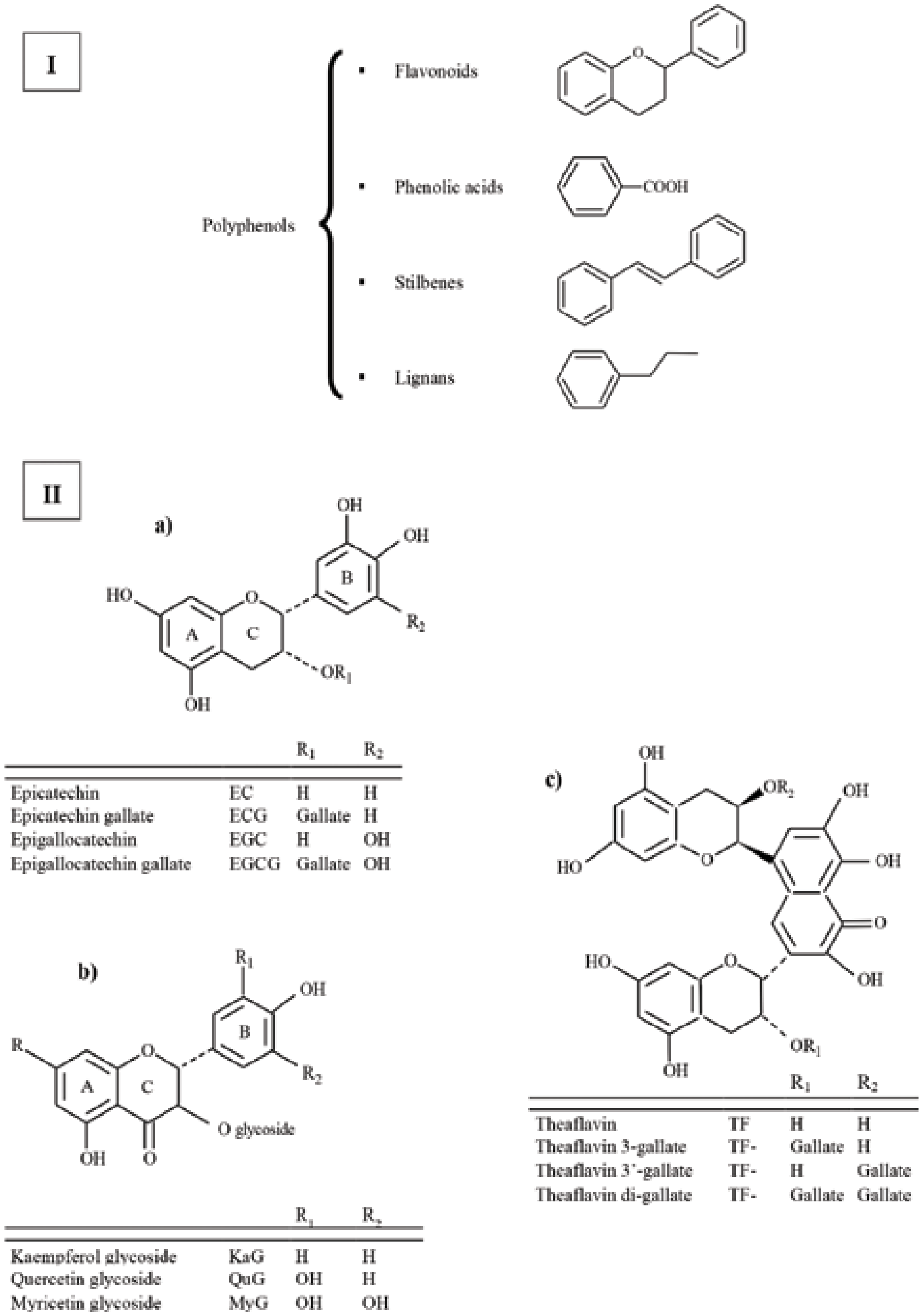

Figure 2. (I) The classification of the phenolic structures of polyphenols. (II) Structure of flavonoids; the core structure contains a diphenylpropane skeleton. The main flavonoids found in fresh green tealeaves are: (a) flavanols, (b) flavonols and (c) theaflavins.

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 $^1O_2$, $\bullet O_2^-$, •OH and peroxyl radicals (ROO•). 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 $^1O_2$ 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), •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 (•OH) generated

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

$$\text{Transition metal ion}(n^{+}) + H_2O_2 \rightarrow \text{Transition metal ion}(n+1) + \bullet OH + OH^{-}$$

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 •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 •OH and $^1O_2$ [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 •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 •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 •OH and guanine [22, 39].

Although the direct relationship between DNA damage and •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 •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].

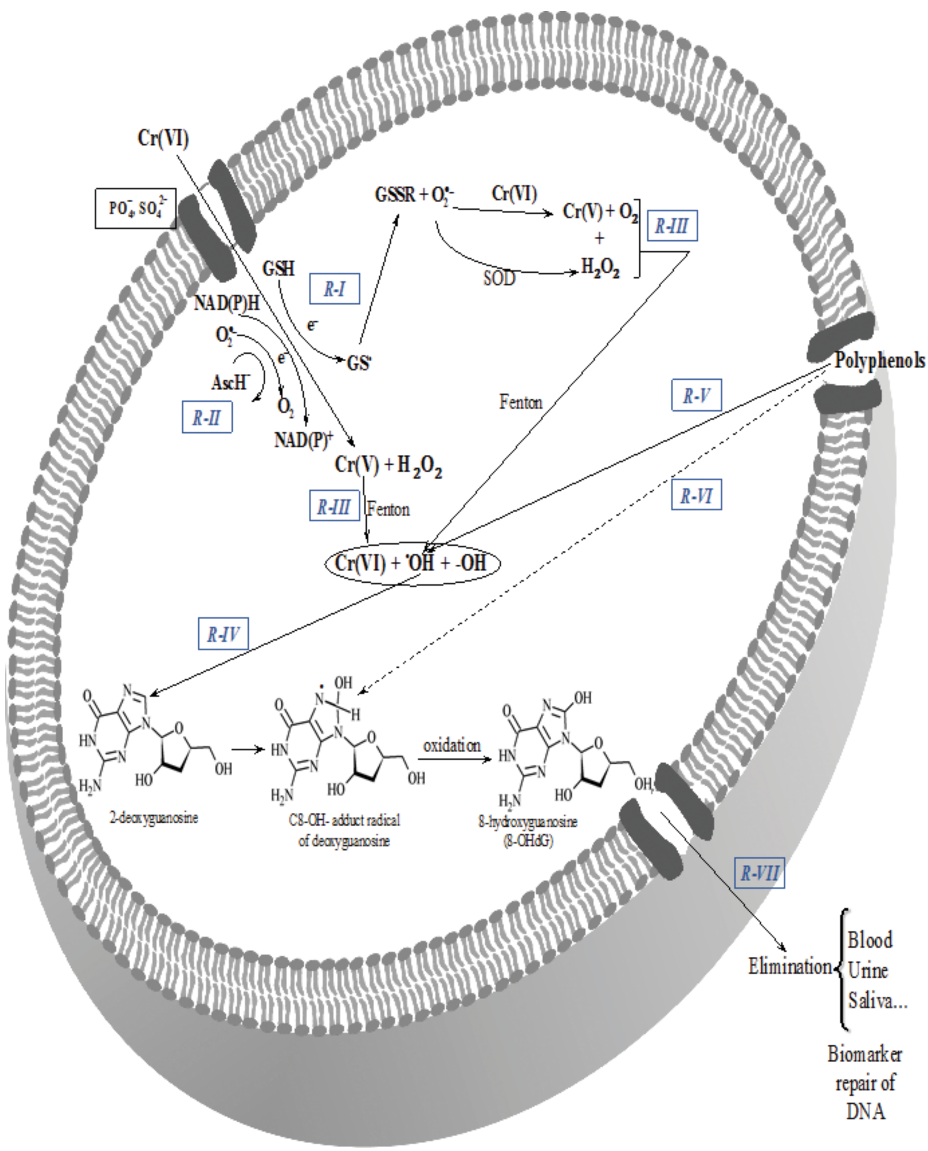

Figure 3. Routes of Cr(VI) and polyphenols in the induction, protection and modulation of DNA oxidative stress damage.

Compounds of Cr are major inorganic environmental pollutants. Its valence states range from −2 to +6 and mainly exists in two different redox forms: (i) Cr(III), an essential micronutrient that plays an important role in protein, sugar and fat metabolism and (ii) Cr(VI), known to be highly toxic, with well-documented carcinogenesis in lung, nasal and sinus tissues in toxicological and epidemiological studies. The increase of Cr(VI) compounds in the environment is caused by anthropogenic sources such as metallurgy, electroplating, inorganic chemical production, pigment and fungicide manufacturing, wood preservation, leather tanning and refractory industry. The high mobility, solubility and bioavailability of Cr(VI) compounds 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 •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 $\bullet O_2^-$ and •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

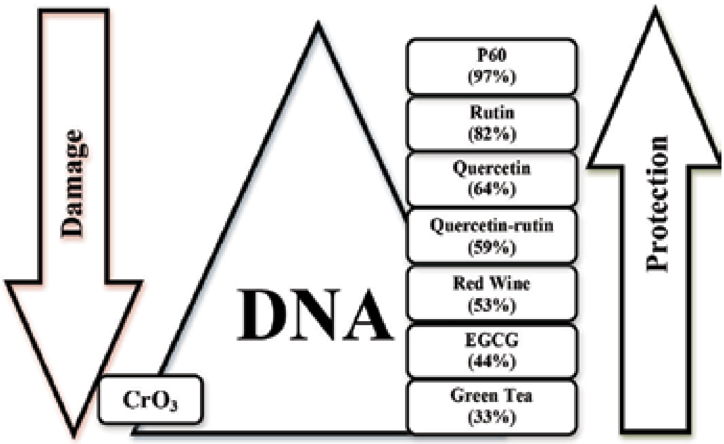

Figure 4. Levels of protection of different compounds from DNA damage caused by CrO_3.

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 •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

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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 •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.

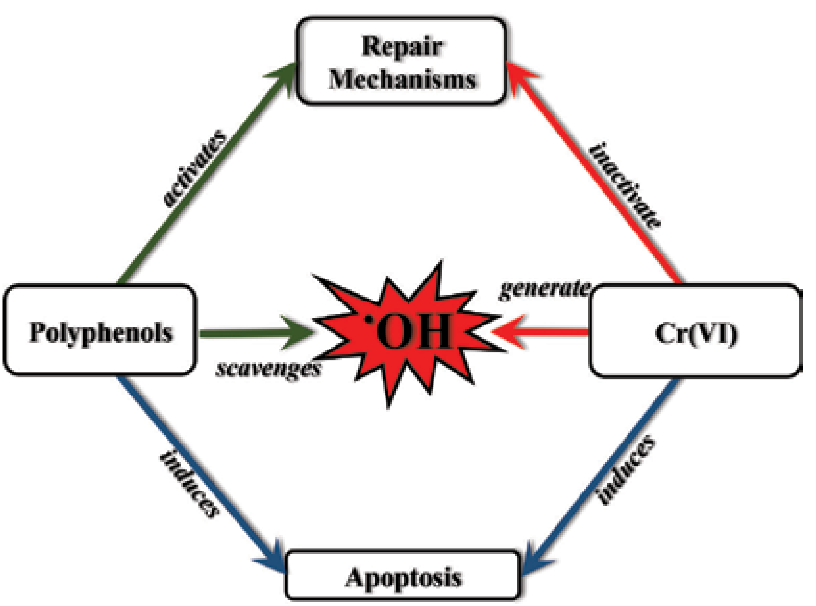

Figure 5. Summary of the interaction between polyphenols and heavy metals.

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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Section 3

Polyphenols and Technology

Chapter 4

Potentials of Polyphenols in Bone-Implant Devices

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Additional information is available at the end of the chapter

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Abstract

Knowledge of bioactive plant-derived polyphenols is growing to such an extent that science interest is looking at development of different applications in regenerative medicine through new and state-of-the-art tissue engineering technologies. Due to their well-established and demonstrated antioxidant and anti-inflammatory beneficial properties, polyphenols have been extensively investigated to the extent that they provide benefits to different pathological conditions, including cardiovascular and bone diseases, neurodegenerative disorders, and cancer. By taking into account the main molecular pathways of polyphenols' action, we want to focus this chapter on applications of polyphenols in bone-implant devices. In particular, results of polyphenols' effects on bone cells and tissues following local delivery from innovative biomaterials will be discussed, together with preliminary in vivo tests. Purpose of the dissertation is to provide the reader new insights into knowledge of polyphenols not only regarding the different molecular mechanisms involved in their action but also the biological responses deriving from local applications.

Keywords: polyphenols, bone regeneration, molecular mechanisms, bone-implant devices, periodontitis

1. Introduction

Plants and their single parts have been employed, for millennia, for healing purposes alone or as adjunct therapy to conventional pharmaceuticals, thanks to their richness in different bioactive compounds, effective on several biological systems [1]. Among them, polyphenols do possess different beneficial properties on health—especially effective on the improvement of chronic pathologies such as cardiovascular disease, osteoporosis, diabetes, and neurodegenerative disorders—which thus strengthens the interest of scientific community [2]. Osteoporosis is a multifactorial degenerative bone disease characterized by an imbalance between bone-forming and bone-resorbing factors, due to the interaction between genes involved in the normal bone metabolism and different external factors, such as vitamin D deficiency, alcohol consumption, smoking, aging, and menopause [3]. Thanks to their wide range of activities exerted on different biological levels, polyphenols have been shown to protect the bone system, starting from bone mass increase to slowdown of bone turnover, due to their well-known combined actions on inflammation, oxidative stress, hormones activity, and aging [4].

In fact, several important molecular pathways involved in bone metabolism are targeted by polyphenols, such as the estrogen (E) signaling pathway, the mitogen-activated protein kinase (MAPK) cascade, sirtuin 1 (Sirt1), Wnt/β-catenin, TGF-β/BMP, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt, and adenosine monophosphate protein kinase (AMPK) [4].

Nowadays, the interest of scientific community toward these phytochemicals is much more increasing, in light of their potential strong clinical impact [5–8], even if the bioavailability aspect has to be strongly taken into account. In fact, bioavailability is an important factor when talking about polyphenols' actions, and this is because it is affected by different environmental, host and food processing-related elements, as well as the chemical structure itself [9]. In addition to bioavailability, the dose is also an aspect to take into consideration and, specifically, the dose at which the phenolic compound is effective. The effective dose is normally different from the ingested dose because, in plants, the common polyphenol chemical structure is the esterified form, absorption of which is markedly reduced in human tissues, while administration of oral doses at supraphysiological concentrations, which could elicit beneficial effects, has been shown to be toxic [10]. Furthermore, the ingested polyphenols can conjugate to proteins and polysaccharides, beyond affecting absorption [11]. Many efforts have been made to overcome the problems derived from oral intake, and so, controlled topical application of polyphenols, by nanoparticulate drug delivery systems, for example, could represent a new era in bone disease management.

A growing body of evidence suggests a role for phytomolecules, such as polyphenols, in bone biology. Molecules such as epigallocatechin-3-gallate (EGCG) from green tea have been positively associated, by multivariate analysis, to a positive trend of increased total body mineral density with tea drinking ($p < 0.05$) in a cohort of almost 5000 multiethnic postmenopausal women [12, 13]. A number of in vitro studies have described polyphenols' interaction with bone regeneration pathways. These molecules have also been investigated as possible therapeutic agents for periodontal inflammation because of their ability to modulate the host inflammatory response [14]. The efficacy of these molecules has been shown in the control of the bone resorption process by osteoimmunological actions, particularly by means of systemic administration in the diet [15].

The possibility of using molecules of this type for treatment and tissue regeneration in particular cases, such as periodontal and peri-implant defects, is obviously of great practical interest [16].

Furthermore, a local use, unlike the systemic approach, could be a solution to overcome some issues related to the biological adsorption and to enhance the beneficial action of polyphenolic molecules at the resorption site. Different delivery systems have been designed to overcome the highly soluble nature and the chemical instability of polyphenols, from polymeric matrices, to surface-coated surfaces, nanoparticles, microemulsions and liposomes. In general, all kinds of drug delivery systems composed of bone-targeting moieties and/or carriers with the therapeutic agent to be delivered can be employed [17].

Being local delivery of polyphenols, for bone regeneration, a quite recent theme, first studies show, however, promising results in terms of enhancement of bone mass and osteoblast proliferation and reduction of the inflammation-related bone resorptive processes [18].

2. Molecular mechanisms of polyphenols in bone protection

Polyphenols are characterized by different properties, which make them able to exert beneficial actions on several biological systems. Such properties include antioxidation, anti-inflammation, antiviral, and antiallergenic activity and are mainly due to their chemical compositions that vary from a compound class to another. This difference is due to the diverse chemical structures that, even though share common phenolic features, vary in both configuration and total number of phenolic hydroxyl groups, able to interact with reactive oxygen species (ROS) and reactive nitrogen species (RNS), thanks to their capacity to donate hydrogens [19]. Polyphenols with the B-ring hydroxyl configuration, such as flavonoids, do show a significant antioxidant action, which increases along with the total number of OH groups and with the presence of the 3,4-catechol structure [20]. As non-enzymatic antioxidant molecules, polyphenols do exert their radical-scavenging activity by interrupting free-radical chain reactions, such as those involved in lipid peroxidation, thus acting as hydrogen donators, reducing agents, superoxide radical scavengers, and singlet oxygen quenchers (**Figure 1**) [21]. This ROS-scavenging activity is particularly evident in icaritin (a flavonoid isolated from *Epimedium pubescens*) and phloridzin-mediated mechanisms, involved in reducing superoxide generation in osteoclasts [22], and, since ROS are responsible for activation of NF-κB signaling, prevention of ROS production has indirect effects on NFATc1, the master TF for promotion of osteoclastogenesis regulated by the NF-κB pathway. This is the case of curcumin that, at 5 μM, does inhibit osteoclast differentiation, by suppressing ROS generation [23].

Furthermore, polyphenols are also metal chelators, in which they have been shown to interact with metals, especially Fe and Zn, in a manner depending on their own concentration and on the metal concentration [24, 25].

In addition to act as direct antioxidants, polyphenols do contribute to activate and regulate antioxidant enzymes, to inhibit oxidases, cyclooxygenases, and other enzymes, such as iNOS, involved in radical generation [26], through upregulation of Nrf2, a nuclear factor that contributes to the enhanced production of antioxidant enzymes [27, 28].

Furthermore, catalase (CAT), an enzyme that detoxifies hydrogen peroxide, and superoxide dismutase (SOD), the major antioxidant defense system against $O2^-$ [29], have been shown to be increased in rats after administration of ellagic acid (EA), thus decreasing the level of lipid peroxidation and accelerating the healing process after tooth extraction [30, 31]. Subsequent elevation of CAT and SOD to heme oxygenase (HO) system activation can be seen with curcumin 10 μM, which upregulates HO-1 expression, important for bone marrow stem cell

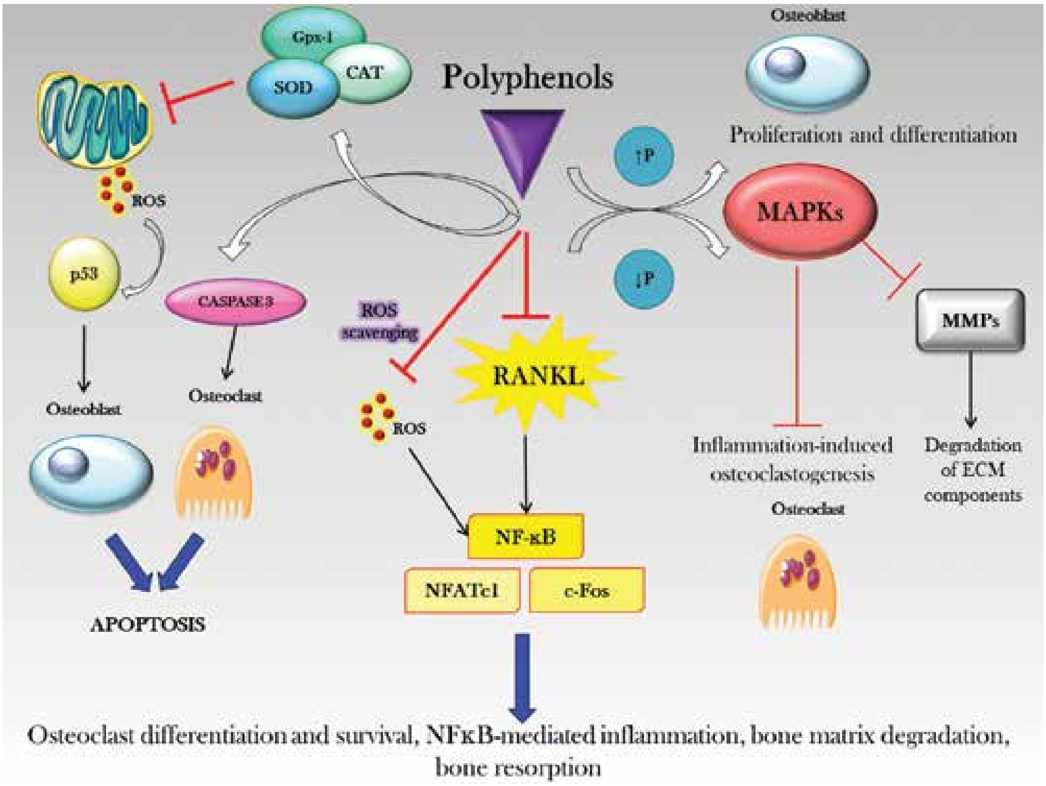

Figure 1. Molecular signaling mechanisms involved in polyphenol-induced bone protection.

differentiation in the osteoblastic lineage [32]. Moreover, curcumin dose-dependently (0.5–4 μM) and resveratrol upregulate the content of the antioxidant enzyme glutathione peroxidase (Gpx)-1 in the osteoclast, thus modulating the ROS levels (**Figure 1**) [33, 34].

Intracellular redox homeostasis is maintained, thanks to the presence of multiple interacting molecular pathways involved in the regulation of genes modulated by transcription factors (TFs) that contain redox-sensitive cysteine residues at their DNA-binding sites. Among them, NF-κB, AP-1, Nrf2, and hypoxia-inducible factor (HIF) are involved in the control of the cell life-or-death mechanism [35]. Downregulation of the prostanoid pathway by polyphenols also gives a negative contribution to bone resorption: in fact, quercetin, quercitrin, icaritin, and phloridzin have been shown to diminish production of prostaglandin E2 (PGE2), through downregulation of COX-2 and HIF-1α pathways [36–39].

Since these phytochemicals also showed pro-oxidant activities under certain conditions, and in a directly proportional manner to the total number of hydroxyl groups, it may be possible that activation of antioxidant enzymes occurs in response to this pro-oxidative feature, particularly evident in the presence of metals such as Cu and Fe, in cancer cells [40] but not in normal cells [41]. Such toxic property determines cytotoxic and pro-apoptotic effects, due to ROS-mediated cellular DNA breakage. In cancer cells, copper ions have been shown to be significantly elevated, compared to normal cells, and polyphenols are able to mobilizate endogenous Cu ions, which then activate a copper-dependent pro-oxidant pathway, leading to breakage of the double helix [42]. Epigallocatechin-3-gallate (EGCG) shows cytotoxic properties on osteoclasts, thanks to its reductive actions on Fe(III) catalyzed through the Fenton reaction, leading to production of hydroxyl radicals [43–45].

Antioxidant actions of polyphenols are not only limited to inhibition of bone resorption but are also directed at promotion of bone formation, through enhancing survival, function, and metabolism of osteoblasts. Specifically, reduction of the apoptosis rate through suppressing p53 signaling in the mitochondrion has been shown to be exerted by proanthocyanidins, thanks to their ROS-scavenging actions (**Figure 1**) [46].

The mitochondrion is the major site of production of ROS and RNS, and with the presence of multiple membranes, oxidative stress due to an easy lipid peroxidation is a common fact [47]. Furthermore, mitochondrial ROS also play a role in different signaling pathways, primarily those involved in cell death and survival signals: for example, mitochondria are essentials for the transactivation of growth factor receptor signaling to downstream mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase 1/2 (ERK1/2), C-Jun N-terminal kinase (JNK), and p38 [48].

It is thus clear that polyphenols, such as antioxidants, are important bone protectors, thanks to their regulation of bone cell proliferation and survival or death [49, 50]. In fact, by decreasing the oxidative status, they do contribute to osteoblast proliferation, activity, and differentiation, through crosstalking with different molecular signaling pathways.

So, activation of MAPK pathway by polyphenols leads to beneficial effects at different levels, with promotion of bone anabolism through phosphorylation of ERK, p38, and JNK [51–57], reduction of bone resorption, inhibition of osteoclast differentiation, and regulation of bone remodeling through suppressing receptor activator of nuclear factor kappa-B (NF-κB) ligand (RANKL) (**Figure 1**) [58–62]. RANKL, expressed by osteoblasts, T cells, and endothelial cells, stimulates osteoclast precursors to differentiate in mature osteoclasts, by binding to its cognate receptor, RANK, expressed on the surface of target cells. Binding of RANKL to RANK leads to TNF receptor-associated factor (TRAF) 6 recruitment and subsequent MAPK activation, as well as PI3K and NF-κB [63].

Hence, it is clear that by targeting the main TF of the inflammatory pathway, polyphenols are able to influence several processes involved in bone resorption, with inhibition of the expression of genes, such as interleukin (IL)-1β [64], monocyte chemotactic protein (MCP)-1 [65], IL-6 [66], tumor necrosis factor (TNF)-α [67], and matrix metalloproteinases (MMPs) [68], and induction of anti-inflammatory cytokines, such as IL-10 [69].

These anti-inflammatory properties have also effect on osteoclast differentiation, with inhibition of NFATc1 gene expression, thus affecting the early stages of osteoclast differentiation [28, 54, 70–74].

Concerning osteoblastic differentiation, several signaling pathways can be activated by polyphenols and, specifically, through transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP), Wnt/β-catenin, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt, and the estrogen (E2) pathway. For example, EGCG 5μM has been shown to positively act on osteoblast differentiation and mesenchymal stem cell (MSC) proliferation by upregulating BMP2 and runt-related transcription factor 2 (Runx2) expression [75]. Also, myricetin is able to promote osteoblast differentiation and activity, by targeting small mother against decapentaplegic (SMAD)1/5/8, downstream of BMP signaling [76, 77]. Osteoblast mineralization through upregulation of alkaline phosphatase (ALP) gene expression has also been shown to be induced by EGCG 25μM, through activation of β-catenin [78], and by hydroxyflavones 20μM, through activation of Akt signaling [57].

Given that polyphenols have also been demonstrated to have estrogen-like biological activities, it can be argued that they can modulate the estrogen-dependent pathway by acting as partial agonists and/or antagonists of the estrogen receptor (ER) in a tissue type and ligand concentration-dependent manner [79]. In this context, they do target the classical ER pathway, by binding to the ER and thus activating the ER-dependent gene transcription leading to inhibition of bone resorption and stimulation of osteoblastic bone formation [37, 80, 81], but they are also able to exert beneficial effects on bone system through the non-classical estrogen pathway, thus eliciting expression of the main osteoblast differentiation genes [82–85].

3. Applications of polyphenols in medicine for bone regeneration

Applications of polyphenols to investigate local bone formation are under current investigation because of the promising results obtained in the still limited in vitro and in vivo studies.

Different strategies have been considered to overtake the several disadvantages concerning polyphenol bioavailability, stability, and biopharmaceutical properties in general. These include different drug delivery systems (DDSs), encapsulation [86], chemical modifications [87], design of colloidal systems [88], use of nanoparticles [89], and implant surface modifications.

DDS can be divided into local and systemic and are characterized by different properties: first, local drug delivery is primarily intended for a local controlled effect, with reduction of the dose and possible side effects, while systemic delivery has the advantage of non-invasiveness, but uncontrollable and non-specific drug release [90].

In the field of bone regeneration, there is a need for the use of local and sustained drug release to avoid the risk of possible occurring infections at the site of bone defect. This is made possible, thanks to a spatial and temporal controlled drug release, leading to an increase in local effectiveness and minimization of toxicity to other tissues [91]. That is why biomedical applications for bone tissue regeneration have been employing carrier scaffolds, surfaces of which are coated by biodegradable polymer coatings loaded with the interested molecule.

3.1. Development of biomaterials functionalized with polyphenols

Within the wide world of bone regeneration, periodontal regeneration has gained particular attention. In the last decade, the development of grafting materials has aroused great interest [92–94]. In particular, synthetic ceramic materials, such as tricalcium phosphate and hydroxyapatite (HA), have been widely used due to their good reproducibility, biocompatibility, non-immunogenicity, but especially because of their similarity to the components of the native bone mineral phase [95, 96]. The most used materials (bone grafts, scaffolds, bone pastes, putties, etc.) available on the market for those applications work mainly through mechanical action, providing a functional scaffold for cell adhesion [97–99]. Some other molecules confer them a biological action, as they are able to stimulate bone regeneration (osteoinduction). For example, bovine bone–based biomaterials are treated to not completely eliminate the organic portion, so that the final product still contains collagen molecules, which play a role in the promotion of new bone formation [100]. There are also synthetic products containing collagen or its sequences, which are designed to favor the interaction with the cellular components and, thus, the regeneration process [101, 102].

Furthermore, growth factors and other biological molecules (such as amelogenin) are present in some materials commonly used in the sector [103–105].

However, loss of soft tissue and resorption of bone tissue around natural teeth are, in many cases, the consequence of a complex infection of bacterial origin, which are known as periodontitis (or peri-implantitis if it occurs after the insertion of a titanium implant) [16]. Therefore, although the mentioned products are widely used with successful results, the development of the scientific knowledge in this field has indicated possible ways of improvement.

For natural reasons, the masticatory apparatus is the interface of a rich in bacteria environment and the soft and skeletal tissues. The cells of the immune system are constantly stimulated by the contact with the microorganisms and are continuously urged to mount an inflammatory response.

Periodontitis is due to a bacterially induced chronic inflammatory disease that destroys the connective tissues and the bone that supports the teeth. Tissue destruction occurs following the cell death induced by the harmful products derived by the bacterial biofilm and, indirectly, following the activation of inflammatory cells, which produce and release cytokines acting as pro-inflammatory and catabolic mediators [106].

It is known that some cytokines produced in response to the inflammatory stimulus, such as interleukin 1, have a powerful effect in stimulating the formation of osteoclasts [107–109]. Formation of new bone and resorption of the existing bone tissue are normally balanced in the body, ensuring the so-called bone homeostasis, but in conditions of prolonged inflammatory response, such as those naturally present in the areas where the teeth emerge in the oral cavity and in the peri-implant areas, the pro-osteoclastogenic action of cytokines leads to the onset of resorption phenomena. These phenomena are favored not only by general factors, such as inadequate oral hygiene, but also and especially by individual factors, such as genetic aspects in the case of periodontitis or periodontal disease [110–112].

The common treatment for patients affected by these diseases is the use of a local debridement in order to eliminate the residual tissue infected, a surface decontamination and the use of classical bone grafting material in combination with a systemic antibiotic therapy. Unfortunately, this approach is not so effective, due to a specific adhesion of bacteria on the biomaterials and to the very low penetration of the antibiotic into the osseous defect [113–115].

Besides stimulation of osteoclastogenesis and bone resorption, prolonged inflammatory stimulation involves destruction of the soft tissue, also because of the so-called oxidative stress, that damages tissues as a result of a loss of control of the defence mechanisms that detoxify the organism from reactive oxygen species (ROS), molecular species generated in inflammatory sites.

The commercially available material currently used in periodontal regeneration has a lack in prevention or in controlling the cause of bone resorption. It would be, thus, desirable to provide an action aimed at controlling specific antiosteoclastogenic actions and effects to prevent the damage derived from oxidative stress, by exerting antioxidant properties.

The use of polyphenols as natural molecules to control and enhance periodontal regeneration, particularly in patient affected by periodontitis, is due to their antioxidant, free-radical scavenging, and antimicrobial properties.

Polyphenol molecules could be synthesized in laboratory or could be extracted from the different plant sources or their residues. Among the wide panorama of the natural sources of these molecules, extraction from wastes for ecological, ethical, and economic reasons has aroused great interest. One of the most abundant residuals with a high percentage of polyphenol content is the grape marc (skin and seeds). Grape, with 63 million of tons of products, is one of the most extensively cultivated crops in the world; most of it is used for wine production, and the consequence is the creation of almost 10 million of tons of by-products [116]. These byproducts represent approximately 20% of the harvested grapes, and they have been for long time undervalued; however, in the last decade, many research groups have been focused on the potential of the winery pomace as source of polyphenols. It is known in the art that polyphenols in complex mixtures may be easily extracted, and in literature, many different techniques are present: classical solvent extraction (using different solvents) [117–121], simulated maceration [122], ultrasound-assisted extraction [123], microwave-assisted extraction [124], and the most recent extraction using supercritical fluid in combination or not with solvent [117, 125, 126].

The phenolic composition of the extract from grape seeds and skin has been extensively analyzed, in terms of quantity and quality [127–129]. Of course, in literature, a wide dispersion of data is present, which is due to the fact that the phenolic spectra depend from the enological practice and, of course, from the variety of grape. It is possible to list different molecules that are present: anthocyanins (in particular for red grape), gallic acid (in particular in seeds), epigallocatechin (in skin), hydroxycinnamic acids (in particular in white pomace skin), proanthocyanidins, quercetin, resveratrol, and so on. However, in general, it is possible to assess that the most abundant compounds are anthocyandins and flavanols [127].

Procyanidins and proanthocyanidins, complex molecules consisting of different repeating units and present in grapes, have been extensively studied in relation to their ability to re-mineralize dental tissue and its potential effect to treat periodontal disease [14, 15, 45, 130–135].

The purpose is to use these molecules for the treatment of specific pathologies, such as periodontitis, in combination with a biomaterial, to locally administrate polyphenols. However, such local use is hindered by the high-water solubility of these molecules, which could rapidly remove them from the site of implantation. The possibility of local use could be achieved if polyphenols were mixed or bonded with a carrier able to exert the effects of the phenolic compounds for a prolonged time, circumventing the problem of the high solubility and instability of polyphenols. For examples, the polyphenolic extracts could be combined with collagen, exploiting the crosslinking effect exerted by some of these molecules [136], particularly the so-called tannins, which are molecules with high-molecular weight derived from the condensation of the repeated units of flavanols [137].

The obtained collagen-gel is stable in aqueous environment and allows a sustained controlled local release [138]. The authors suggest to combine it with granular ceramic fillers and to fill the peri-implant bone defects. This solution, for example, combines the mechanical scaffolding properties with the biological and pro-osteogenic actions of collagen and with an effective and locally concentrated anti-inflammatory, antioxidant, and antiosteoclastogenic action of polyphenols (see **Figure 2**) [139]. In vitro assays showed a high antioxidant effect and a controlled release of polyphenols (such as gallic acid, proanthocyanidins, catechins, and epicatechins). Interesting results show that, in presence of a compound that generates free radicals (sodium nitroprusside), the presence of polyphenols released from the bone filler protects the cells. Furthermore, osseointegration efficacy was evaluated through animal studies, by implanting the material in the medial condyle of the femur bone of rabbits, which showed an increase in the percentage of new formed bone area, compared with the bone filler without polyphenols.

The role of polyphenols was also studied to investigate their inhibitory properties on collagen degradation caused by collagenase enzymes. In particular, bacteria could enhance the production of enzymes that may degrade the collagenous matrix of soft and hard tissues [140]. Hence, during inflammation, the over production of collagenase enhances the disruption of the supporting tissue [141]. It has also been shown that catechin and epigallocatechin gallate-treated collagen exhibit between 56 and 95% resistance against collagenolytic hydrolysis by collagenases [142, 143]. These kinds of properties could be effective only if polyphenols were locally administrated, in order to be directly in contact with the tissue and to protect the collagen matrix from degradation.

MMP-8 (collagenase-2) and MMP-9 (gelatinase-B) are considered the main responsible for collagen degradation in inflamed tissues, for example, during gingivitis and periodontitis. It has been demonstrated that bacterial infections increase the expression of those enzymes [144]. Those MMPs are produced by Gram (−) periodonto-pathogens and play a crucial role in tissue destruction during periodontitis; it has also been demonstrated that grape seed extracts, rich in proanthocyanidin molecules, inhibit their activity, suggesting that polyphenol extracts could be used in the development of novel strategies for the treatment of periodontitis [14, 135].

The success rate of implant installation depends on the quantity and quality of bone, which is present in the extraction site [145, 146]. However, the quality of the new formed bone and its osseointegration also depends on the reaction caused by the implant itself. Dental replacement by using titanium dental implants is nowadays a quite common procedure in oral surgery [147]. Millions of dental implants are placed worldwide per year, and this number is

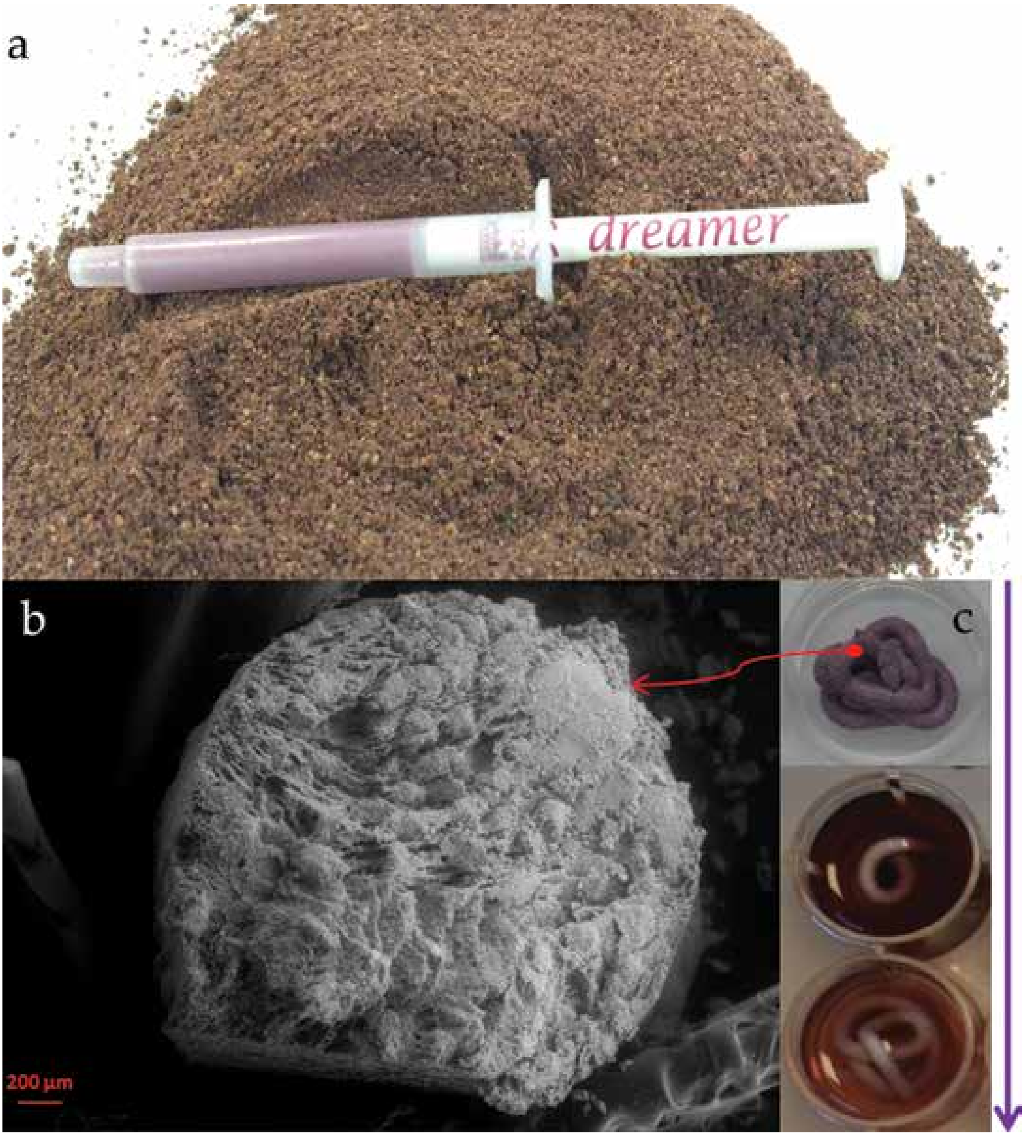

Figure 2. Composition for filling bone and periodontal defects combined with polyphenols rich extract. (a) Bone filler paste. (b) Scanning electron microscopy of the composite bone filler. (c) Optical image of the bone filler paste during the sustained release of polyphenols.

expected to increase [148]. Titanium is used not only in dentistry, but also in orthopedics, since it is characterized by a bioinertia, which promotes tissue regeneration. Periprosthetic infection is a consequence of implant insertion procedures, and it is due to the formation of microbial plaque accumulation, which promotes reaction of the inflammatory cells around the implant. Furthermore, macrophage cells consider the implant as a foreign body, and thus, they act by increasing the expression of pro-inflammatory cytokines and chemokines, which lead to the generation of chronic inflammation [113, 149].

3.2. Development of Ti surfaces functionalized with polyphenols

In dentistry, research has been focusing its attention on titanium (Ti) implants coated with osteoinductive and/or antibacterial agents, thus promising an improvement of the success rate of implants [150]. In order to control the post-implantation infection, a stable coating bonded on the surface of the implant is much more desirable rather than a releasing form, since a stable and durable interface between implant and tissue could avoid biofilm formation, reduce inflammation, and promote osseointegration.

In this respect, polyphenols, thanks to their well-known antibacterial properties [151, 152], are now largely considered in bone regeneration applications and aimed at inhibiting biofilm formation [153], as it has been demonstrated, for example, for flavonoids from propolis [154], proanthocyanidins [155, 156], and chlorogenic acid [157]. Among all polyphenols, EGCG is largely studied for its antibacterial and anti-inflammatory properties, which makes it a promising compound to be employed in different treatments [158] and, in particular, for the improvement of the periodontal status, through EGCG-containing slow-release local delivery systems, such as hydroxypropyl cellulose strips, applied in the periodontal pockets [132].

The inflammatory properties of polyphenols are also an important aspect to take into consideration, because of their potential in playing a role in all the mechanisms that control bone resorption and, consequently, bone loss. In fact, bone resorption and inflammation are strictly linked, as the main inflammatory pathways are also involved in osteoclastogenesis and bone remodeling [159]. Furthermore, a situation of chronic inflammation also leads to a continuous efflux of the mediators of inflammation [160], a fact that can be easily observed in ulcers and periodontitis. Periodontal disease is a chronic inflammatory disease characterized by the progressive destruction of the tooth supporting tissues, following a chronic inflammatory response to the accumulation of bacterial plaque on and around the teeth [161]. Therapeutic approaches aimed at modulating the host response, did involve polyphenols from Cranberries (*Vaccinium macrocarpon*) extracts, and led to beneficial effects slowing the periodontal disease progression [162].

In this field, polyphenols have also been considered for applications [163] in helping to control the oral hygiene, through the use of toothpastes enriched with 0.1% extracts containing naringenin and quercetin [164] and 0.5% extracts containing baicalein, baicalin, and wogonin [165] and through the use of polyphenol-containing gels [166].

Direct osteopromotive effects of polyphenols on osteoblast differentiation, proliferation, and protection are also well documented, so different bioactive polyphenol-coated biomaterials have been engineered, from the development of Ti surfaces functionalized with flavonoids [167, 168] conferring them osteopromotive, anti-inflammatory, and antibacterial properties, to tea polyphenol-modified calcium phosphate nanoparticles, which have been shown to enhance remineralization of preformed enamel lesions on bovine incisors [169].

Modifications of Ti surfaces, with quercitrin nanocoatings, allowed Córdoba et al. to engineer a polyphenol-functionalized biomaterial with enhanced mineralization properties, compared to Ti surfaces alone [170].

Improvement of hydroxyapatite (HA) deposition has been shown for grade 5 Ti silica-based bioactive glasses functionalized with extracts from green tea or red grape skin, making them suitable for bone contact applications [171].

Testing a mixture of 0.2mg EGCG with alpha tricalcium phosphate particles in rat calvarial defects has led to encouraging results of enhancement of bone formation [172], while conjugation of 4.2μg EGCG with a gel showed ability to induce differentiation of a mouse mesenchymal stem cell line toward the osteoblast lineage [18].

Employ of scaffolds enriched with polyphenols is, thus, considered as a promising tool for bone regeneration bioengineering, as functionalization of scaffold surface with polyphenols increases the bone regeneration ability, compared to a scaffold alone [173, 174]. Biodegradable soybean-based biomaterials (SBs), in a granulated form, have been employed as bone filler, in vitro, to investigate the biological properties for bone applications. Specifically, inhibition of osteoclast activation following incubation with SB has been observed, with a parallel inhibitory effect on monocyte/macrophage activity and, thus, a general anti-inflammatory action ascribed to the two main soy phytoestrogens genistein and daidzein. Furthermore, SBs have also been shown to induce mineralization in osteoblasts in vitro [175]. The same group of researchers then investigated the morphology of bone in response to SB granules in rabbits, and confirming the previous in vitro experiment [175], they showed bone repair with features distinct from that associated with sham-operated non-treated defects [176].

Widely used in a bone regeneration context, hydrogels do show many advantages compared to other kinds of scaffolds [177] because they can easily encapsulate bioactive substances, such as polyphenols too, which are able to induce mineralization. Increase of mineralization, through the use of gellan gum (GG) hydrogels enriched with Seanol®, an antioxidative food supplement containing Ecklonia cava-derived phlorotannins, has been observed in vitro [178], even if in some cases, the mineralization process has not been observed [179].

Lack of statistical differences, following implantation of a combination of a bovine-derived HA and *Cissus quadrangularis* extracts, has been also observed in a clinical trial involving 20 patients with intrabony defects [180]. Among the several medicinal plants exhibiting osteoprotective properties, safflower (*Carthamus tinctorius*) has been investigated in light of its numerous beneficial effects on bone formation, and, in particular, its seed extracts (SSE) have been combined with a collagen sponge (SSE/Col) functioning as a bone filler for the regeneration of periodontal tissue in beagle dogs. Results, at 8-week, showed increase of bone formation in the groups having received the SSE/Col, compared to control groups [181]. The same results of bone regeneration on dogs have been showed, by the same authors, after implantation of a polylactide glycolic acid bioabsorbable barrier membrane (PLGA) containing SSE [182].

4. Conclusions

Thanks to their demonstrated multiple health beneficial properties, polyphenols are increasingly considered for employ in different fields, from medicine, to nutraceutical and cosmeceutical industries. That is why the general interest, particularly in the field of medicine, is drawing attention to the development of next-generation biomaterials, functionalized with bioactive molecules, such as polyphenols. Thanks to the obtained promising results, polyphenol-containing bone designed biomaterials could represent a new era in bone disease management, with a high impact on bone regeneration quality.

Conflict of interest

Two of the authors (CC and MM) own shares of Nobil Bio Ricerche srl, while ET and GI are Nobil Bio Ricerche employees. The company is involved in R&D of polyphenol-containing bone-implant devices for commercial use.

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Chapter 5

Polyphenols of Red Grape Wines and Alcohol-Free Food Concentrates in Rehabilitation Technologies

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Abstract

The chapter presents the results of development and testing of products, rich in polyphenols (wine, drink, extract, food grape concentrate), which has a high antioxidant capacity, and which was confirmed by research both in vitro and in vivo. It was shown that oral intake of innovative products block the development of metabolic syndrome and ischemic myocardial damage in experimental animals. Clinical studies have proved, that patients with coronary heart disease and hypertension can significantly improve their health conditions, if they add innovative products saturated with grape polyphenols into their diet, during treatment at sanatorium or resort. The chapter estimates the vectors of development in viticulture and winemaking industry for further production of healthy food for the population.

Keywords: grape polyphenols, wine, wine beverage, extract, grape food concentrates, innovative products, antioxidant capacity, metabolic syndrome, ischemia

1. Introduction

Polyphenols of cultivated grape (*Vitis vinifera*) are famous for their biologically active features, which give them broader potential in prophylactic nutrition [1–7]. In this respect, they were included in “Russian Standards” as functional ingredients determining both antioxidant- and cardiovascular-supported effects [8, 9].

Grape polyphenols are subdivided into flavonoid and nonflavonoid phenols [2–4]. Flavonoid polyphenols include anthocyanins, catechins, proanthocyanidins, and quercetins. At the same time, anthocyanins contain mainly malvidin, delphinidin, and peonidin glycosides. (+)-D-catechins, (−)-epicatechin, and (−)-epicatechin-3-O-gallate were found. In quercetin groups, quercetin itself and quercetin glycosides were determined. Proanthocyanidins, or polymerized catechins-oligomers (2–6 units) and tannins (7 and more units), compose up to 95% of polyphenols in red grape wines and polyphenolic concentrates. Non-flavonoid polyphenols include phenolic acids - trans-caftaric acid, trans-coutaric acid, gallic acid, syringic acid, also stilbenes: trans-resveratrol and viniferin as well.

Red grape wines and food concentrates rich in polyphenols contain significant amounts of high-molecular phenolic compounds, consisting of the named proanthocyanidins, their condensates with anthocyanins and protein-tannate labile agglomerates with antioxidant potential. Among wide spectrum of the anhtocyanins biological activity, ability to increase elasticity of blood vessels and improve eye vision is remarkable [10, 11]. Catechins and their polymerized forms—proanthocyanidins are the most powerful antioxidants, with activity exceeding that of C and E vitamins, and are able to inhibit prostaglandins' synthesis, being catalyzed by cyclooxygenase-2. It leads to the inhibition of inflammatory processes [12]. (−)-Epicatechin and (−)-epigallocatechin gallate are able to induce tumor cells' apoptosis [13]. Oligomeric proanthocyanidins, coming into blood, delay low-density lipoprotein oxidation in blood plasma, preventing cardiovascular pathology [14]. Besides this, they decrease cholesterol concentration, preventing development of atherosclerosis. [15]. Tannins, without penetration into blood, nevertheless, normalize intestinal microflora due to their tanning features [16].

Quercetin and quercetin glycosides as well as trans-resveratrol improve both elasticity and permeability of blood capillaries and coronary flow as well [17]. Trans-resveratrol activates cells' regulatory processes leading to apoptosis of damaged or abnormal cells and, hence, demonstrates antineoplastic activity [18].

Total polyphenols of grapes show high antioxidant activity by 2 orders of magnitude greater than the index of antioxidant activity in human's blood serum [2].

Positive experimental and clinical results are known due to the usage of total polyphenols of grapes in nonalcoholic polyphenolic food concentrate "Enoant," reducing the intoxication in a case of cytostatic therapy by cisplatin [19, 20].

American dietologists in order to reduce cardiovascular risk recommend red grape wines with total polyphenol concentration of 2.5 g/L in the dosage of 150–300 mL/day [21]. Moderate and regular consumption of red wines is favorable for the prevention of cardiovascular disaster [22–25]. At the same time, it was shown that ethanol consumption, exceeding 31 mL/day, affects circulatory system negatively. [26–28]. In this connection, it becomes essential to determine both effective and safe dosages of red grape wines, which besides polyphenols contain from 9 to 15% of ethanol. The solution of the mentioned problem is relevant both for scientific and practical enotherapy, in order to prove its effective side. Besides, standard winemaking technologies do not set norms for grape polyphenol content. This complicates the ration of daily wine consumption according to recommended norms of biologically active polyphenols about 505 mg/day [29].

2. Aims of the present study

The aims of this study are as follows:

1. Monitoring of quantitative and qualitative composition of polyphenols and antioxidant capacity of the traditional dry still red wines and grape food concentrates.
2. Monitoring of quantitative and qualitative composition of polyphenols and antioxidant capacity of the sparkling wines.
3. Evaluation of biological activity of both dry still red wines and sparkling wines using experimental models in vivo.
4. Clinical testing of experimental products rich in red grape polyphenols.
5. Clinical probation of products rich in red grape polyphenols at sanatorium rehabilitation of cardiovascular diseases.

The study was performed using the Crimean and Kuban winemaking raw materials.

2.1. Monitoring of quantitative and qualitative composition of polyphenols and antioxidant capacity in the traditional dry still red wines and grape food concentrates

Industrial samples of Cabernet Sauvignon, Saperavi, Merlot wines and grape food concentrates "Enoant," "Enoant Premium," "Fenokor" were applied for the study. Producers used the samples of 2004 vintage year [30].

Quantitative and qualitative compositions of polyphenols in juices, wines, polyphenol extracts were estimated by the method of high-performance liquid chromatography with diode array detector (LC-DAD) using a chromatographic system (Agilent 1100 series; Agilent Technologies Inc.), an autosampler, and a control module. For the separation of substances, reversed-phase C18 column (Zorbax SB, 3.5 μm, 2.1, and 150 mm; Agilent Technologies Inc.) was used. A gradient solvent system was used consisting of 0.6% aqueous solution of trifluoroacetic acid (solvent A) and water/methanol/trifluoroacetic acid, 30:70:0.4, v/v. (solvent B). The elution profile had the following proportions (v/v) of the solvent B: 0 min, 8%; 0–8 min, 8–38%; 8–24 min, 38–100%; 24–29 min, 100%; 29–33 min, 8%. The solvent flow rate was 0.25 mL/min. The column temperature was fixed at 45°C and the injection volume was chosen to be 2 μL. Quantitation was performed using an external calibration curve monitoring peak areas at 280 nm for gallic acid, (+)-D-catechin, (−)-epicatechin, and proanthocyanidins; 313 nm for the derivatives of hydroxycinnamic acids; 371 nm for quercetin; and 525 nm for anthocyanins, respectively. Compounds found in the study were identified by comparison to standards as well as by comparison with standards previously characterized on the HPLC system by both relative elution times and spectral matching. [12, 14, 31]. The content of anthocyanins was determined in terms of oenin chloride, the contents of trans-caftaric acid in terms of caffeic acid, and the content of polymeric and oligomeric proanthocyanidins produced in terms of (+)-D-catechin. To ensure uniformity of results, the content of substances was calculated in mg/kg dry pomace.

Standards for each phenolic class were prepared from commercial sources. Gallic acid, caffeic acid, (+)-D-catechin, oenin chloride, quercetin dihydrate, isoquercitrin was obtained from Fluka (Fluka Chemie AG, Switzerland) and trans-resveratrol, (-)-epicatechin, syringic acid was obtained from Sigma-Aldrich a (Sigma-Aldrich, Switzerland).

The mass concentration of total polyphenols in wine and concentrates was determined by a colorimetric method by Folin-Ciocalteu reagent [32]. Grape food concentrates were diluted 100 times. One milliliter of the tested solution was placed in a volumetric flask with a capacity of 100 mL, adding 1 mL of Folin-Ciocalteu reagent and 10 mL of sodium carbonate solution; it was adjusted to the mark with distilled water at a temperature of 20 ± 0.5°C and stirred.

In 30–40 min, the optical density of the solutions was measured in 10-mm cuvette, at a wavelength of 670 nm against control solution on the "CFC-2" photoelectric colorimeter (ZOMZ, Russia). The value of the mass concentration of phenolic substances in mg/L for gallic acid was determined according to the calibration curve. The result was taken as the arithmetic mean of two parallel definitions, with permissible difference between them not exceeding (measuring range 0.1–90 g/L) 5%.

For the evaluation of antioxidant activity of wine and concentrates, amperometric method was used for measuring mass concentrations of antioxidants according to the standard antioxidant trolox on the device "Tsvet-Yauza 01-AA" (NPO "Khimavtomatika," Russia) GOST R 54037 [33]. All determinations were carried out in three replicates. The research results were processed by standard methods of mathematical statistics [34].

The obtained experimental data on qualitative and quantitative composition of polyphenols, indicator of antioxidant capacity for still dry red wines, and food concentrates of polyphenols are presented in **Tables 1** and **2**.

As shown in **Table 1**, qualitative composition of polyphenols in red wines, produced in the Crimea and the Kuban areas from Cabernet Sauvignon, Merlot, Saperavi grape varieties, is represented by anthocyanins, quercetin-3-O-glucoside, quercetin, (+)-D-catechin, (−)-epicatechin, trans-caftaric acid, trans-coutaric acid, gallic acid, syringic acid, oligomeric and polymeric proanthocyanidins. The proanthocyanidins compose 94–95% of the total amount of polyphenols in the Crimean wines and 75–86% in the wines of the Kuban. The polyphenols in red wines are identical in their composition to polyphenols of Cabernet Sauvignon, found in grape pomace, forming the total polyphenols of wine [35].

However, it should be noted that trans-resveratrol detected in the concentration 9–92 mg/kg of dry weight pomace, is almost not present in red wines. The concentration of total polyphenols in the wines of the Crimea and the Kuban region varies in the range of 2.8–4.2 g/L (measured by high-performance liquid chromatography) and 3.8–4.5 g/L (measured by Folin-Ciocalteu), which exceeds the average content of phenolic compounds in red wines (2.5 g/L) recommended to reduce the risk of cardiovascular pathology [21]. Indicator of antioxidant capacity of red grape wine corresponds to 2.36–2.75 g/L standard antioxidant trolox, which indirectly proves high biological activity of red wines.

Index	Product manufacturers					
	Brand					
	"Massandra"			"Kubanvino"		
	Cabernet	**Merlot**	**Saperavi**	**Cabernet**	**Merlot**	**Saperavi**
Anthocyans, mg/L						
Total anthocyans	20.3	23.8	23.4	133.3	167.5	556.2
Flavones, mg/L						
Quercetin-3-O-glycoside	8.5	15.9	11.5	15.7	36.9	9.8
Quercetin	2.8	1.6	1.2	0.3	4.1	0.7
Flavan-3-ols, mg/L						
(+)-D-catechin	34.7	44.8	26.8	60.8	83.5	58.6
(−)-Epicatechin	34.5	47.4	29.7	52.9	78.8	71.2
Oxycinnamic acids, mg/L						
Trans-caftaric acid	45.6	58.0	44.3	29.9	52.7	69.6
Trans-coutaric acid	7.5	10.0	7.4	3.5	5.4	11.8
Oxybenzo acids, mg/L						
Gallic acid	39.3	42.6	33.8	78.1	67.8	63.0
Syringic acid	7.0	5.3	9.0	8.4	4.0	4.3
Proanthocyanidins, mg/L						
Oligomeric	187	222	200	221	222	212
Polymeric	3045	3723	3525	2068	2072	2380
Integrated indices, g/L						
Total phenolic substances (by HPLC)	3.43	4.2	3.91	2.67	2.79	3.44
Total phenolic substances (by Folin-Ciocalteu)	4.35	4.56	4.25	3.89	3.85	4.13
Antioxidant capacity (trolox)	2.36	2.75	2.38	2.37	2.49	2.69

Table 1. Qualitative and quantitative composition of polyphenols in industrial samples of red grape wine.

The potential technological supply of polyphenols in the grape pomace, being extracted by water-alcohol extraction at 50% moisture, is 60 g per 1 kg of pomace [35]. It means that it retrieves about 40% of polyphenols in wet grape pomace based on the concentration of polyphenols in wine (4.5 g/L) in winemaking traditional "red" method.

Thus, a large part of the total polyphenols in the production of red wines remains in the pomace, which leads to significant losses of grape polyphenols, valued at the market in the range of 2.0–2.5 USD per 1 g.

Index	Enoant	Enoant Premium	Fenokor
Total anthocyans, mg/L	18.9 ± 0.4	28.5 ± 0.6	—
Flavones, mg/L			
Quercetin-3-O-glycoside	3.1 ± 0.1	3.5 ± 0.1	15.4 ± 0.03
Quercetin	49.6 ± 1.1	81.2 ± 1.1	10.2 ± 0.2
Flavan-3-ols, mg/L			
(+)-D-catechin	177.6 ± 4.0	208.5 ± 5.1	1752.6 ± 35.1
(−)-Epicatechin	118.4 ± 2.7	127.3 ± 3.1	1374.2 ± 27.5
Oxycinnamic acids, mg/L			
Trans-caftaric acid	11.7 ± 0.3	16.9 ± 0.4	—
Trans-coutaric acid	1.8 ± 0.0	2.4 ± 0.1	—
Oxybenzoic acids, mg/L			
Gallic acid	341.1 ± 7.7	465.2 ± 11. 3	1119.2 ± 22.4
Syringic acid	22.6 ± 0.5	26.2 ± 0.6	—
Proanthocyanidins, mg/L			
Oligomeric proanthocyanidins	603 ± 14	1614 ± 39	4598 ± 92
Polymeric proanthocyanidins	28,155 ± 634	38,436 ± 932	172,662 ± 3455
Integrated indices			
Total phenolic substances (by HPLC), g/L	29.50 ± 0.70	41.01 ± 1.00	181.53 ± 3.6
Total phenolic substances (by Folin-Ciocalteu), g/L	18.51 ± 0.49	21.81 ± 0.59	82.69 ± 2.29
Antioxidant capacity (trolox), g/L	24.72 ± 0.73	36.48 ± 0.92	196.22 ± 4.92

Table 2. Qualitative and quantitative composition of polyphenols in industrial samples of grape food concentrates.

The possibility of deeper extraction of total polyphenols from pomace and other secondary raw materials of industrial grape processing is being implemented in innovative products containing polyphenols as the target components of biological activity. The number of such products, produced in different countries, including Russia, involves alcohol-free food concentrate of grape polyphenols. Their composition is shown in **Table 2** [30].

Comparative analysis of the experimental data presented in **Tables 1** and **2** shows that the integral indicators of nonalcoholic grape food concentrates by an order of magnitude greater than similar data of red grape wines. At the same time, "Fenokor," derived from grape seed, lacks monomeric anthocyanins, oxycinnamic acids, and syringic acid. However, the qualitative composition of polyphenols in "Enoant" and "Enoant Premium" produced from the pomace of Cabernet Sauvignon variety demonstrates no difference from the composition of polyphenols in red wines.

As in red grape wines, in the food concentrate of grape polyphenols, only traces of trans-resveratrol are found, but in "Fenokor," the proportion of proanthocyanidins to the total amount of polyphenols increases up to 97.6%.

Apart from certain differences in the qualitative composition of polyphenols in red wines from different varieties of the Crimea and Kuban and concentrates, obtained from pomace and grape seed, approximation between the mass concentration of total polyphenols of grapes and indicator of antioxidant capacity of wines and concentrates is observed with high correlation coefficient R = 0.9952 [30]:

$$Y = 0.536 + 0.139X + 0.0804\,X^2 - 0.0006\,X^3 \qquad (1)$$

where Y is the value of antioxidant capacity, g/L in terms of trolox and X is the mass concentration of total polyphenols in grapes, g/L reagent Folin-Ciocalteu.

Eq. (1) is valid in the limits of variation of 1,0-82,7 g/L and phenolic substances 0,76 - of 196,2 g/L in terms of antioxidant capacity.

Eq. (1) allows assessing the antioxidant capacity of products by the concentration data of grape polyphenols in wine and concentrates.

The given monitoring data prove that both polyphenols of quiet table red wines of Crimea and Kuban region and alcohol-free polyphenol concentrates, "Enoant" and "Fenokor," are qualitatively identical. Their biological activity potential is represented by the dependence of antioxidant capacity indicator from the concentration of total polyphenols in products. This dependence is supposed to be the general both for the wines and for concentrates.

2.2. Monitoring of the quantitative and qualitative composition of polyphenols and indicator of sparkling wines antioxidant capacity

In reference sources, there is absence of information about the qualitative and qualitative composition of polyphenols, and indicator of antioxidant capacity of sparkling wines in Russia. Apparently, as in the case with still grape wines, it is connected with lack of appropriate requirements in the regulations [36].

In this connection, the aim of our study was to determine the qualitative and quantitative composition of polyphenols and index of antioxidant capacity of sparkling grape wines, and evaluate the potential consumption of those as a product for healthy diet [37].

The object of our research was white, pink, red sparkling wines, delivered into the market network by their Russian manufacturers. All 26 samples of sparkling wine correspond to the regulated requirements of the normative document [36]. To determine the qualitative and quantitative composition of polyphenols and the index of antioxidant capacity in samples of sparkling wines, methods were applied, as described in Section 2.1.

Table 3 shows experimental data on the total amount of polyphenols and the level of antioxidant capacity in samples of sparkling wines.

No.	The name of the sample	Vintage year	Date of bottling	Mass concentration of phenolic substances in Folin-Ciocalteu in terms of gallic acid, g/L	Antioxidant capacity in terms of trolox, g/L
1	Wine sparkling brut white "Pinot Zolotaya Balka"	2016	June 06, 2016	0.29	0.505
2	Wine sparkling brut white "Chardonnay Zolotaya Balka"	2015	August 31, 2015	0.322	0.514
3	Wine sparkling semidry white "Zolotaya Balka"	2016	March 14, 2016	0.27	0.500
4	Wine sparkling semisweet red "Zolotaya Balka"	2015	August 07, 2015	1.744	0.933
5	Wine sparkling semisweet white "Zolotaya Balka"	2016	July 01, 2016	0.286	0.504
6	Wine sparkling brut white "Zolotaya Balka"	2016	June 01, 2016	0.284	0.505
7	Wine sparkling semisweet wine "Muscat Sparkling"	2015	August 06, 2015	0.416	0.536
8	Wine sparkling semisweet pink "Golden Beam"	2015	August 07, 2015	0.412	0.530
9	Wine sparkling semisweet "Muscat Sparkling"	2015	August 04, 2015	0.442	0.539
10	Wine sparkling semisweet white "Crimean" Trademark "Sevastopol sparkling"	2015	December 24, 2015	0.286	0.506
11	Wine sparkling semidry white "Crimean" Trademark "Sevastopol sparkling"	2015	December 25, 2015	0.304	0.511
12	Wine sparkling semidry white "Crimean" Trademark "Chersonese Tavrichesky"	2015	December 24, 2015	0.393	0.524
13	Wine sparkling brut white "Crimean" Trademark "Chersonese Tavrichesky"	2015	December 25, 2015	0.384	0.521
14	Wine sparkling semisweet red "Crimean" Trademark "Sevastopol sparkling"	2015	June 17, 2015	1.659	0.872
15	Wine sparkling aged brut red "Novyi Svet. Crimean sparkling"	2012	January 26, 2016	1.280	0.843
16	Wine sparkling aged semisweet red "Novyi Svet. Crimean sparkling"	2012	February 19, 2016	1.412	0.860
17	Russian champagne aged brut white "Novyi Svet"	2012	February 13, 2016	0.271	0.567

No.	The name of the sample	Vintage year	Date of bottling	Mass concentration of phenolic substances in Folin-Ciocalteu in terms of gallic acid, g/L	Antioxidant capacity in terms of trolox, g/L
18	Russian champagne aged white semisweet "Novyi Svet"	2013	February 12, 2016	0.289	0.560
19	Russian champagne aged semisweet pink "Novyi Svet"	2013	February 12, 2016	0.415	0.636
20	Russian champagne aged semidry white "Novyi Svet"	2012	January 27, 2016	0.284	0.588
21	Russian champagne aged semidry pink "Novyi Svet"	2012	February 18, 2016	0.330	0.607
22	Wine sparkling aged semisweet red "Premium Cabernet" Abrau-Durso	2009	November 29, 2014	2.346	1.420
23	Wine sparkling aged semisweet red "Premium Cabernet" Abrau-Durso	2009	January 10, 2015	0.313	0.580
24	Wine sparkling aged brut pink "Premium Pink"	2013	March 01, 2015	0.250	0.568
25	Russian champagne brut white "Abrau-Durso"	2013	October 06, 2014	0.207	0.566
26	Russian champagne semidry white "Abrau-Durso"	2013	December 24, 2014	0.231	0.593

Table 3. Quantitative and qualitative composition of polyphenols and indicator of sparkling wines antioxidant capacity.

Data analysis for the quantitative content of total polyphenols in grapes (**Table 3**) shows that in white sparkling wines, the massive concentration of polyphenols varies in the range from 0.21 to 0.42 g/L; in pink 0.31–0.44 g/L; and in red 0.84–2.35 g/L. The indicator of antioxidant capacity varies in the range from 0.51 to 1.42 g/L for trolox, increasing with higher content of polyphenols in sparkling wine. This dependence is well approximated by Eq. (1) obtained earlier for still red dry wines and concentrates of grape polyphenols (Section 2.1).

Thus, the range of application of Eq. (1) is expanded at the lower end to 0.21 g/L total polyphenols in Folin-Ciocalteu and to 0.51 g/L in trolox index of antioxidant capacity. Of all tested brands, white sparkling wines are characterized by the low content of grape polyphenols and, consequently, low values of the antioxidant capacity indicator.

As shown in our study of the qualitative composition of polyphenols, whole spectrum of anthocyanins is absent in white sparkling wines, due to the specific technology of its production, which greatly diminishes biologically active substances in sparkling wines.

Table 4 shows experimental data obtained in the qualitative and quantitative composition of polyphenols of grapes in pink and red sparkling wines.

Index	Wines sparkling pink Samples No.					Wines sparkling red Samples No.				
	8	9	19	21	23	4	14	15	16	22
Anthocyans, mg/L										
Total Anthocyans, mg/L	1.4	2.2	0.6	0	0	49.1	4.9	10.1	8.2	2.7
Flavones, mg/L										
Quercetin-3-O-glycoside	0.7	2.4	0.3	2.2	2.1	6.9	3.0	1.5	1.7	3.4
Quercetin	3.1 ± 0.1	3.5 ± 0.1	15.4 ± 0.03	0.1	0.2	0.3	0.5	0.2	0.4	1.2
Flavan-3-ols, mg/L										
(+)-D-catechin	5.1	4.6	10.1	6.3	18.1	29.2	15.4	20.0	16.1	18.1
(−)-Epicatechin	7.7	10.5	6.8	6.8	2.5	34.5	18.9	16.2	15.7	8.4
Oxycinnamic acids, mg/L										
Trans-caftaric acid	28.1	68.8	18.6	30.1	12.1	75.4	41.9	36.1	35.7	6.0
Trans-coutaric acid	1.8	1.5	2.1	3.2	1.6	2.9	2.2	5.0	4.7	1.4
Oxybenzoic acids, mg/L										
Gallic acid	17.7	11.1	13.3	10.1	6.4	34.9	40.5	33.6	34.9	66.8
Syringic acid	1.8	1.1	10.1	0.3	—	5.7	4.5	3.0	3.2	2.4
Proanthocyanidins, mg/L										
Oligomeric	144.0	116.7	46.0	63.0	76.0	185.7	151.0	109.0	104.0	105.0
Polymeric	470.7	240.0	240.0	236.0	309.0	2555.2	2353.6	1243.0	1412.0	3021.0
Integrated indies, g/L										
Total phenolic substances (by HPLC)	0.68	0.68	0.31	0.33	0.40	2.68	2.64	1.41	1.57	3.2
Total phenolic substances (by Folin-Ciocalteu)	0.41	0.41	0.41	0.33	0.31	1.77	1.66	1.28	1.41	2.30
Antioxidant capacity (trolox)	0.53	0.54	0.64	0.61	0.58	0.93	0.87	0.84	0.86	1.42

Table 4. Qualitative and quantitative composition, antioxidant capacity of pink and red sparkling wines.

Composition of phenolic complex in both red sparkling wines and still dry red wines include anthocyanins, flavones, flavan-3-ols, hydroxy-cinnamic and hydroxybenzoic acid, oligomeric and polymeric proanthocyanidins. Proanthocyanidins are 91.5–96.8% of total amount of total polyphenols in red sparkling wines; this content is several times lower than that in still dry red wines. However, index of antioxidant capacity of red sparkling wines indirectly indicates the potential biological activity. The index is at a high level and is 0.84–1.42 g/L by trolox, which allows to assess biological effects of this product in vivo in experimental models.

Hence, there is an obvious correlation between antioxidant capacity indicator and the concentration of total polyphenols in products. That fact in a case of red wines proves the high potential of their biological activity.

2.3. Evaluation of the biological activity of still dry red wines and sparkling wines on experimental models in vivo

The results of numerous epidemiological studies have shown that the consumption of grape polyphenols at a dose of 300–400 mg daily (with an estimated average of 2.5 mg/L of polyphenols in European red wines) reduces cardiovascular mortality in France by 36–56% if compared to that in the US, taking into account the same level of saturated fat consumption and cholesterol level in blood for both countries [38].

The biological activity of the total polyphenols of red grape wine in relation to cardiovascular pathology manifests itself in the cardioprotective effect which is achieved due to the following factors: the reduction of low-density lipoprotein in the blood, the inhibition of atherosclerotic transformation of the blood vessel walls, stability augmentation of cell membranes, prevention of cytolysis, and inhibition of platelet aggregation [3].

Protective properties of grape polyphenols allow us to avoid the risk of the development of arterial hypertension and atherosclerosis provoked by the metabolic syndrome which has become extremely widespread over the past few decades [39].

Biological activity of polyphenols in red still dry wines was evaluated using wine material of Cabernet Sauvignon variety with mass concentration of total polyphenols 2.5 g/L, by Folin-Ciocalteu on the model of metabolic syndrome induced by fructose feeding [39]. Experimental studies were carried out on 50 white male rats, with weight 180–200 g, and the age of 10–12 weeks. Animals of experimental groups with metabolic syndrome received standard food and 10% fructose solution as drinking water for 8 weeks. Animals of the control group received standard food and water. Fructose feeding for 8 weeks contributed to the development of metabolic syndrome: visceral adiposity, hyperglycemia, hypercholesterolemia, dyslipoproteinemia (metabolic syndrome group). Since the fifth week, the animals of the three experimental groups with correction of the metabolic syndrome received wine in the dose of 0.7 mL (300 mL of wine per 70 kg of body weight). Wine material was diluted with water, reaching a concentration of polyphenols as 0.5; 1.0; and 2.5 g/L. Each group consisted of 10 animals.

To assess biological activity, the concentration of products reacting with thiobarbituric acid (TBA-AP), peroxidase-like activity (PLA), catalase-like activity (CLA), an intracellular antioxidant superoxide dismutase activity (SOD) were studied in blood serum of the animals.

Statistical data processing was carried out using methods of variation statistics; the data were considered to be reliable at $P < 0.05$. Biochemical parameters obtained on model of the metabolic syndrome are shown in **Table 5**.

As shown in **Table 5**, the full normalization of biochemical parameters of blood in animals with metabolic syndrome, to the level of normal animals, was achieved by using wine materials containing 2.5 g/L of polyphenols, i.e., with a daily intake of 750 mg of polyphenols per 70 kg of body weight. It appears that this dose of the consumption of total polyphenols of grapes can be taken as an estimate in the determination of the cardioprotective effectiveness of polyphenols in the correction of the metabolic syndrome negative impact by dry red table wines.

The research into the biological effects of sparkling grape wine polyphenols was performed on the sample of the red sparkling wine (**Table 3**, sample No.22) with the mass concentration of phenolic substances of 2.35 g/L (Folin-Ciocalteu grade) and the trolox equivalent of antioxidant capacity of 1.42 g/L. The research was performed in compliance with the model of circulatory hypoxia [40, 41] on white male rats of Wistar line (25 animals) as its object, each aged 10–12 weeks and weighing 180–200 g. For the experiment, which lasted for 2 weeks, they were divided into four experimental groups.

The control group of animals (K) received standard food and drinking water. The animals of the second experimental group (K/P) received standard food and drinking water and were to undergo bloodletting within the first week of the experiment. Animals of the third experimental group (K/P + W) also underwent bloodletting in the first week of the experiment and received sparkling red wine diluted with water as a daily drink. Animals of the fourth experimental group (W) did not undergo bloodletting and received sparkling red wine diluted with water as a daily drink. Red sparkling wine was given to the animals of the third and fourth groups at a dose of 0.5 mL per 100 g of body weight, which correspond to 880 mg of total polyphenols per 70 kg of body weight. In the control and experimental groups of animals, blood samples for studies were collected by decapitation under ether anesthesia. Statistical data processing was carried out using methods of variation statistics, with the data considered to be reliable at $P < 0.05$.

Index	**Metabolic syndrome**	**Metabolic syndrome + correction**			**Control**
		Polyphenols concentration, g/L			
		0.5	**1.0**	**2.5**	
SOD, U/mL	116.12 ± 6.28	126.94 ± 5.54	130.55 ± 4.13	136.20 ± 5.28	136.43 ± 5.178
TBA-AP nM MDA/mL	178.11 ± 4.45	151.24 ± 15.2	143.08 ± 13.47	130.85 ± 4.69	118.43 ± 2.79
PLA mcM/gHb•s	2.75 ± 0.09	2.80 ± 0.12	3.28 ± 0.37	3.56 ± 0.13	3.11 ± 0.11
CLA mM/gHb•s	0.17 ± 0.01	0.18 ± 0.02	0.20 ± 0.02	0.23 ± 0.01	0.22 ± 0.01

Table 5. Biochemical indices of animals' blood in the correction of metabolic syndrome by red wines.

The antioxidant capacity of the blood was estimated on the basis of the content of intracellular antioxidant enzyme superoxide dismutase (SOD), peroxidase-like activity (PLA), and catalase-like activity (CLA). The intensity of free radical lipid oxidation in the blood serum was assessed by the concentration of active products that react with thiobarbituric acid (TBA-AP).

The main results of the simulated hypoxia correction by the polyphenols of red sparkling wine are presented in **Table 6**.

As shown in **Table 6**, modeling of hypoxia by bloodletting had led to a marked increase in indicators of oxidative-antioxidant homeostasis of the blood of animals—TBA-AP, PLA, SOD indicators in the group with hypoxia (K/P) have increased if compared to the control group of healthy animals (K) that demonstrates a high level of free radical oxidation stress and exhausting of antioxidant systems. The group (K/P + W), treated with the polyphenols of red sparkling wine, demonstrates the decrease of TBA-AP by more than 8 times if compared to the same indicator group K/P with bloodletting. This index was decreased by 10 times in the group (W) which received only red sparkling wine without bloodletting.

Thus, the application of red sparkling wine polyphenols for the correction of free radical damage during hypoxia proved to be effective. The application of total polyphenols of red sparkling and quiet wines in dosages of 750–880 mg/kg on models of both metabolic syndrome and hypoxia confirms the high efficiency of the named substances. It allows stating the polyphenol concentration of 2.5 g/dm^3 adequate for the requested biological effects of red wines.

2.4. Elaboration of experimental products rich in red grape polyphenols

Reducing the risk of cardiovascular diseases and other abnormal body conditions becomes possible with a more extensive use of enotherapy potential, derived from the biological activity of the total polyphenols of red grapes, in health care practice. Despite numerous epidemiological, experimental and clinical studies, there is still no numerical index governing the content of total polyphenols in red wine varieties at a level that ensures the biological efficacy of wine as a product of functional purpose.

Given these circumstances, technical conditions and technological instructions for dry red table wine “Health,” wine beverage “Health,” and the extract of grape polyphenols were

Index	Experimental groups			Control group
	K/P	K/P + W	W	K
SOD, U/mL	233.68 ± 5.54	413.25 ± 9.79	345.55 ± 8.19	136.43 ± 5.178
TBA-AP nM MDA/mL	250.33 ± 5.92	28.56 ± 0.89	23.55 ± 0.78	118.43 ± 2.79
PLA mcM/gHb•s	5.98 ± 0.06	5.83 ± 0.06	6.84 ± 0.07	3.11 ± 0.03
CLA mM/gHb•s	1.94 ± 0.02	6.10 ± 0.02	3.68 ± 0.01	0.22 ± 0.01

Table 6. Biochemical indicators of animal blood in the correction of hypoxia by red sparkling wine polyphenols.

developed; an experimental batch of products was produced. The testing of experimental samples of the products for their biological activity in vitro and in vivo was conducted [42, 43].

Physicochemical characteristics governing the performance of these products include the increased amounts of phenolic compounds at a level of not less than 2.5 g/L for wine and beverage wine, and not less than 20.0 g/L for the extract of grape polyphenols.

Achieving a normalized indicator for the phenolic substances in wine is provided for by the traditional methods of mash fermentation; whereas in the wine beverage, blending wine material with an alcohol extract of grape polyphenols is allowed for that purpose. In the extract of grape polyphenols, the concentration of polyphenols is achieved by alcohol extraction, fermented pomace, and subsequent dealcoholization of the extract resulting in the concentration of alcohol not exceeding 15% vol.

Studies of experimental samples of wine, wine beverage, and the extract conducted in the season of grape processing of the vintage of 2015 showed that the content of polyphenols was 2.53–3.02 g/L in wine, 2.5–3.02 g/L in the wine beverage, and 21.5 g/L in the extract. Physicochemical characteristics of dry red table wine "Health," wine beverage "Health," and the extract of grape polyphenols are shown in **Tables 7** and **8**.

Normative index	**Sample***	**Actual index**		
		Grape variety		
		Cabernet Sauvignon	**Merlot**	**Saperavi**
Ethyl alcohol, % vol. from 10.5 to 15	1	10.8 ± 0.1	11.7 ± 0.1	12.0 ± 0.1
	2	10.9 ± 0.1	11.8 ± 0.1	11.9 ± 0.1
Sugar, g/L, no more than 4	1	2.5 0 ± 0.2	1.5 ± 0.2	2.7 ± 0.2
	2	2.5 ± 0.2	2.9 ± 0.2	2.8 ± 0.2
Titratable acids, g/L, at least 3.5	1	5.8 ± 0.1	5.3 ± 0.1	4.4 ± 0.1
	2	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1
Volatile acids, g/L, not more than 1.0	1	0.60 ± 01	0.53 ± 0.1	0.68 ± 0.1
	2	0.68 ± 0.1	0.68 ± 0.1	0.65 ± 0.1
Given extract, g/L, not less than 18.0 g/L	1	22.9 ± 1	20.5 ± 1	19.8 0 ± 1
	2	20.9 ± 1	20.0 ± 1	19.9 ± 1
Citric acid, g/L, not more than 1.0	1	0.10	0.19	0.10
	2	0.10	0.10	0.10
Total sulfur dioxide, mg/L, not more than 200	1	65.0	69.0	75.0
	2	75.0	75.0	74.0
Phenolic compound, g/L, not less than 2.5	1	2.53 ± 0.1	2.56 ± 0.1	3.02 ± 0.1
	2	3.02 ± 0.1	2.56 ± 0.1	3.00 ± 0.1

*1—Dry red table wine "Health" and 2—wine beverage "Health."

Table 7. Physicochemical characteristics of experimental samples of dry red table wine "health" and wine beverage "health".

Normative index	Actual index
Ethyl alcohol, % vol. from 10.5 to 15	10.6 ± 0.1
Titratable acids, g/L, at least 3.5	7.4 ± 0.2
Phenolic compound, g/L, not less than 2.5	21.5 ± 0.8
Mass fraction of dry substances, %, not less than 3.0	10.0

Table 8. Physicochemical characteristics of the experimental sample of extract of grape polyphenols.

The qualitative composition of polyphenols in experimental samples did not differ from the typical polyphenol composition of industrial samples of wines of the Crimea and Kuban (**Table 1**) and industrial samples of grape food concentrates (**Table 2**).

The index of antioxidant capacity in the experimental samples of wines and wine beverages corresponded to 1.51–1.72 g/L trolox equivalent and that of extract of grape polyphenols equaled 33.74 g/L trolox equivalent, which confirmed the high potential of the biological activity of experimental samples.

Evaluation of biological activity of experimental samples of red wine “Health” and the extract of grape polyphenols was performed in vivo on models of ischemic myocardial damage in rats. The conditions under which the animals were kept and the research experiments were carried out are in accordance with the requirements of international regulations “Guide for the Care and Use of Laboratory Animals” [43].

Investigations were carried out on 40 sexually mature male rats of Wistar line, each weighing 180–200 g. The animals were divided into 4 groups, with 10 animals in each group, 1 group was a control one and 3 others were experimental. Animals of experimental groups were given an aqueous solution of cobalt chloride ($CoCl_2$) for 7 days orally for the modeling of hypoxia and ischemic damage of the myocardium. For the correction of myocardial ischemia, experimental animals of two groups were administered:

- 0.25 mL/kg of the extract of grape polyphenols together with 0.5 mL/kg of water for 7 days (third experimental group);
- 2.5 mL/kg of red wine “Health” within 7 days (fourth group).

Seven days after decapitation of the animals, the components of free radical oxidation and antioxidants—TPK-AA, PLA, KLA, SOD—were detected in the serum.

Electron microscopical study of ischemic myocardial damage in heart sections of animals was carried out with the help of the electron microscope Selmi-125 at an accelerating voltage of 125 kV.

Biochemical indicators of blood on the experimental model of ischemia with cobalt chloride under the correction by polyphenols of the experimental produce are presented in **Table 9**. Statistical processing of the data was carried out by standard methods of variation statistics with the confidence probability of P = 0.95.

№	Index / group	TBA-AP nM MDA/mL	CLA mM/gHb•s	PLA mcM/gHb•s	SOD, U/mL
1	Control	120.67 ± 4.52	0.21 ± 0.01	3.17 ± 0.13	140.25 ± 6.20
2	$CoCl_2$	231.25 ± 6.17	0.15 ± 0.01	2.16 ± 0.12	97.08 ± 5.10
3	$CoCl_2$ + grape polyphenols extract	152.23 ± 4.76	0.20 ± 0.02	2.57 ± 0.09	127.42 ± 4.38
4	$CoCl_2$ + wine beverage "Health"	167.80 ± 5.06	0.17 ± 0.01	2.54 ± 0.14	111.17 ± 5.18

Table 9. Biochemical indicators of blood of animals for the correction polyphenols products experimental model of ischemia with cobalt chloride ($CoCl_2$).

As shown in **Table 9**, cobalt chloride leads to a decrease in the activity of intracellular antioxidant enzyme superoxide dismutase (SOD), catalase (CLA), peroxidase (PLA), and an increase in free radical oxidation of lipids in the concentration of active products that react with thiobarbituric acid (TBA-AP). These negative effects are corrected by daily oral administration of the total red grape polyphenols with red wine and extracts of grape polyphenols in the amount of 448 and 376 mg per 70 kg of body weight. Noticeable correction has already been detected within the first week of grape polyphenol consumption.

Electron microscopical examination of slices of the hearts of animals revealed such marked pathological effects of cobalt chloride ($CoCl_2$) on the myocardium in sexually mature male rats as: intracellular edema, focal disintegration, fragmentation of muscle fibers, destruction of endothelial cells, and contractile cardiomyocytes, lysis of myofibrils, the development of anemia. The result of the influence of cobalt chloride on the heart of animals was the development of ischemic cardiomyopathy.

Morphological changes of the myocardium in the groups of animals with the correction by grape polyphenols reflect the trend toward the minimal volume of inflicted damage and the preservation of myocardial structure, manifested in the normalization of the cell structures and fibers of muscle tissue, which demonstrates the cytoprotective properties of total grape polyphenols that are promising for the prevention of cardiomyopathy.

The application of both experimental red wines and red wine drink "Health" with polyphenols concentration at least 2.5 g/dm^3 as well as alcohol-containing concentrate with polyphenols concentration at least 20 g/dm^3 had demonstrated the high efficiency in the model of myocardial ischemic damage. That opens up broad prospects of the clinical use of the named products.

2.5. The clinical testing of products rich in grape polyphenols at the health resort rehabilitation of cardiovascular diseases

The successful testing of the biological activity of total polyphenols in experimental samples of red wine and extracts of grape polyphenols in vivo on models of ischemic damage of myocardium allowed to apply them for clinical trials of cardioprotective activity at the health resort (sanatorium) "AI-PETRI" in Yalta from May to October 2016. Two groups of patients were formed: a group of patients with coronary heart disease (96 people) and a group of patients with hypertension (163 people). The research protocol was approved by the local Ethics Committee [42–44]. All patients received identical treatment as prescribed by the doctors of the institution. Additionally, they were given either red wine "Health" or the extract of grape polyphenols calculated as 10 mg of total polyphenols of grapes per 1 kg of body weight or an average of 280 mL of wine and 36 mL of extract per day.

Patients were examined both at the beginning and at the end of the course of treatment of 14 days. The functional state of the cardiovascular system, blood biochemical parameters, functional tests of the hemodynamic function of the heart, the state of lipid peroxidation and antioxidants in the blood, and the overall state of patients were studied.

The positive dynamics of the health condition was registered in all patient groups, but in the groups receiving the wine or the extract of grape polyphenols, a greater number of parameters were improved when compared with control groups.

The positive effect manifested itself to a greater extent in the rehabilitation of patients with a coronary heart disease. As shown in the histograms of **Figure 1**, the significant decrease in total cholesterol, atherogenic quotient and the amount of fibrinogen in the blood were registered, which corresponds to the reduction of both thrombosis and cholesterol plaque formation risk.

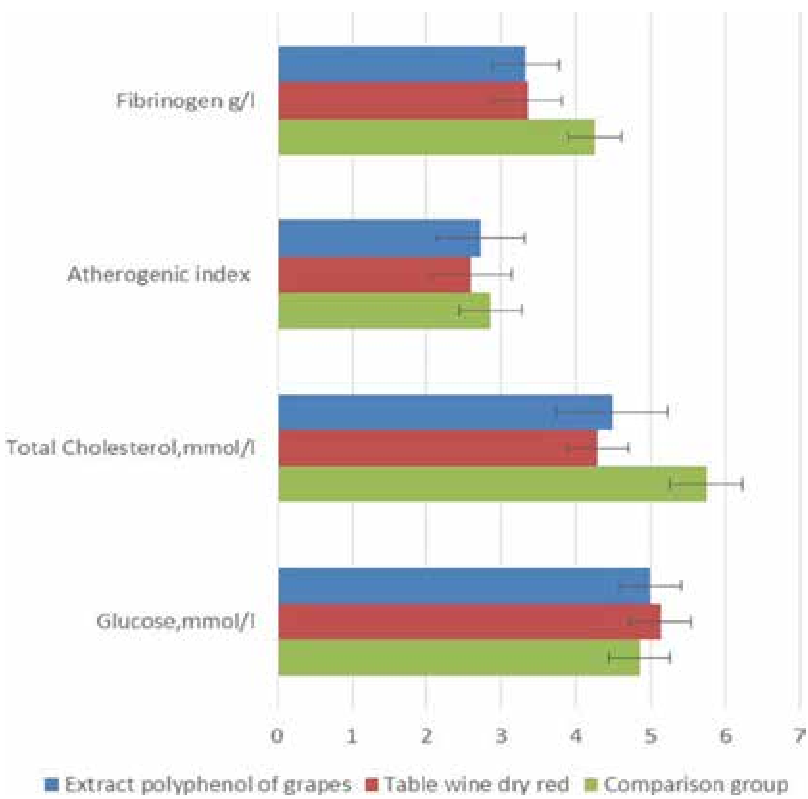

Figure 1. Changes in biochemical parameters of blood on the background of total polyphenols red wine extract in patients with coronary heart disease.

As clinically proved, the patients with the ischemic heart disease showed the twofold decrease in the need for nitroglycerin intake; the quarter of the patients had restrictions from any forms of physical activity removed; in more than 85% patients, there was both a marked decline in fatigue and the rise of tolerance to physical activity registered if compared to other patients who only received basic institutional treatment. The obtained results give us grounds to be optimistic about the possibility of using saturated total polyphenols of red grape wine and extracts of grape polyphenols for the rehabilitation of patients with a coronary heart disease and hypertension when receiving treatment at health resorts or sanatoria.

The successful rehabilitation of patients with ischemic heart disease and arterial hypertension was accomplished due to total polyphenols in red wines and polyphenols extracts despite the presence of alcohol in concentration of 10.6–12.0% ABV.

3. Conclusions

The polyphenol complex of red grape wines and grape concentrates, produced in Russia traditionally and by using innovative techniques, contains the identical groups of phenolic compounds and flavonoids, has an integral antioxidant capacity which increases with the rise of total polyphenol concentration in the products (Eq. (1)). It should be noted that 75.0–97.6% of total grape polyphenols are represented by oligomeric and polymeric proanthocyanidins.

The use of total polyphenols of grape red wines and grape concentrates calculated respectively as 376 mg, 448 mg, 750 mg, 870 mg per 70 kg of body weight in the experimental models of ischemia, hypoxia, and metabolic syndrome confirmed the high biological activity of the total polyphenols.

The possibility of successful rehabilitation of patients suffering from arterial hypertension and a coronary heart disease by taking total polyphenols of wine and the concentrate in the amount of 700 mg per 70 kg of body weight in the course of a 14-day health resort/sanatorium treatment was shown.

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Polyphenols, which mostly contain phenol structures, are phytochemicals found naturally in many types of food such as nuts, cocoa, berries, tea, and red wine. Emerging studies have shown that polyphenols can have multiple beneficial effects on the human body, such as antioxidative, anticancer, and antidiabetic effects. The present book encompasses many key aspects of polyphenolsfrom classification, dietary sources, health benefits, to possible mechanisms of action. “Polyphenols” is intended for a wide range of readers, hoping to provide readers with the recent scientific findings of why and how polyphenols can exert multiple health benefits.

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