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Insight into DNA protection ability of medicinal herbs and potential mechanisms in hydrogen peroxide damages model

Zahra Sabahi[1], Fatemeh Soltani[2], Mahmoodreza Moein[1,3]✉

[1]*Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[2]*Biotechnology Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran*

[3]*Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

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ABSTRACT

DNA damage is one of the most important consequences of oxidative stress in the cells. If DNA repair is unable to modify these inducible DNA damages, genomic instability may lead to mutation, cancer, aging and many other diseases. Single cell gel electrophoresis or comet assay is a common and versatile method to quantify these types of DNA damages. DNA damages induced by hydrogen peroxide (H_2O_2) are one of the proper models for measurement of protective ability of different compounds. So the main aim of this review is to provide an overview about protection ability of medicinal plants and their potential mechanism against H_2O_2 induced DNA damages. In this review, relevant researches on the effect of medicinal plants on DNA damages induced by H_2O_2 and possible molecular mechanisms are discussed. It seems that, medicinal plants are considered as therapeutic key factors to protect DNA from consequences caused by oxidative stress. Sufficient *in vitro* evidences introduce them as DNA protective agents through different mechanisms including antioxidant activity and some other cellular mechanisms. Moreover, in order to correlate the antigenotoxicity effects with their potential antioxidant property, most of medicinal plants were evaluated in term of antioxidant activity using standard methods. This review highlights the preventive effects of herbal medicine against oxidative DNA damages as well as provides rational possibility to engage them in animal studies and future clinical investigations.

1. Introduction

Throughout the lifespan of the cells, they expose to reactive oxygen species (ROS) generated by both endogenous metabolisms and exogenous resources. The overproduction of ROS which is defined as oxidative stress has serious pathological impacts on important biomacromolecules such as DNA and proteins[1]. Although the cells adopt compensatory mechanisms to overcome the deleterious effects of oxidative stress, but in some circumstances, DNA damages cannot be prevented and eventually correlate with etiology, resulting in different diseases such as cancer[2–4].

DNA damages can be measured by various methods such as high-performance liquid chromatography, gas chromatography, mass spectrometry, immunoassays and comet assay (single cell gel electropho resis)[5]. Among these methods, comet assay is most popular for DNA damage detection. It is a simple and useful

✉Corresponding author: Mahmoodreza Moein, Medicinal Plants Processing Research Center, Shiraz, Iran; Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
Tel: +98 71 32424127; 32424128; 32425305; 32425374
Fax: +98 71 32424126
E−mail: mrmoein@sums.ac.ir

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method to evaluate the DNA damage and repair as well as DNA-protective effects of different compounds[6].

According to the previous researches, two-thirds of the world's plant species have therapeutic value and many plants can protect cells against oxidative stress[7]. Although, significant role of bioactive natural products was clear, their application in modern drug discovery has been considerable since the 19th century. About 50% of the available drugs, directly or indirectly, are originated from natural products[8]. Additionally, natural products have been suggested as adjutant therapy for free radicals associated diseases, because of successful preclinical investigations which confirmed the antioxidant and cellular protective effects of natural compounds[9].

This review aimed to document the value of herbal medicines as protective agents against hydrogen peroxide (H_2O_2) induced DNA damages which were investigated by comet assay.

2. ROS

ROS are reactive chemical molecules containing oxygen and are products of different cellular pathways[10]. Endogenously, they are natural byproducts of oxidative metabolism and mitochondrial aerobic respiration. Approximately 5% of oxygen is converted to endogenous ROS[11-15]. Bacterial phagocytosis, virus infected cells and degradation of fatty acids by peroxisome are other cellular sources of ROS[13]. In addition, they can be produced through the activity of several cellular enzymes including nicotinamide adenine dinucleotide phosphate-oxidases, xanthine oxidase, and uncoupled endothelial nitric oxide synthase[15]. However, various stress such as ionizing and ultraviolet radiation[11,12,16] as well as environmental and some of therapeutic agents can dramatically increase the level of ROS[11]. Although, the important role of ROS in cell signaling and homeostasis is well documented but they are considered as potential cause of several biomacromolecules (lipids, proteins and DNA) damage[11,17,18]. Accordingly, in normal situation the cells adopt various enzymatic and non-enzymatic protection mechanisms in order to protect themselves against such harmful effects. In this concept, the excessive amount of ROS is defined as oxidative stress which is the result of an imbalance between the production and the scavenging of free radicals[4,19,20].

Oxidative stress has contributed to etiology of ageing and several diseases such as cancer, atherosclerosis, rheumatoid arthritis, Alzheimer and Parkinson[3,16,18,21-24]. In spite of different issues related to ROS overproduction, one may assume that complete ROS elimination is useful, however this strategy is not recommended due to their critical role in different cellular pathways such as activation of guanylate cyclase and cGMP formation, interleukin-2 and transcription nuclear factor κB[15] and induction of apoptosis[25]. Another strategy that can be adopted is using antioxidants, either natural or synthetic ones, to boost the defense system against oxidative stress[26-28].

Most ROS include superoxide, hydroxyl radical, H_2O_2, nitric oxide, and hydroxyl ion. The most important ROS among these molecules is H_2O_2, regarding to the mitogenic effect, cell cycle regulation and DNA damage. Based on this, in this review we only focus on chemistry and biological effects of H_2O_2 and hydroxyl radical.

Mitochondria synthesize adenosine triphosphate through electron transport chain and it is related to reduction-oxidation (redox) reactions. This chain is made up of a series of compounds that transfer electrons from electron donors to electron acceptors. Molecular oxygen is the last electron acceptor in this transport chain[19]. Throughout these (and other) electron transfer reactions, high reactive metabolites of oxygen, namely superoxide anion (O_2^-), H_2O_2 and hydroxyl radical (OH^-) are produced[19,29,30]. Peroxisomes are the other natural sources of H_2O_2 in cytosol[31].

These species have single and unpaired electron, which makes them highly chemically reactive and capable to donate another electron[32,33]. Half-life of ROS is affected by environmental factors such as pH and other radical species[32]. Among oxygen derivatives, H_2O_2 is a non-radical compound but highly reactive, and is produced in high concentration in the living cells[32].

H_2O_2 is product of $O_2^{\bullet-}$ dismutation and other reactions mediated by different enzymes including monoamine oxidase, xanthine oxidase, urate oxidase and D-amino acid oxidase. It is also detectable in expired breath, human urine, blood and some other body fluids. In general, H_2O_2 is cytotoxic in different cell types at concentration equal or higher than 50 μM; however this is affected by various factors such as cell type, cell iron content, length of exposure, H_2O_2 concentration and the media of cell culture. Side effects of H_2O_2 are reduced by the activity of catalases, glutathione peroxidases and thioredoxin-linked systems[34]. H_2O_2 participates in oxidation reaction through a non-radical pathway[35]. Furthermore, the interaction of the superoxide ($O_2^{\bullet-}$) radical and H_2O_2 results in the formation of highly reactive hydroxyl radicals[30,36], which can be a source of more harmful species, such as hydroxyl radicals (OH) or hypochlorous acid[32]. Peroxidases and catalases can scavenge H_2O_2 in most organisms[30,37].

It is accepted that decomposition of H_2O_2 depends on the presence of transition metal ions (especially Fe^{2+}) in Fenton reaction[35,36]. In this non-enzymatic reaction, Fe^{2+} reacts with H_2O_2 and produces $OH^{\bullet-}$, and Fe^{3+}, then this ferric ion participates in Haber-Weiss reaction to form Fe^{2+}[38].

Hydroxyl radical is a small, highly portable, water-soluble and extremely reactive radical[38] which is able to react as soon as formation[39]. Hydroxyl radicals are produced under cell stress conditions such as inflammations, embryo teratogenesis, and pathogen-defense reactions[40].

The rate of hydroxyl radical formation is about 50 hydroxyl radical per second, so that means each cell may produce 4 million hydroxyl radicals every day, which can be deactivated or may attack different biomacromolecules[37]. Hydroxyl radicals are able to carry out three different types of reactions including hydrogen abstraction, addition, or electron transfer, leading to produce new radicals and propagating some chain reactions.

Its prefer to react with electron density molecule site is due to electrophilic nature of this radical[41].This radical and hydroperoxyl are the most dominant ROS initiator in lipid peroxidation because of adequate energy to remove hydrogen atoms from unsaturated fatty acids. This attack can generate free radicals from polyunsaturated fatty acids [38]. Lipid peroxidation not only leads to loss of membrane property but also the resulting products can react with proteins, enzymes, and nucleic acids; which are associated with etiology of

different diseases[42]. Nucleic acids, DNA and/or RNA, are the other targets of $OH^{\bullet}$. Hydroxyl radical preferentially attacks to guanine base to form a C-8 OH-adduct, as discussed in the next section.

Some of DNA damages are results of reaction between DNA and lipid proxidation products such as malondialdehyde (MDA), 4-hydroxynonenal, acrolein and isoprostanes. It seems that MDA is the most mutagenic product of lipid peroxidation[16,43]. 4-hydroxynonenal and MDA are powerful electrophile agents which are able to react with proteins and DNA and form protein carbonyls and etheno DNA adducts[16].

3. DNA damage measurement

During past decades, different methods and techniques have been engaged to assess DNA damage. These are comprised of comet assay[44], mass spectrometry in combination with gas chromatography[44-48] or liquid chromatography[44,46,48,49] immunochemical techniques, post-labeling assays, and enzyme linked immunosorbent assay[48]. The principal way among these methods is screening of analysis and single product of DNA damages. For instance, in the HPLC-ECD technique the 2′-deoxyribonucleoside form of 8-hydroxyguanine (8-OH-Gua, also called 8-oxoGua) is detected. Several previous reviews have documented the advantages and limitation of these methods[4,44-46,48,50]. According to the aim of our paper, we focus on comet assay as a valuable method to detect DNA damages.

Over a period of past decade, single cell gel electrophoresis or comet assay[51] has become one of the standard, simple and reliable methods for examining the DNA damages and repair. It has been employed in a variety of genetic toxicology area including human biomonitoring, ecotoxicology, and fundamental genotoxicity studies[52-54]. In addition, it can be considered as a powerful tool for analyzing the effects of different nutrients, antioxidants, supplements and *etc*. on DNA damage and also pathologies such as cancer related to DNA damage[55-58].

Technically, in this assay the isolated cells are suspended in agarose and layered on a microscope slide. The embedded cells are then lyzed by exposure to a high salt solution containing detergent which destroys the cell membrane and other components except the supercoiled DNA nucleoid. The prepared slides containing the embedded cells, so called "cell sandwich", are submitted to electrophoresis which allows migration of DNA fragments (if there is any DNA strand breaks) toward the anode. In electric field, the velocity of DNA fragments varies based on their sizes. As there are DNA fragments with different sizes a typical comet shape having a head and a tail will be formed. In general, the amount of DNA damage is proportional to the percent of DNA in tail. The higher intensity of DNA in tail, the higher DNA break has occurred. Several comprehensive reviews are available in respect to come assay methodology[59]. Determining the specific type of DNA damage is likely by using protocol variants. The pH at which the immobilized nucleoids are electrophoresed can partly determine the type of strand breaks, namely single or double strand breaks. It was stated that by using alkaline (pH>13) version of comet assay, both single and double strand breaks are detected, and on the other hand in neutral conditions only double strand breaks can be detected[60]. However, this belief is not completely true because even at neutral comet assay, single strand breaks will be present in the tail. Alkali- labile sites, apurinic and apyrimidinic (AP) sites are also appeared as strand breaks in denaturation condition at pH>13. These sites are formed during base repair process and also in response to some chemical alterations. Accordingly, at neutral pH, AP sites cannot be revealed as a break. Beside these types of DNA damage, oxidized bases also occur in the cells after exposure to oxidative stress; however they cannot be detected by using common comet assay procedure. These forms of damage can be readily converted to strand breaks by incorporating lesion-specific enzymes such as formamidopyrimidine DNA glycosylase and endonuclease III after the cells are lyzed[58,61]. Therefore, in order to evaluate the mechanism and also the sort of DNA damage the procedure has to be adjusted. The common alkaline comet assay can represent basal DNA damage and strand breaks. It seems that the potential of antioxidants, either natural or chemical to prevent oxidized base formation, cannot be assessed unless specific enzymes such as EndoII are included in the designed experiment. Otherwise, the results might be misinterpreted because the tail of a comet would be a mixture of AP sites, double and single strand breaks not exactly the breaks resulting from oxidized bases.

Regarding to the cells that researchers widely used, it should be noted that it depends on the goal of a study. For instances, if someone is looking for an antioxidant capable to protect normal cells against genotoxicity lymphocytes, normal cell lines such as fibroblasts are appropriate options[56,57,62-66]. In addition, some other cells such as PC12 or HepG2 can be utilized as neuronal or hepatic models respectively[67-69].

Although the comet assay is a simple and valuable technique, often there is large variability in the published results. This can be related to the presence of variations in different steps (cell isolation, treatment, lysis, electrophoresis), materials and also environmental conditions. In general, it seems that by standardization of different protocols these variations might be minimized. Furthermore, there are several parameters such as % DNA in tail, tail length and tail moment that can be used to report the final results of a comet assay. The lack of a unique appropriate parameter is another limitation of comet assay because it is almost unlikely to compare the results of different studies reporting various parameters. According to the literature, % DNA in tail, tail moment and also tail length were the most frequent parameters that were preferred for interpreting in a vast majority of toxicology studies.

4. Medicinal plants as protective agents

Due to the great importance of DNA damages in genome integrity, researchers have made great efforts to prevent or diminish DNA damage in particular oxidative damage. In this sense, numerous strategies have been engaged[70].

To prevent or delay ROS-driven oxidative damage the cells utilize different enzymatic and non-enzymatic antioxidant systems. Because of narrow endogenous antioxidant capacity, the cells have to protect themselves by exogenous antioxidants especially those from natural herbs[41,71]. Moreover, the side effects of synthetic

antioxidant, namely toxic and/or mutagenic effects, highlight the importance of natural antioxidant[26,30]. Medicinal plants are one of the most important protective agents in this issue, as they possess various natural substances having different pharmacological effects, and used as long ago as 3 000 BC[72]. On the other hand, natural compatibility and less side effects of herbal medicine are reasons for consideration in health care[30,73]. Certainly, these therapeutic effects are related to the presence of different determined compounds[72,74].

One of the strategies that can be adopted to protect the cells against stress oxidative and nearly have no deleterious effects on cell components such as DNA is using natural-based antioxidants. With the aim to find efficient antigenotoxic agents, several studies have been conducted to assess the protective effects of different plant species and their secondary metabolites against oxidative DNA damage. H_2O_2, the most common genotoxic agent, was used as the oxidative damaging agent; however other genotoxic compounds such as methyl ethanesulfonate have been used in a few reports (in the current review we excluded the latter studies).

In order to find a relation between the type of plant or phytocompounds and their potential protective effects, herein we have focused on the most relevant studies that reported the antigenotoxic activity of various plants and/or metabolites against H_2O_2 induced DNA damages.

Based on the emerging interest of using comet assay as a simple and reliable method to evaluate antigenotoxic/genotoxic effects, we considered only the reports that applied this method. The comet assay can be used in two versions, neutral and alkaline. In both protocols, the breakage in the DNA strands can be detected. However, in order to determine whether or not oxidative damage occurs, an extra step has to be carried out. It is likely to evaluate oxidative damage by adding specific enzymes, capable of converting oxidized base to a break. Accordingly, it is helpful to include these enzymes in studies that aimed to analyze the effects on anti-oxidants on H_2O_2-induced DNA damages.

Our literature review showed that about 80 different species belonging to 38 families were used as the subject of studies in which their protective effects against H_2O_2 induced DNA damage were evaluated (complete data were not shown). Interestingly, the most of plants possessing protective effects belong to Lamiaceae family (22%), and the two other families namely, Asteraceae and Apiaceae are in the second and third ranking (about 5%), respectively (Figure 1). The details of plants belong to these three families are explained in Table 1. Other plants which are introduced as protective in H_2O_2 models belong to families comprise Plantaginaceae (*Plantago asiatica*, *Bacopa monniera*)[75,76], Rosaceae (*Plantago asiatica*, *Bacopa monniera*)[77-79], Solanaceae (*Lycium chinense*, *Withania somnifera*)[80,81], Anacardiaceae (*Mangifera indica*, *Rhus coriaria* L.)[82,83], Apocynaceae (*Hemidesmus indicus*, *Gymnema montanum*)[76,84], Brassicaceae (*Armoracia rusticana*, *Moricandia arvensis*)[85,86], Combretaceae (*Terminalia arjuna*, *Terminalia bellerica* Roxb.)[87], Fabaceae (*Ceratonia siliqua*, *Lupinus luteus* L.)[88,89], Leguminosae (*Acacia salicina*, *Glycyrrhiza glabra*)[90,91] and so on.

In case of the part of plant used as protective agents is very imperative factor. Based on our bibliography, the most common parts of plants used in such studies were the leaves (28%), roots (15%) and aerial parts (12 %) respectively (Figure 2).

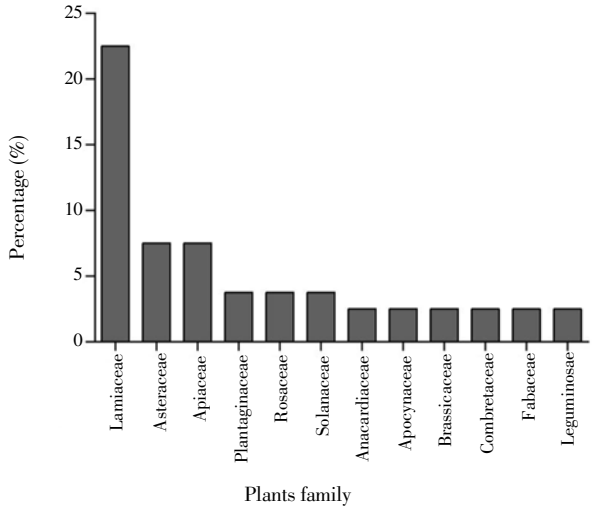

Figure 1. Frequency of use of the most plant families in protection assay against oxidative DNA damage induced by H_2O_2.

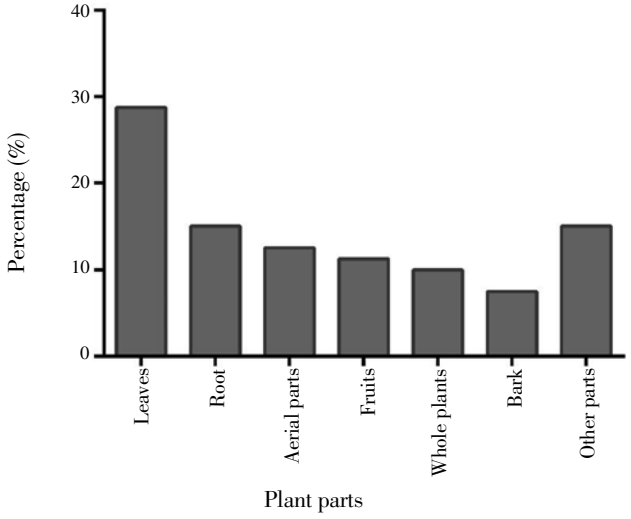

Figure 2. Frequency of plant parts used in protection against oxidative DNA damages induced by H_2O_2.

Apparently, in the most studies, plant extracts were used to determine protection activity, however plant extracts may not be proper sources for biological assay. It seems that several factors during extraction process might result in variation. For examples, length of the extraction time, pH, solvent, temperature, the size of plant tissue and the solvent-to-sample ratio may affect the extraction efficiency[58]. Furthermore, the seasonal and geographical factors may be considered as other reasons of variations[59]. Accordingly, reproducibility of biological activities of herbal extracts can be influenced by both biochemical differences and variability of extraction methods.

Besides, inconsistent reports about activity and efficacy of extracts might be related to different versatile compounds which may have synergistic or antagonistic activities. In addition, the observed results might be because of some unknown substances[60,61].

Table 1

Literature review of plants used to protect cells against DNA damages induced by H_2O_2.

Family	Scientific name	Antioxidant assay	Part (s) used	Isolated compound	Cell line	Comet assay type	References
Lamiaceae	*Mentha arvensis L. var. piperascens*	Yes[92]	Whole plant/Acid hydrolysates methanolic extract	Morin	Human lymphocytes	Alkaline	[92]
	Melissa officinalis	No	Leaves/ Ethanolic Extract	NI	Human lymphocytes	Alkaline	[93]
	Mesona procumbens Hemsl	Yes[94]	Whole plant/Aqueous extracts	NI	Human lymphocytes	Alkaline	[94]
	Origanum vulgare	Yes[95]	Extract	NI	Caco-2 cells	Alkaline	[95]
	Origanum Heracleoticum	No	Flower / Ethanol extraction	NI	Human lymphocytes	Alkaline/ endonuclease III	[96]
	Orthosiphon staminus	No	Leaves/ Methanol extract	NI	SH-SY5Y human neuroblastoma cell	Alkaline	[97]
	Rosmarinus officinalis Linn.	Yes[98]	Leaves/ Ethanol extract	NI	Caco-2 cells	Alkaline	[98]
	Salvia aurea L.	Yes[100]	Aerial parts/Essential oil	NI	Human melanoma cells	Alkaline	[99]
	Salvia judaica Boiss.	No	Aerial parts/Essential oil	NI	Human melanoma cells	Alkaline	[99]
	Salvia viscosa	Yes[101]	Aerial parts/Essential oil	NI	Human melanoma cells	Alkaline	[99]
	Salvia officinalis	Yes[102]	Extract	NI	Caco-2 cells	Alkaline	[95]
	Salvia officinalis	Yes[102]	Leaves/ Ethanol extract	NI	HepG2 cells	Alkaline	[102]
	Teucrium ramosissimum	Yes[103]	Leaf methanol extract and fractions	NI	Human lymphocytes	Alkaline	[103]
	Thymus piperella L	No	Aerial parts/ Ethanol extraction	NI	Human lymphocytes	Alkaline/ endonuclease III	[96]
	Thymus vulgaris	Yes[102]	Leaves/Ethanol extract	NI	HepG2 cells	Alkaline	[102]
Asteraceae	*Bidens alba L. var. minor*	Yes[92]	Whole plant/Acid hydrolysates methanolic extract	NI	Human lymphocytes	Alkaline	[92]
	Crepis vesicaria L.	No	Leaves/Ethanol extract	NI	Human lymphocytes	Alkaline/ endonuclease III	[92]
	Echinacea purpurea	Yes[104]	Extract	NI	Caco-2 cells	Alkaline	[95]
	Lactuca sativa L.	Yes[105]	Leaves / Water fraction from the hydroalcoholic extract	NI	Mouse neuroblastoma cell line	Alkaline	[105]
	Scolymus hispanicus L	No	Raquis /Ethanol extraction	NI	Human lymphocytes	Alkaline/ endonuclease III	[96]
	Rhaponticum carthamoides	Yes[106]	Root/ Aqueous methanol extract	NI	CHO Cells	Alkaline/ pretreatment and repair study	[106]
Apiaceae	*Centella asiatica*	Yes[107]	-Leaves /Methanol extract -Whole plan/Acid methanol extract[90]	- Castasterone - NI[90]	Human lymphocytes	Alkaline	[108]
	Daucus carota ssp. sativus var. atrorubens Al	Yes[109]	Whole plan/Acid methanol extract	NI	Human cells of colonic mucosa	Alkaline	[109]
	Ferula persica	No	Root	Persicasulfide A	Rat lymphocyte	Alkaline	[110]
	Ferula szowitsiana	No	Root	-Auraptene[111]	Human Lymphocytes	Alkaline	[111]
	Scandix australis	No	Aerial parts/Ethanol extraction	NI	Human lymphocytes	Alkaline/ endonuclease III	[95]

*NI: not isolated.

4.1. Bioactive compounds

According to our literature review (Table 1) about 90% of plants were examined for their potential antioxidant properties using different methods. It is well-documented that antioxidants can prevent the pro-oxidation process, or biological oxidative damage[112]. It seems that DNA protection against oxidative stress could be related to the antioxidant property of the forementioned plants[112].

This consistency may be due to the presence of phenolic compounds as strong antioxidants, which are introduced in previous studies. It is assumed that there is a strong connection between the content of phenolic compounds and antioxidant activities[113-117].

These compounds are secondary metabolites and products of pentose phosphate, shikimate, and phenyl propanoid pathways in plants. They have a significant role in physiological and morphological characteristics[113]. In addition, they are involved in a variety of functions including reproduction, growth, defense and also plant color. On the other hand, these phytochemicals possess chemopreventive activities[118].

The base of their structures is an aromatic ring having one or more hydroxyl group. Their structure varies from simple phenolic molecules to polymer compounds. In some cases, natural phenolic compounds conjugate with polysaccharides, or conjugate with one or more phenolic groups or functional groups such as esters and methyl esters. They can be classified as phenolic acids, flavonoids, tannins and the less common stilbenes and lignans[119,120]. The antioxidant activity of phenolic compounds is associated with several mechanisms: scavenging radical species, donating hydrogen atoms or electron, enzyme inhibition, chelating metal cations and upregulating or boosting antioxidant.

Their structure is a key cause of their antioxidant properties[113,114]. Two chemical parts of phenolic compounds play an important role in free radical scavenging: phenolic hydroxyl groups and dihydroxy groups. Phenolic hydroxyl groups are able to donate a hydrogen atom or an electron to radical species, hydrocarbon backbone which delocalizes an unpaired electron[114], while dihydroxy groups can conjugate to transition metals such as Cu^{+} or Fe^{2+} to inhibit free radical formation by these metals in Fenton reaction. As mentioned previously, these metal ions interact with hydrogen peroxide in Fenton reaction to produce hydroxyl radicals[114]. Stoichiometry and kinetic of these reactions vary based on the different structures, for instances, hydroxyl groups, glycosylation and amount of steric hindrance of proton H abstraction[121].

4.2. Flavonoids

Flavonoids are of Latin originate "*flavus*" which means yellow. They are secondary metabolites contributing different colors (red, blue and purple) to the different parts of plants. They are a large group of phenolic compounds in which three rings (A, B and C) form their basic structure (Figure 3). It seems that the hydroxyl groups attached to these rings are responsible for many biological activities[54,118]. Results of many *in vitro* and *in vivo* studies confirm their possible health benefits, mainly due to potential antioxidant and free radical scavenging activities[122]. More than 5 000 flavonoids and 13 subclasses are defined so far. Flavonoid structure possesses phenyl benzopyrone containing A and B aromatic rings, attached to 3 carbons of C ring which is usually pyran ring. Based on saturating level and opening of the central pyran ring, flavonoids are subdivided into several subgroups.

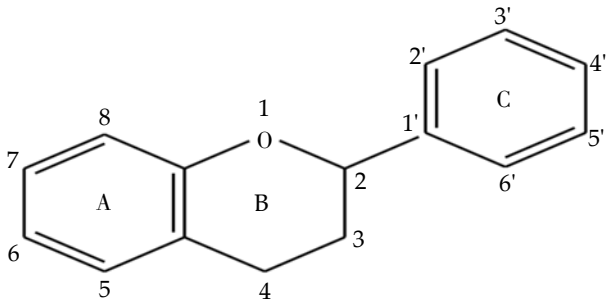

Figure 3. Basic structure of flavonoids.

Occasionally, it was shown that glycosylation of flavonoids diminishes the free radical scavenging activities, however the water solubility is increased. 7-hydroxyl position is common position for sugar attachment in flavones and isoflavones. However, 3- and 7-hydroxyl positions are targets of glycosylation in flavonols. In addition, 3- and 5-hydroxyl in anthocyanidins are attached to the sugars more commonly[41,118,119].

The ability of flavonoids to reduce harmful effects of free radicals might be through different mechanisms. One direct approach is by free radical scavenging activity. Free radicals oxidized flavonoids generate more stable and less-reactive flavonoid radicals. On the other hand, hydroxyl groups which are highly reactive react with free radicals and produce stable radicals[114,123]. This facts can be defined by thermodynamic rules which imply that low redox potential of hydroxyl in flavonoids would reduce free radicals such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals through hydrogen donation[123]. Some flavonoids are superoxide scavenger, while the others are recognized as peroxynitrite scavengers.

The other approach that flavonoids apply is via inhibition of xanthine oxidase and protein kinase C. Xanthine oxidase has an important role in oxidative injury particularly after ischemia-reperfusion. This enzyme participates in metabolism of xanthine to uric acid and superoxide as well as free radicals[123]. Oxidation of xanthin leads to produce H_2O_2 and superoxide anion, which are the causes of oxidative damages[124]. The other enzymes that are inhibited by flavonoids include cyclooxygenase, lipoxygenase, microsomal succinoxidase and nicotinamide adenine dinucleotide oxidase[122,125]. Further, more antioxidant activity of some phenolic compounds is related to dihydroxy groups in their structure. They are able to be conjugated with transition metals so that free radical formation is inhibited. Through Fenton reaction, hydrogen peroxide interacts with Cu^{+} or Fe^{2+} to form OH, which is an initiator of free radical chain reactions such as lipid peroxidation[114,123]. The metal chelating activity of flavonoids is related to hydroxy-keto group (a 3-OH or 5-OH plus a 4-C = O) and large number of catechol/gallol groups in their structures[114].

The results of several studies implied the strong relationship between flavonoid consumption and antioxidant capacity of plasma. This increased capacity may be due to increased level of uric acid, although the exact mechanism is unclear[122,126]. Another mechanism

of phenolic antioxidant is through cellular modulation. They improve the phase Ⅱ detoxification activity of glutaion s-transferase and quinone reductase which possess a critical role in detoxification of chemical toxins[127].

Also, phenolics modulate various molecular targets in cellular signaling machinery systems. Mitogen-activated protein kinase, protein kinase C, and serine/threonine protein kinase Akt/PKB can be activated by phenolics. Pro-inflammatory enzymes (COX-2 and iNOS) genes can be down regulated and tyrosine kinases, NF-$_{\kappa}$B, c-JUN can be inhibited by phenolic compounds[128]. Phenolics are also able to reduce Bax and Bad protein production, which results in opening mitochondrial transition pore complex to inhibit oxidative stress[128,129]. So, it seems that phenolics can mainly inhibit or induce key factors of cell signaling cascades rather than being just radical scavengers[73].

5. Conclusion

ROS are products of endogenous and exogenous induction. In normal conditions, ROS play a role of signaling messenger in different cellular pathways but in higher concentrations will lead to reversible and nonreversible cellular damages. Oxidative stress resulting from increased ROS production has trace in etiology of different disease such as cancer.

Among various ROS, H_2O_2 is more permeable and is known as an intercellular signaling molecule which can be converted to the other ROS. Reaction between $O_2^{\bullet-}$ and H_2O_2, which are mediated by transition metal ions, generates OH which is able to attack DNA and trigger DNA damage. In many cases, unrepaired damages lead to genetic instability, which is responsible for many disease processes. This review has summarized some herbal medicines participate in DNA protection against H_2O_2-induced DNA damage detecting by comet assay.

Also, in this article, some of the natural compounds were described which have critical roles as radical scavengers and potent protective antioxidants. Distinct compounds or/and their combination were able to show these protective effects. Since several of these studies were conducted on plant extracts, it seems that this field of research needs more investigations to discover the defined substances and their distinct cellular mechanisms responsible for such antigenotoxicity effects. Certainly, *in vivo* studies should be carried out to confirm these effects and to introduce them in clinic as proper candidates to diminish DNA damages side effects. Furthermore, effectiveness, safety and possible adverse effects of herbal medicines should be considered for further developments. In addition, as several investigations in regard to the antigenotoxic effects were carried out as primary studies, further *in vivo* and mechanistic studies will be required to clarify the real DNA protective activity.

Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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