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Anti-Oxidants and their Role in Disease Management

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ABSTRACT

Free radicals are produced in the body because of exposure to radiation, some environmental pollutants and as byproducts of normal metabolism. They can damage components of cell and result in various types of diseases because of their highly reactive nature. Free radicals are neutralized by different types of systems in the body which include antioxidant enzymes like glutathione peroxidase, catalase, and superoxide dismutase and small molecules of nutrient-derived antioxidant (vitamin C, flavonoids, vitamin E, carotenes, glutathione, taurine, and uric acid,). A delicate balance exists between antioxidants and free radicals in healthy individuals. In the present review, different methods of antioxidant activity and their role in the management of different diseases have been discussed. In some pathologic conditions like cancer and diabetes, the level of antioxidants falls below normal due to oxidative stress. Supplements of antioxidant for such conditions are expected to be beneficial. Regular consumption of antioxidant rich foods is the best preventive measure for certain diseases.

Keywords: Free radicals, Metabolism, Antioxidant activity, Antioxidant enzymes

INTRODUCTION

Oxygen is vital for aerobic life process, however about 5% or more of the inhaled O_2 is converted to reactive oxygen species (ROS). A free radical (FR) can be defined as a chemical species possessing an unpaired electron. FR can be positively charged, negatively charged or electrically neutral. When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids, and carbohydrates and this leads to a number of physiological disorders [1]. Free radicals have been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, and neurological disorders and in the process of aging [2,3].

Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. They may reduce the energy of the free radical or suppress radical formation or break chain propagation or repair damage and reconstitute membranes [4].

Antioxidants have gained considerable interest because of their protective role in inhibiting free radical reactions and damage caused by ROS. It was proved that bioavailability of antioxidants from natural sources are considerably high compared to that of synthetic antioxidants [5-7]. Scientific evidence suggests that antioxidants reduce the risk of chronic diseases including cancer and heart diseases. Primary sources of naturally occurring antioxidants are whole grains, fruits, and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, morin and licorice flavonoids, have strong antioxidant activity [8,9], while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease [10-12]. Antioxidant compounds like phenolic acids,

polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers [8,13-15]. The leaf extract of *M. koenigi* also show strong antioxidant activity [16-18]. The free radical scavenging activity of antioxidants in foods was substantially investigated and reported by some researchers [19,20].

METHOD CONSIDERATIONS

Various methods have been used to monitor and compare the antioxidant activity of different foods. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum, and other biological fluids. These methods require special equipment and technical skills for the analysis. The different types of methods published in the literature for the determination of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods [21]. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O_2), the hydroxyl radical (OH), or the peroxyl radical (ROO). The various methods used to measure antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant [22]. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc., and may not be practical when large numbers of samples are involved [23].

The role of free radicals in many ailments has been well established. Several biochemical reactions in our body generate reactive oxygen species, which, if not effectively scavenged by cellular constituents, may lead to various disease conditions. Much research into free radicals has confirmed that foods or plants rich in antioxidants play an essential role in the prevention of free radical related diseases [24-26]. A wide range of antioxidants of synthetic origin such as butylated hydroxytoluene (BHT) has been proposed for use in the treatment of various free radicals related diseases, but it has been proven that these compounds also show toxic effects like liver damage and mutagenesis. Hence, nowadays the search for natural antioxidants source is gaining much importance. A high antioxidant potential observed in many tropical plants is obviously part of their natural defense mechanism against noxious events causing oxidant damage, e.g. microbial infections [15].

List of *In vitro* Antioxidant Methods

Hydrogen Atom Transfer methods (HAT)

Oxygen radical absorbance capacity (ORAC) method: The oxygen radical absorbance capacity (ORAC) assay is a method that measures the antioxidant capacity of a substance [27]. The ORAC assay measures a fluorescent signal from a probe that is quenched in the presence of ROS. Addition of an antioxidant absorbs the generated ROS, allowing the fluorescent signal to persist. Trolox® (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) is a vitamin E analogue and a known antioxidant. It is used as a standard by which all unknown antioxidants are compared. Modifications of the ORAC assay include the use of fluorescein as the fluorescent probe (ORACFL), the separation of hydrophilic and lipophilic antioxidants to obtain total antioxidant capacity and an adaptation to a high-throughput platform.

The ORAC assay is unique and in that its ROS generator, AAPH (2,2'-azobis (2-methylpropionamidine) dihydrochloride), produces a peroxyl free radical upon thermal decomposition. This free radical is commonly found in the body, making this reaction biologically relevant. Furthermore, AAPH is reactive with water and lipid soluble substances, so it can measure total antioxidant potential [28].

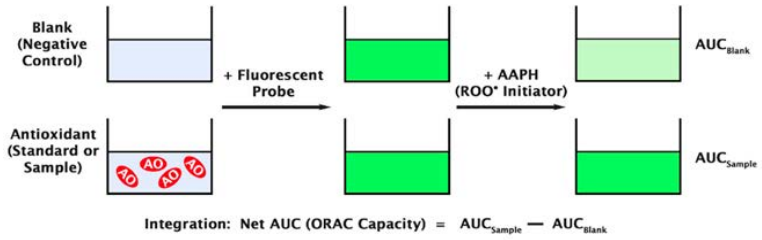

Figure 1 Oxygen radical absorbance capacity method.

Lipid peroxidation inhibition capacity (LPIC) assay: The lipid peroxidation inhibition capacity (LPIC) method measures the ability of both lipophilic and hydrophilic antioxidants to protect a lipophilic fluorescent probe 4, 4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid, incorporated in the membrane, from 2,2′-azobis(2-amidinopropane)hydrochloride generated radicals in the surrounding aqueous solution [29]. Antioxidant activities of test compounds are measured either after they are mixed with preformed liposomes (LPICMixed) or after they are incorporated into liposomes (LPICInco) as they are made. The results are analysed to determine how the method of mixing and the structures of the antioxidants influenced their protection of the membrane from free radical attack. The LPICMixed values are larger than the LPICInco values.

Total radical trapping antioxidant parameter (TRAP): Another assay which has been applied in human plasma is the total radical trapping antioxidant parameter (TRAP). In this assay, the rate of peroxidation induced by AAPH (2'-azobis(2-amidinopropane) hydrochloride) is monitored through the loss of fluorescence of the protein R-phycoerythrin (R-PE). In the TRAP assay the lag-phase induced by plasma is compared with that induced by Trolox in the same plasma sample [30].

Hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline): Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. The model used is ascorbic acid-iron- EDTA model of $HO^{\cdot}$ generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals. The reaction mixture (1.0 ml) consist of 100 µl of 2-deoxy-Dribose (28 mM in 20 mM KH_2PO_4 -KOH buffer, pH 7.4), 500 µl of the extract, 200 µl EDTA (1.04 mM) and 200 µM $FeCl_3$ (1:1 v/v), 100 µl of H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM) which is incubated at 37°C for 1 h. 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) are added and incubated at 100°C for 20 min. After cooling, absorbance is measured at 532 nm, against a blank sample. Gallic acid, mannitol, catechin, vitamin E, quercetin, BHA, α- tocopherol, rutin or ascorbic acid can be used as a positive control [31].

Scavenging of H_2O_2 radicals: Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper, and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure [32]. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20-60 µg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ Scavenged } (H_2O_2) = (A_0 - A_1/A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test. Ascorbic corrosive, rutin BHA, α-tocopherol or quercetin can be utilized as a positive control.

ABTS {2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)} radical scavenging method: This assay is based on the principle that when 2,2'-azinobis-(3- ethyl-benzothiazoline-6-sulphonic acid) {ABTS} is incubated with a peroxidase (such as metmyoglobin and H_2O_2, a relatively stable radical cation (ABTS+). The formation of ABTS+ on interaction with ferryl myoglobin produces a relatively stable blue-green color, measured at 600nm. Antioxidants in the fluid sample suppress this color production to a degree that is proportional to their concentrations [33].

In this equation,

HX-FeIII=metmyoglobin, X-[FeIV=0]=ferrylmyoglobin, ABTS=2,2'-azino-di-[3-ethylbensthiazoline sulphonate]

Scavenging of super oxide radical formation by alkaline (SASA): Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Numerous biological reactions generate superoxide anions which are highly toxic

species. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT [34]. The decrease of absorbance at 560 nm with antioxidants, thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide anion radicals are generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). The reaction is started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and then the absorbance is measured at 560 nm against a blank sample. Gallic acid, BHA, ascorbic acid, α-tocopherol, curcumin, quercetin or trolox can be used as a positive control.

Electron Transfer methods (ET)

Trolox equivalent antioxidant capacity (TEAC): The $ABTS^{\cdot}+$ formed from the reaction $ABTS\text{-}e^{-} \rightarrow ABTS^{\cdot}+$ reacts quickly with ethanol/hydrogen donors to form colourless 2, 2'-azinobis (3-ethylbenzothiazoline 6- sulfonate (ABTS). The reaction is pH-independent. A decrease of the $ABTS^{\cdot}+$ concentration is linearly dependent on the antioxidant concentration [35]. The radical cation ABTS. + is generated by persulfate oxidation of ABTS. A mixture (1:1, v/v) of ABTS (7.0 mM) and potassium persulfate (4.95 mM) is allowed to stand overnight at room temperature in dark to form radical cation ABTS +. A working solution is diluted with phosphate buffer solution to absorbance values between 1.0 and 1.5 at 734 nm. An aliquot (0.1 ml) of each sample is mixed with the working solution (3.9 ml) and the decrease of absorbance is measured at 734 nm after 10 min at 37°C in the dark. Aqueous phosphate buffer solution (3.9 ml, without ABTS.+ solution) is used as a control. The ABTS+ scavenging rate is calculated. Trolox, BHT, rutin, ascorbic acid or gallic acid can be used as a positive control [36].

Ferric reducing antioxidant power (FRAP) assay: FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}- TPTZ complex with an absorption maximum at 593 nm [37]. This reaction is pH dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content. 0.2 ml of the extract is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3$. $6H_2O$ solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or trolox can be used as a positive control [38].

DPPH free radical scavenging assay: The molecule 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; (DPPH) is characterized as stable free radical having delocalization of the spare electron over the molecule, in order to avoid the dimerization of the molecule, which would be the case with most of the other free radicals [39,40]. The delocalization gives rise to deep violet color, which is characterized by an absorption band at about 517 nm (in methanol solution). When a solution containing a substance that can donate a hydrogen atom is mixed with DPPH, it gives rise to the reduced form by losing violet color (if picryl group is still present in the mixture then there will be a chance of residual pale-yellow color to be obtained) [41]. The primary reaction representing the DPPH radical by Z• and the donor molecule by AH, is

Z• + AH = ZH + A•

Where ZH is the reduced form and A• is free radical produced in this first step

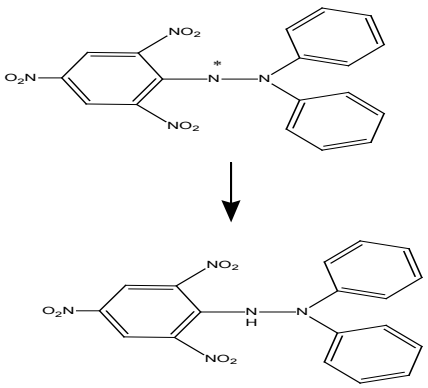

Figure 2 DPPH free radical scavenging method

Copper (II) reduction capacity: CUPRAC method is a novel hydroxyl radical scavenging antioxidant activity assay for water-soluble antioxidants. ROS may attack biological macromolecules giving rise to oxidative stress-originated diseases. Since OH is very short-lived, secondary products resulting from OH attack to various probes are measured. Although the measurement of aromatic hydroxylation with HPLC/ electrochemical detection is more specific than the low-yield TBARS test, it requires sophisticated instrumentation. As more convenient and less costly alternative, we can use p-aminobenzoate, 2,4- and 3,5-dimethoxybenzoate probes for detecting hydroxyl radicals generated from an equivalent mixture of Fe(II) + EDTA with hydrogen peroxide. The produced hydroxyl radicals attacked both the probe and the water-soluble antioxidants in 37-incubated solutions for 2 h. The CUPRAC absorbance of the ethyl acetate extract due to the reduction of Cu (II)-neocuproine reagent by the hydroxylated probe decreased in the presence of OH scavengers, the difference being proportional to the scavenging ability of the tested compound. A rate constant for the reaction of the scavenger with hydroxyl radical can be deduced from the inhibition of color formation. The second-order rate constants of the scavengers were determined with competition kinetics by means of a linear plot a A_0/A as a function of C scavenger/C probe where A_0 and A are the CUPRAC absorbencies of the system in the absence and presence of scavenger, respectively and C is the molar concentration of relevant species. The 2,4– and 3,5-dimethoxybenzoates were the best probes in terms of linearity and sensitivity [27]. Iodide, metabisulfite, hexacyanoferrate (II), thiourea, formate, and dimethyl sulfoxide are shown by the modified CUPRAC assay to be more effective scavengers than mannitol, glucose, lysine, and simple alcohols as in the TBARS assay. The developed method is less lengthy, more specific, and of a higher yield than the classical TBARS assay. The hydroxyl radical scavenging rate constants of ascorbic acid, formate and hexacyanoferrate (II) that caused interference in other assays could be easily found with the proposed procedure. Apricots as five varieties of Malatya region are screened for antioxidant capacity by using CUPRAC. The novel reagent for the CUPRAC total antioxidant capacity assay, bis(neocuproine) copper(II) chloride, is easily accessible, stable, selective and responding to all antioxidants. Sulphite (normally contributing to the color formed in the CUPRAC assay) is removed prior to assay on a strongly basic anion exchanger at pH 3 in the form of HSO. The CUPRAC findings correlated well with the results of ABTS/TEAC and Folin assays.

DMPD (N,N-dimethyl-p-phenylene diamine dihydrochloride) Method: This assay depends on the reduction of buffered solution of colored DMPD in acetate buffer and ferric chloride. The strategy includes measurement of decrease in absorbance of DMPD in presence of scavengers at its absorption maxima of 505 nm. The antioxidant activity of wines is estimated by utilizing this technique. The activity is expressed as percentage reduction of DMPD [28].

Role of Antioxidants in Human Health

According to the literature, antioxidants have impact on health. Many antioxidants that penetrate the body through ingestion, inhalation, or skin can be harmful. These substances can generate free radicals that are accumulated. This accumulation can cause damage and even death due to the biological consequences [41]. Currently, the main causes for reducing the plasma level are due to antioxidants that are produced during smoking and chronic alcoholism [42,43]. For example, in the skin there is a defense antioxidant against UV radiation which is formed by melanin and antioxidant enzymes. This defense prevents swelling, wrinkling, and skin cancer. The benefit of antioxidant uptake has been demonstrated in the course of some diseases and certain conditions such as diabetes, asthma, hemodialysis, thalassemia, rheumatoid arthritis, systemic attack, post-menopause, schizophrenia, depression, and leukemia [12].

Antioxidants in cancer therapy

Earlier studies suggest that cancer cells have a higher level of oxidative stress compared to normal cells. This stress is due to an increased production of ROS and some changes in the metabolic activity related to oncogenic transformation [44-47]. Therefore, tumor cells may be more sensitive to drugs that generate big amounts of ROS, or drugs that damage the ROS scavenging capacity of cells, that leads to apoptosis [48]. Apoptosis is conducted by proteases called caspases which acts by causing cellular DNA damage and disruption of microtubules [49,50]. In a multifactorial disease such as cancer, it is important to note the relation between antioxidants and gene expression. Tumor cells show elevated levels of ROS, which alter pro oncogenic signaling pathways that contribute to the malignant phenotype of cells. These signaling pathways are studied by Nrf2 and p53 routes [51]. Nrf2 belongs to an important signaling pathway that controls the expression of genes involved in the neutralization of oxidant agents [52], and the p53

pathway protects the DNA from the oxidation induced by ROS [53-55]. Many signaling pathways associated with carcinogenesis are related directly or indirectly to ROS metabolism.

These pathways may also be affected by the presence of antioxidants [56]. Increasing ROS during cancer development makes tumor cells highly reliant on antioxidant agents [57]. For this reason, low concentrations of free radicals due to an excessive administration of antioxidants may promote the proliferation of harmful cells in the neo-plastic state, upgrading the development of cancer rather than interrupting it [58]. Another view to consider is that the intense generation of ROS in tumor cells could damage DNA, promoting the genetic instability and the development of drug resistance. However, it appears interesting to develop new therapeutic strategies to eliminate tumor cells using ROS-mediated mechanism [59]. Radiation therapy is based on the ability of the ionizing radiation to kill cells. This therapy precisely involves the generation of ROS, including hydroxyl radicals, superoxide anion, and other organic radicals, and also producing lipid per oxidation [60]. In the presence of oxygen, these radicals cause increased formation of other ROS such as peroxides [61]. Consequently, adverse radiation effects may be influenced by these increased radicals, affecting the cellular antioxidant status [62]. In the trial conducted by Bairati, et al. with head and neck cancer patients, who were treated with radiotherapy and supplemented with high doses of vitamin C and E, seemed to improve the adverse effects, but also a loss of effectiveness of the treatment was observed along with an increased mortality in patients who received the treatment with antioxidants [63]. There are several studies that have linked the consumption of vitamins with improved adverse effects during chemotherapy and radiotherapy [64]. However, other trials showed that the intake of vitamins does not improve the side effects and could even reduce the efficacy of the treatment. As for chemotherapy, there are numerous agents that induce cell death by oxidative stress directly, leading to the disruption of redox signaling and ROS scavenging, or indirectly by reducing intracellular levels of antioxidants and deactivating the cellular defense [65]. Numerous articles have reported on many chemotherapeutic agents whose effects involve the induction of oxidative stress. Some of them are new molecules such as Meroxest, a synthetic merosesquiterpene derivative of the trans-communic acid, plentiful in *Cupressus sempervirens* [66] or Jadomycin, which is synthesized by the bacteria *Streptomyces venezuelae* [67]. Other compounds are part of the current therapeutic repertoire, like oxaliplatin, bleomycin, gemcitabine, cyclophosphamide, celecoxib, capecitabine, bortezomib (a proteasome inhibitor, approved for the treatment of multiple myeloma), and arsenic trioxide (ATO). ATO, which is used in the treatment of acute promyelocytic leukemia (APL), can produce a loss of permeability of the outer mitochondrial membrane and impair the function of the respiratory chain, leading to an increase in superoxide anion. However, many of the agents that induce oxidative stress have hardly any studies about the interaction between their antineoplastic activity and antioxidants [68]. Then, we present the information about various antitumor drugs which have been selected according to their utility, therapeutic efficacy, and involvement in studies that were focused on the evaluation of the interaction with antioxidants during chemotherapy.

In other cases, antioxidant supplements have shown positive effects, without affecting the effective treatment. The combination of 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC) appears to involve a decrease in antioxidant levels, as a result of the lipid per oxidation produced in the cell membrane [69]. In a clinical trial conducted to test the effectiveness of *Uncaria tomentosa* with patients treated with FAC who were in stage II of invasive ductal carcinoma of breast showed that the patients who received chemotherapy along with 30 mg/day of the extract of the plant experienced a decrease in the adverse effects from chemotherapy such as neutropenia, without affecting the effectiveness of drugs. Similarly, tannins (a type of polyphenols) administered during the treatment with doxorubicin showed their capacity of lowering the cardio toxicity caused by the drug, without reducing its antitumor efficacy.

Adjuvant in Anticancer Therapy

A very important topic in antitumor therapy based on doxorubicin is the development of drug resistance. In this case, the relationship between this resistance and the presence of endogenous antioxidants was recently described. McDonald, et al. managed to demonstrate the involvement of peroxiredoxins (Prdx) in the doxorubicin resistance of MCF-7 breast tumor cells. Prdx are a family of six proteins expressed in mammals which are thiol-specific antioxidants. This trial showed that MCF-7 had elevated levels of Prdx compared to nontumor cells MCF-10A, and the levels of these proteins in line MCF-7 resistant to doxorubicin were higher. This study also stated that the suppression of the expression of four of these six Prdx led to an increase in the apoptotic effect of doxorubicin. Taxanes are anticancer cytotoxics that include paclitaxel, which is a natural antitumor drug used to treat various types of tumors. Numerous studies have indicated that it induces ROS and alters the permeability of the mitochondrial membrane producing H_2,

and O_2. A recent study reported a reduction of glutathione levels in blood samples collected from patients treated with Paclitaxel, which implies that there was a decrease of the antioxidant potential of cells [70]. *In vitro* studies also point in the same direction. T47D and MDA-MB231 breast tumor cells, treated with scavengers (NAC, catalase, or SOD), were able to maintain their viability. It was discovered by another agent, 2-deoxy-D-glucose (a competitive inhibitor of glycolysis), was able to promote a prooxidant effect of paclitaxel [71]. In other trials, it was shown that the administration of resveratrol, during the treatment with paclitaxel, decreased its antineoplastic action against breast tumor cells both *in vitro* and *in vivo* [72].

Docetaxel (Taxotere) is a derivative of paclitaxel that is often used as a first-line drug to treat prostate cancer and other types of tumors. According to Ravin, et al. study, its way of inducing cell death would be due to microtubule depolymerisation [73]. It has also been reported that this drug is able to induce oxidative stress by activating caspase 3 [74]. Recently, the prooxidant effect of docetaxel on breast tumor cells (MDA-231 and MCF-7) was demonstrated, which could be enhanced with the addition of C6 ceramide (a cell-permeable-short-chain ceramide), increasing the drug toxicity. Attending to reduce the side effects of this drug, a reduction of oxidative stress in blood levels of mice with breast tumor cells xenografts was found, due to the supplementation of a nitro-oxide (3-carbamoylpyrroline nitroxyl derivative pirolin) when they were treated with docetaxel and doxorubicin. It also was found that this compound did not interfere with the antitumor activity of these drugs [75].

Cisplatin was the first heavy metal used for treating cancer and it has been widely used to treat solid tumors of lung, ovary, testes, lymphoma, etc., [76]. Its mechanism of action involves not only the generation of an intense oxidative stress but also causes numerous side effects due to their toxicity. Itis also associated with the expression of p53 (tumor suppressor gene), antiapoptotic Bax proteins, p21 protein (cell cycle regulator), and the cleavage of PARP and caspases 3 and 9. After an extensive review, it has come to our attention that there is a large literature focused on the study of interactions between treatment with cisplatin and antioxidant supplementation, so this fact may be a reflection of the importance of this drug in the treatment of cancer [77].

The role of quercetin is remarkable, since it has been reported in several studies that it seemed to act as an adjuvant in the treatment with cisplatin. In a recent study on the treatment with cisplatin in ovarian tumor cells (C13∗ and SKOV3), it was found that high concentrations of quercetin (40 μM-100 μM) appeared to have a proapoptotic effect, while low concentrations (5 μM-30 μM) seemed to reduce the damage caused by ROS. This reduction of the damage was due to the increase of SOD, and therefore the antineoplastic effect of cisplatin was attenuated. Similarly, the interaction of quercetin with commonly used drugs in the treatment of ovarian cancer (5-FU, taxol, and pirarubicin) was analyzed and the results were alike. Moreover, *in vivo* studies using athymic nude mice with C13∗ cells xenografts showed that low doses of quercetin could cause inefficiency in the treatment with cisplatin, 5-FU, taxol, or pirarubicin. Considering all the results discussed above, we conclude that antioxidant intake seems to influence the effectiveness of antitumor therapy and its adverse effects. However, we believe that at this moment it is not possible to give a general recommendation or not to take antioxidants during treatment. This is because the final effect which depends on the type of cancer, the mechanism of action of the drug or drugs used in the treatment, and the type of antioxidants [78].

Antioxidants in the treatment of various cardiovascular diseases

Cardiovascular disorders or cardiovascular diseases (CVD) are major illness associated with heart and blood vessels. Reactive oxygen species (ROS), generated during excessive oxidative stress, are responsible for the pathophysiology of various cardiovascular disorders including atherosclerosis, cardiac hypertrophy, cardiomyopathy, heart failure, ventricular remodeling, ischemia/reperfusion injury and myocardial infarction. Cellular "redox homeostasis" generally maintains the healthy physiology in cardiac myocytes and endothelial cells. However, during excessive oxidative stress body's endogenous system fails to maintain normal physiology hence antioxidant supplementation is necessary, which could scavenge the free radicals and other toxic radicals. Several antioxidants such as CoQ10, beta carotene, lycopene, quercetin, reserveterol, vitamin C and vitamin E have shown preventive and therapeutic benefits in different forms of cardiovascular diseases [79]. However, poor biopharmaceutical properties and variable pharmacokinetics of several antioxidants limits their use as therapeutic agents. Hence delivery of stable antioxidants at the site of action is a need of current scenario. Several novel carriers-based approaches have shown considerable benefits for the systemic and site-specific delivery of antioxidants for the preventive and therapeutic treatment of

several cardiovascular diseases. In the present review, conventional as well as novel antioxidants have been discussed with special emphasis for the treatment of CVD.

Novel Antioxidant-Based Therapies in Ischemia-Reperfusion

Cardiac Preconditioning with Omega 3 *in vitro* studies, animal experiments, observational studies and randomized clinical trials have examined the cardiovascular effects of seafood consumption and long-chain omega-3 polyunsaturated fatty acids. These types of fatty acids are composed of eicosapentaenoic acid (EPA; 20:5 n-3), docosahexaenoic acid (DHA; 22:6 n-3) and α-linolenic acid (ALA; 18:3 n-3). Alpha-linolenic acid is a plant-derived omega 3 found in a relatively limited set of seeds, nuts, and their oils. Alpha-linolenic acid cannot be synthesized in humans and it is an essential dietary fatty acid [80]. There are biochemical pathways to convert ALA to EPA and EPA to DHA, but such endogenous conversion is limited in humans: between 0.2% and 8% of ALA is converted to EPA (with conversion generally higher in women) and 0% to 4% of ALA to DHA (10-14). Thus, tissue and circulating EPA and DHA levels are primarily determined by their direct dietary consumption [81]. Recent studies suggest that the beneficial effects of fish oil are due, in part, to the generation of various free radical-generated non-enzymatic bioactive oxidation products from omega 3, although the specific molecular species responsible for these effects have not been identified. It is of interest to note that the beneficial effects of EPA and DHA could arise from both direct short-term or long-term effects mediated by changes in some intracellular pathways as discussed below. Direct actions of omega 3 have been confirmed by experimental studies of sudden cardiac death in a reliable dog model, showing that these compounds electrically stabilize heart cell membranes through the modulation of the fast voltage-dependent Na^+ currents and the L type Ca^{2+} channels. Derived from this effect, cardiac cells become resistant to arrhythmias [23,82-84]. Moreover, it has been pointed out that n-3 polyunsaturated fatty acids (PUFA) can exert a reversible modulation in the kinetics of several ion channels by binding to specific sites on channel proteins and by non-specifically incorporating them into lipid cell membranes [85]. These changes are consistent with the type of fatty acids incorporated into the cardiac tissue membrane. With regard to diet supplementation, it has been noted that diet rich in omega-3 polyunsaturated fatty acids are associated with decreased incidences of cardiovascular disease [86]. The extent to which these beneficial fats are incorporated into and distributed throughout body tissues is uncertain. In some animals supplemented for more than two weeks with diets enriched with omega 3 as Nutrients 2017, 9, 966 of 23 fish oil, the incorporation kinetics of both EPA and DHA have been measured, and this might be associated with the tissue response profile to an injury [87]. In the case of the heart and blood vessels, this would determine the type of hemodynamic response to a pro-inflammatory and pro-oxidant injury. For example, controlled ischemia in an *ex vivo* model may induce a greater recovery of ventricular function if the supplementation has a high incorporation of DHA in the cardiac tissue [4,88]. These kinetics would also allow a more efficient anti-oxidant and anti-inflammatory [89] response at the heart tissue level [90]. However, consumption of dietary flaxseed appears to be an effective means to increase ALA content in body tissues, but the degree will depend upon the tissues examined. In relation to chronic consumption, it has been reported that omega 3 is selectively incorporated into cardiac cell membranes in a dose-related manner after 8 weeks of supplementation [91]. Also, omega 3 can improve post-ischemic functional recovery in the *ex vivo* Langendorff perfusion of rat heart, also suggesting the benefit of a diet highly enriched with omega 3 content [92]. Regular intake can slow the heart rate, reduce myocardial oxygen consumption, and increase coronary reserve. These properties contribute to preconditioning-like effects of resistance to myocardial infarction and improved post-hypoxic recovery. These effects can be demonstrated in isolated hearts, regardless of the effects of omega 3 on neural or blood parameters. Also, the enrichment of myocardial membranes with omega 3 reduces vulnerability to cardiac arrhythmias, particularly ventricular fibrillation, and attenuates heart failure and cardiac hypertrophy.

Antioxidant Mechanism Induced by Omega 3

Experimental evidence demonstrates that antioxidant effects of omega 3 are related to the incorporation of these compounds into the cell membrane and the modulation of redox signaling pathways. In this view, omega 3 supplementation increases the expression and activity of the antioxidants enzymes and attenuates thiobarbituric acid-reactive substances (TBARS) increased in rats [93]. Oxidized omega 3 reacts directly with Keap1, a negative regulator of Nrf2, initiating Keap1 dissociation with Cullin3 and thereby inducing Nrf2-dependent target antioxidant genes such as heme oxygenase-1 [94]. This omega 3-antioxidant reinforcement is associated with a reduction in the susceptibility of myocytes to ROS-induced IR injury, and to an increase in SOD and GSH-Px expressions [95]. Animal studies

showed that the cardio protective effects of PUFA can be exerted through the up regulation of heat shock protein 72, a key preconditioning protein, and higher omega 3 content of myocardial membranes, which appears to facilitate the protective response to hypoxic injury [96,97]. Recently, hearts supplemented with omega 3 showed lower infarct size and a higher left ventricular pressure compared with non-supplemented rats. Hearts in the supplemented group with omega 3 showed lower levels of oxidative stress markers and higher antioxidant activity, decreased activity and NF-κβ and Nrf2 activation, compared with the non-supplemented group.

Antioxidants in the Treatment of Neurological Disorders

Free radical or oxidative injury may be a fundamental mechanism underlying a number of human neurological diseases. Therapy using free radical scavengers (antioxidants) has the potential to prevent, delay, or ameliorate many neurological disorders. However, the biochemistry of oxidative path biology is complex and optimum antioxidant therapeutic options may vary and need to be tailored to individual diseases. *In vitro* and animal model studies support the potential beneficial role of various antioxidant compounds in neurological diseases. However, the results of clinical trials using various antioxidants, including vitamin E, tirilazad, N-acetylcysteine, and ebselen, have been mixed. Potential reasons for these mixed results include lack of pretrial dose-finding studies and failure to appreciate and characterize the individual unique oxidative processes occurring in different diseases. Moreover, therapy with antioxidants may need to be given early in chronic insidious neurological disorders to achieve an appreciable clinical benefit. Predisease screening and intervention in at-risk individuals may also need to be considered in the near future. Although many animal studies support the importance of oxidative mechanisms in neurological disorders, potential publication bias may favor positive studies, a problem less likely to occur with human studies. Randomized controlled clinical trials of compounds with antioxidant properties have yielded positive, negative, marginal, or conflicting results, both in neurological and non-neurological disorders. Compounds tested in neurological diseases include vitamin E, tirilazad, N-acetylcysteine, epsilon, selegiline, idebenone, and extract of Gingko biloba. Diseases studied includes Parkinson disease, Alzheimer disease, multi-infarct dementia, amyotrophic lateral sclerosis (ALS), Huntington disease, acute ischemic stroke, subarachnoid hemorrhage, head and spinal cord injury, and intractable childhood epilepsy. Unfortunately, none of the clinical trials performed to date has measured markers of oxidative injury as a surrogate marker of drug efficacy, either in pretrial dose-finding studies or in subgroups of patients during the trials. This is important because some of the compounds used may not be effective antioxidants *in vivo* in humans, or alternatively the compounds may be effective but have been given in suboptimal doses. Some compounds (e.g. vitamin E, probucol) may have antioxidant or pro-oxidant effects depending on dosage, concomitant treatments, and study models [49,50]. These considerations underscore the importance of pretrial dose finding studies using novel markers of free radical injury now available, such as isoprostanes [40,41], 8-hydroxy-2-deoxyguanosine [42], or 3-nitrotyrosine [43], reliable markers of lipid, DNA, and protein oxidation, respectively. Measurement of potential reduction of biochemical indices of free radical injury should be made in subgroups of patients as a surrogate marker of antioxidant effectiveness in future controlled trials [98]. The objective measurement of antioxidant effectiveness could also provide critical information in individual treatment trials of putative antioxidants in rare neurologic disorders in which large prospective studies are not possible [99].

Oxidative Stress in Alzheimer's Disease

Oxidative stress in AD patients occurs due to various factors such as genetic factors (apolipoprotein E ε4 allele), germ line mutations (amyloid-β protein precursor gene, presenilin-1 gene, and presenilin-2 gene), environmental causes, lifestyle-related factors (smoking) and certain health conditions such as diabetes, brain injury and hypercholesterolemia [100]. Oxidative stress is found in various *in vitro* (cells in culture) and *in vivo* models (transgenic animals) as well as in tissues and fluids from patients with AD (living and postmortem brains) and cognitive diseases such as MCI and Down syndrome. Oxidative stress affects AD patients at four different levels; proteins, nucleic acids, lipids, and enzymes [34]. Increased nitrative stress in human AD brains has been reported in the form of increased levels of protein oxidation [67], protein nitration, 3-nitrotyrosine, 3,3′-dityrosine in hippocampus and major regions of the brain including inferior parietal lobule (IPL), neocortical regions and ventricular cerebrospinal fluid [77]. Both nuclear and mitochondrial DNA has been modified by oxidative stress to increase levels of 8-hydroxy-2-deoxyguanosine and oxidized bases in cerebral cortex and cerebellum of AD patients as compared to age-matched control subjects [21,71]. Increased levels of malondialdehyde, a measure of lipid peroxidation, are found in human AD brains [25]. Butterfield

and colleagues recently demonstrated that brain synaptosomes in AD and MCI patients had oxidative stress-mediated increased modification of phosphatidylserine, a key lipid necessary for membrane integrity [24]. Early-stage as well as late-stage AD brains expressed decreased antioxidant enzyme activities for key anti-oxidant enzymes such as: superoxide dismutase, catalase, glutathione peroxides and glutathione reductase [65].

Brain tissue from a transgenic mouse model (APPsw) of human familial AD having a "Swedish" mutant amyloid-β protein precursor (AβPP) and peripheral leukocytes MCI patients have shown increased lipid peroxidation, increased oxidative damage to DNA and decreased plasma total antioxidant capacity [75]. The underlying oxidative stress in AD is mediated via various marker proteins and is supported by many preclinical investigations. Aβ/AβPP can directly induce reactive nitrogen species in cell culture models, as well as in *in vivo* models. Astroglial cells isolated from brains of AD patients had increased levels of heme oxygenase-1 (HO-1), a marker of oxidative stress [47]. Moreover, transgenic mouse and C. elegant models of AD amyloidosis exhibit compromised antioxidant defense, increased protein oxidation and lipid peroxidation [67]. Similarly, the frontal, neurons, astroglial cells and blood vessels of postmortem AD brains had increased levels of nitric oxide synthase enzymes and hydroxyl radicals leading to indicative of increased production of nitrotyrosine and nitrative stress. Additionally, mitochondrial problems, energy deprivation and compromised antioxidant defense are associated with increased free radical burden in AD brains. Numerous evidences for involvement of mitochondrial problems with AD came from early defects in Glucose utilization and deregulation of key mitochondrial enzymes such as α-ketoglutarate dehydrogenize, pyruvate dehydrogenize and more commonly for cytochrome c oxidize (COX) [101].

Although these evidences suggest that diagnosis of AD has an oxidative stress component to pathology, it is not still known whether oxidative stress in AD is a cause (damage) or an effect (response to the damage). This information is crucial for designing the preclinical, as well as clinical studies for these agents to develop effective anti-AD therapies.

Antioxidant therapies for Alzheimer's disease

Currently available anti-AD therapies can be classified as follows:

• Treating cognitive and behavioral symptoms (anti-cholinesterase, anti-oxidants);

• Treatments for sleep changes; and

• Alternative treatments such as behavioral training [83]

Adjunct therapies include pharmacological agents such as non-steroidal anti-inflammatory drugs (NSAIDs) [57] which are widely used throughout the world because they not only serve as anti-inflammatory drugs but also as analgesics and antipyretics [102], metals such as copper (stabilizing the Cu/Zn SOD activity) and metal chelator, e.g., clioquinol [84]. Earlier neurotransmitter theory by Bartus in 1982 led to the development of the very first anti-AD agents which consisted of cholinesterase inhibitors; glutamine, tacrine, donepezil, and rivastigmine [88] and later to the development of memantine, a NMDA-receptor antagonist [85]. Large clinical trials by the U.S. Foods and Drug Administration (FDA) in 1995 for cholinesterase inhibitors and for memantine in 2002 showed modest symptomatic benefits on cognitive, behavioral, and global measures [102]. Most of the anti-cholinesterase-based therapies produce very moderate symptomatic relief and poor prognosis; therefore, the development of novel interventions which can target fundamental early changes (such as oxidative stress) has been the focus of recent anti-AD therapeutics. Antioxidant strategies are divided into three categories namely:

• Free radical scavengers, e.g. vitamin C and E, β-carotene;

• Preventive antioxidants such as metal chelators, glutathione peroxides and SOD enzymes; and

• *de novo* and repair enzymes such as lipases, proteases, and DNA repair enzymes.

Nonspecific antioxidants include melatonin, omega-3 polyunsaturated fatty acid (docosahexaenoic acid), curcumin, ubiquinone, and α-lipoic acid.

Various dietary supplements have also been shown to provide treatment for AD. For instance, S-adenosyl methanone (SAM) supplementation in apolipoprotein E (ApoE) deficient mice improved neuropathological features of AD. Chan, et al. observed neuroprotection by dietary supplementation of apple juice concentrate, rich source of SAM, in AD ApoE deficient mice. Moreover, in this same mouse model, folate and vitamin E deficiency led to increased

presenilin-1 expression (processes amyloid) which was later attenuated by apple juice concentrate in both juvenile and adult mice. Many other dietary components, e.g., caffeine (500 mg or 5-6 cups of coffee a day [88] epigallocatechin-gallate esters from green tea and red wine (Cabernet Sauvignon) have been shown to inhibit amyloidosis and Aβ production in both cell culture and animal models. Various life-style factors such as calorie restriction high activity in environmental enrichment and voluntary exercise have shown synergistic effects to antioxidants in mitigating AD neuropathophysiology [89].

CONCLUSIONS

Oxidative stress and free radicals induced diseases can be managed by introducing clinically proven naturally occurring antioxidants or antioxidant supplements which can prevent onset of many cardiovascular diseases, neurodegenerative disorders and cancers. These antioxidants have a strong potential in the conventional treatments of diseases especially in, inflammatory disease, neurodegenerative diseases, cancer, and diabetes. Hence, with balanced diet and good supplementation of fruits, vegetables, grains, oils, and nuts having adequate essential antioxidants such as vitamin A, E, C, lipoic acid etc., are sufficient to improve our body's immune system and to prevent many diseases, and premature aging.

DECLARATIONS

Conflict of Interest

The authors have disclosed no conflict of interest, financial or otherwise.

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