

## NATURAL ANTIOXIDANTS AS DEFENSE SYSTEM AGAINST CANCER

ANTERPREET CHAHAL<sup>1</sup>, ADESH K SAINI<sup>1</sup>, ANIL KUMAR CHHILLAR<sup>2</sup>, REENA V SAINI<sup>1\*</sup>

<sup>1</sup>Department of School of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan – 173 229, Himachal Pradesh, India. <sup>2</sup>Centre for Biotechnology, MD University, Rohtak – 124 001, Haryana, India. Email: reenasaini@shooliniuniversity.com

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## ABSTRACT

In living cells, the production of free radicals that comprise both reactive oxygen species (ROS) and reactive nitrogen species is highly regulated that help the cells to sustain redox homeostasis. Overproduction of ROS from mitochondrial electron transport chain leakage or excessive stimulation of xanthine oxidase and other oxidative enzymes leads to the uncontrolled production of free radicals leading to oxidative stress that can mediate damage to cell structures. This damage can be repaired by the antioxidant defense system. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cellular components such as DNA, proteins, and lipids. The use of antioxidants in cancer prevention is a rapidly evolving research area where antioxidants scavenge free radicals and thus, indirectly help in the prevention of cancer. A wide range of antioxidants such as glutathione, N-acetylcysteine, coenzyme Q10, lycopene, flavonoids, and isoflavones when used in combination with chemotherapy and radiotherapy, result in the reduction of drug toxicity and enhanced efficacy of anticancer agents. This review aims at the use of these exogenous antioxidants as disease-oriented therapy and elucidating the relation of antioxidant enzymes with different types of cancers to overcome the harmful effects of cancer treatment.

**Keywords:** Antioxidants, Cancer, Reactive oxygen species, Glutathione, Flavonoids, Tumor.

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## INTRODUCTION

Free radicals are unstable molecules that are formed as natural by-products in the body during biological processes and lead to oxidative stress. This imbalance is repaired by the body's endogenous antioxidant defense system and by ingesting exogenous antioxidants [1]. In living cells, free radicals that comprise both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in a regulatory manner that helps to sustain redox homeostasis at the cellular level in the normal healthy tissues [2]. ROS and RNS, which are together referred as ROS and RNS have an important role in gene regulation through signaling mechanisms [3,4].

Most cells can produce superoxide ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), and nitric oxide (NO) on demand and these free radicals play a key role in cellular processes as in the generation of adenosine triphosphate (ATP) during oxidative phosphorylation [5]; detoxification of xenobiotics by cytochrome P450 [6]; apoptosis of defective cells, killing microorganisms, and cancer cells by macrophages and cytotoxic lymphocytes [7,8]. Overproduction of ROS from mitochondrial electron transport chain leakage or excessive stimulation of xanthine oxidase and other oxidative enzymes leads to uncontrolled production of free radicals leading to oxidative stress that can mediate damage to cell structures, including lipids and membranes, proteins, and nucleic acids and form harmful products such as lipid peroxides and other lipid adducts. The consequent protein damage results in loss of enzyme activity, while DNA damage can result in mutagenesis and carcinogenesis [9-11]. These consequences, thus, make free radicals responsible for causing several human diseases [12,13]. Excess of free radicals are scavenged endogenously by enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxin reductase [14-16], and non-enzymatic antioxidants such as uric acid, Vitamin A (retinoids), carotenoids, alpha-tocopherol (Vitamin-E), flavonoids, and other related polyphenols such as quercetin, catechins, isoflavones, lignans, flavanones, and ellagic acid [17,18].

Antioxidants act as a radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS [19]. Supplementation of antioxidants in cancer treatment is a rapidly evolving area as they have been widely studied for their ability to prevent cancer in humans and decreasing side effects of existing cancer treatments including chemotherapy and radiotherapy [20,21].

## ROS AND RNS

ROS is a broader term; it includes many reactive species, e.g., superoxide ( $O_2^{\cdot-}$ ), hydroxyl ( $OH^{\cdot}$ ), peroxy ( $ROO^{\cdot}$ ), alkyl radical, alkoxy ( $RO^{\cdot}$ ) radicals, singlet oxygen ( $O$ ) and semiquinone radical ( $HQ^{\cdot}$ ), and ozone ( $O_3$ ) [22]. There are two types of ROS, (a) which contain one or more unpaired electron(s) in their outer molecular orbitals, for example, superoxide, nitric oxide and hydroxyl radicals, and (b) non-radical ROS, which include hydrogen peroxide, ozone, peroxy nitrate and hydroxide that do not have unpaired electron(s) but are chemically reactive and can be converted to radical ROS [23]. Hydroxyl radicals are formed in the presence of metals and hydrogen peroxide (Fenton reaction); peroxy nitrite might play a small role in hydroxyl radical formation. In this process, certain non-radicals are also produced that are either oxidizing agents or easily converted into radicals, such as HOCl (hypochlorous acid), ozone,  $H_2O_2$ , and lipid peroxides with no unpaired electrons.  $H_2O_2$  and lipid peroxides also serve as a source of highly reactive  $^{\cdot}OH$ ,  $ROO^{\cdot}$ , and  $RO^{\cdot}$  radicals. The  $O_2^{\cdot-}$  reacts quickly with very few molecules, whereas hydroxyl radical  $OH^{\cdot}$  has an extremely high rate of reactivity [22]. Superoxide anion can be generated both enzymatically, e.g., during the NADPH phagocytes oxidase reaction in neutrophils, and non-enzymatically in the mitochondrial respiratory chain. The superoxide anion plays an important role in the formation of other ROS such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems [24].

RNS is a collective term that includes nitric oxide radical (NO<sup>•</sup>), peroxyxynitrite (ONOO<sup>-</sup>), nitrogen dioxide radical (NO<sub>2</sub><sup>•</sup>), and other oxides of nitrogen and products arising when NO<sup>•</sup> reacts with O<sub>2</sub><sup>-•</sup>, RO<sup>•</sup>, H<sup>•</sup>NO<sup>•</sup> (HNO, Reduced form of nitric oxide; HNO<sub>2</sub>, nitrous acid) [25]. The superoxide anion can react with nitric oxide (NO<sup>•</sup>) and form peroxyxynitrite (ONOO<sup>-</sup>), which can generate toxic compounds such as hydroxyl radical and nitric dioxide [26]. NO<sup>•</sup> plays a major role in cellular signaling, vasodilation, insulin secretion, peristalsis, neural development, and immune response [27]. It is a highly reactive small uncharged molecule containing one unpaired electron, therefore, considered a free radical. Endogenous NO<sup>•</sup> is formed in the biological tissues through the action of nitric oxide synthase where L-arginine and oxygen are converted into NO<sup>•</sup> and citrulline through a five-electron oxidative process. The reaction requires the presence of many cofactors such as flavin adenine dinucleotide, flavin mononucleotide, nicotinamide adenine dinucleotide phosphate, tetrahydrobiopterin, and heme [28,29]. L-arginine gets converted into L-citrulline and nitric oxide by the action of NOS, but under uncoupling conditions, these enzymes also produce superoxide. Unregulated production of nitric oxide can be damaging to tissues due to its potential cytotoxicity [30].

Under steady state conditions, the ROS molecules are scavenged by various antioxidative defense mechanisms [31]. Enhanced generation of ROS can overcome cell's intrinsic antioxidant defenses resulting in a condition known as "oxidative stress." The equilibrium between the production and the scavenging of ROS may be agitated by various biotic and abiotic stress factors [32]. Excessive or sustained ROS production, when exceeding the available antioxidant defense systems, produces oxidative stress, (Fig. 1) that damages cell structure and disrupts function through lipid peroxidation of cell membranes and degrades nucleic acids [33].

Oxidative damage to cells and tissues also leads to aging and other chronic diseases such as atherosclerosis, heart failure, and cancer [34]. Humans are exposed to many anthropogenic factors like toxic metals (lead, cadmium, mercury, and arsenic) that are widely found in our environment including contaminated air, water, soil, and food. Exposure to arsenic increases free radical generation and cause damage to the biological membrane through increased lipid peroxidation and protein carbonyl content followed by decreased antioxidant defense system [35,36]. Glutathione (GSH) is an important biomolecule involved in the antioxidant defense system against toxicants and arsenic showed high affinity toward GSH leading to decreased levels of GSH [37]. Chronic arsenic exposure has been associated with apoptosis of lymphocytes and involved in immunotoxic responses [38]. It has been reported that arsenic exposure causes increased generation of free radicals coupled with enhanced oxidative stress leading to thymic atrophy in a mouse model [37,39].

Recent studies indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules; therefore, the toxicities associated with these metals might be due to oxidative tissue damage [40]. Redox-active metals, such as iron, copper, and chromium, undergo redox cycling whereas redox-inactive metals, such as lead, cadmium, mercury, and other metals deplete the major antioxidants, particularly thiol-containing antioxidants and enzymes of the cell [36].

## ANTIOXIDANT DEFENSES

Antioxidants act as a radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and non-enzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS. Endogenous antioxidants play a crucial role in maintaining optimal cellular functions and thus, systemic health and well-being. However, under conditions, which promote oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions [19].

Two principal mechanisms of action have been proposed for antioxidants [41]. The first is a chain breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the system. The second mechanism involves removal of ROS/RNS initiators (secondary antioxidants) by quenching chain initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms, including electron donation, metal ion chelation, or by gene expression regulation [42].

Many antioxidants have aromatic ring structures and are able to delocalize the unpaired electrons. Antioxidant mechanisms of polyphenolic compounds are based on hydrogen donation abilities and chelating metal ions [33]. After donating a hydrogen atom, phenolic compounds become resonance-stabilized radicals, which do not easily participate in other radical reactions.

Endogenous protein antioxidants with enzymatic activity such as GPx, SOD, and CAT also play a critical role in reduction of oxidative stress [43]. GPx exist in two forms: Selenium-dependent and selenium independent, each with different subunits and different active sites [44,45]. GPx catalyzes the reduction of H<sub>2</sub>O<sub>2</sub> or organic peroxide (ROOH) to water or alcohol [46,47]; this process occurs in the presence of GSH, which is converted into GSSG (oxidized glutathione) during this reaction. The reaction has special significance in the protection of the polyunsaturated fatty acids located within the cell membranes where the enzyme functions as a part of a multi-component antioxidant defense system within the cell [48]. There are four isoforms in humans, cytosolic and mitochondrial (GPx1), cytosolic (GPx2), extracellular (GPx3), and the phospholipid peroxide (GPx4) [49,50]. The kidney and liver have the highest amount of GPx [51]. GPx enzyme plays an important role as a first line of defense against oxidative stress as it is the first enzyme that is activated under high levels of ROS in various body parts and tissues including dorsal root ganglion [52,53]. Recent studies have shown the involvement of GPx4 in an endogenous tumor suppressive mechanism known as ferroptosis which can be triggered by small molecules or conditions inhibiting the biosynthesis of glutathione or GPx4 [54,55]. Cells tend to be indefatigably exposed to the threat of ROS-mediated destruction, as inhibition of GPx4 activity leads to the rapid accumulation of L-ROS and cell death in cell culture, and deletion of Gpx4 in mice is embryonic lethal [56,57]. RSL3 (RAS-selective lethal 3, Type II) mediated inactivation of GPx4 is essential to induce ferroptosis and overexpression of GPx4 blocks RSL3-induced cell death [56].

SODs are a group of key enzymes functioning as the first line of antioxidant defense with the ability to convert highly reactive superoxide radicals into hydrogen peroxide and molecular oxygen [58]. There are four isozymes of SOD: (1) SOD1 (associated with Cu/Zn) requires Cu and Zn for its biological activity; the loss of Cu results in its complete inactivation and is the cause of multiple diseases in human and animals [59]. (2) SOD2, it has been shown to be involved in inflammatory response [60-62]. The SOD3 enzyme has many physiological effects; studies have reported reduced cardiovascular damage by administration of recombinant SOD3 [63,64]. The SOD4 associated with Ni was discovered in *Streptomyces* [65] but has also been found in some genera of actinobacteria and cyanobacteria [66].

Catalase is a tetrameric porphyrin containing an enzyme that is located mainly in peroxisomes. It catalyzes the conversion of H<sub>2</sub>O<sub>2</sub> to water and molecular oxygen [47]. Catalase along with other enzymes such as GPx and SOD have been considered as biomarkers of oxidative stress in various organs; for example, in streptozotocin-induced diabetic rats, hepatic levels of these enzymes are dramatically reduced, although treatment with red palm oil (*Elaeis guineensis*) and Rooibos tea extract (*Aspalathus linearis*) can improve this effect [67-69].

Another major thiol antioxidant is the tripeptide GSH, a multifunctional intracellular antioxidant which is considered as the major thiol-disulfide redox buffer of the cell [70]. Free form of this antioxidant

is present mainly in reduced form (GSH), which is converted into its oxidized form (GSSG) by enzyme glutathione reductase [71,72]. The main protective roles of glutathione against oxidative stress are that it can act as a cofactor for several detoxifying enzymes, participate in amino acid transport across the plasma membrane, scavenge hydroxyl radical and singlet oxygen directly, and regenerate Vitamins C and E back to their active forms [70].

### FREE RADICALS AND CANCER

The major targeting site of free radicals is genetic material carried by the cells. The types of damages include strand breaks (single or double strand breaks), various forms of base damage yielding products such as 8-hydroxyguanosine, thymine glycol or abasic sites, damage to deoxyribose sugar as well as DNA protein cross-linkages [73]. These damages can result in inheritable mutations that can yield a cancer in somatic cells or fetal malformations in the germ cells. The involvement of free radicals with tumor suppressor genes and proto-oncogenes recommend their role in the development of different human cancers [74]. Constant activation of transcription factors such as NF- $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B-cells) and activator protein-1 appears to be one functional role of elevated ROS levels during tumor progression [75].

Compared with normal cells, malignant cells seem to function with higher levels of endogenous oxidative stress in culture and *in vivo* [76,77]. For example, leukemia cells freshly isolated from blood samples from patients with chronic lymphocytic leukemia or hairy-cell leukemia showed increased ROS production compared with normal lymphocytes. It has been shown that ROS have toxic effects to both normal and abnormal cells (infected by intracellular pathogens and malignant cells). It has been shown that increased oxidative stress could enhance prevalence of malignancies by direct cellular damage, [78-80] however, oxidative stress when applied as immune system arms could protect organisms from invading pathogens and malignant cells [81].

Although the precise pathways leading to ROS stress in cancer cells remain unclear, several intrinsic and extrinsic mechanisms are thought to cause oxidative stress during cancer development and disease progression. Activation of oncogenes, anomalous metabolism, mitochondrial dysfunction and loss of functional p53 are some of the intrinsic factors known to cause increased ROS production in cancer cells [82-85]. Mitochondrial DNA (mt-DNA) mutations have also been shown to be correlated with increased ROS levels in certain types of cancer cells, including those in solid tumors and leukemia [15,16]. Several protein components of the electron transport chain are encoded by mt-DNA. Thus, mutations of mt-DNA are likely to cause impairments in electron transfer, leading to leakage of electrons and the generation of superoxide, which can subsequently be converted to other types of ROS [84].

At an advanced disease stage, cancer cells usually exhibit genetic instability and show a significant increase in ROS generation, that results in gene mutations induced by ROS (especially in the mitochondrial genome) leading to further metabolic malfunctions and abnormal ROS generation. Increased ROS stress in cancer cells correlates with the aggressiveness of tumors and poor prognosis. Normal ROS levels are necessary for the progression of several basic biological processes including cellular proliferation and differentiation [86]. Cancer cells have an intrinsic elevated ROS level compared to normal body cells. Therefore, elevated oxidative signaling may be implicated in the promotion and progression of a number of different cancers. ROS can affect cellular proteins, lipids, and DNA, leading to genomic instability and activation of various signaling cascades related to tumorigenesis (Fig. 2). The formation of new blood vessels out of pre-existing capillaries, referred to as angiogenesis, is an essential component of tumor growth, survival, and metastasis [87,88]. Proliferation, migration, and tube formation in endothelial cells are some of the key events in tumor angiogenesis which are mediated by ROS [88]. As cancer cells exhibit a greater ROS levels than normal cells, so these ROS

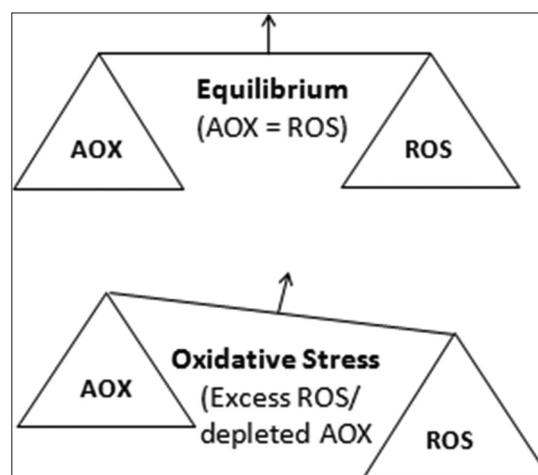


Fig. 1: Disturbance in the equilibrium between ROS and antioxidants leads to Oxidative stress

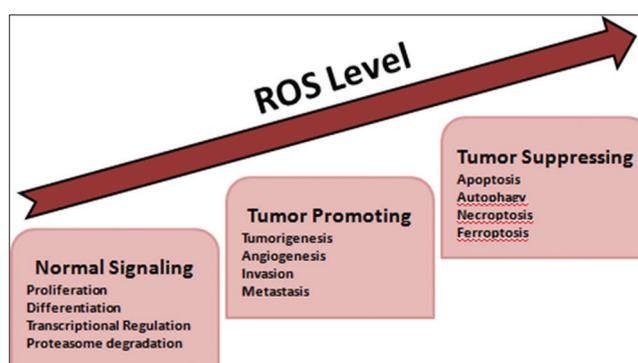


Fig. 2: Elevated ROS levels have been linked to various tumorigenic processes which leads to biochemical and molecular changes.

levels are counteracted by an increased activity of antioxidant enzymes in cancer cells which leads to activation of different cell death pathways, therefore, limiting the cancer progression [86].

### ANTIOXIDANTS AGAINST CANCER

Cancer is a growing health problem in both developing and developed countries. At present, the on-going treatments for cancer are chemotherapy, radiotherapy, and surgery. Some of the most used chemotherapy drugs include antimetabolites (e.g., methotrexate), DNA interactive agents (e.g., cisplatin and doxorubicin), anti-tubulin agents (taxanes), hormones, and molecular targeting agents [89,90]. However, clinical uses of these drugs are accompanied with several side effects such as hair loss, suppression of bone marrow, drug resistance, gastrointestinal lesions, neurologic dysfunction, and cardiac toxicity [91,92]. Therefore, there is a need for new anticancer agents with better effectiveness and lesser side effects.

Endogenous antioxidant enzymes like SOD that provide the first line of defense in human cancers have been studied in human cancers. In human patient samples, Cu-Zn SOD activity is decreased in breast carcinoma [93]. In human esophageal cancers, studies have shown that decreased Mn-SOD levels are associated with increased incidences of esophageal adenocarcinoma [94]. In human oral cancers, a high expression level of Mn-SOD was associated with better disease-specific survival, especially for patients with moderate or poor differentiation of squamous cells of buccal cavity, and early stage buccal mucosal squamous cell carcinomas [95].

Catalase enzyme has also been studied for its role in cancer disease. Decreased catalase activity due to the inflammation in lung leads to increase hydrogen peroxide intracellularly and create an intracellular

environment favorable to DNA damage and the promotion of cancer [96]. Another study showed higher oxygen-free radical production and decreased catalase activity, supporting the oxidative stress in breast cancer [97].

Loss of heterozygosity of cytosolic GPx1 gene was implicated in lung cancer patients by Moscow *et al.* [98]. Ratnasinghe *et al.* investigated the association between the proline to leucine polymorphism at codon 198 of hGPx1 (human cellular GPx1) and lung cancer risk. They showed that due to the high prevalence of leucine residue, the hGPx1 variant contributes significantly to lung cancer risk among the Caucasians but not among the ethnic Chinese who do not exhibit this polymorphism [99].

There are other non-enzymatic antioxidants with beneficial effects in medical practice, especially in cancer [100]. One of them is quercetin, a plant-derived aglycone form of flavonoid glycosides, which has been used as a nutritional supplement and may be beneficial against a variety of diseases, including cancer [101]. It has been reported that quercetin has a higher reduction potential compared with curcumin, that it reduced LPS-induced ROS/NO production to near normal levels [102]. It has also been reported that long-term exposure of cancer cells to quercetin may prevent cell proliferation and survival, and the interference of quercetin with cell-cycle progression diminishes the efficacy of microtubule-targeting drugs such as taxol and nocodazole to arrest cells at G2/M [103].

It has also been revealed that some antioxidants (e.g., quercetin and naringenin) are able to inhibit cytochrome P450 enzymes (CYP1A1 and CYP3A4, respectively) involved in the bioactivation of chemical carcinogens [28], constituting another proposed chemopreventive mechanism of polyphenols against cancer development including lung cancer [104,105].

Preclinical studies have shown that large doses of ascorbic acid (Vitamin C) show significant anticancer effects in animal models and tissue culture investigations [106-108]. These include direct cytotoxic effects in certain cancer cell lines at micromolar ( $\mu\text{M}$ ) to millimolar (mM) concentrations [109]. Early clinical studies suggested that intravenous (i.v.) and oral ascorbic acid may diminish symptoms and possibly prolong survival in terminal cancer patients [110-112].

Other antioxidants such as isoflavones and indole-3-carbinol (I3C), and its *in vivo* dimeric product 3,3-diindolylmethane (DIM) exhibit a promising effect on the inhibition of ROS accumulation [113-116]. The main sources of isoflavones are soy and other plants in the Legume family and Brassica family. The isoflavones include genistein, daidzein, glycitein, formononetin, biochanin A, desmethylangolensin, and equol. The Brassica family is the main source of I3C and DIM. The isoflavones, I3C and DIM, have been shown to inhibit NF- $\kappa$ B activation stimulated by ROS [117,118] suggesting their potent ability as antioxidants. The inhibition of cancer growth by isoflavones could be mediated through induction of apoptosis and the modulation of expression of the genes related to the cell growth and apoptotic processes [119-123].

#### RELATION OF ANTIOXIDANT THERAPY WITH OTHER THERAPIES

A widespread research has been done in the area of cancer prevention and therapeutic and it has been shown that wide range of antioxidants such as glutathione, N-acetylcysteine, coenzyme Q10, lycopene flavonoids, and isoflavones when used in combination with chemotherapy and radiotherapy result in the reduction of drug toxicity and increase the survival time of patients by increasing the tumor response to these therapies [20]. The modulating effects of antioxidants in treatment depend on a wide range of factors, including the metabolic state of the patient, the stage and site of the disease, and the modality being used [124]. The cellular changes would ideally, enhance tumor cell killing, largely by apoptosis, and reduce the probability of normal cell death. Antioxidant enzymes and detoxifiers have the ability to inhibit tumor initiation and promotion *in vivo* and *in vitro* [125].

Combinations of antioxidants have been shown synergistic anti-tumor effects *in vivo*. Dasari *et al.* reported that significant upsurge

was observed in antioxidant levels between the patients treated with radiotherapy and chemotherapy than the patients treated with chemotherapy alone. Hence, the radiotherapy along with chemotherapy kills and decreases the size of cancer cells which facilitate the significant alterations (increased) in the development of the antioxidant system, which is not possible in case of chemotherapy alone [126]. The efficacy of anticancer drugs used in chemotherapy is limited by the fraction of actively dividing cells because these drugs do not kill resting cells unless those cells divide soon after exposure to the drug. Some of the anticancer drugs including bleomycin, doxorubicin (adriamycin), and cisplatin rely on ROS as they produce free radicals that play a role in treatment [127]. Even when the mechanism of the chemotherapeutic drug is independent of free radical action, antioxidants help to maintain the health of normal tissues and protect them from the toxic effects of free radical producing cytokines that circulate in cancer patients and increase with the severity of the disease [128]. Another therapy, i.e., radiotherapy uses ionizing radiations (x- and  $\gamma$ -rays) to induce cancer cell death through free radical formation. This therapy includes two mechanisms: First mechanism is apoptosis, resulting in cell death within a few hours of radiation and second mechanism is a radiation-induced failure of mitosis and the inhibition of cellular proliferation which kills cancer cells [20]. About two-thirds of x- and  $\gamma$ -ray damage is caused by free radicals that kill tumor cells but intimidate the reliability and endurance of surrounding normal cells. Response to radiation depends on the type, dosage and time intervals of radiation, inherent tissue sensitivity, and intracellular factors that include position in the cell cycle, concentration of oxygen, thiols, and other antioxidants [85].

#### CONCLUSION

The abnormal production of free radicals has been known to cause several human diseases. The antioxidant defense system can only protect the body when the normal physiological level of the free radicals is maintained. When there is a high level of ROS, increasing their burden in the body, it leads to oxidative stress, tissue injury and subsequent diseased conditions. The balance between the two has to be maintained as low antioxidant status lead to enhanced oxidative stress in cancer patients, even before oncology treatment starts. Data from several experiments done both *in vivo* and *in vitro* conditions have illustrated the importance of antioxidants in cancer prevention therapies. The combination of radiotherapy and chemotherapy has shown beneficial outputs and has demonstrated the role of enhanced oxidant system. Radiotherapy along with chemotherapy kills and decrease the size of cancer cells which facilitate the significant alterations in the development of the antioxidant system, which is not possible in case of chemotherapy alone. Therefore, the need of the hour is to put more efforts for clinical validation of the promising antioxidant agents. There is a need to prove whether antioxidant therapies can prevent or overcome the damaging effects of ROS in life-threatening situations. The compounds must be tested for their safety, toxicity, selectivity, bioavailability, and therapeutic efficacy. In this context, full execution of clinical trials in well-identified and best-suited populations need to be done to determine the efficacy of antioxidant agents in cancer prevention as well as cancer therapeutics. Combination therapy with these agents can also be tried to achieve synergistic clinical effects.

Newer approaches utilizing collaborative research and modern technology in combination with established traditional health principles will yield dividends in the near future in improving health, especially among those who do not have access to the use of costlier western systems of medicine, thus decreasing death rates.

#### CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

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