ANTICANCER RESEARCH *41*: 5881-5902 (2021)
doi:10.21873/anticanres.15408

Review

Targeting Production of Reactive Oxygen Species as an Anticancer Strategy

GRIGORIA-KALLIOPI MARIOLI-SAPSAKOU[1] and MALAMATI KOURTI[1,2]

[1]*European University Cyprus Research Center, Nicosia, Cyprus;*
[2]*Angiogenesis and Cancer Drug Discovery Group, Basic and Translational Cancer Research Center, Department of Life Sciences, European University Cyprus, Nicosia, Cyprus*

Abstract. *Cancer remains the second leading cause of death worldwide. Research is currently focused on finding novel anticancer therapies and elucidating their mechanisms of action. Cellular redox balance is a promising target for new therapies, as cancer cells already have elevated levels of oxidizing agents due to hypermetabolism and genetic instability. Although free radicals are actively involved in vital cellular signaling pathways, they have also been implicated in certain diseases, including cancer. The aim of this review was to highlight the involvement of oxidative stress in the mechanism of action of anticancer agents. The difference in cellular redox balance between normal and cancer cells is discussed as a potential anticancer target, along with various examples of approved or experimental drugs that may alter the redox state. These drugs are presented in relation to their pro-oxidant or antioxidant mechanisms, with the consequent goal of underscoring the importance of such mechanisms in the overall efficacy of anticancer drugs.*

Oxidative stress is defined as the imbalance between the production and elimination of free radicals and reactive oxygen species (ROS), favoring the former, and is usually a result of weak or depleted antioxidant defense mechanisms or of the overproduction of ROS (1, 2). The reactive nature of free radicals is a direct consequence of one or more unpaired electrons in their valence shell, so they can readily react with vital biomolecules such as proteins, lipids and nucleic acids. Free radicals containing oxygen are defined as ROS, and can include radical or non-radical molecules (3, 4).

Free radicals and ROS play important yet complicated roles in the body, as they participate in functions such as the activation of the immune system against bacterial infections and the activity of hormones such as insulin (5). ROS are also produced as a response to cytokine and growth factor signaling, as well as to exogenous factors, including smoking and atmospheric pollution (6).

Most endogenous ROS are byproducts of the electron transport chain (ETC) and the nicotinamide adenine dinucleotide phosphate oxidase complex (NADPH-oxidase) (7, 8). The ETC comprises a sequence of complexes transporting electrons from donor to recipient molecules through redox reactions, eventually flowing to molecular oxygen in aerobic respiration, where several ROS are produced (Figure 1) (7, 9). The NADPH-oxidase is mainly activated during respiratory stress and produces superoxide radicals and H_2O_2, participating in immunological responses as signaling molecules (10, 11).

For the proper function of ROS, the key is a tightly regulated redox balance, which enables their supportive role in cellular proliferation and survival but limits their redox misbalancing properties (12). Redox balance is maintained by several endogenous antioxidant defense systems, including glutathione (GSH), peroxiredoxins, thioredoxin (TRX), superoxide dismutase (SOD) and catalase (13, 14). Another important factor is nuclear factor erythroid 2-related factor 2 contributing to the counterbalancing of free radical overproduction (15, 16). This factor controls both the basic and induced expression of a series of antioxidant response elements-dependent genes in order to regulate the physiological and pathophysiological consequences of oxidative exposure. Thus, it is a regulator of cellular resistance to oxidative molecules (17).

Thus, with oxidative stress and ROS signaling being implicated in many normal functions, oxidative stress has been linked to certain diseases as a potential mechanism of toxicity.

Correspondence to: Malamati Kourti (ORCID iD: 0000-0002-4176-8425), 6 Diogenous str., Egkomi, Nicosia, 2404, Cyprus. E-mail: M.Kourti@euc.ac.cy

Key Words: Free radicals, ROS, redox homeostasis, oxidative stress, cancer, antioxidants, pro-oxidants, anticancer drugs, review.

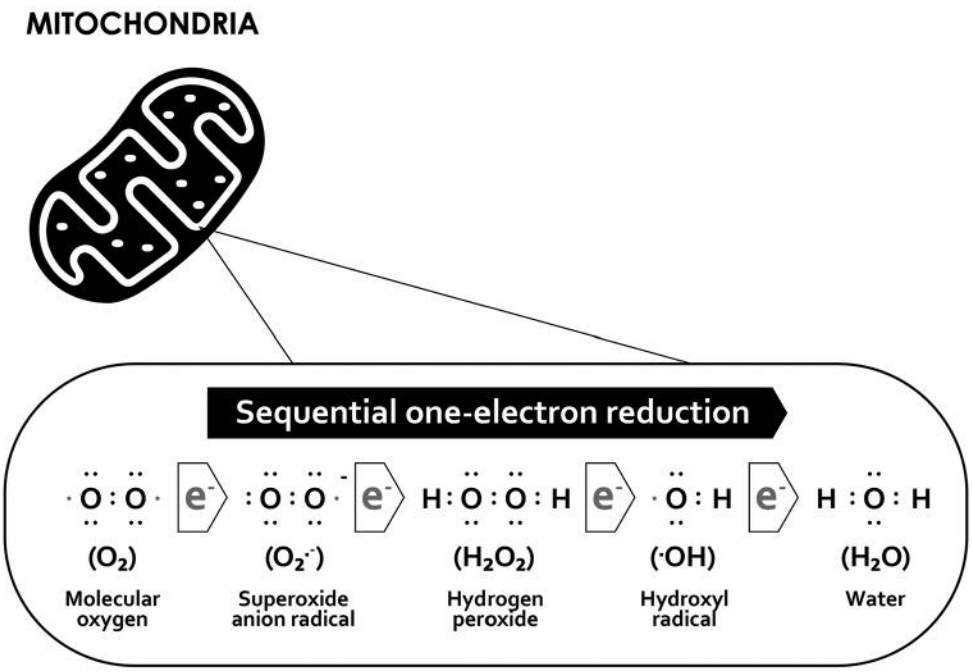

Figure 1. *Monovalent reduction of molecular oxygen to water in biological systems, with the intermediate production of reactive oxygen species. This process occurs mainly within the inner mitochondrial membrane. As shown in the figure, molecular oxygen (O_2) is a free radical with two unpaired electrons. A reduction of O_2 by one electron produces the superoxide anion radical ($O_2^{\cdot -}$), which can undergo another one-electron reduction to produce hydrogen peroxide (H_2O_2). A further reduction of H_2O_2 by one electron generates the hydroxyl radical ($^{\cdot}OH$), which can eventually produce water by a final one-electron-reduction step.*

These diseases range from cancer and atherosclerosis to neurodegenerative diseases and bacterial infections (5, 12, 18). Chronic ROS overproduction depletes the antioxidant defense systems, leading to DNA damage and mutagenesis (19), eventually contributing to carcinogenesis, neoangiogenesis and metastasis (12, 20). Aging has also been linked to oxidative stress and ROS overproduction (1).

Redox balance in normal and cancer cells. As is now widely accepted, homeostasis is a fundamental property of living cells (21). Redox homeostasis, defined as the tight balance between oxidized and reduced molecules (2) within the body, is achieved by enzymatic systems (including catalase, SOD, peroxiredoxins), non-enzymatic systems (such as GSH, cysteine and thioredoxin) and metal-binding proteins (ferritin, metallothioneins, ceruloplasmin *etc.*), that bind and eliminate transition metals and inhibit their involvement in redox reactions (22). Damage to redox homeostasis by excessive production of oxidized biomolecules is associated with cellular toxicity, such as DNA damage, lipid peroxidation, sulfhydryl protein depletion, and oxidative protein stress (2).

Cancer comprises a group of diseases with the common characteristics of dysregulated cell proliferation and survival, as well as the potential to invade and metastasize to adjacent or distant organs and tissues (23). Cancer cells have been shown to have elevated ROS levels mainly due to hypermetabolism (24). The augmented ROS concentrations and the alteration of redox balance lead to the establishment of redox signaling which enables cancer cells to survive even in these harsh conditions, and to resist cell death (16).

There are several metabolism-related mechanisms that promote ROS production in cancer cells. These involve hypermetabolism, overactivated cellular signaling, increased peroxisomal activity, altered mitochondrial function, oncogene expression and elevated enzymatic activity, for example for thymidine oxidases, cyclo-oxygenases, lipoxygenases and phosphorylases (16). These alterations help cancer cells adapt to an oxidative environment more efficiently than normal cells (19). They are also part of the biochemical and molecular changes that are necessary for the initiation, promotion and progression of a tumor, along with its resistance to chemotherapy (25). In the early stages, cancer cells grow and proliferate uncontrollably, a process facilitated by transcription factors, and are therefore vulnerable to DNA damage. In later stages, metastatic cancer undergoes the above metabolic changes, which allows tumors to survive in the presence of intense oxidative stress at the expense of normal cells, by acquiring increased levels of endogenous antioxidant enzymes (6, 12, 26). Elevated ROS facilitates cancer growth in many

ways, causing DNA damage and genomic instability and ultimately leading to the reprogramming of cancer cell metabolism for the benefit of cancer cell survival (16). Mitochondria, as well as glycolysis, glutaminolysis and the oxidation of fatty acids, play a key role in these interactions between redox homeostasis and altered metabolism in tumor cells (27). Tumor invasion and metastasis are also benefited by increased ROS production (28).

Free radicals and ROS are also considered as the most important mutagens in cancer stem cells, where increased levels inhibit the process of self-renewal and stimulate their differentiation into primary tumor cells, while several signaling pathways linked to oxidative stress support the malignant transformation of stem cells (19).

Anticancer strategies related to oxidative stress. Although oxidative stress is detrimental to most normal cell types, it also appears to be an important regulatory mechanism for cancer cells, as mentioned above (29). Thus, these differences in the redox balance between normal and cancer cells can be used as a target for new anticancer therapies.

Cancer cells are more sensitive to pro-oxidant agents and to the inhibition of their antioxidant systems due to the excessive ROS already present (12). On one hand, elevated ROS levels are responsible for cancer progression. On the other hand, when produced in excessive amounts, ROS can jeopardize not only the viability of normal cells but also of cancer cells. Therefore, therapeutic anticancer strategies related to the regulation of free radicals and ROS can be divided into two broad categories: Therapies that are based on the induction of further oxidative stress, and therapies that eliminate these free radicals in an effort to inhibit malignant transformation in compromised cells. The first category can be further subdivided into therapies that inhibit a specific antioxidant system and those that induce ROS production even further, the two of them usually combined together (22, 30).

The first category mainly includes ionizing radiation therapy (31, 32) and certain chemotherapeutic pro-oxidant agents used in conventional cancer regimens. These promote the overproduction of ROS, which eventually reduces the cellular antioxidant capacity and leads to selective targeting, apoptosis and cancer cell death. Elevated levels of ROS induced by pro-oxidant drugs together with a weak cellular defense system lead to a significant imbalance between pro-oxidant and antioxidant molecules, allowing for greater cell damage and cancer cell elimination (12, 33) (Figure 2). Some examples of anticancer drugs that primarily act as pro-oxidants include cisplatin, doxorubicin, imexon, motexafin gadolinium (MGd) and buthionine sulfoximine (BSO), which induce oxidative stress and inhibit antioxidant defense mechanisms in cancer cells. As Watson emphasized, *"The vast majority of all agents used to directly kill cancer cells (ionizing radiation, most chemotherapeutic agents, and some targeted therapies) work through either directly or indirectly generating ROS that block key steps in the cell cycle"* (34).

The second category mainly includes antioxidant molecules that act *via* slowing down or inhibiting oxidation and binding free radicals in order to eliminate them (13). Normally, antioxidants focus on the neutralization of toxic oxidative substances. From this point of view, antioxidants can help prevent and treat cancer by reducing oxidative stress and radical signaling (22). Another potential role for antioxidant supplementation during cancer treatment is for the prevention of toxic side-effects of chemotherapy on normal tissues and organs (35). Medicinal and aromatic plants are usually used as health supplements and also as active components of cosmetics but their use in chemotherapy patients relies on their potential to reinforce endogenous antioxidant mechanisms by removing free radicals or by stimulating intracellular antioxidant enzymes (36). These antioxidants include, apart from dietary antioxidants, also endogenous antioxidant systems and enzymes (37).

Several antioxidants have been tested during the last decades for their proven or anticipated beneficial effects against oxidative stress and cancer, such as vitamin E, vitamin C, carotenoids, flavonoids and polyphenols (1, 38). Other synthetic antioxidant molecules comprise among others butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and *tert*-butylhydroquinone (12). The difference in antioxidant sensitivity between normal and cancer cells, causing great variability in cellular uptake, accumulation in specific tissues and the subsequent cellular responses induced, should also be taken into consideration (6).

In most chemotherapy regimens, antioxidant supplements are combined with pro-oxidant chemotherapeutic agents (39). However, it is still under review whether dietary antioxidant supplementation enhances conventional cancer treatments (40).

From this point of view, antioxidants can in fact interfere with the effectiveness of pro-oxidative chemotherapy, for example by completely eliminating free radicals and ROS (41). As Watson again pointed out: *"Free-radical-destroying antioxidative nutritional supplements may have caused more cancers than they have prevented"* (34). On the other hand, by diminishing free radicals and ROS, antioxidants can alleviate the unwanted toxicity to normal tissues caused by chemotherapy, thus allowing for increased dosing of chemotherapies, and limit ROS within a restricted range in order to protect normal cells (22). In some cases, of course, the reduced cell proliferation rate caused by some anticancer agents can alter the effectiveness of other drugs aimed at rapidly proliferating cells. In such cases, low levels of antioxidants help with cancer cell apoptosis, as a synergistic effect of antioxidants with certain chemotherapeutic agents has been shown (6). Therefore, better guidelines on antioxidant supplementation

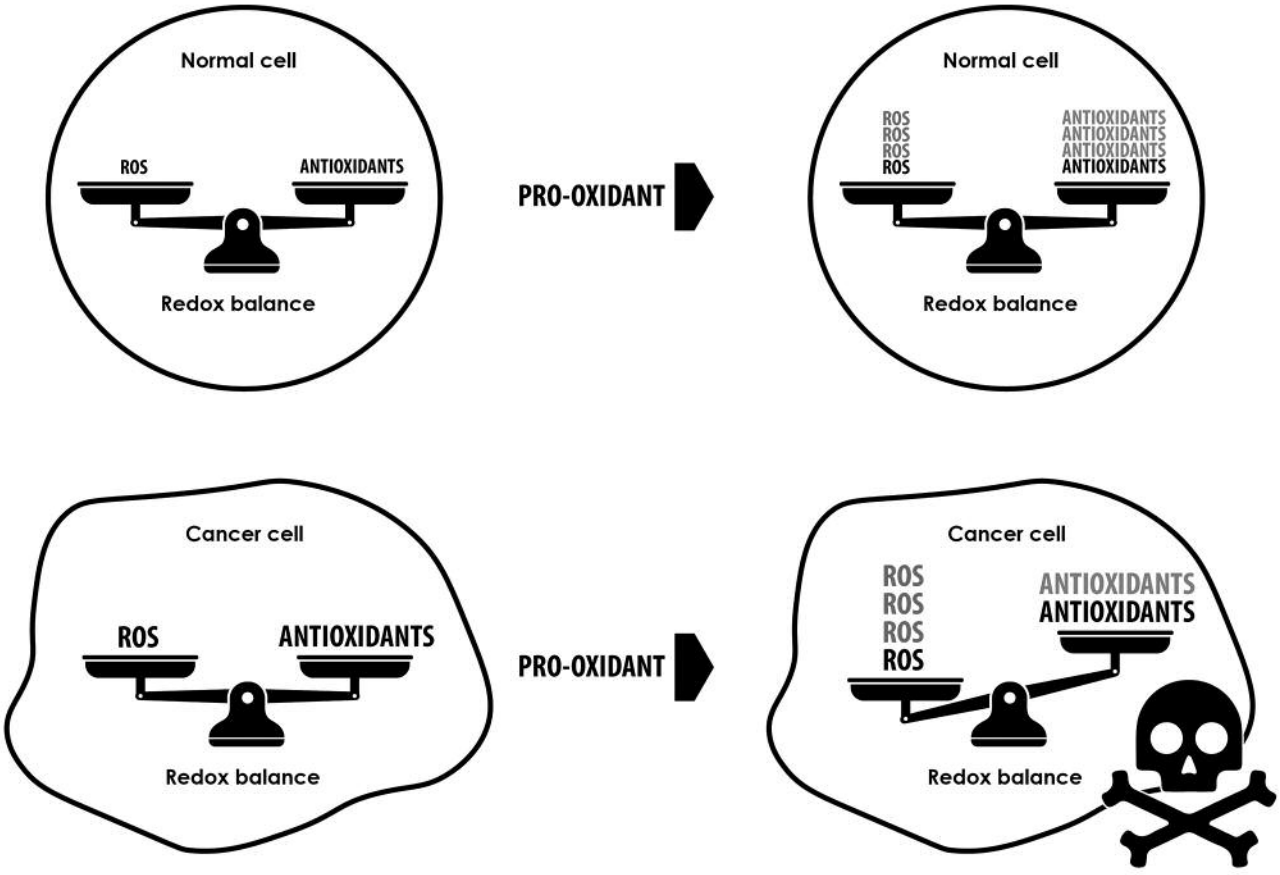

Figure 2. *Redox balance in normal and cancer cells, before and after treatment with pro-oxidant agents. Normal cells have lower basal reactive oxygen species (ROS) and antioxidant levels than cancer cells. In normal cells, ROS participate in signaling pathways in order to promote proliferation and survival, whereas an elevated ROS level can have detrimental effects, such as tumor progression and invasion. The redox balance within normal cells is easily maintained by calibrating the antioxidant processes. On the other hand, due to hypermetabolism, cancer cells have an elevated basal ROS level, tightly counterbalanced by an elevated level of antioxidant agents. However, once the ROS level exceeds the redox capacity of cancer cells after treatment with a pro-oxidant agent, severe oxidative stress occurs, resulting in the apoptosis of cancer cells due to irreparable oxidative damage.*

during chemotherapy are needed in order to achieve the best possible result and avoid dangerous treatments for patients with cancer (35).

Based on the above, this literature review aimed to highlight redox properties as a key point of the mechanism of action of anticancer drugs, whether they act pro-oxidatively or more as antioxidants. These abilities may be the primary mechanism of action but may also have additional roIes, assisting a more targeted anticancer mechanism. The aim of this review is not to provide an exhaustive list of anticancer drugs and molecules that affect the production of ROS but rather to attempt to demonstrate the importance of such redox mechanisms, giving a few representative examples of such studies for both pro-oxidant and antioxidant agents. There are already several literature reviews presenting comprehensive lists of pro-oxidant and antioxidant anticancer agents which are particularly interesting (6, 12, 24, 30). Thus, in this review, selected chemotherapeutic agents are presented that have been tested in the context of different cancer types, emphasizing the part of their mechanism of action concerning the regulation of free radical production by giving relevant research examples. We feel that a summary of this knowledge will lead to useful conclusions regarding anticancer therapies and may clarify when antioxidant supplementation is useful, either as a monotherapy in some cancer types or as part of a more comprehensive chemotherapy regimen.

Cisplatin. Cisplatin (Figure 3) is one of the most commonly used drugs in the chemotherapy of various types of cancer, such as testicular, ovarian, non-small-cell lung, head and neck, bladder and stomach cancer (42).

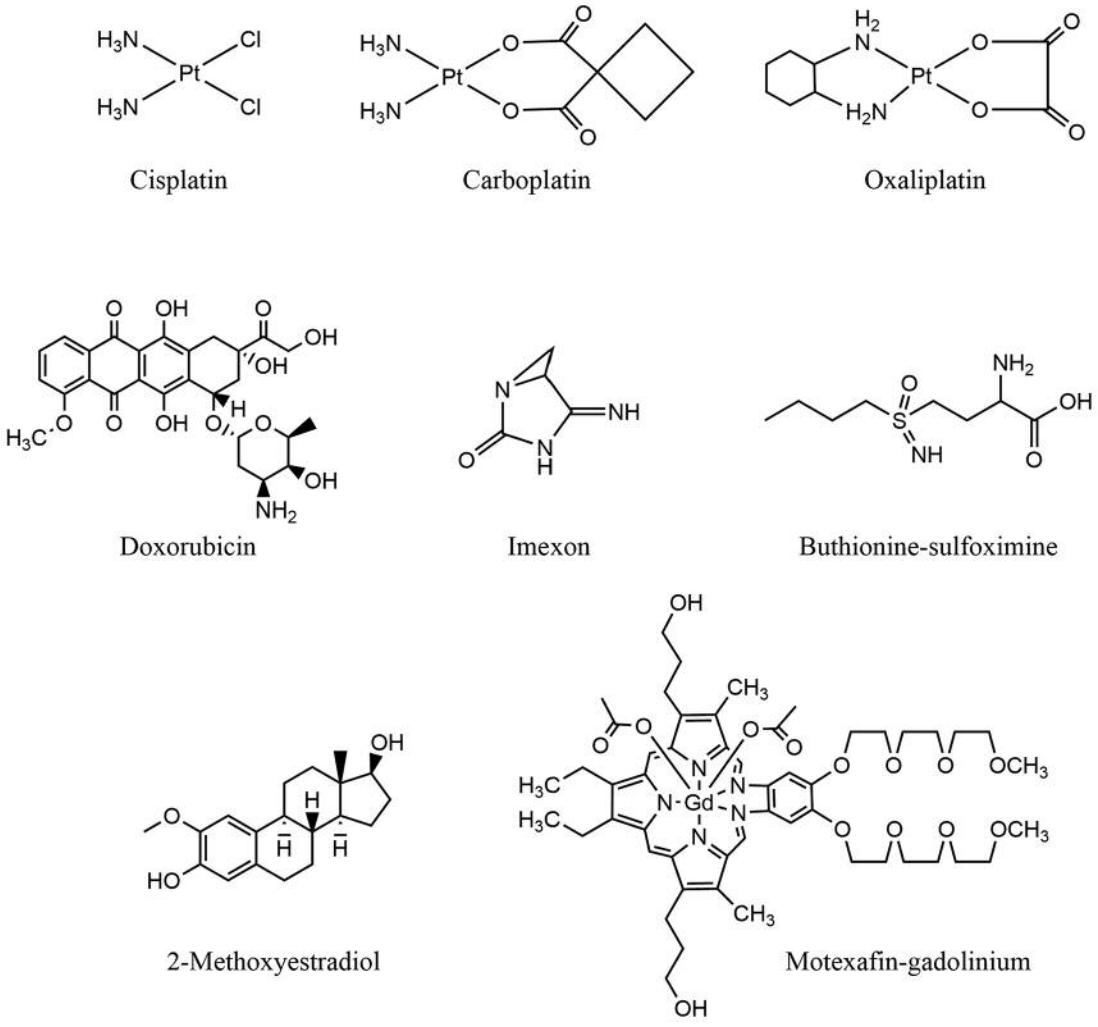

Figure 3. *Molecular structures of some pro-oxidant anticancer drugs.*

However, cisplatin is also characterized by extensive toxicity, provoking side-effects such as renal damage (43), deafness (44) and peripheral neuropathy (45), limiting its overall effectiveness. Cisplatin has also been linked to significant inflammatory responses (46, 47). These observations have led to the development of platinum analogs that would be clinically effective without possessing the toxicity of cisplatin. Carboplatin and oxaliplatin, as shown in Figure 3, are the most popular analogs, approved by the Food and Drug Administration (FDA) in 1986 and 1996, respectively. Other more recent analogs include ormaplatin and enloplatin (42, 48).

The primary mechanism of action of cisplatin involves its ability to bind to purine bases of DNA and to interfere with its repair mechanisms, eventually causing irreparable DNA damage and apoptosis in cancer cells. In general, DNA is the most important target for cisplatin, and this mechanism leads to general cytotoxicity and hypersensitivity effects in both prokaryotic and eukaryotic cells, due to inadequate DNA repair pathways (48). However, cisplatin is also a pro-oxidant, as it promotes the overproduction of free radicals and ROS. This high level of ROS ultimately reduces the antioxidant capacity of cancer cells and leads to selective targeting and apoptosis. The induced oxidative stress additionally enhances the DNA-damaging effects of cisplatin, further reinforcing its mechanism of anticancer activity (12).

These pro-oxidant effects have been shown in many studies using cisplatin. Berndtsson *et al.*, using the colon cancer cell line HCT116 and the melanoma cell line 224, found that even at a low dose of 10 μM, cisplatin caused DNA damage, while higher doses of 20 and 30 μM caused

the formation of peroxide radicals, and eventually led to the apoptosis of cancer cells. The relationship between radical production and cisplatin's anticancer activity was more evident when they showed that this anticancer effect was inhibited by the addition of peroxide scavengers (Tiron), and was independent of the DNA damage caused in general. As for its overall anticancer ability, cisplatin at 10 μM rapidly induced apoptosis in 224 and HCT116 cells within the first 24 hours, whereas concentrations below 10 μM induced significant apoptosis at 72 hours, as evidenced by the loss of mitochondrial membrane permeability, annexin V apoptotic test and caspase activation. Cisplatin concentrations below 10 μM induced slight caspase-3 activation (49).

The implication of ROS in the mechanism of anticancer activity of cisplatin was also evident in another study, where v3 integrin-negative and v3 integrin-positive human laryngeal cancer cells (HEp2) were investigated for their resistance to cisplatin, along with other pro-oxidant anticancer drugs. It was shown that v3 integrin-expressing HEp2 cells were resistant to cisplatin, while also overexpressing B-cell lymphoma 2 apoptosis regulator (BCL2) protein and GSH. BCL2 was found not to be involved in the explored resistance mechanism of these cells, but GSH played a key role, not by directly detoxifying cisplatin but rather by eliminating ROS after exposure to cisplatin. Cisplatin stimulated ROS overproduction in v3 integrin-negative HEp2 cells even 30 minutes after the addition of the drug, whereas v3 integrin-positive cells had a lower level of ROS that was enhanced by the GSH depleter BSO. Hence, it was observed that ROS play an important role in cisplatin's mechanism of action and that cells that managed to reduce ROS overproduction became cisplatin-resistant (50).

Moreover, ROS generation after cisplatin treatment was also evident in testicular germ cell tumors, where the role of p53-independent apoptotic pathways was explored. It was found that apart from ROS production, the activation of mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK), along with caspase-3, were correlated with cisplatin-induced cell death, even though the MAPK–ERK signaling pathway was not specific for cisplatin-induced apoptosis (51).

Unfortunately, one side-effect of cisplatin, namely hepatotoxicity, has also been attributed to ROS production, since it was found that treatment with this drug alters mitochondrial permeability in rat liver cells, thereby altering the process of oxidative phosphorylation and causing further mitochondrial oxidative stress, eventually affecting the viability of liver cells (52).

Given the above observations for monotherapy, cisplatin has also been tested in combination with other pro-oxidants from very early on, for example with doxorubicin. This combination was effective and well-tolerated, and was used for its synergistic effects in order to relieve symptoms in patients with diffuse malignant pleural mesothelioma (53). In addition, a chemotherapy regimen with cisplatin, doxorubicin and cyclophosphamide for advanced salivary gland carcinomas showed encouraging results (54), similarly to other combinations of cisplatin with pro-oxidants such as everolimus (55).

In a study by He *et al*., WZ35, a new curcumin analog, was shown to have potential anticancer effects both *in vitro* and *in vivo*, and it was investigated whether WZ35 enhanced the effectiveness of cisplatin in gastric cancer cells. Cellular apoptosis and ROS levels were measured by flow cytometry. The activity of the antioxidant enzyme thioredoxin reductase 1 (TRXR1) in normal gastric cells and cancer tissues was quantified by the endpoint insulin reduction assay, whereas western blot analysis and a mouse xenograft model were also used to test the effects of the combination of WZ35 and cisplatin on the growth of gastric malignancies. The results showed that WZ35 significantly improved apoptotic effects of cisplatin in gastric cancer cells, enhancing its pro-oxidant effects by inhibiting the activity of TRXR1. A significant elevation in ROS production was observed, followed by activation of the p38 and c-Jun *N*-terminal kinase signaling pathways, which ultimately induced apoptosis. This combination additionally suppressed *in-vivo* tumor growth in a gastric cancer xenograft model, where TRXR1 activity was successfully reduced. Thus, this synergistic effect between a curcumin analog and cisplatin, which both affect the redox balance in gastric cancer cells, could be considered as a new strategy towards the therapy of stomach cancer (56).

Doxorubicin. Doxorubicin is an anthracycline antibiotic (Figure 3) widely used in the treatment of various cancer subtypes, such as breast, stomach, lung, ovarian and thyroid cancer, soft-tissue sarcoma, multiple myeloma and Hodgkin's lymphoma (57, 58). Doxorubicin·HCl was the first liposome-incorporated anticancer drug that received FDA approval (Doxil®).

The exact mechanism of action of doxorubicin is complex and remains somewhat unclear. Doxorubicin interferes with DNA by inhibiting its biosynthesis and by intercalation. In particular, it inhibits the enzyme topoisomerase II, which relaxes DNA supercoils before transcription, stabilizing the topoisomerase II–DNA complex during the replication process. This leads to a problematic resealing of the double DNA strand, hence inhibiting replication and transcription (58, 59).

Another aspect of its mechanism of action is its ability to generate free radicals and ROS that cause DNA and cell membrane damage. A study by Pilco-Ferreto *et al*. in 2016 confirmed that doxorubicin is active against breast cancer through a pro-oxidant mechanism of action, testing three breast cancer cell lines MCF-10F, triple-positive MCF-7 and the triple-negative cell line MDA-MB-231 (60). The effect of doxorubicin on apoptotic mechanisms and generation of

ROS was examined in this study. The results showed that doxorubicin was able to reduce expression of the anti-apoptotic protein BCL2, which is particularly interesting as a target in anticancer therapy (61, 62), as well as the expression of the nuclear factor kappa B (NF-κB) gene and protein, especially in MDA-MB-231 cells. Moreover, doxorubicin caused an increase in BCL2-associated X, apoptosis regulator (BAX), caspase-8 and caspase-3. The results also showed elevated production of oxidative molecules, such as H_2O_2, suggesting oxidative stress as a possible mechanism for the effects of doxorubin. SOD2 is a mitochondrial protein that catalyzes the conversion of superoxide radicals to hydrogen peroxide and oxygen. Overexpression of *SOD2* gene, and therefore of its protein, led to the suppression of the oxidative damage caused after doxorubicin treatment. Therefore, doxorubicin-induced apoptosis in cancer cells was initiated by an increase in pro-apoptotic and a decrease in anti-apoptotic regulators, that is by causing inactivating proteolytic processing of the BCL2 family of proteins and activation of caspases, as well as increased oxidative stress leading to DNA damage (60).

Another study combined thymoquinone (a potential pro-oxidant of natural origin) with low concentrations of doxorubicin, and the anticancer effects against adult T-cell leukemia/lymphoma (ATLL) were determined in both *in vitro* and *in vivo* models. The main cell lines used were human T-lymphotropic virus 1 (HTLV-1)-positive HuT-102 cells and HTLV-1 negative Jurkat cells. $CD4^+$ ATLL cells were treated both with monotherapies of thymoquinone and doxorubicin, and with their various combinations. The results showed that the combination of thymoquinone and doxorubicin was more potent in inhibiting cell viability and provoking cell-cycle arrest in sub-G_1 phase than the monotherapies of doxorubicin or thymoquinone for both cell lines. Their combination caused apoptosis, increased production of free radicals and ROS, and disrupted mitochondrial membrane potential. The vital role of oxidative stress in the observed apoptotic result was shown using pretreatment with the radical scavenger *N*-acetyl-cysteine (NAC). This significantly hindered the apoptotic response, suggesting that cell death was indeed dependent on ROS overproduction. For the *in vivo* studies, an ATLL mouse xenograft model was used and it was shown that the combination of thymoquinone and doxorubicin reduced tumor growth more significantly than the two monotherapies, without affecting the survival rates of the mice. Therefore, this combination regimen might lead to allowing use of lower doses of doxorubicin against ATLL while the anticancer effects remain unchanged (63).

Imexon. Imexon (4-imino-1,3-diazabicyclo-hexan-2-one) (Figure 3) is an aziridine-derived iminopyrrolidone. Its main mechanism of action is related to the production of free radicals and ROS through the opening of the aziridine ring, resulting in apoptosis (64, 65). It has been shown to induce immediate cytotoxicity by binding to free sulfhydryl groups within the cells, depleting molecules such as GSH, increasing ROS and reducing mitochondrial membrane potential (64). Imexon has been studied against hematological and solid cancers and has been shown to be active without provoking significant toxicity. Preclinical data has shown good anticancer activity against a variety of cancer subtypes, including breast, non-small-cell lung, and prostate cancer (66).

According to two studies by the group of Dvorakova *et al.* (67, 68), *in vitro* imexon treatment increased the level of free radicals, and eliminated mainly cancer cells already compromised by elevated ROS levels, as in the case of various multiple myeloma cells. Pretreatment of cancer cells with the antioxidant thenoyltrifluoroacetone, which inhibits the production of peroxide radicals in complex II of the mitochondrial ETC, partially reduced the cytotoxicity of imexon, underlying the importance of ROS overproduction as the main anticancer mechanism. This was also observed after pretreatment of cancer cells with the antioxidant agent NAC. Loss of mitochondrial membrane potential, mitochondrial DNA damage and mitochondrial swelling were also observed. It was hypothesized that the levels of thiols in cancer cells sensitive to imexon were inherently lower than in other cell types, and that the antioxidant defense systems of these cells were relatively less effective in controlling ROS overproduction. As a proof of concept, the group created an imexon-resistant cell line, where increased BCL2, TRX2 and GSH protein levels were observed, while no loss of mitochondrial membrane potential or oxidative stress were detected, even after imexon treatment. Moreover, the resistant cell line created showed significant changes in mitochondrial morphology, while no other morphological changes were detected in other cellular organelles.

In another study on the pancreatic cancer cell line MiaPaCa-2, the effects of imexon on producing endoplasmic reticulum (ER) stress were investigated, since it is well-known that oxidative folding of proteins within the ER requires strict regulation of redox homeostasis. It was shown that acute exposure to imexon elicited an ER stress response and inhibited protein synthesis. Using RNAi techniques, the group found that the eIF2B5 translation initiation factor was the main target of imexon, enabling the inhibition of cancer cell growth, but this factor appeared not to be significant for the effects of imexon on protein synthesis. Concomitant reduction of intracellular thiols with NAC reversed the activity of imexon. However, co-treatment with peroxide scavengers did not have an effect, suggesting that thiol binding may be the major player in the oxidative effects of imexon. Thus, this study suggested that disruption of the redox balance in the ER may be another feature of the anticancer effects of imexon, highlighting it as a potential therapeutic target against pancreatic cancer (69).

Imexon was also shown to eliminate dexamethasone-sensitive and -resistant myeloma cells, both time- and dose-dependently, through a mechanism involving caspase-8 activation. Although ROS production and low GSH levels were found to play a key role after treatment of myeloma cells with high imexon concentrations, low concentrations induced apoptosis by a caspase-8-dependent pathway instead of producing an increased pro-oxidant state. The myeloma cell lines involved in this study were C2E3 (dexamethasone-sensitive), 1-310 and 1-414 (dexamethasone-resistant), RPMI-8226 (chemotherapy-sensitive) and DOX-1V and DOX-10V (chemotherapy-resistant). It was observed that 48 h after treatment with imexon, C2E3 and 1-414 cells underwent caspase-8 dependent apoptosis, whereas RPMI-8226 cells were depleted of thiols, cysteine and GSH. High concentrations of imexon resulted in elevated ROS levels in C2E3, RPMI-8226 and 1-310 cell lines but other oxidative stress biomarkers such as 8-hydroxydeoxyguanosine were not elevated. Therefore, imexon was shown to induce cytotoxicity through both a caspase-8-dependent pathway at low concentrations in dexamethasone-sensitive and resistant myeloma cells, and through a mechanism of ROS overproduction at higher concentrations (64).

Motexafin gadolinium. Motexafin gadolinium (Xcytrin) (MGd), shown in Figure 3, is a member of a class of rationally designed porphyrin-like molecules called texaphyrins. It was designed as an inhibitor of TRXR and ribonucleotide reductase, and its use in the treatment of cancer was based on the fact that it selectively accumulates in cancer cells. However, the FDA did not approve the new drug application filed in 2006 concerning the chemotherapeutic use of MGd against brain metastases from lung cancer based on the drug's failure to meet the pre-specified primary goal in clinical trials (70, 71).

The anticancer mechanism of MGd is tightly linked to redox imbalance (72), resulting in the apoptosis of cancer cells. MGd is actively involved in redox reactions, as it was shown to oxidize intracellular thiols and endogenous reducing molecules, such as GSH, ascorbic acid and nicotinamide adenine dinucleotide phosphate ($NADP^+$) (73). Through a process known as futile redox cycling, MGd catalyzes electron transport directly to molecular oxygen resulting in free radical and ROS production (74). The combination of oxidized proteins and metabolites with ROS formation causes cancer cell apoptosis and lowers the cytotoxicity threshold for many chemotherapeutic agents currently in use (75). Therefore, MGd could be used both as a monotherapy or in combination with other chemotherapies and radiation (74).

As redox mechanisms have been shown to play vital roles in multiple myeloma cells, Evans *et al*. (76) hypothesized that disruption of the redox balance by MGd would result in cytotoxicity in myeloma cells. Therefore, the effects of MGd on cytotoxicity, apoptosis, ROS production and intracellular drug accumulation were investigated in a variety of chemotherapy-sensitive and -resistant cells (C2E3, 1-310, 1-414, RPMI 8226 and DOX-10V). MGd cytotoxicity was evident in all the tested cell lines within 24 hours after the co-treatment of MGd with ascorbic acid. Ascorbic acid was necessary for the cytotoxic effect since it enhanced MGd cellular uptake and it had been previously found to deplete GSH in myeloma cells (77) and help with the overproduction of hydrogen peroxide (73), although when added alone in this study it conferred the expected antioxidant activity. The mechanism of cytotoxicity of MGd was associated with changes in mitochondrial membrane potential and increased expression of annexin V. This was accompanied by depletion of intracellular GSH and increased intracellular ROS production. Cells showed substantial MGd uptake. On the other hand, catalase administration inhibited MGd-induced cell death. Finally, patient-derived multiple myeloma cells were also found to be sensitive to MGd (76).

In a different cancer context, MGd was again evaluated for its ROS-producing, GSH-depleting and DNA-damaging effects in breast cancer cells (EMT6). This study also evaluated the ability of MGd to increase radiosensitivity and inhibit DNA-repair mechanisms after X-ray treatment. Similar to the previous study, the results showed that cells co-treated with MGd plus equimolar concentrations of ascorbic acid had significantly increased ROS and lower GSH levels compared to controls. This led to free radical and ROS generation, which affected GSH homeostasis and caused DNA strand breaks. This alteration of GSH level increased the hypoxic, but not the aerobic, sensitivity of EMT6 cells to radiotherapy. In this context, MGd altered the DNA-repair kinetics of the single-strand breaks immediately after irradiation but failed to completely inhibit EMT6 cell repair (78).

Buthionine sulfoximine. L-(*S,R*)-Buthionine sulfoximine (BSO) (Figure 3) was developed as a selective irreversible inhibitor of glutamate-cysteine ligase, the first rate-limiting enzyme involved in the biosynthesis of GSH. Glutathione depletion is a common sensitizing technique *in vitro* and *in vivo* in order to intensify cancer cell damage induced by radiation or chemotherapy. BSO has also been shown to increase the efficacy of oxidative antiparasitic drugs (79, 80).

BSO was proven to act synergistically with the new low-molecular-weight regulator 5681014 that selectively enhanced expression of the multidrug resistance-associated protein ATP binding cassette subfamily C member 1 (ABCC1, previously MRP1) leading to reduced levels of GSH. Using high ABCC1-expressing cell lines of lung cancer, ovarian cancer and neuroblastoma, the group showed that 5681014 successfully reduced the intracellular GSH level. The combination of BSO and this regulator depleted intracellular GSH, increased free radical and ROS

production, and abolished the clonogenic capacity of these cells. NAC pretreatment was able to re-establish clonogenic capacity, suggesting that reduced GSH production was involved in the mechanism of action. Moreover, 5681014 in combination with BSO strongly sensitized cancer cells to chemotherapeutic agents which are substrates of ABCC1, especially arsenic trioxide, and was more effective than either the ABCC1 regulator 5681014 or BSO alone. Thus, BSO and GSH-reducing ABCC1 regulators can be considered for the treatment of chemoresistant cancers which overexpress ABCC1 (81).

Immunoresistance is a key step for hepatocellular carcinoma (HCC) metastasis. In their study, Lee *et al*. demonstrated *in vitro* and *in vivo* anticancer effects for a combination therapy of BSO and 3-bromopyruvate (3-BP) in anoikis-resistant (AR) HCC cells. AR HCC cells were significantly chemoresistant and showed high glycolysis and low ROS levels. A combination therapy of BSO with 3-BP effectively suppressed the viability of AR HCC cells, provoking apoptosis, inhibiting glycolysis and enhancing the ROS level. Similar results were also obtained in a mouse xenograft model, where tumor originated from AR HCC cells was significantly reduced in the BSO/3-BP-treated group compared with groups treated with 3-BP or sorafenib alone (82).

2-Methoxyestradiol. 2-Methoxyestradiol (2-ME) (Figure 3) is a natural estrogen derivative and metabolite of estradiol and 2-hydroxyestradiol. It is a very weak partial agonist for the estrogen receptor and as an anticancer drug, it inhibits complex I of the ETC. It also acts as an anti-angiogenic agent (83-85). Due to its low bioavailability and metabolism problems, it failed to succeed in clinical trials, so new analogs are under development (86).

It appeared that the establishment of oxidative stress is a vital part of the cytotoxic effects of 2-ME against chronic lymphocytic leukemia (CLL), a disease characterized by the aggregation of mainly inactive B-cells. CLL cells are already compromised by inherent oxidative stress which makes them even more sensitive to pro-oxidant agents. This study showed that treatment of CLL cells with 2-ME, which underwent effective cellular uptake, led to the accumulation of superoxide anion radicals by inhibiting the action of SOD, resulting in oxidative damage in mitochondrial membranes and activation of apoptotic pathways. This meant that the combination of 2-ME with other potent pro-oxidants may lead to even more cytotoxic and sensitizing results (87).

The bone marrow microenvironment is a key factor in the development and progression of another type of leukemia, acute myeloid leukemia (AML). Leukemia stem cells are hypoxic, which leads to the expression of hypoxia inducible factor 1α (HIF1α), an important prognostic factor for patients with AML. In a study by Zhe *et al*., 2-ME was tested *in vitro* as a candidate inhibitor of HIF1α for the treatment of AML. Suppression of HIF1α caused significant apoptosis of AML cells and 2-ME was superior to traditional chemotherapeutic drugs in this context. In addition, 2-ME was cytotoxic for AML patient-derived bone marrow stem cells, whereas little toxicity was reported against normal cells. The pro-oxidant activity of 2-ME led to the accumulation of free radicals and ROS within AML cells, which activated mitochondrial apoptotic pathways (88).

Rituximab. In the late 1980s, the idea of using monoclonal antibodies that recognize tumor-related antigens in order to treat hematological malignancies became a reality, and rituximab became a well-tolerated and highly effective option initially used for patients with lymphoproliferative disorders (89). Rituximab is an IgG1-κ chimeric mAb binding CD20 antigen on the membrane of B-lymphocytes (90). Since CD20 is also expressed in normal B cells, there was a strong rationale for using rituximab to eradicate abnormal antibodies in autoimmune diseases, such as rheumatoid arthritis in combination with methotrexate (91), as well as to treat cancer subtypes including non-Hodgkin's lymphoma (92) and CLL (93, 94).

CD40-stimulated CLL cells are generally chemoresistant. However, CD40 stimulation was shown to sensitize CLL cells to rituximab-induced cell death. This was a rapid effect (within hours of rituximab treatment) and was independent of caspase and p53 activation. On the contrary, rituximab-induced CLL apoptosis was found to be dependent on extracellular calcium concentration and ROS overproduction. Hence, by establishing oxidative stress conditions, stimulation by CD40 may sensitize CLL cells to rituximab (95).

Vitamin E. 'Vitamin E' refers to the group of all biologically active tocopherols, tocotrienols and their derivatives. These natural lipid-soluble compounds are found in a variety of foods and have a wide range of biological activities. The basic structure of vitamin E contains a polar chromanol head group with a long isoprenoid side chain. There are eight forms of vitamin E found naturally, namely α-, β-, γ- and δ-tocopherol, and α-, β-, γ-, and δ-tocotrienol (96) (Figure 4).

All forms of vitamin E are thought to have strong antioxidant activity because they have similar phenolic moieties and intercept lipid peroxyl radicals by donating hydrogen from their phenolic group to the chromanol ring. These peroxyl radicals are formed instantaneously during lipid peroxidation but vitamin E reacts with peroxyl radicals before they can attack polyunsaturated fatty acids. The product of this reaction is a tocopheroxyl radical that will be eventually reduced mainly by ascorbic acid (vitamin C). Forms of vitamin E with unsubstituted position 5, including γ-tocopherol, can also react with reactive nitrogen species, such as NO_2 or peroxynitrides, to form 5-nitro-γ-tocopherol (96, 97).

In addition to its well-documented antioxidant activity, vitamin E also has multiple therapeutic properties, including anticancer activity against breast, prostate and colon cancer (98).

In the case of prostate cancer, γ-tocopherol was found to suppress the production of protein kinase C and collagenase in *in vitro* experiments on the prostate cancer cell line DU-145. The results also showed a reduction in the production of cyclin D1 and cyclin E, therefore documenting an inhibitory effect on cell-cycle development, reducing progression into the S-phase. A similar effect was also observed for CaCo-2 colon adenocarcinoma cells, where DNA synthesis was inhibited as shown by 5-bromo-2'-deoxy-uridine incorporation assay. For both cancer subtypes, γ-tocopherol was more potent than α-tocopherol. Therefore, a risk reduction after consumption of high concentrations of γ-tocopherol was proposed for colon and prostate cancer (99).

Camptothecin is a potent anticancer agent tested against breast, ovarian, colon, lung and stomach cancer, although it is not water-soluble and has been associated with considerable side-effects (100). Another interesting study investigated the combined effect of vitamin E with camptothecin. HeLa cells were treated with different concentrations of camptothecin either as a monotherapy or in the presence of 100 μM vitamin E. The results obtained showed camptothecin induced DNA, protein and lipid damage, which was not inhibited in the presence of vitamin E. Hence, vitamin E did not interfere with camptothecin mechanism of anticancer activity (101). Additionally, this antioxidant supplementation proved beneficial in relieving the side-effects associated with camptothecin both *in vivo* (102-104) and *in vitro* (105), proposing a potential combination between chemotherapeutic and antioxidant agents as a means of eliminating adverse reactions of chemotherapy.

Vitamin C. Vitamin C (Figure 4) is a key dietary component, with many physiological roles linked to its ability to donate electrons. It is a powerful antioxidant and serves as a co-factor for a group of biosynthetic and gene regulatory enzymes. Vitamin C also contributes to immunological responses, supports the function of the epithelial barrier against pathogens and protects against environmental and endogenous oxidative stress. Especially for its immunomodulatory role, vitamin C accumulates in phagocytes, where it enhances chemotaxis, phagocytosis, ROS scavenging, and ultimately microbial killing [reviewed in (106)].

A study by Sant *et al*. communicated that a reduction in 5-hydroxymethylcytosine (5hmC) production was linked to malignant transformation in breast cancer cells. Given the co-factory role of vitamin C for ten-eleven translocation methylcytosine dioxygenase 1 (TET1), an important regulator of DNA demethylation and gene transcription, it was hypothesized that increased vitamin C levels would help increase 5hmC production by this enzyme. Firstly, it was shown that mRNA expression of sodium-dependent vitamin C transporter was reduced in both human breast cancer specimens and in breast cancer cell lines. Administration of low concentrations of vitamin C increased the production of 5hmC in three breast cancer cell lines and induced their death. This apoptotic effect of vitamin C was mediated by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a well-known pro-apoptotic agent. Vitamin C increased *TRAIL* transcription and translation more than twofold. The overexpression of TRAIL induced by vitamin C was largely eliminated by siRNA targeting TETs and an antibody to TRAIL, which inhibited apoptosis. Moreover, the pro-apoptotic effect of vitamin C was associated with BAX and caspase activation, BCL-xL binding, and cytochrome *c* release. Thus, vitamin C could be considered both as a preventative agent, and for the treatment of breast cancer (107).

At this point, it should be mentioned that vitamin C is one of the molecules that possesses a dual role, both as an antioxidant and as a pro-oxidant, depending on the cellular conditions. Given this, catalytic therapy is a type of cancer treatment based on the observation that certain antioxidants, such as vitamin C, upon co-administration with medicinal herbal extracts and a transition metal, most often copper or iron, can act in a pro-oxidant manner (108). This observation will also be discussed below for vitamins and dietary polyphenols, which follow a similar pattern. This dual role has been extensively researched and discussed in several works (109-112).

Vitamin D. Vitamin D (Figure 4) is an antioxidant agent that can regulate gene expression by binding to specific intracellular receptors. As an anticancer agent, it has been tested in the context of a variety of cancer subtypes including bladder (113), breast (114), colorectal (115), gastric (116), lung (117) and prostate (118) cancer among others.

In a pre-clinical study carried out in Hungary (118), vitamin D_3 supplementation (3,000-3,300 IU) was administered to 42 volunteers with prostate cancer grouped according to laboratory parameters and tumor markers, and its redox and metal homeostasis regulatory effect was explored. The trigger for this study was the increase in the recommended daily dose of vitamin D_3 from 200 IU to 2,000 IU in Hungary. Several essential and non-essential elements were quantified and the concentrations of Fe, Cr and Pb were found to be significantly increased in the erythrocytes of patients with prostate cancer after vitamin D_3 administration. Vitamin D_3 supplementation also had a beneficial regulatory role for the most important essential elements such as Ca, Cu, Mn, Mg and Ni, as their concentrations returned to normal ranges. However, Li deficiency enhanced by vitamin D_3 in patients with prostate cancer needs to be considered further. Therefore, vitamin D_3

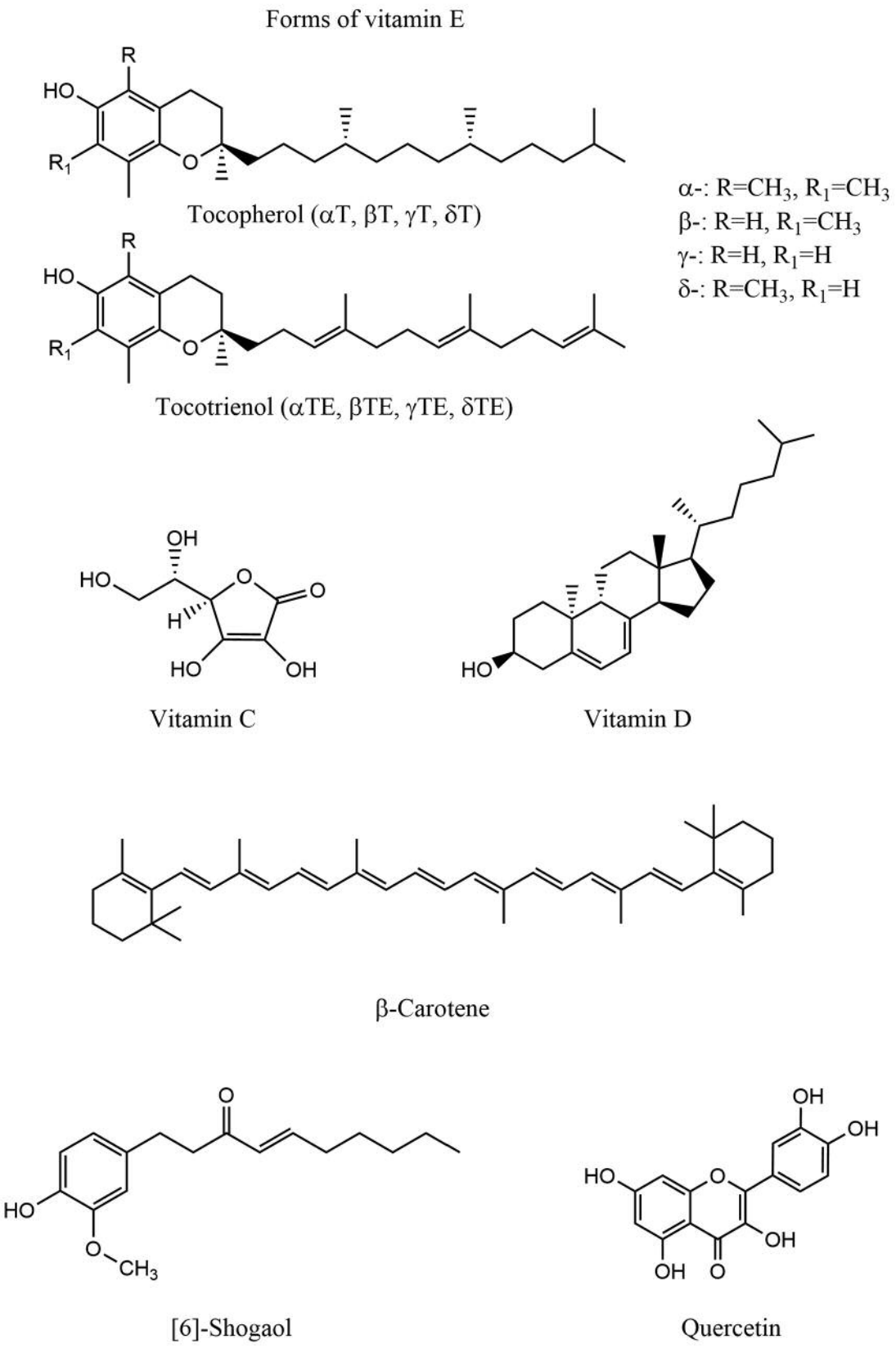

Figure 4. *Molecular structures of some antioxidant anticancer drugs*.

appears to help balance redox homeostasis, which could positively affect prostate cancer outcome (118).

In another study, cisplatin was combined with vitamin D against leukemia and colon cancer. A 3-day pretreatment of the human promyelocytic leukemia cell line HL-60 with calcitriol or new vitamin D_3 analogs significantly enhanced its *in vitro* susceptibility to cisplatin, doxorubicin and genistein. In addition, a synergistic antiproliferative effect with vitamin D was observed for the three cytotoxic agents explored, leading to a significant reduction in the inhibitory concentrations for each cytotoxic agent tested when combined with calcitriol or its analogs, compared to the respective monotherapy (48, 119).

β-Carotene and carotenoids. β-Carotene is a tetra-terpenoid consisting of two β-ionone rings, as shown in Figure 4. Along with lycopene, it is one of the most commonly consumed dietary carotenoids in humans, resulting in high concentrations in blood plasma. β-Carotene is the most important precursor of vitamin A, producing two molecules of vitamin A when broken down. β-Carotene supplementation enhances normal vision (120), growth and tissue differentiation (121) and helps reducing the risk of diseases such as esophageal cancer (122), although increased risk of lung cancer was associated with β-carotene supplementation (123), an effect not found in a long post-trial follow-up though (124).

Sowmya *et al*. attempted to evaluate the molecular mechanism for the anticancer activity of β-carotene isolated from *Spinacia oleracea* in MCF-7 human breast cancer cells tested in low concentrations, since high concentrations of β-carotene have been associated with a pro-oxidant and carcinogenic activity (125). β-Carotene led to the dose-dependent apoptosis of MCF-7 cells, which correlated well with their morphological changes. An increased caspase-3 activity was observed for these apoptotic cells. Protein expression quantification showed a decrease in the expression of the anti-apoptotic proteins BCL2 and poly (ADP-ribose) polymerase, as well as the survival protein NF-κB. It also hindered the activation of important cellular signaling proteins such as AKT serine/threonine kinase 1 and ERK1/2, inhibiting the subsequent activation of the downstream signaling pathway. In addition, β-carotene down-regulated the antioxidant enzyme *SOD2*, along with nuclear factor erythroid 2-like 2 and X-box binding protein 1, an ER stress marker. Therefore, the observed anticancer mechanism was correlated with the antioxidant properties of this carotenoid, which at low physiological concentrations predominantly exerts these activities instead of having a pro-oxidant cancer-inducing role (125).

From this discussion, dietary antioxidants that act through pro-oxidant anticancer mechanisms cannot be omitted. Even though these compounds are not currently prescribed to patients with cancer as part of standard chemotherapeutic regimens, they have shown excellent anticancer properties with mechanisms quite distinct from the abovementioned antioxidant vitamins. So even though they are mainly phenolic compounds with reported antioxidant properties, in the case of cancer, they have shown a pro-oxidant potential, which eventually leads to apoptotic results, similar to the case of ascorbic acid in catalytic therapy mentioned above (126-129). Therefore, a few exemplary studies of dietary phenols will be discussed here, with the aim of highlighting the potential of these compounds to act also through the generation of ROS, which is basically opposite to their expected antioxidant effect. This is not uncommon for antioxidant compounds, where their antioxidant or pro-oxidant behavior heavily depends on the surrounding environment or the type of cells tested. Especially in the presence of transition metals such as Fe or Cu, phenolic compounds may act as pro-oxidants through Fenton and related reactions, and this was observed not only for [6]-shogaol, quercetin and flavonoids discussed below, but also for other dietary phenols such as curcumin, resveratrol and carnosol, among others (130-133).

[6]-Shogaol. [6]-Shogaol (Figure 4) is the main bioactive ingredient in the popular food spice ginger (*Zingiber officinale*). It has been attributed antioxidant, antiproliferative and anticancer activities (134). The interesting part of its mechanism of anticancer activity is that although it is a phenolic compound with good antioxidant properties, among other mechanisms, its cytotoxicity is linked to the elevated production of ROS and the implication of oxidative stress and oxidative damage (135, 136).

Similar studies by Pan *et al*. (137) and Annamalai *et al*. (138) suggested that [6]-shogaol induced cancer cell apoptosis through a pro-oxidant mechanism. In the first study (137), COLO 205 colon cancer cells were shown to be inhibited by this compound, which provoked mitochondrial changes with subsequent cytochrome *c* release, caspase pathway activation and DNA damage. ROS overproduction preceded these apoptotic results. Several pro-apoptotic proteins were found upregulated, such as BAX, apoptosis antigen 1 (FAS) and gene product of growth arrest and DNA damage-inducible gene 153 (GADD153), whereas other anti-apoptotic counterparts were found to be down-regulated, such as BCL2 and BCL-xL. The well-known antioxidant NAC was able to reverse cancer cell apoptosis, but this was not observed with other antioxidants.

In the second study (138), [6]-shogaol was tested in Hep-2 cells. Cell viability assays showed a dose-dependent cytotoxic effect of [6]-shogaol, which was well-correlated with a dose-dependent pro-oxidant activity through elevated ROS production. Other pro-oxidant observations included increased levels of thiobarbituric acid reactive substances (a lipid

peroxidation biomarker) and reduced levels of antioxidant systems such as SOD, GSH and catalase. Moreover, mitochondrial dysfunction, morphological changes in Hep2 cells and nuclear damage were implicated in the anticancer mechanism of this compound. Similar to previous results for COLO 205 cells (137), caspases-3 and -9, BAX and cytochrome *c* were found to be up-regulated in [6]-shogaol-treated Hep2 cells, following a dose-dependent pattern (138). Other similar pro-oxidant and cytotoxic activities for [6]-shogaol were also reported for other cancer subtypes, such as gastric, ovarian, lung and breast cancer (139-142).

Quercetin. The oxidative capacity of quercetin (Figure 4), as one of the main polyphenols present in extracts of *Gingko biloba,* was studied by Babich *et al*. (143), where the aim was to prove the pro-oxidant anticancer mechanism of this compound also described in other studies (126, 144). Oral carcinoma HSC-2 cells were found to be inhibited by *G. biloba* leaf extracts in a dose-, pH- and time-dependent manner. Cell culture medium enriched with antioxidant scavengers such as catalase, SOD, pyruvate, NAC or divalent cobalt interfered with this pro-oxidant mechanism, and lower concentrations of ROS (H_2O_2 and $O_2^{\cdot -}$) were evident in these cells, which were not apoptotic. Moreover, depleted levels of GSH were reported for HSC-2 cells treated with this extract, potentiating the cytotoxic effect of elevated ROS production, since GSH is a well-reported cellular antioxidant molecule. Further to this observation, co-administration with GSH depleters such as 1-chloro-2,4-dinitrobenzene, BSO and 1,3-*bis*[2-chloroethyl]-1-nitrosourea further enhanced the cytotoxic result of *G. biloba* extract, highlighting the pro-oxidant mechanism underlying its anticancer properties. In conclusion, the apoptosis of HSC-2 cells was confirmed by different assays, as well as by the observation of apoptotic morphological changes in cells treated with *G. biloba* leaf extract. This apoptosis was well-associated with the overproduction of ROS caused in these cells, which was abrogated by antioxidant scavengers and enhanced by GSH depleters (143).

Another recent study found a synergistic anticancer effect of quercetin with maleic anhydride derivatives against liver cancer. Maleic anhydride derivatives have shown potent anticancer effects, being pro-oxidants themselves (145). The pretreatment of HepG2 cells with two novel maleic anhydride derivatives (C1 and C2) followed by quercetin treatment resulted in reduced GSH level, also reducing the ratio of reduced/oxidized GSH (GSH/GSSG index). Treatment of HuH7 and HepG2 with either maleic anhydride derivative alone or each separately combined with quercetin led to increased ROS production, as well as depletion of GSH. Another interesting result of this study was that the timing of quercetin treatment had a significant impact on the observed result, since co-administration of the agents, and pretreatment with quercetin first resulted in opposite observations. When administered alone, quercetin mainly exhibited antioxidant effects, even though mitochondrial apoptosis was also induced (146).

Finally, the pro-oxidant properties of quercetin were validated in another study, where gold nanoparticles loaded with quercetin were found to induce ROS production, cause cell-cycle arrest in the sub-G phase, activate mitochondrial apoptosis and lead to p53 down-regulation (147).

Other flavonoids. Dietary flavonoids also belong to the group of potential anticancer agents with a dual mechanism of action. Their pro-oxidant activity was evident in lung (A549), myeloid (HL-60) and prostate (PC-3) cancer cells, where GSH levels were found to be significantly reduced, especially in mitochondria. Hydroxychalcone and dihydroxychalcone flavonoids were the most potent in reducing GSH expression in lung and myeloid cancer cells by half, at a low concentration (25 μM). On the other hand, chrysin and apigenin were the most potent GSH depleters in prostate cancer cells. These flavonoids were also tested for their potential to act synergistically with other pro-oxidants, such as etoposide and 2-ME, where most of them were reported to potentiate the overall cytotoxicity effect. Moreover, some flavonoids also showed mitochondrial dysfunction and cytochrome *c*-releasing properties. Finally, several flavonoids were reported as potent inducers of GSH efflux, probably mediated by MRPs (148).

The pro-oxidant anticancer activity of flavonoids was also found in other studies. In one study 5, 7-dimethoxyflavone was found to reduce the viability of HepG2 cancer cells through elevated ROS production and subsequent sub-G_1 cell-cycle arrest in a concentration-dependent manner (149). In a similar study, catechin, epicatechin and naringenin in particular, were shown to inhibit colon cancer *via* increased ROS production leading to caspase activation, decreased protein kinase C action, G_2/M cell-cycle arrest and autophagy in a ROS-dependent way (150). Therefore, flavonoids are continuously being evaluated as potential pro-oxidant anticancer agents.

Other dietary polyphenols. The anticancer properties of dietary polyphenols are now well-documented in different cancer subtypes (151-155). The involvement of a ROS-mediated anticancer mechanism was also evident in numerous studies, such as for caffeic acid against fibrosarcoma or for apigenin against colorectal adenocarcinoma (156, 157). In more detail, in two studies by the group of Hadi *et al*. (158, 159), plant polyphenols such as genistein, luteolin, apigenin and resveratrol were tested for their pro-oxidant anticancer properties in relation to copper concentration, which is generally elevated in cancer cells (160, 161). In the first study, endogenous copper mobilization resulting in the overproduction of ROS was observed, followed by oxidative

DNA damage and apoptosis (159). In the second study, apigenin, luteolin, epigallocatechin-3-gallate and resveratrol were tested for their anticancer and pro-oxidant properties. Apigenin and luteolin were potent in inhibiting breast, pancreatic and prostate cancer cell growth and provoking apoptosis in a concentration-dependent manner. ROS scavengers such as catalase, SOD and thiourea abrogated this effect, suggesting that this ROS overproduction was indeed important in the anticancer effect observed. The other two polyphenols, epigallocatechin-3-gallate and resveratrol, were also reported to inhibit prostate cancer cell growth. The interference of copper was shown *via* the use of neocuproine, a copper-specific chelator, whereas zinc and iron chelators did not show similar results. Again, for these compounds, ROS scavengers eliminated the anticancer effects. Another important finding was that normal breast epithelial cells (MCF-10A) failed to undergo growth inhibition by these compounds, an observation reversed when copper enrichment of the culture media was tested (158). Therefore, a pro-oxidant copper-involving mechanism was proposed for plant polyphenols, further strengthening the hypothesis that indeed antioxidant compounds may also present pro-oxidant anticancer effects, as also suggested in other studies by the same group (162).

A short outline of the above-mentioned anticancer agents and their mechanisms of action according to the articles discussed here are presented in Table I.

Discussion

Cancer cells have been shown to have elevated ROS levels mainly due to hypermetabolism. The augmented ROS concentration and the alteration of redox balance lead to the establishment of redox signaling, which is partly responsible for the further progression of cancer. It is now widely accepted that the altered redox balance enables cancer cells to cope with the elevated levels of ROS present, which makes them dependent on certain antioxidant mechanisms. There are several mechanisms promoting ROS overexpression such as hypermetabolism, altered mitochondrial function, overactivated signaling and increased enzymatic activity. Therefore, there is rationale for targeting this distinct characteristic of cancer cells and dysregulate it even further in order to promote apoptosis, since when produced in large excess, ROS can endanger even cancer cell viability.

In general, anticancer drugs that work by affecting free radicals provide positive expectations in the prevention and treatment of cancer. Anticancer pro-oxidants work by increasing the production of free radicals and ROS thereby disrupting the redox balance of cancer cells, resulting in apoptosis. According to their mechanism of action, they may bind lipids, proteins and DNA and induce the production of free radicals or they can activate signaling pathways involving protein kinases and thus initiate an apoptotic cascade. Pro-oxidant anticancer drugs can also target the mitochondria, as they are the main organelles responsible for the formation of ROS, and can alter mitochondrial membrane permeability or disrupt oxidative phosphorylation and lead to leakage of ROS causing further mitochondrial oxidative stress. Moreover, pro-oxidant anticancer drugs can down-regulate genes associated with cancer cell survival, for example genes for antioxidant enzymes, such as SOD, or for small non-protein antioxidant biomolecules, such as GSH. This depletion of intracellular antioxidants can cause apoptosis of cancer cells.

On the other hand, the role of anticancer antioxidants is also under investigation, since they can bind free radicals and prevent oxidative stress, thus helping to balance the altered redox homeostasis found in cancer cells. In addition, some known antioxidants may also act by enhancing ROS production, when in the presence of transition metals that help initiate redox reactions. These antioxidants, based on their investigated behavior as anticancer drugs, can be administered as monotherapies or even prophylactically, but they may also be combined with pro-oxidant anticancer agents, in order to mitigate the adverse reactions of pro-oxidants, or synergize with them for more effective anticancer regimens.

These redox imbalances are exploited by pro-oxidant anticancer drugs, such as cisplatin, doxorubicin, imexon, MGd, BSO, 2-ME and rituximab, which increase ROS and thus lead to excessive oxidative stress and cancer apoptosis. Exogenous antioxidants, such as vitamin E, vitamin C, vitamin D, B-carotene, [6]-shogaol, flavonoids and dietary polyphenols, can also play an important role in the treatment of cancer, sometimes by slowing down or inhibiting oxidation by binding free radicals and reducing oxidative stress or other times abrogating ROS overproduction and acting pro-oxidatively. In all these cases, tumor cells appear to have an even more disrupted redox balance relative to untreated cancer cells, and this demonstrates how the manipulation of free radicals and ROS may be a potential target for new cancer therapies.

Conclusion

In conclusion, free radicals and ROS are components of cellular signaling pathways and play an important role not only in normal cell physiology but also in the pathophysiology of certain diseases, such as cancer. In the early stages of cancer, redox signaling is enabled for further progression, but later the increased ROS level comprises a favorable target for promoting apoptosis through an oxidative mechanism. Therefore, antioxidant supplementation may have better results when administered as a preventive measure against the initiation of cancer or in early stages in order to re-program the disrupted redox balance present in cancer cells and eliminate potential side-effects from other anticancer agents, whereas pro-oxidant chemotherapeutics should be

Table I. *Chemotherapeutic agents that manipulate ROS production and their mechanisms of action based on the studies used.*

Drug	Main mechanism of action	ROS-related mechanism of action	Cancer subtype	Reference
Cisplatin	DNA damage *via* binding with purines and interference with DNA-repair mechanisms	Free radical and ROS production	Colon, melanoma	(49)
			Laryngeal	(50)
			Testicular germ	(51)
			Gastric	(56)
Doxorubicin	Inhibition of DNA biosynthesis and topoisomerase II for replication and transcription	Free radical and ROS production	Breast	(60)
			Adult T-cell leukemia/ lymphoma	(63)
Imexon	Binding to thiol groups *e.g.,* GSH and reduction of mitochondrial membrane potential	Opening of the aziridine ring and induction of oxidative stress	Multiple myeloma	(67, 68)
			Pancreatic	(69)
			Multiple myeloma	(64)
Motexafin Gadolinium	Inhibitor of TRXR1 and ribonucleotide reductase and oxidation of endogenous reducing molecules *e.g.*, GSH, ascorbic acid, $NADP^+$	Free radical and ROS production through futile redox cycling	Multiple myeloma	(76)
			Breast	(78)
Buthionine sulfoximine	Selective irreversible glutamate-cysteine ligase inhibition and GSH depletion	Redox imbalance *via* ROS production	Lung, ovarian, neuroblastoma	(81)
			Hepatocellular	(82)
2-Methoxyestradiol	Partial agonist of estrogen receptor and inhibition of ETC complex I	Free radical and ROS production	Chronic lymphocytic leukemia	(87)
			Acute myeloid leukemia	(88)
Rituximab	Chimeric mAb binding to CD20 of B-lymphocyte membrane	Free radical and ROS production, sensitization of CD40-stimulated CLL cells	Chronic lymphocytic leukemia	(95)
Vitamin E	Termination of lipid peroxidation	Reaction with peroxyl radical and RNS	Prostate, colon	(99)
			Cervical	(101)
Vitamin C	Co-factor of biosynthetic and gene regulatory enzymes, support of immunological responses	ROS scavenging	Breast	(107)
Vitamin D	Binding to intracellular receptors and regulation of gene expression	Balance redox homeostasis	Prostate	(118)
			Promyelocytic leukemia, colon	(119)
β-Carotene and Carotenoids	Precursor of vitamin A	Antioxidant activity	Breast	(125)
[6]-Shogaol	Reduction of endogenous antioxidants *e.g.,* SOD, GSH, CAT	ROS production	Colon	(137)
			Laryngeal	(138)
Quercetin	Depletion of GSH	ROS production	Oral	(143)
			Hepatocarcinoma	(146)
			Hepatocarcinoma	(147)
Other flavonoids	Depletion of GSH	Pro-oxidant activity	Lung, myeloid, prostate	(148)
	Sub-G_1 cell-cycle arrest	ROS production	Hepatocarcinoma	(149)
	Increased caspase and decreased PKC activation, decreased CAM expression, G_2/M cell-cycle arrest, autophagy	ROS production	Colon	(150)
Other dietary polyphenols	Endogenous copper mobilization, oxidative DNA damage and apoptosis	Copper-related overproduction of ROS	Lymphocytic	(159)
			Breast, pancreatic, prostate	(158)

CAM: Cell adhesion molecules; CAT: catalase; CLL: chronic lymphocytic leukemia; ETC electron transport chain; GSH: glutathione; mAb: monoclonal antibody; $NADP^+$: nicotinamide adenine dinucleotide phosphate; PKC: protein kinase C; RNS: reactive nitrogen species; ROS: reactive oxygen species; SOD: superoxide dismutase; TRXR1: thioredoxin reductase 1.

considered in later stages in order to further increase ROS production and push cells towards oxidative damage, DNA breakage and apoptosis. Finally, some antioxidant anticancer drugs may also act pro-oxidatively when oxidative stress is already present in cancer cells, hence their use can also be considered in later stages of cancer.

Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

Both Authors contributed equally to the writing of this review article.

References

1 Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J: Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol *39(1)*: 44-84, 2007. PMID: 16978905. DOI: 10.1016/j.biocel.2006.07.001

2 Valko M, Morris H and Cronin MT: Metals, toxicity and oxidative stress. Curr Med Chem *12(10)*: 1161-1208, 2005. PMID: 15892631. DOI: 10.2174/0929867053764635

3 Li R, Jia Z and Trush MA: Defining ROS in Biology and Medicine. React Oxyg Species (Apex) *1(1)*: 9-21, 2016. PMID: 29707643. DOI: 10.20455/ros.2016.803

4 Phaniendra A, Jestadi DB and Periyasamy L: Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem *30(1)*: 11-26, 2015. PMID: 25646037. DOI: 10.1007/s12291-014-0446-0

5 Lushchak VI: Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact *224*: 164-175, 2014. PMID: 25452175. DOI: 10.1016/j.cbi.2014.10.016

6 Athreya K and Xavier MF: Antioxidants in the treatment of cancer. Nutr Cancer *69(8)*: 1099-1104, 2017. PMID: 29043851. DOI: 10.1080/01635581.2017.1362445

7 Bedard K and Krause KH: The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev *87(1)*: 245-313, 2007. PMID: 17237347. DOI: 10.1152/physrev.00044.2005

8 Liu Y, Fiskum G and Schubert D: Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem *80(5)*: 780-787, 2002. PMID: 11948241. DOI: 10.1046/j.0022-3042.2002.00744.x

9 Anraku Y: Bacterial electron transport chains. Annu Rev Biochem *57*: 101-132, 1988. PMID: 3052268. DOI: 10.1146/annurev.bi.57.070188.000533

10 Sahoo S, Meijles DN and Pagano PJ: NADPH oxidases: key modulators in aging and age-related cardiovascular diseases? Clin Sci (Lond) *130(5)*: 317-335, 2016. PMID: 26814203. DOI: 10.1042/CS20150087

11 Slauch JM: How does the oxidative burst of macrophages kill bacteria? Still an open question. Mol Microbiol *80(3)*: 580-583, 2011. PMID: 21375590. DOI: 10.1111/j.1365-2958.2011.07612.x

12 Kim SJ, Kim HS and Seo YR: Understanding of ROS-inducing strategy in anticancer therapy. Oxid Med Cell Longev *2019*: 5381692, 2019. PMID: 31929855. DOI: 10.1155/2019/5381692

13 Pisoschi AM and Pop A: The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem *97*: 55-74, 2015. PMID: 25942353. DOI: 10.1016/j.ejmech.2015.04.040

14 Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M: Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact *160(1)*: 1-40, 2006. PMID: 16430879. DOI: 10.1016/j.cbi.2005.12.009

15 DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, Scrimieri F, Winter JM, Hruban RH, Iacobuzio-Donahue C, Kern SE, Blair IA and Tuveson DA: Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature *475(7354)*: 106-109, 2011. PMID: 21734707. DOI: 10.1038/nature10189

16 Kumari S, Badana AK, G MM, G S and Malla R: Reactive oxygen species: A key constituent in cancer survival. Biomark Insights *13*: 1177271918755391, 2018. PMID: 29449774. DOI: 10.1177/1177271918755391

17 Ma Q: Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol *53*: 401-426, 2013. PMID: 23294312. DOI: 10.1146/annurev-pharmtox-011112-140320

18 Ray PD, Huang BW and Tsuji Y: Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal *24(5)*: 981-990, 2012. PMID: 22286106. DOI: 10.1016/j.cellsig.2012.01.008

19 Dayem AA, Choi HY, Kim JH and Cho SG: Role of oxidative stress in stem, cancer, and cancer stem cells. Cancers (Basel) *2(2)*: 859-884, 2010. PMID: 24281098. DOI: 10.3390/cancers2020859

20 Gupta SC, Kim JH, Prasad S and Aggarwal BB: Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev *29(3)*: 405-434, 2010. PMID: 20737283. DOI: 10.1007/s10555-010-9235-2

21 Modell H, Cliff W, Michael J, McFarland J, Wenderoth MP and Wright A: A physiologist's view of homeostasis. Adv Physiol Educ *39(4)*: 259-266, 2015. PMID: 26628646. DOI: 10.1152/advan.00107.2015

22 Angeloni C, Maraldi T and Vauzour D: Redox signaling in degenerative diseases: from molecular mechanisms to health implications. Biomed Res Int *2014*: 245761, 2014. PMID: 25057480. DOI: 10.1155/2014/245761

23 Iorio F, Knijnenburg TA, Vis DJ, Bignell GR, Menden MP, Schubert M, Aben N, Gonçalves E, Barthorpe S, Lightfoot H, Cokelaer T, Greninger P, van Dyk E, Chang H, de Silva H, Heyn H, Deng X, Egan RK, Liu Q, Mironenko T, Mitropoulos X, Richardson L, Wang J, Zhang T, Moran S, Sayols S, Soleimani M, Tamborero D, Lopez-Bigas N, Ross-Macdonald P, Esteller M, Gray NS, Haber DA, Stratton MR, Benes CH, Wessels LFA, Saez-Rodriguez J, McDermott U and Garnett MJ: A landscape of pharmacogenomic interactions in cancer. Cell *166(3)*: 740-754, 2016. PMID: 27397505. DOI: 10.1016/j.cell.2016.06.017

24 Gurer-Orhan H, Ince E, Konyar D, Saso L and Suzen S: The role of oxidative stress modulators in breast cancer. Curr Med Chem *25(33)*: 4084-4101, 2018. PMID: 28699501. DOI: 10.2174/0929867324666170711114336

25 Galadari S, Rahman A, Pallichankandy S and Thayyullathil F: Reactive oxygen species and cancer paradox: To promote or to suppress? Free Radic Biol Med *104*: 144-164, 2017. PMID: 28088622. DOI: 10.1016/j.freeradbiomed.2017.01.004

26 Assi M: The differential role of reactive oxygen species in early and late stages of cancer. Am J Physiol Regul Integr Comp Physiol *313(6)*: R646-R653, 2017. PMID: 28835450. DOI: 10.1152/ajpregu.00247.2017

27 Panieri E and Santoro MM: ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell Death Dis *7(6)*: e2253, 2016. PMID: 27277675. DOI: 10.1038/cddis.2016.105

28 Morry J, Ngamcherdtrakul W and Yantasee W: Oxidative stress in cancer and fibrosis: Opportunity for therapeutic intervention with antioxidant compounds, enzymes, and nanoparticles. Redox Biol *11*: 240-253, 2017. PMID: 28012439. DOI: 10.1016/j.redox.2016.12.011

29 Nisha K and Deshwal RK: Antioxidants and their protective action against DNA damage. Int J Pharm Pharm Sci *3*: 28-32, 2011.

30 Firczuk M, Bajor M, Graczyk-Jarzynka A, Fidyt K, Goral A and Zagozdzon R: Harnessing altered oxidative metabolism in cancer by augmented prooxidant therapy. Cancer Lett *471*: 1-11, 2020. PMID: 31811907. DOI: 10.1016/j.canlet.2019.11.037

31 Cheng K, Sano M, Jenkins CH, Zhang G, Vernekohl D, Zhao W, Wei C, Zhang Y, Zhang Z, Liu Y, Cheng Z and Xing L:

Synergistically enhancing the therapeutic effect of radiation therapy with radiation activatable and reactive oxygen species-releasing nanostructures. ACS Nano *12(5)*: 4946-4958, 2018. PMID: 29689158. DOI: 10.1021/acsnano.8b02038

32 Leach JK, Van Tuyle G, Lin PS, Schmidt-Ullrich R and Mikkelsen RB: Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res *61(10)*: 3894-3901, 2001. PMID: 11358802.

33 Martin-Cordero C, Leon-Gonzalez AJ, Calderon-Montano JM, Burgos-Moron E and Lopez-Lazaro M: Pro-oxidant natural products as anticancer agents. Curr Drug Targets *13(8)*: 1006-1028, 2012. PMID: 22594470. DOI: 10.2174/138945012802009044

34 Watson J: Oxidants, antioxidants and the current incurability of metastatic cancers. Open Biol *3(1)*: 120144, 2013. PMID: 23303309. DOI: 10.1098/rsob.120144

35 Ozben T: Antioxidant supplementation on cancer risk and during cancer therapy: an update. Curr Top Med Chem *15(2)*: 170-178, 2015. PMID: 25496272.

36 Kurutas EB: The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J *15(1)*: 71, 2016. PMID: 27456681. DOI: 10.1186/s12937-016-0186-5

37 Chahal A, Saini A, Chhillar A and Saini R: Natural antioxidants as defense system against cancer. Asian Journal of Pharmaceutical and Clinical Research *11(5)*: 38, 2018. DOI: 10.22159/ajpcr.2018.v11i5.24119

38 Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D and Bitto A: Oxidative stress: Harms and benefits for human health. Oxid Med Cell Longev *2017*: 8416763, 2017. PMID: 28819546. DOI: 10.1155/2017/8416763

39 Antoliková N, Talian I, Bober P, Tomková Z, Chripková M and Sabo J: The controversial effect of vitamin c combined with traditional oncology therapy. Int J Pharm Sci Rev Res *37(2)*: 22-26, 2016.

40 Giorgio M: Oxidative stress and the unfulfilled promises of antioxidant agents. Ecancermedicalscience *9*: 556, 2015. PMID: 26284120. DOI: 10.3332/ecancer.2015.556

41 Sotler R, Poljšak B, Dahmane R, Jukić T, Pavan Jukić D, Rotim C, Trebše P and Starc A: Prooxidant activities of antioxidants and their impact on health. Acta Clin Croat *58(4)*: 726-736, 2019. PMID: 32595258. DOI: 10.20471/acc.2019.58.04.20

42 Amable L: Cisplatin resistance and opportunities for precision medicine. Pharmacol Res *106*: 27-36, 2016. PMID: 26804248. DOI: 10.1016/j.phrs.2016.01.001

43 Yao X, Panichpisal K, Kurtzman N and Nugent K: Cisplatin nephrotoxicity: a review. Am J Med Sci *334(2)*: 115-124, 2007. PMID: 17700201. DOI: 10.1097/MAJ.0b013e31812dfe1e

44 Guthrie TH Jr and Gynther L: Acute deafness. A complication of high-dose cisplatin. Arch Otolaryngol *111(5)*: 344-345, 1985. PMID: 4039131. DOI: 10.1001/archotol.1985.00800070096018

45 Kurian A, Babu B, Punnoose B, Chacko C, Rao M, Chand DS, Bc V and P N: Cisplatin-induced peripheral neuropathy: An observational descriptive study. Int J Res Pharm Sci *11(3)*: 3585-3589, 2020. DOI: 10.26452/ijrps.v11i3.2515

46 A A Aly H and G Eid B: Cisplatin induced testicular damage through mitochondria mediated apoptosis, inflammation and oxidative stress in rats: impact of resveratrol. Endocr J *67(9)*: 969-980, 2020. PMID: 32507773. DOI: 10.1507/endocrj.EJ20-0149

47 Pabla N and Dong Z: Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int *73(9)*: 994-1007, 2008. PMID: 18272962. DOI: 10.1038/sj.ki.5002786

48 Dasari S and Tchounwou PB: Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol *740*: 364-378, 2014. PMID: 25058905. DOI: 10.1016/j.ejphar.2014.07.025

49 Berndtsson M, Hägg M, Panaretakis T, Havelka AM, Shoshan MC and Linder S: Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA. Int J Cancer *120(1)*: 175-180, 2007. PMID: 17044026. DOI: 10.1002/ijc.22132

50 Brozovic A, Majhen D, Roje V, Mikac N, Jakopec S, Fritz G, Osmak M and Ambriovic-Ristov A: alpha(v)beta(3) Integrin-mediated drug resistance in human laryngeal carcinoma cells is caused by glutathione-dependent elimination of drug-induced reactive oxidative species. Mol Pharmacol *74(1)*: 298-306, 2008. PMID: 18441044. DOI: 10.1124/mol.107.043836

51 Schweyer S, Soruri A, Heintze A, Radzun HJ and Fayyazi A: The role of reactive oxygen species in cisplatin-induced apoptosis in human malignant testicular germ cell lines. Int J Oncol *25(6)*: 1671-1676, 2004. PMID: 15547704. DOI: 10.3892/ijo.25.6.1671

52 Custódio JB, Cardoso CM, Santos MS, Almeida LM, Vicente JA and Fernandes MA: Cisplatin impairs rat liver mitochondrial functions by inducing changes on membrane ion permeability: prevention by thiol group protecting agents. Toxicology *259(1-2)*: 18-24, 2009. PMID: 19428939. DOI: 10.1016/j.tox.2009.01.022

53 Ardizzoni A, Rosso R, Salvati F, Fusco V, Cinquegrana A, De Palma M, Serrano J, Pennucci MC, Soresi E and Crippa M: Activity of doxorubicin and cisplatin combination chemotherapy in patients with diffuse malignant pleural mesothelioma. An Italian Lung Cancer Task Force (FONICAP) Phase II study. Cancer *67(12)*: 2984-2987, 1991. PMID: 2044044. DOI: 10.1002/1097-0142(19910615)67:12<2984::aid-cncr2820671208>3.0.co;2-q

54 Dreyfuss AI, Clark JR, Fallon BG, Posner MR, Norris CM Jr and Miller D: Cyclophosphamide, doxorubicin, and cisplatin combination chemotherapy for advanced carcinomas of salivary gland origin. Cancer *60(12)*: 2869-2872, 1987. PMID: 2824016. DOI: 10.1002/1097-0142(19871215)60:12<2869::aid-cncr2820601203>3.0.co;2-y

55 Pinto-Leite R, Arantes-Rodrigues R, Palmeira C, Colaço B, Lopes C, Colaço A, Costa C, da Silva VM, Oliveira P and Santos L: Everolimus combined with cisplatin has a potential role in treatment of urothelial bladder cancer. Biomed Pharmacother *67(2)*: 116-121, 2013. PMID: 23433853. DOI: 10.1016/j.biopha.2012.11.007

56 He W, Xia Y, Cao P, Hong L, Zhang T, Shen X, Zheng P, Shen H, Liang G and Zou P: Curcuminoid WZ35 synergize with cisplatin by inducing ROS production and inhibiting TrxR1 activity in gastric cancer cells. J Exp Clin Cancer Res *38(1)*: 207, 2019. PMID: 31113439. DOI: 10.1186/s13046-019-1215-y

57 Kalender Y, Yel M and Kalender S: Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin. Toxicology *209(1)*: 39-45, 2005. PMID: 15725512. DOI: 10.1016/j.tox.2004.12.003

58 Rivankar S: An overview of doxorubicin formulations in cancer therapy. J Cancer Res Ther *10(4)*: 853-858, 2014. PMID: 25579518. DOI: 10.4103/0973-1482.139267

59 Tacar O, Sriamornsak P and Dass CR: Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol *65(2)*: 157-170, 2013. PMID: 23278683. DOI: 10.1111/j.2042-7158.2012.01567.x

60 Pilco-Ferreto N and Calaf GM: Influence of doxorubicin on apoptosis and oxidative stress in breast cancer cell lines. Int J Oncol *49(2)*: 753-762, 2016. PMID: 27278553. DOI: 10.3892/ijo.2016.3558

61 Hamdy R, Ziedan NI, Ali S, Bordoni C, El-Sadek M, Lashin E, Brancale A, Jones AT and Westwell AD: Synthesis and evaluation of 5-(1H-indol-3-yl)-N-aryl-1,3,4-oxadiazol-2-amines as Bcl-2 inhibitory anticancer agents. Bioorg Med Chem Lett *27(4)*: 1037-1040, 2017. PMID: 28087272. DOI: 10.1016/j.bmcl.2016.12.061

62 Ziedan NI, Hamdy R, Cavaliere A, Kourti M, Prencipe F, Brancale A, Jones AT and Westwell AD: Virtual screening, SAR, and discovery of 5-(indole-3-yl)-2-[(2-nitrophenyl)amino] [1,3,4]-oxadiazole as a novel Bcl-2 inhibitor. Chem Biol Drug Des *90(1)*: 147-155, 2017. PMID: 28067996. DOI: 10.1111/cbdd.12936

63 Fatfat M, Fakhoury I, Habli Z, Mismar R and Gali-Muhtasib H: Thymoquinone enhances the anticancer activity of doxorubicin against adult T-cell leukemia *in vitro* and *in vivo* through ROS-dependent mechanisms. Life Sci *232*: 116628, 2019. PMID: 31278946. DOI: 10.1016/j.lfs.2019.116628

64 Evens AM, Prachand S, Shi B, Paniaqua M, Gordon LI and Gartenhaus RB: Imexon-induced apoptosis in multiple myeloma tumor cells is caspase-8 dependent. Clin Cancer Res *10(4)*: 1481-1491, 2004. PMID: 14977852. DOI: 10.1158/1078-0432.ccr-1058-03

65 Kuehl PJ, Hoye WL and Myrdal PB: Preformulation studies on imexon. Drug Dev Ind Pharm *32(6)*: 687-697, 2006. PMID: 16885124. DOI: 10.1080/03639040600599830

66 Moulder S, Dhillon N, Ng C, Hong D, Wheler J, Naing A, Tse S, La Paglia A, Dorr R, Hersh E, Boytim M and Kurzrock R: A phase I trial of imexon, a pro-oxidant, in combination with docetaxel for the treatment of patients with advanced breast, non-small cell lung and prostate cancer. Invest New Drugs *28(5)*: 634-640, 2010. PMID: 19499186. DOI: 10.1007/s10637-009-9273-1

67 Dvorakova K, Payne CM, Tome ME, Briehl MM, Vasquez MA, Waltmire CN, Coon A and Dorr RT: Molecular and cellular characterization of imexon-resistant RPMI8226/I myeloma cells. Mol Cancer Ther *1(3)*: 185-195, 2002. PMID: 12467213.

68 Dvorakova K, Waltmire CN, Payne CM, Tome ME, Briehl MM and Dorr RT: Induction of mitochondrial changes in myeloma cells by imexon. Blood *97(11)*: 3544-3551, 2001. PMID: 11369649. DOI: 10.1182/blood.v97.11.3544

69 Sheveleva EV, Landowski TH, Samulitis BK, Bartholomeusz G, Powis G and Dorr RT: Imexon induces an oxidative endoplasmic reticulum stress response in pancreatic cancer cells. Mol Cancer Res *10(3)*: 392-400, 2012. PMID: 22275514. DOI: 10.1158/1541-7786.MCR-11-0359

70 Malani A, Bembom O and van der Laan M: Accounting for heterogeneous treatment effects in the FDA approval process. Food Drug Law J *67(1)*: 23-50, i, 2012. PMID: 24624647.

71 Richards GM and Mehta MP: Motexafin gadolinium in the treatment of brain metastases. Expert Opin Pharmacother *8(3)*: 351-359, 2007. PMID: 17266469. DOI: 10.1517/14656566.8.3.351

72 Sessler JL and Miller RA: Texaphyrins: new drugs with diverse clinical applications in radiation and photodynamic therapy. Biochem Pharmacol *59(7)*: 733-739, 2000. PMID: 10718331. DOI: 10.1016/s0006-2952(99)00314-7

73 Magda D, Gerasimchuk N, Lecane P, Miller RA, Biaglow JE and Sessler JL: Motexafin gadolinium reacts with ascorbate to produce reactive oxygen species. Chem Commun (Camb) *(22)*: 2730-2731, 2002. PMID: 12510321. DOI: 10.1039/b208760j

74 Magda D, Lepp C, Gerasimchuk N, Lee I, Sessler JL, Lin A, Biaglow JE and Miller RA: Redox cycling by motexafin gadolinium enhances cellular response to ionizing radiation by forming reactive oxygen species. Int J Radiat Oncol Biol Phys *51(4)*: 1025-1036, 2001. PMID: 11704327. DOI: 10.1016/s0360-3016(01)01810-7

75 Scott J, Dorr RT, Samulitis B and Landowski TH: Imexon-based combination chemotherapy in A375 human melanoma and RPMI 8226 human myeloma cell lines. Cancer Chemother Pharmacol *59(6)*: 749-757, 2007. PMID: 17333195. DOI: 10.1007/s00280-006-0329-z

76 Evens AM, Lecane P, Magda D, Prachand S, Singhal S, Nelson J, Miller RA, Gartenhaus RB and Gordon LI: Motexafin gadolinium generates reactive oxygen species and induces apoptosis in sensitive and highly resistant multiple myeloma cells. Blood *105(3)*: 1265-1273, 2005. PMID: 15388578. DOI: 10.1182/blood-2004-03-0964

77 Bahlis NJ, McCafferty-Grad J, Jordan-McMurry I, Neil J, Reis I, Kharfan-Dabaja M, Eckman J, Goodman M, Fernandez HF, Boise LH and Lee KP: Feasibility and correlates of arsenic trioxide combined with ascorbic acid-mediated depletion of intracellular glutathione for the treatment of relapsed/refractory multiple myeloma. Clin Cancer Res *8(12)*: 3658-3668, 2002. PMID: 12473574.

78 Donnelly ET, Liu Y, Paul TK and Rockwell S: Effects of motexafin gadolinium on DNA damage and X-ray-induced DNA damage repair, as assessed by the Comet assay. Int J Radiat Oncol Biol Phys *62(4)*: 1176-1186, 2005. PMID: 15990023. DOI: 10.1016/j.ijrobp.2005.04.014

79 Cilurzo F, Cristiano MC, Da Pian M, Cianflone E, Quintieri L, Paolino D and Pasut G: Overcoming cancer cell drug resistance by a folic acid targeted polymeric conjugate of buthionine sulfoximine. Anticancer Agents Med Chem *19(12)*: 1513-1522, 2019. PMID: 31241440. DOI: 10.2174/1871520619666190626114641

80 Defty CL and Marsden JR: Melphalan in regional chemotherapy for locally recurrent metastatic melanoma. Curr Top Med Chem *12(1)*: 53-60, 2012. PMID: 22196271. DOI: 10.2174/156802612798919187

81 Gana CC, Hanssen KM, Yu DMT, Flemming CL, Wheatley MS, Conseil G, Cole SPC, Norris MD, Haber M and Fletcher JI: MRP1 modulators synergize with buthionine sulfoximine to exploit collateral sensitivity and selectively kill MRP1-expressing cancer cells. Biochem Pharmacol *168*: 237-248, 2019. PMID: 31302132. DOI: 10.1016/j.bcp.2019.07.009

82 Lee M, Jo A, Lee S, Kim JB, Chang Y, Nam JY, Cho H, Cho YY, Cho EJ, Lee JH, Yu SJ, Yoon JH and Kim YJ: 3-bromopyruvate and buthionine sulfoximine effectively kill anoikis-resistant hepatocellular carcinoma cells. PLoS One *12(3)*: e0174271, 2017. PMID: 28362858. DOI: 10.1371/journal.pone.0174271

83 Huang P, Feng L, Oldham EA, Keating MJ and Plunkett W: Superoxide dismutase as a target for the selective killing of cancer cells. Nature *407(6802)*: 390-395, 2000. PMID: 11014196. DOI: 10.1038/35030140

84 LaVallee TM, Zhan XH, Johnson MS, Herbstritt CJ, Swartz G, Williams MS, Hembrough WA, Green SJ and Pribluda VS: 2-methoxyestradiol up-regulates death receptor 5 and induces apoptosis through activation of the extrinsic pathway. Cancer Res *63(2)*: 468-475, 2003. PMID: 12543804.

85 Pribluda VS, Gubish ER Jr, Lavallee TM, Treston A, Swartz GM and Green SJ: 2-Methoxyestradiol: an endogenous antiangiogenic and antiproliferative drug candidate. Cancer Metastasis Rev *19(1-2)*: 173-179, 2000. PMID: 11191057. DOI: 10.1023/a:1026543018478

86 Potter BVL: Sulfation pathways: Steroid sulphatase inhibition *via* aryl sulphamates: clinical progress, mechanism and future prospects. J Mol Endocrinol *61(2)*: T233-T252, 2018. PMID: 29618488. DOI: 10.1530/JME-18-0045

87 Zhou Y, Hileman EO, Plunkett W, Keating MJ and Huang P: Free radical stress in chronic lymphocytic leukemia cells and its role in cellular sensitivity to ROS-generating anticancer agents. Blood *101(10)*: 4098-4104, 2003. PMID: 12531810. DOI: 10.1182/blood-2002-08-2512

88 Zhe N, Chen S, Zhou Z, Liu P, Lin X, Yu M, Cheng B, Zhang Y and Wang J: HIF-1α inhibition by 2-methoxyestradiol induces cell death *via* activation of the mitochondrial apoptotic pathway in acute myeloid leukemia. Cancer Biol Ther *17(6)*: 625-634, 2016. PMID: 27082496. DOI: 10.1080/15384047.2016.1177679

89 Press OW, Appelbaum F, Ledbetter JA, Martin PJ, Zarling J, Kidd P and Thomas ED: Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood *69(2)*: 584-591, 1987. PMID: 3492224.

90 Hansen PB and Hasselbalch HC: [Rituximab. A monoclonal CD20 antibody-hematological indications and possible treatments for immunoinflammatory diseases]. Ugeskr Laeger *166(6)*: 466-469, 2004. PMID: 15045710.

91 Wang Z, Bao HW and Ji Y: A systematic review and meta-analysis of rituximab combined with methotrexate *versus* methotrexate alone in the treatment of rheumatoid arthritis. Medicine (Baltimore) *99(8)*: e19193, 2020. PMID: 32080104. DOI: 10.1097/MD.0000000000019193

92 Harrison AM, Thalji NM, Greenberg AJ, Tapia CJ and Windebank AJ: Rituximab for non-Hodgkin's lymphoma: a story of rapid success in translation. Clin Transl Sci *7(1)*: 82-86, 2014. PMID: 24528902. DOI: 10.1111/cts.12111

93 Brown JR, Cymbalista F, Sharman J, Jacobs I, Nava-Parada P and Mato A: The role of rituximab in chronic lymphocytic leukemia treatment and the potential utility of biosimilars. Oncologist *23(3)*: 288-296, 2018. PMID: 29212732. DOI: 10.1634/theoncologist.2017-0150

94 Lucchini E, Zaja F and Bussel J: Rituximab in the treatment of immune thrombocytopenia: what is the role of this agent in 2019? Haematologica *104(6)*: 1124-1135, 2019. PMID: 31126963. DOI: 10.3324/haematol.2019.218883

95 Jak M, van Bochove GG, van Lier RA, Eldering E and van Oers MH: CD40 stimulation sensitizes CLL cells to rituximab-induced cell death. Leukemia *25(6)*: 968-978, 2011. PMID: 21403646. DOI: 10.1038/leu.2011.39

96 Traber MG: Vitamin E. Adv Nutr *12(3)*: 1047-1048, 2021. PMID: 33684201. DOI: 10.1093/advances/nmab019

97 Jiang Q: Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med *72*: 76-90, 2014. PMID: 24704972. DOI: 10.1016/j.freeradbiomed.2014.03.035

98 Alqahtani S and Kaddoumi A: Vitamin E transporters in cancer therapy. AAPS J *17(2)*: 313-322, 2015. PMID: 25466495. DOI: 10.1208/s12248-014-9705-5

99 Gysin R, Azzi A and Visarius T: Gamma-tocopherol inhibits human cancer cell cycle progression and cell proliferation by down-regulation of cyclins. FASEB J *16(14)*: 1952-1954, 2002. PMID: 12368234. DOI: 10.1096/fj.02-0362fje

100 Wang XH, Huang M, Zhao CK, Li C and Xu L: Design, synthesis, and biological activity evaluation of campthothecin-HAA-Norcantharidin conjugates as antitumor agents *in vitro*. Chem Biol Drug Des *93(6)*: 986-992, 2019. PMID: 30218487. DOI: 10.1111/cbdd.13397

101 Bhori M, Singh K, Marar T and Chilakapati MK: Exploring the effect of vitamin E in cancer chemotherapy-A biochemical and biophysical insight. J Biophotonics *11(9)*: e201800104, 2018. PMID: 29770585. DOI: 10.1002/jbio.201800104

102 Singh K, Bhori M and Marar T: α-Tocopherol mediated amelioration of camptothecin-induced free radical damage to avert cardiotoxicities. Hum Exp Toxicol *34(4)*: 380-389, 2015. PMID: 25304969. DOI: 10.1177/0960327114533577

103 Singh K and Marar T: Acute toxicity of camptothecin and influence of alpha-tocopherol on hematological and biochemical parameters. J Cell Tissue Res *11*: 2833-2837, 2011.

104 Singh K and Marar T: Prophylactic role of vitamin e on camptothecin induced oxidative stress in the small intestine of rats. J Pharm Res *5*: 5480-5484, 2012.

105 Singh K, Mhatre V, Bhori M and Marar T: Vitamins E and C reduce oxidative stress and mitochondrial permeability transition caused by camptothecin – an *in vitro* study. Toxicological & Environmental Chemistry *95(4)*: 646-657, 2019. DOI: 10.1080/02772248.2013.805013

106 Carr AC and Maggini S: Vitamin C and immune function. Nutrients *9(11)*: 1211, 2017. PMID: 29099763. DOI: 10.3390/nu9111211

107 Sant DW, Mustafi S, Gustafson CB, Chen J, Slingerland JM and Wang G: Vitamin C promotes apoptosis in breast cancer cells by increasing TRAIL expression. Sci Rep *8(1)*: 5306, 2018. PMID: 29593282. DOI: 10.1038/s41598-018-23714-7

108 Rozanova Torshina N, Zhang JZ and Heck DE: Catalytic therapy of cancer with porphyrins and ascorbate. Cancer Lett *252(2)*: 216-224, 2007. PMID: 17275175. DOI: 10.1016/j.canlet.2006.12.026

109 Bhat SH, Azmi AS, Hanif S and Hadi SM: Ascorbic acid mobilizes endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: a putative mechanism for anticancer properties. Int J Biochem Cell Biol *38(12)*: 2074-2081, 2006. PMID: 16861029. DOI: 10.1016/j.biocel.2006.05.017

110 Said Ahmad M, Fazal F, Rahman A, Hadi SM and Parish JH: Activities of flavonoids for the cleavage of DNA in the presence of Cu(II): correlation with generation of active oxygen species. Carcinogenesis *13(4)*: 605-608, 1992. PMID: 1315626. DOI: 10.1093/carcin/13.4.605

111 Sakagami H and Satoh K: Modulating factors of radical intensity and cytotoxic activity of ascorbate (review). Anticancer Res *17(5A)*: 3513-3520, 1997. PMID: 9413196.

112 Ullah MF, Khan HY, Zubair H, Shamim U and Hadi SM: The antioxidant ascorbic acid mobilizes nuclear copper leading to a

prooxidant breakage of cellular DNA: implications for chemotherapeutic action against cancer. Cancer Chemother Pharmacol *67(1)*: 103-110, 2011. PMID: 20213077. DOI: 10.1007/s00280-010-1290-4

113 Liao Y, Huang JL, Qiu MX and Ma ZW: Impact of serum vitamin D level on risk of bladder cancer: a systemic review and meta-analysis. Tumour Biol *36(3)*: 1567-1572, 2015. PMID: 25359617. DOI: 10.1007/s13277-014-2728-9

114 Buono G, Giuliano M, De Angelis C, Lauria R, Forestieri V, Pensabene M, Bruzzese D, De Placido S and Arpino G: Pretreatment serum concentration of vitamin D and breast cancer characteristics: A prospective observational Mediterranean study. Clin Breast Cancer *17(7)*: 559-563, 2017. PMID: 28601383. DOI: 10.1016/j.clbc.2017.05.007

115 Zhang X and Giovannucci E: Calcium, vitamin D and colorectal cancer chemoprevention. Best Pract Res Clin Gastroenterol *25(4-5)*: 485-494, 2011. PMID: 22122765. DOI: 10.1016/j.bpg.2011.10.001

116 Durak Ş, Gheybi A, Demirkol Ş, Arıkan S, Zeybek ŞÜ, Akyüz F, Yaylım İ and Küçükhüseyin Ö: The effects of serum levels, and alterations in the genes of binding protein and receptor of vitamin D on gastric cancer. Mol Biol Rep *46(6)*: 6413-6420, 2019. PMID: 31549372. DOI: 10.1007/s11033-019-05088-9

117 Liu J, Dong Y, Lu C, Wang Y, Peng L, Jiang M, Tang Y and Zhao Q: Meta-analysis of the correlation between vitamin D and lung cancer risk and outcomes. Oncotarget *8(46)*: 81040-81051, 2017. PMID: 29113365. DOI: 10.18632/oncotarget.18766

118 Süle K, Szentmihályi K, Szabó G, Kleiner D, Varga I, Egresi A, May Z, Nyirády P, Mohai M Jr and Blázovics A: Metal- and redox homeostasis in prostate cancer with vitamin D_3 supplementation. Biomed Pharmacother *105*: 558-565, 2018. PMID: 29886377. DOI: 10.1016/j.biopha.2018.05.090

119 Siwińska A, Opolski A, Chrobak A, Wietrzyk J, Wojdat E, Kutner A, Szelejewski W and Radzikowski C: Potentiation of the antiproliferative effect *in vitro* of doxorubicin, cisplatin and genistein by new analogues of vitamin D. Anticancer Res *21(3B)*: 1925-1929, 2001. PMID: 11497279.

120 Yanai K, Ehara T, Sakaguchi S and Sueno O: The effect of beta-carotene derived from microalga dunaliella bardawil intake on japanese visual function - randomized double-blind placebo comparison study. Jpn Pharmacol Ther *46*: 1579-1590, 2018.

121 Haghighat M, Iranbakhsh A, Baharara J, Ebadi M and Sotoodehnejadnematalahi F: Effect of β-carotene on the differentiation potential of ciliary epithelium-derived MSCs isolated from mouse eyes on alginate-based scaffolds. Exp Eye Res *202*: 108346, 2021. PMID: 33147471. DOI: 10.1016/j.exer.2020.108346

122 Li K and Zhang B: The association of dietary β-carotene and vitamin A intake on the risk of esophageal cancer: a meta-analysis. Rev Esp Enferm Dig *112(8)*: 620-626, 2020. PMID: 32543872. DOI: 10.17235/reed.2020.6699/2019

123 Middha P, Weinstein SJ, Männistö S, Albanes D and Mondul AM: β-carotene supplementation and lung cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study: The role of tar and nicotine. Nicotine Tob Res *21(8)*: 1045-1050, 2019. PMID: 29889248. DOI: 10.1093/ntr/nty115

124 Virtamo J, Taylor PR, Kontto J, Männistö S, Utriainen M, Weinstein SJ, Huttunen J and Albanes D: Effects of α-tocopherol and β-carotene supplementation on cancer incidence and mortality: 18-year postintervention follow-up of the Alpha-tocopherol, Beta-carotene Cancer Prevention Study. Int J Cancer *135(1)*: 178-185, 2014. PMID: 24338499. DOI: 10.1002/ijc.28641

125 Sowmya Shree G, Yogendra Prasad K, Arpitha HS, Deepika UR, Nawneet Kumar K, Mondal P and Ganesan P: β-carotene at physiologically attainable concentration induces apoptosis and down-regulates cell survival and antioxidant markers in human breast cancer (MCF-7) cells. Mol Cell Biochem *436(1-2)*: 1-12, 2017. PMID: 28550445. DOI: 10.1007/s11010-017-3071-4

126 Azam S, Hadi N, Khan NU and Hadi SM: Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties. Toxicol *In Vitro 18(5)*: 555-561, 2004. PMID: 15251172. DOI: 10.1016/j.tiv.2003.12.012

127 Cooper R, Morré DJ and Morré DM: Medicinal benefits of green tea: part II. review of anticancer properties. J Altern Complement Med *11(4)*: 639-652, 2005. PMID: 16131288. DOI: 10.1089/acm.2005.11.639

128 Nemeikaite-Ceniene A, Imbrasaite A, Sergediene E and Cenas N: Quantitative structure-activity relationships in prooxidant cytotoxicity of polyphenols: role of potential of phenoxyl radical/phenol redox couple. Arch Biochem Biophys *441(2)*: 182-190, 2005. PMID: 16111645. DOI: 10.1016/j.abb.2005.07.002

129 Yang CS, Landau JM, Huang MT and Newmark HL: Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr *21*: 381-406, 2001. PMID: 11375442. DOI: 10.1146/annurev.nutr.21.1.381

130 Hadi SM, Bhat SH, Azmi AS, Hanif S, Shamim U and Ullah MF: Oxidative breakage of cellular DNA by plant polyphenols: a putative mechanism for anticancer properties. Semin Cancer Biol *17(5)*: 370-376, 2007. PMID: 17572102. DOI: 10.1016/j.semcancer.2007.04.002

131 Hadi SM, Ullah MF, Shamim U, Hanif S, Azmi AS and Bhat S: A prooxidant mechanism of red wine polyphenols in chemoprevention of cancer. Int J Canc Prev *3(3)*: 175-186, 2009.

132 Lee KW and Lee HJ: Biphasic effects of dietary antioxidants on oxidative stress-mediated carcinogenesis. Mech Ageing Dev *127(5)*: 424-431, 2006. PMID: 16519920. DOI: 10.1016/j.mad.2006.01.021

133 Valdés A, García-Cañas V, Koçak E, Simó C and Cifuentes A: Foodomics study on the effects of extracellular production of hydrogen peroxide by rosemary polyphenols on the anti-proliferative activity of rosemary polyphenols against HT-29 cells. Electrophoresis *37(13)*: 1795-1804, 2016. PMID: 26842614. DOI: 10.1002/elps.201600014

134 Ozkan T, Hekmatshoar Y, Pamuk H, Ozcan M, Yaman G, Yagiz GC, Akdemir C and Sunguroglu A: Cytotoxic effect of 6-Shogaol in Imatinib sensitive and resistant K562 cells. Mol Biol Rep *48(2)*: 1625-1631, 2021. PMID: 33515349. DOI: 10.1007/s11033-021-06141-2

135 Nazim UM and Park SY: Attenuation of autophagy flux by 6-shogaol sensitizes human liver cancer cells to TRAIL-induced apoptosis *via* p53 and ROS. Int J Mol Med *43(2)*: 701-708, 2019. PMID: 30483736. DOI: 10.3892/ijmm.2018.3994

136 Nedungadi D, Binoy A, Vinod V, Vanuopadath M, Nair SS, Nair BG and Mishra N: Ginger extract activates caspase independent paraptosis in cancer cells *via* ER stress, mitochondrial dysfunction, AIF translocation and DNA

damage. Nutr Cancer *73(1)*: 147-159, 2021. PMID: 31690139. DOI: 10.1080/01635581.2019.1685113

137 Pan MH, Hsieh MC, Kuo JM, Lai CS, Wu H, Sang S and Ho CT: 6-Shogaol induces apoptosis in human colorectal carcinoma cells *via* ROS production, caspase activation, and GADD 153 expression. Mol Nutr Food Res *52(5)*: 527-537, 2008. PMID: 18384088. DOI: 10.1002/mnfr.200700157

138 Annamalai G, Kathiresan S and Kannappan N: [6]-Shogaol, a dietary phenolic compound, induces oxidative stress mediated mitochondrial dependant apoptosis through activation of proapoptotic factors in Hep-2 cells. Biomed Pharmacother *82*: 226-236, 2016. PMID: 27470359. DOI: 10.1016/j.biopha.2016.04.044

139 Ishiguro K, Ando T, Maeda O, Ohmiya N, Niwa Y, Kadomatsu K and Goto H: Ginger ingredients reduce viability of gastric cancer cells *via* distinct mechanisms. Biochem Biophys Res Commun *362(1)*: 218-223, 2007. PMID: 17706603. DOI: 10.1016/j.bbrc.2007.08.012

140 Rhode J, Fogoros S, Zick S, Wahl H, Griffith KA, Huang J and Liu JR: Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. BMC Complement Altern Med *7*: 44, 2007. PMID: 18096028. DOI: 10.1186/1472-6882-7-44

141 Sang S, Hong J, Wu H, Liu J, Yang CS, Pan MH, Badmaev V and Ho CT: Increased growth inhibitory effects on human cancer cells and anti-inflammatory potency of shogaols from Zingiber officinale relative to gingerols. J Agric Food Chem *57(22)*: 10645-10650, 2009. PMID: 19877681. DOI: 10.1021/jf9027443

142 Tan BS, Kang O, Mai CW, Tiong KH, Khoo AS, Pichika MR, Bradshaw TD and Leong CO: 6-Shogaol inhibits breast and colon cancer cell proliferation through activation of peroxisomal proliferator activated receptor γ (PPARγ). Cancer Lett *336(1)*: 127-139, 2013. PMID: 23612072. DOI: 10.1016/j.canlet.2013.04.014

143 Babich H, Ackerman NJ, Burekhovich F, Zuckerbraun HL and Schuck AG: Gingko biloba leaf extract induces oxidative stress in carcinoma HSC-2 cells. Toxicol *In Vitro 23(6)*: 992-999, 2009. PMID: 19560534. DOI: 10.1016/j.tiv.2009.06.023

144 Lee KW, Hur HJ, Lee HJ and Lee CY: Antiproliferative effects of dietary phenolic substances and hydrogen peroxide. J Agric Food Chem *53(6)*: 1990-1995, 2005. PMID: 15769125. DOI: 10.1021/jf0486040

145 Andrade-Jorge E, Godínez-Victoria M, Sánchez-Torres LE, Fabila-Castillo LH and Trujillo-Ferrara JG: Aryl maleimides as apoptosis inducers on L5178-Y murine leukemia cells (in silico, *in vitro* and *ex vivo* study). Anticancer Agents Med Chem *16(12)*: 1615-1621, 2016. PMID: 27141879. DOI: 10.2174/1871520615666160504094417

146 Carrasco-Torres G, Baltiérrez-Hoyos R, Andrade-Jorge E, Villa-Treviño S, Trujillo-Ferrara JG and Vásquez-Garzón VR: Cytotoxicity, oxidative stress, cell cycle arrest, and mitochondrial apoptosis after combined treatment of hepatocarcinoma cells with maleic anhydride derivatives and quercetin. Oxid Med Cell Longev *2017*: 2734976, 2017. PMID: 29163752. DOI: 10.1155/2017/2734976

147 Bishayee K, Khuda-Bukhsh AR and Huh SO: PLGA-loaded gold-nanoparticles precipitated with quercetin downregulate HDAC-Akt activities controlling proliferation and activate p53-ROS crosstalk to induce apoptosis in hepatocarcinoma cells. Mol Cells *38(6)*: 518-527, 2015. PMID: 25947292. DOI: 10.14348/molcells.2015.2339

148 Kachadourian R and Day BJ: Flavonoid-induced glutathione depletion: potential implications for cancer treatment. Free Radic Biol Med *41(1)*: 65-76, 2006. PMID: 16781454. DOI: 10.1016/j.freeradbiomed.2006.03.002

149 Li H, Zhang X and Wang W: Anticancer activity of 5, 7-dimethoxyflavone against liver cancer cell line HepG2 involves apoptosiS, ROS generation and cell cycle arrest. Afr J Tradit Complement Altern Med *14(4)*: 213-220, 2017. PMID: 28638884. DOI: 10.21010/ajtcam.v14i4.24

150 Dükel M, Tavsan Z and Kayali H: Flavonoids regulate cell death-related cellular signaling *via* ROS in human colon cancer cells. Process Biochemistry *101*: 11-25, 2021. DOI: 10.1016/j.procbio.2020.10.002

151 Alshatwi AA, Periasamy VS, Athinarayanan J and Elango R: Synergistic anticancer activity of dietary tea polyphenols and bleomycin hydrochloride in human cervical cancer cell: Caspase-dependent and independent apoptotic pathways. Chem Biol Interact *247*: 1-10, 2016. PMID: 26800624. DOI: 10.1016/j.cbi.2016.01.012

152 Chatterjee K, AlSharif D, Mazza C, Syar P, Al Sharif M and Fata JE: Resveratrol and pterostilbene exhibit anticancer properties involving the downregulation of HPV oncoprotein E6 in cervical cancer cells. Nutrients *10(2)*: 243, 2018. PMID: 29485619. DOI: 10.3390/nu10020243

153 Jeong H and Lee J: Effects of dietary fiber from Mozuku (Cladosiphon novae-caledoniae kylin) residue on antioxidant activity and anticancer in HT-29 human colon cancer cells according to extraction condition. Applied Chemistry for Engineering *25(4)*: 363-367, 2017. DOI: 10.14478/ace.2014.1039

154 Moiseeva EP, Almeida GM, Jones GD and Manson MM: Extended treatment with physiologic concentrations of dietary phytochemicals results in altered gene expression, reduced growth, and apoptosis of cancer cells. Mol Cancer Ther *6(11)*: 3071-3079, 2007. PMID: 18025290. DOI: 10.1158/1535-7163.MCT-07-0117

155 Szliszka E and Krol W: The role of dietary polyphenols in tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis for cancer chemoprevention. Eur J Cancer Prev *20(1)*: 63-69, 2011. PMID: 20861738. DOI: 10.1097/CEJ.0b013e32833ecc48

156 Banerjee K and Mandal M: Oxidative stress triggered by naturally occurring flavone apigenin results in senescence and chemotherapeutic effect in human colorectal cancer cells. Redox Biol *5*: 153-162, 2015. PMID: 25965143. DOI: 10.1016/j.redox.2015.04.009

157 Rajendra Prasad N, Karthikeyan A, Karthikeyan S and Reddy BV: Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Mol Cell Biochem *349(1-2)*: 11-19, 2011. PMID: 21116690. DOI: 10.1007/s11010-010-0655-7

158 Khan HY, Zubair H, Faisal M, Ullah MF, Farhan M, Sarkar FH, Ahmad A and Hadi SM: Plant polyphenol induced cell death in human cancer cells involves mobilization of intracellular copper ions and reactive oxygen species generation: a mechanism for cancer chemopreventive action. Mol Nutr Food Res *58(3)*: 437-446, 2014. PMID: 24123728. DOI: 10.1002/mnfr.201300417

159 Khan HY, Zubair H, Ullah MF, Ahmad A and Hadi SM: Oral administration of copper to rats leads to increased lymphocyte cellular DNA degradation by dietary polyphenols: implications for a cancer preventive mechanism. Biometals *24(6)*: 1169-1178, 2011. PMID: 21717118. DOI: 10.1007/s10534-011-9475-9

160 Gupte A and Mumper RJ: Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. Cancer Treat Rev *35(1)*: 32-46, 2009. PMID: 18774652. DOI: 10.1016/j.ctrv.2008.07.004

161 Kaiafa GD, Saouli Z, Diamantidis MD, Kontoninas Z, Voulgaridou V, Raptaki M, Arampatzi S, Chatzidimitriou M and Perifanis V: Copper levels in patients with hematological malignancies. Eur J Intern Med *23(8)*: 738-741, 2012. PMID: 22920946. DOI: 10.1016/j.ejim.2012.07.009

162 Zubair H, Khan HY, Sohail A, Azim S, Ullah MF, Ahmad A, Sarkar FH and Hadi SM: Redox cycling of endogenous copper by thymoquinone leads to ROS-mediated DNA breakage and consequent cell death: putative anticancer mechanism of antioxidants. Cell Death Dis *4*: e660, 2013. PMID: 23744360. DOI: 10.1038/cddis.2013.172

Received August 17, 2021
Revised September 13, 2021
Accepted September 17, 2021