

Biological Effects of Secondary Plant Metabolites on the Expression of Key Factors Involved in DNA Damage Response Signaling Pathway and Apoptosis in Cancer Cell Lines

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Abstract: *Various types of external events, as well as internal processes in the cell continuously create conditions for DNA damage. For protection, cells developed DNA damage response network (DDR), representing a cascade of linked cellular and molecular events to eliminate critical and dangerous conditions in order to maintain genome stability. Disturbances in DDR are a causative factor in the occurrence of cancer. Study in proteins involved in DDR can be used for therapeutic purposes for detecting, predicting and treating cancer [1]. Plant extracts, and in particular, the secondary metabolites in which they are rich, show great modulatory effects of major components of DDR. In this review, we aim to show the molecular mechanism of action of some secondary metabolites studied in recent years on the DDR network, and summarize whether common principles of their action can be found.*

Keywords: bioactive compounds, cell death, DDR, oxidative stress, plants

1. Introduction

More than 5000 years people used plants for medicinal purposes; about 70 % of the global population in developing countries uses ancient medicines based on plant extracts for their primary healthcare [2]. Worldwide, cancer is one of the main causes of death. With the development of modern methods of surgery, radiotherapy and drug treatment, the prognosis for survival of patients has significantly improved. Since the mid - 1990s, scientists have suggested that in many types of cancer, there is a defect in the way cells monitor and repair damaged DNA. Numerous clinical studies have reported plants several medical properties, including antioxidant, anti - inflammatory, antimicrobial, anticancer, antibiotic and analgesic properties [3], which make them a suitable source of substances for the development of innovative therapies. Plants naturally produce a variety of products named primary metabolite, used for the processes of growth and development of plants, such as photosynthesis, translocation and respiration. The products obtained from primary metabolites, not directly involved in growth and development, are considered secondary metabolites. These bioactive compounds are found in fruits, vegetables and many other plants in small quantities, they are substances with nutrition value and therapeutic potential [4]. They supply health protection in addition to their basic nutritional values [5] and vary considerably in their structures and functions. [6 - 8]. Many secondary metabolites protect against oxidative or UV stress, and can affect cell signaling pathways. They take action as defense mechanisms by interposing with molecular targets within the cell in herbivores, microbes, and plants [9].

2. Classification of Secondary Metabolites

The extensive study of secondary metabolites was initiated in the middle of the 19th century. They can be classified according to chemical composition (containing nitrogen or not), structure (e. g., containing a sugar, having rings), their solubility in various solvents, or the pathway of biosynthetic. Their main division is in three large groups based on the biosynthetic pathway namely phenolics, terpenes, and alkaloids [10].

2.1. Polyphenolic Compounds.

Polyphenolic compounds represent a diverse group of natural compounds with numerous phenolic functions. A vast number of clinical studies have been carried out to clarify their positive effects in protecting against cardiovascular diseases, metabolic dysfunction, aging and anticarcinogenic properties (such as suppressing tumor growth, metastasis, and angiogenesis) [11 - 13]. By diverse biochemical processes, such as initiation of immune system, inhibition of oxidation, anti - inflammatory properties, modulating effects on cellular signaling pathways involved in DNA damage and repair, dietary polyphenols have been proven to prevent carcinogenesis. [14 - 17]. According to the number of aromatic rings and molecular structure, they are classified into four subgroups: flavonoids, lignans, stilbenes and phenolic acids.

2.1.1. Flavanoids are a classic type of polyphenols with two aromatic rings linked together by a three carbon bridge. They are subdividing into anthocyanidins, flavonols, flavones, isoflavones, flavanones and flavan - 3 - ols.

2.1.1.1. Anthocyanidins provided color of flowers, leaves and fruits. They protect plants against UV radiation [18], have antioxidant activity, increase the levels of enzymes with anti-inflammatory and carcinogen-deactivating properties and can inhibit the growth of cancer cells [19].

2.1.1.2. Flavanol and flavan-3-ols mainly produced in cocoa and tea, have anticancer effect, influence gene expression and cell signaling [20].

2.1.1.3. Kaempferol and quercetin are most representative flavonols. They inhibit proliferation of various cancers and can induce apoptosis and DNA damage in cancer cells [21, 22].

2.1.1.4. Flavones are contained in apples, cabbages, herbs and carrots. Luteolin and apigenin are the main representatives of the group. Luteolin can inhibit the development of cancer cells and induce apoptosis, demonstrate antioxidative and anti-inflammatory effects [23]. Apigenin has anti-cancer potential by modulating key signaling pathways [24].

2.1.1.5. Soybean and soybean products are the main dietary source of isoflavones, especially daidzein and genistein. They demonstrated antioxidant, chemoprotective and anti-inflammatory effects. Isoflavones inhibit the proliferation and metastasis of cancer cells by influence gene expression [25].

2.1.2. Some of representatives of lignans family are arctigenin, honokiol, magnolol, they have key role in cancer prevention and management by decrease proliferation and induce apoptosis. In cell studies, increase the levels of enzymes with antioxidant, anti-inflammatory and carcinogen-deactivating effects [26].

2.1.3. Resveratrol is mainly included in grapes skin, red wine and peanuts. It is part of stilbenes and has antioxidant, anti-inflammatory and anti-tumor properties. Resveratrol acts as a chemopreventive agent in the four major stages of carcinogenesis, namely initiation, promotion, progression, and metastasis of various cancers, such as breast, prostate, colorectal and lung cancers [27].

2.1.4. Hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids; they are presented in green and black tea, coffee, walnuts and cereals. Phenolic acids exert proapoptotic and antiproliferative effects [28, 29]. Other phenolic acids with proven antioxidant and antimutagenic effects are gallic, ferulic, chlorogenic, p-Coumaric and caffeic acids [30 - 32].

2.2. Terpenoids form the largest class of all secondary metabolites. They can be found in almost all living organisms many of which are of plant origin. The diversity of all plant terpenoids had been derived from mevalonic acid and containing five carbons isoprene units. Many terpenoids are essential for plant growth and development [33]. Generality studies have concentrated on the molecular biochemistry and genomic terpenoid biosynthesis. Since 1990 terpenoids have become indispensable in treatment of ovarian, breast and other cancers [34].

2.3. Alkaloids are a class of naturally occurring nitrogen-containing compounds with at least one nitrogen as a heteroatom in their heterocyclic ring. They are conveniently extracted in water because they are mainly soluble in aqueous solutions. Currently, more than 8000 natural compounds are classified as alkaloids [35, 36]. Some of the most famous of them are caffeine, nicotine, cocaine and morphine. Several biological activities of alkaloids have been reported - analgesic, antibacterial, antifungal, anti-inflammatory, anticancer, and antiviral activity [37 - 41].

3. Extraction of plant metabolites

The composition and concentration of biologically active ingredients in plants are mainly influenced by external physical factors (pH, temperature, light, etc.), soil composition and plant growth factors [42]. It also has a huge impact on the conditions in which the herbal material is dried and stored. Initial step and key in achieving quality outcome is preparation of plants for analysis, to obtain a final product is used complete plants or fractions [43]. Plant extraction is a procedure of separating active plant materials or secondary metabolites from inactive material using an appropriate solvent and standard procedure for extraction.

There have been several techniques used in the extraction including conventional and new hi-tech techniques. The extraction includes quite numbers of procedures such as maceration, infusion, decoction, percolation, digestion and Soxhlet extraction. The choice of organic solvents (hexane, methanol, acetone, ethanol, etc.) and/or water for the dissolution of the soluble metabolites depends on the type of the plant fragments for extraction and nature of the bioactive compounds. Different chromatography techniques used in purification and separation of the secondary metabolites. Several novel methods have been developed, such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE). These techniques improve yield, reduce the extraction time, minimizing the solvent volume [44 - 46].

4. Oxidative stress and its importance for living organisms

Reactive oxygen species (ROS) are byproducts produced by oxidation reactions in organisms, participate in many cellular processes vital for normal cell growth and proliferation. ROS are group of reactive chemicals derived from oxygen [47], mainly include hydrogen peroxide (H_2O_2), superoxide anion radical ($O_2^{\cdot-}$), hypochlorous acid (HOCl), singlet oxygen (1O_2), hydroxyl radical ($\cdot OH$), alkoxy radical ($RO\cdot$), and the peroxy radical ($ROO\cdot$) [48]. ROS can be classified into two groups of compounds: radicals (contain at least one unpaired electron in the shells around the atomic nucleus and have the ability of independent existence) and non-radicals (not free radicals but can easily take to free radical reactions) [49]. Sources of ROS can be both endogenous and exogenous. Different cellular organs, where the oxygen consumptions are high, such as mitochondria, peroxisomes and endoplasmic reticulum, are endogenous sources of ROS. The exogenous

origin of ROS can be effects of physical factors such as different chemical compounds (as xenobiotics and pesticides), α - , β - , γ - , UV and X - radiation, ultrasound, temperature, ozone, cigarette smoke [50, 51]. An imbalance in ROS levels is defined as Oxidative stress [52], and may damage many biological macromolecules, such as DNA, RNA, proteins and lipids and results in genomic instability, followed by the activation of a series signaling pathways and changes in cell growth, that lead to degenerative diseases. Oxidative stress may promote tumor transformation of cells [53]; inhibit the cell cycle, and regulate via different pathways tumor cell death [54].

Antioxidants are naturally present in plants, animals, microorganisms or be chemically synthesized. The bioactive compounds have been reported to assist in the adjustment of redox signaling pathways by modulating the levels of Reactive oxygen species (ROS).

Cells have a variety of endogenous antioxidant substances to maintain optimal levels of ROS. According to their mechanism of action, they can be separated into enzymatic and non - enzymatic ones. Operate together they set up antioxidant protection system of every organism. Starring are antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidases (GPx), catalase (CAT), xanthine oxidoreductase (XOR), and glutathione reductase (GR). SODs are metalloenzymes with metal ions in their active centers. Regarding the metal cofactor, there are three isoforms of SOD: cytoplasmic, extracellular, and mitochondrial. SOD catalyzed the reaction of dismutation superoxide (O_2^-) to hydrogen peroxide and molecular oxygen [55, 56]. GPx reduce H_2O_2 and organic peroxides with the help of reduced glutathione [55 - 57]. CAT is responsible for the dismutation of hydrogen peroxide [55, 57]. XOR catalyze the oxidation of hypoxanthine to xanthine and xanthine to uric acid. XOR exists in two inter converting alternative isoforms with opposing effects – xanthine dehydrogenase has antioxidant effects, while xanthine oxidase has pro - oxidative effects [58]. GR is found in the mitochondria and cytosol, it is enzyme related to glutathione, and also participates in reactions responsible for oxygen detoxification [55 - 57]. Non - enzymatic antioxidants are glutathione, ascorbic acid, tocopherol, ubiquinol, carotenoids and others [59, 60]. Knowledge of the concentration levels and activity of antioxidant enzymes allows for estimation of the possibility of developing selected diseases founded on extreme formation of ROS and free radicals [61]. Notably, there exist exogenous antioxidants, which are not producing in the cells, but can be received via antioxidant rich vitamins, spices, foods and herbs [60]. Plants provide a rich source of compounds with antioxidant properties and free - radical scavenging ability, associated mostly with secondary metabolites (polyphenols, tocopherols), ascorbic acid, carotenoids, macromolecules (such as peptides and polysaccharides) and essential oils [62]. There are various methods for the assessment of antioxidant activity, which can generally be divided into three distinct categories: spectrometry, electrochemical assays and chromatography. It is difficult to compare one method with another, so it is necessary to determine the proper selection of method (s) for valid evaluation of antioxidant potential of the compound of interest [63].

Antioxidants can be used as effective agents for cancer treatment by reducing oxidative stress. Numerous studies of plant extracts rich in secondary metabolites in genotoxic damage to cells have been carried out, proving, in addition to their antioxidant properties, radioprotective, anticlastogenic and and carcinogenic properties. Orientin and vicenin (Ocimum flavonoids) at an optimal concentration of 17.5 μ M reduced chromosomal damage in human peripheral lymphocytes exposed to 4Gy gamma radiation through free radical scavenging mechanism [64]. Higher concentrations (>20%) of alcoholic extract of *Alpiniazarumbet* prevent chromosomal damage caused by irradiation due to the radical scavenging activity of flavonoids and phenolics [65]. Pretreatment with *Haberlearhodopensis* (resurrection plant) methanol extract, increased the antioxidant activity of SOD and CAT enzymes, decreased the level of malonic aldehyde (MDA), and decreased oxidative stress by scavenging the free radicals in irradiated lymphocytes of New Zealand rabbits [66 - 68], thereby provided radioprotection and increased lipid peroxidation. Other studies by the authors prove more an anticlastogenic effect of *Haberlearhodopensis* extract, expressed in reduction of percentage of aberrant metaphases before and after gamma irradiation (2.0 Gy) [67], and antimutagenic potential by inhibited the frequency of sister chromatid exchanges [69].

5. DNA damage response (DDR)

Oxidative DNA damage can cause many changes in DNA molecule, such as lesions of bases and pentoses, chain breaks, cross - links between DNA and proteins, regions without nucleobases, etc. The multiple types of DNA lesions that result from oxidative damage are rapidly recognized by the cell, subsequently activating a complex of signaling pathways known as DNA damage response (DDR). In addition to oxidative stress, other endogenous factors (cellular metabolism, replication errors) and exogenous pressures (e. g. chemical exposure, UV light, ionizing radiation, genotoxic agents), constantly damaged cellular DNA and cause single or double strand breaks [70]. The DDR is a hierarchical process that occurs through a cascade of distinct steps carefully controlled by G1, S and G2/M phase checkpoints and cyclin dependent kinases (CDKs), with subsequent activation of different DNA repair systems according to the DNA damage. If the DNA damage is irreparable, the cellular response enters the cell in apoptosis phase or cell death [71]. Repair systems are divided to mismatch repair (MMR), nucleotide excision repair (NER), base excision repair (BER), non - homologous - end - joining (NHEJ), homologous recombination (HR):

5.1. Mismatch repair (MMR) system recognize and repairs insertion - deletion loops (IDLs) and base - base mismatches, that occurring in the genome during replication, recombination, and DNA damage [72].

5.2. Nucleotide excision repair (NER) pathway resolves numerous DNA lesions, that distort the normal helical structure of duplex DNA. NER removes bulky adducts such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine - (6, 4) - pyrimidone photoproducts (6 - 4PPs) generated by UV radiation; base adducts created by chemotherapeutic

drugs such as cisplatin and benzopyrene; base lesions produced by reactions with endogenous lipid peroxidation products, and ROS - induced base modifications such as the cyclopurines [73].

5.3. Base excision repair pathway is highly conserved, cellular multi - enzyme DNA repair process and required for repair a wide range of exogenous and endogenous DNA base damage [74], commonly caused by oxidation, deamination or alkylation. BER process remove small and often non - helix distorting base lesions, as well as abasic sites and single strand DNA breaks. Any disruption of DDR network leads to genomic instability, accumulation of mutations and subsequent oncogenic transformation [75]. One of the most harmful forms of DNA damage are double strand breaks (DSB). In eukaryotes, DSBs are repaired through the concerted action of two mechanisms:

5.4. Homologous recombination (HR) - resolves DSBs in dividing cells during the S and G2 phases of cell cycle. It requires a homologous sister chromatid for implementation. The recognition of the DSB by MRN complex initiated HR [76]. MRN complex is comprised of the MRE11, RAD50, and Nijmegen breakage syndrome (NBS1) proteins, it recruit the protein kinase ataxia telangiectasia mutated (ATM) to DSB sites [77]. ATM is master regulator of the DNA damage response, and it coordinates checkpoint activation and DNA repair mechanisms [77]. The ATM activation triggers by phosphorylating one of histone variant H2AX, producing γ H2AX - functions as a signal for DNA damage. In single strand, DNA damages (ssDNA), replication protein A (RPA) and RAD9/RAD1/HUS1 act as signals and activate ATR pathway [78].

5.5. Nonhomologous end joining (NHEJ) recombination is the major DSB repair system in high eukaryotes, can operate during all phases of cell cycle, but is most active during G1 [79]. To initiate NHEJ, the Ku70/Ku80 heterodimer binds directly to two DSB ends,, DNA - PKcs is recruited in complex with the nuclease Artemis to Ku - bound DNA ends. Pol X family polymerases, Pol μ and Pol λ function to fill in the small gap segments, DNA ligase IV complex, including XLF - Cernunnos (XLF) and XRCC4 carries out the critical ligation step of repair process. In addition, cells maintain an alternative end - joining pathway and engage various factors, such as MRN complex, PARP - 1, WRN and LIG1 that also functioned in HR and single strand break repair [80].

The normal physiological form of cell death, called apoptosis, has been identified as critical process involved in the regulation of cellular homeostasis. Perturbations in programmed cell death regulatory pathways play an important role in the pathogenesis and progression of cancer, as well as in tumor responses to therapeutic interventions. Apoptosis mainly befalls in two pathways: the extrinsic (stimulated by activation of death receptors, such as TNFR1, Fas, DR4/5) and the intrinsic (mediated by mitochondria and Bcl - 2 family proteins) pathways. Activation of either pathway triggers a cascade of caspases, thus inducing caspase - dependent nucleosome fragmentation. In addition, essential role in regulating cell death play NF - κ B, Jak - STAT3 and MARKs signaling pathways [81].

6. Molecular mechanisms of the anticarcinogenic action of secondary plant metabolites.

Numerous researches have evaluated anticancer effect of isolated phytochemicals, because of their impacts on modulating signal pathways of DDR and apoptosis, which shows great potential in cancer detection, prediction and treatment [83]. Of particular interest to us is the molecular mechanism of action of various secondary plant metabolites in regulating DDR signaling pathways, as well as their modulatory effects of the major components of DDR.

In the present review, summarize some of the studies in last years of plant secondary metabolites, and their effect on the relative expression levels of key factors participating in cell cycle control, DDR signaling pathway, and apoptosis in cancer cell lines, looking for common principles of their action.

Karimanet. Al (2022) [83] evaluate the expression levels of key factors of DDR in 3 human cancer cell lines (Breast cancer MCF - 7, Lung cancer A - 549 and Prostate cancer A - 549) in response of treatment with bioflavonoids quercetin and thymoquinone. The expression levels of p53, Rad51, Ku70, XRCC1 and H2AX genes in treated cell were evaluated. H2AX increased in all cell lines after treatment, significantly lower relative expression levels of Rad51, compared with control cells have been evaluated. In all cell lines, quercetin exerted more potent inhibitory effects on the expression of Rad51. Phosphorylation of H2AX and degradation of Rad51 are signs of DNA DSBs [84]. Both bioflavonoids enhanced the expression levels of p53, but quercetin exerted potent stimulatory effects. The p53 is leading cell cycle and apoptosis regulator by induces expression of genes involved in different stages of the two processes. The increase of p53 and the significant decrease of Rad51 could enhance DNA damage checkpoints and induction of DSB incancer cells [85]. Ku70 is a key mediator of non - homologous end joining (NHEJ) repair and negatively regulates apoptosis by sequestration of Bax. In treated cell lines, the expression levels significantly decreased. Induction of apoptosis is the result after inhibition of Ku70 of p53, and dissociation of Bax from Ku70. XRCC1 gene is with critical function in base excision repair (BER), it's involved in cell protection against ionizing radiation. Quercetin and thymoquinone treatment significantly inhibit mRNA expression levels of the gene. Kariman et al. concluded that quercetin and thymoquinone significantly increased DNA damage and suppressed the expression levels of DNA repair genes in cancer cells.

Costa et al. evaluate molecular effects of methoxyruugenol (natural phenolic compound in spices and herbs, such as nutmeg (*Myristicafragrans*)) on signaling pathways in human endometrial cancer cell line (Ishikawa) [86]. The relative expression of five genes involved in the control of cell cycle was evaluated. The results showed an upregulation in p53 and p21 gene expression and a down regulation in CDK4 and CDK6 genes. Methoxyruugenol did not change the expression levels of p16 gene. These results showed triggered p53 - p21 pathway, reduction in cell number and

the induction of G1/S phase cell cycle arrest. The activation of p53 increases the expression of p21, which inhibit cyclin - CDK complexes, regulated G1 - S checkpoint and suppressed the expression of proliferation associated genes by preventing the phosphorylation of pRb. Debacq - Chainiaux et al. exhibited, that rise ROS can promote cellular senescence by activation of p38^{MARK}, leading to upregulation of p53 and p21 [88]. The Ishikawa cells treated with methoxyruugenol increased the ROS levels [86], that point the role of ROS as key factor in signaling pathways.

Zang et al. explored molecular mechanism of anti - cancerous potential of flavonoid Morin extracted from mulberry leaves in Hela cells [87]. The study indicated good anti - cancerous abilities of Morin. The flavonoid decreased mRNA expression of CDK1, Cdc25c, Survivin, cyclin B1 and CHK2 genes, and increased the expression of p53, p21 and Wee 1 genes, that could induce G2/M arrest in Hela cells. The cell apoptosis is biologically regulated by two major pathways: mitochondria - mediated intrinsic and death receptor - mediated extrinsic pathways. To determine which pathway is involved in the Morin - induced apoptosis authors measured the mRNA expression of several genes related with death receptor pathway and with mitochondrial pathway. The result showed that Morin derived apoptosis was regulated via multiple pathways. The mRNA expression of Bax, Bad, cytochrome c, Apaf - 1, caspases - 9, DR3, DR5, FasL, FADD, caspases - 10, PARP, PI3K, AKT, mTOR, P70S6K and Smacc genes were increased and the levels of Bcl - 2, Bcl - xL, AMPK, cIAP - 1, cIAP - 2, PKCε and NF - kb were decreased. The increased levels of intracellular ROS in treated with Morin Hela cells are intermediate signaling molecules. ROS provoked series of biological consequences, including apoptosis [87].

Another study explored the effect of flavonoid Apigenin isolated from *P. villosa* plant on cell cycle regulation of HepG2 cells line [88]. Apigenin treatment upregulate significantly p21 expression. p21 is a downstream protein of p38 MARK pathway and is important for cell cycle regulation. Li et al. found that inhibition of p38 MARK could reverse the p21 upregulation at mRNA and protein level significantly. The team evaluated the expression levels changes of CDK4 and cyclinD1 for further explain the regulation mechanism of Apigenin. CyclinD1 was expressed at higher levels, and CDK4 was downregulated by Apigenin, which induced G1 phase arrest in HepG2 cells [88]. These results suggested, that p38 - MARK - p21 pathway, together with cyclinD1 - CDK4 complex formation, might be involved in the regulation of HepG2 cell proliferation by Apigenin.

The phytochemical zerumbone is a terpenoid extracted from the rhizome of *Zingiber zerumbet*, the species is known as Asian ginger or spicy ginger. Zerumbone (ZER) have antioxidant and antiproliferative properties. Rondina et al. evaluated how ZER influenced the expression of mRNA genes related to cell cycle, cell death, DNA damage and endoplasmic reticulum (ER) stress in HepG2/C3A cells [89]. The authors showed that ZER causes cell cycle arrest in G2/M. The mRNA expression of CDKN1A, which encodes the p21 protein is increased, ZER reduced TP53 mRNA expression, suggesting independent p21 activation. ZER

upregulated GADD45A mRNA expression in response to DNA damage, inhibiting the PARP1 gene, which participates in the DNA repair system. Analysis of apoptosis regulation genes showed increased BBC3 mRNA expression, which encodes proapoptotic protein PUMA, and decreases BIRC5 mRNA expression, which encodes surviving protein that acts as preventing apoptosis. Upregulation of the ERN1 gene, which acts as a transcription factor for ER stress response genes, shows that ZER - induced apoptosis may be related to ER stress. LC3 protein is a reliable marker for detecting autophagy [90], the MAP1LC3A gene, which encoded LC3 protein is down regulated by ZER. The antiproliferative effects of ZER can be attributed to the reduced NF - kb, MYC and IGF - 1 mRNA expression.

One of the most intensively investigated plant polyphenols is resveratrol (3, 5, 4' - trihydroxy - trans - stilbene). It is small molecule stilbene compound with two aromatic rings. Resveratrol is naturally present in food products such as grapes, mulberries and peanuts. Numerous studies have demonstrated its apoptotic, antioxidant and anti - proliferative effects in various cancer cell lines [91 - 93]. Lin Li et al. investigated the effect of resveratrol in HeLa cervical carcinoma cells. The results revealed that resveratrol suppresses cell proliferation and elevates apoptosis by activating caspase - 3 and caspase - 9, upregulating the expression of the pro - apoptotic Bcl - 2 - associated X (BAX) protein, p21, p27, and p53 and down regulating the expression of the anti - apoptotic proteins Bcl - 2, Bcl - XL and Cyclin B1 in HeLa cells [94]. Resveratrol inhibit the proliferation of gastric cancer MGC - 803 cell line, induces apoptosis and inhibit the mRNA expression of proliferation factors β - catenin, c - myc, and cyclin D [95]. In the MCF - 7 cell line, resveratrol interrupt the Rad 51, BRCA1 and BRCA2 expression involved in ATR - CHK1 activation [96].

Chien, C. et al. examined the mechanism of phytochemical naphtho [1, 2 - b] furan - 4, 5-dione (NFD) presenting in *Avicennia marina* induced DNA damage and apoptosis in NSCLC (non - small - cell lung carcinoma) cells. Authors used H1299 cell line. DNA damage sensors ATM and ATR were significantly increased mRNA expression levels, but not DNA dependent protein kinase. DDR downstream - related proteins Chk1 and Chk2 were significantly activated at 3 μM NFD treatment. Down regulated were also the expression levels of pro - survival proteins Bcl - 2, Bcl - xL and Mcl - 1, in contrast to up - regulated expression in pro - apoptotic protein Bax. Decreased levels of endogenous inhibitors of apoptosis, proteins surviving and XIAP were also observed. These results showed that NFT - induced apoptosis is mediated through a DNA damage - dependent pathway [97].

7. Conclusion

In the review studies cited, the scientists found both a large variation in the mechanism of action of secondary plant metabolites and a tendency to overlapping effects in terms of their antioxidative, antigenotoxic and anti - carcinogenic action. Differences in the mechanism of action affecting the expression of various genes involved in distinct stages of the

DDR network are observed. The targeting of tumor cells to apoptosis is also carried out through several signaling pathways. In general, extensive studies of the molecular mechanism of action of each of the secondary metabolites are needed to elucidate the detailed mechanism by which they exert their anticarcinogenic and antioxidant effects. All this requires the application of an integrated approach of tests and methods to capture their added effect of the action on the one hand, and the protective effect of secondary plant metabolites on the other. This emphasizes the need for an in-depth study of the complex molecular mechanisms in order to use their full potential to develop innovative methods to combat malignant diseases in man. From the overview, we can categorically say we agree that some secondary plant metabolites showed great potential for development of new methods in the treatment of cancer, thanks to the different levels of action on the complex and multi-stage DDR system.

Competing interests

The authors have declared that no competing interests exist.

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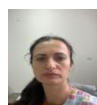
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