Chapter 16

# Targeting the Cytoskeleton with Plant-Bioactive Compounds in Cancer Therapy

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**Abstract**

In this overview we describe the main plant-derived bioactive compounds used in cancer therapy which has the cell cytoskeleton as therapeutic target. Three major classes of these compounds are described: antimitotics with microtubule-destabilizing and—stabilizing effects, plant-bioactive compounds that interact with intermediate filaments/actin, and plant-bioactive compounds that interact with intermediate filaments like keratins and vimentin. We also focus on the molecular aspects of interactions with their cellular targets: microtubules, intermediate filaments, and microfilaments. Some critical aspects of cardiac side effects of cancer chemotherapy are also discussed, focusing on cardiac cytoskeleton and protective effect of plant-derived compounds. The application of plant bioactives in the treatment of cancer has resulted in increased therapeutic efficacy through targeting the cytoskeleton, respectively, prevention of the injury of cytoskeletal components elicited by chemotherapeutics.

**Keywords:** plant-derived compounds, cancer therapy, microtubules, intermediate filaments, microfilaments

## 1. Introduction

Chemotherapy is routinely used for cancer treatment. Since tumor cells lose many of the regulatory pathways of the normal cells, they continue to divide without control. Chemotherapeutic drugs try to solve these abnormalities, but sometimes the toxicity of allopathic treatments creates a significant problem.

The cytoskeleton constitutes the supporting framework of the cell, and it is composed of three types of cytosolic filaments: microtubules, intermediate filaments, and microfilaments. The entire cytoskeletal network is a dynamic structure which regulates the cell structure, and it

is involved in many cellular functions such as movement, transport, or cell division [1]. The cytoskeleton is one of the main therapeutic targets in cancer cells [2].

Various cancer therapies use plant-derived bioactive products. There are four classes of plant-derived anticancer drugs currently used in oncotherapy: vinca alkaloids (vinblastine, vincristine), epipodophyllotoxins (etoposide and teniposide), taxanes (paclitaxel and docetaxel), and camptothecin derivatives (camptothecin and irinotecan) [3]. To date, new generations of vinca alkaloids, camptothecins, and epothilones as well as a novel class of taxanes have been developed. Some of these are in clinical use, others in clinical trials.

The major inconvenience in using antimicrotubule agents in oncotherapy is that these compounds cause significant side effects such as neutropenia and neurotoxicity and because of their limited efficacy as single agents [3].

This review describes the main natural compounds identified in the last year as potential anticancer agents, which have cell cytoskeleton as therapeutic target. We focus on the interactions of plant-derived anticancer drugs with all three types of cytosolic filaments: microtubules, intermediate filaments, and microfilaments. In addition, we summarize the most recent advances in the understanding of the molecular aspects of these interactions.

Some critical aspects of cardiac side effects of cancer chemotherapy are also discussed, focusing on cardiac cytoskeleton and protective effects of plant-derived compounds.

## 2. Microtubules as chemotherapeutic targets of plant-derived bioactives

Microtubules are dynamic structures involved in different cellular processes including cell division, where they are the most important constituents of the mitotic spindle apparatus during the M phase of cell division [4]. They are polymers composed of $\alpha$- and β-tubulin heterodimers, characterized by high dynamics of polymerization/depolymerization, resulting in the elongation or shrinkage of the filaments. Polymerization of microtubules occurs when $\alpha$- and β-tubulin monomers bind to a GTP at the nucleotide exchangeable site (E-site) in β-tubulin and the non-exchangeable site (N-site) in $\alpha$-tubulin. Once GTP is hydrolyzed, it becomes non-exchangeable, which matches the addition of the next tubulin dimer to the plus (+) end of the microtubule. Upon depolymerization, the GTP cap is detached, allowing the microtubules depolymerize releasing the $\alpha$-/β-tubulin heterodimers into the cytoplasm. Subsequently, the GDP attached to another free β-tubulin and can exchange to GTP at the E-site, before another polymerization cycle begins [4, 5].

Dynamic instability is regulated by a number of microtubule-associated proteins (MAPs), which bind to stabilize the microtubules [6]. MAP phosphorylation induces its dissociation leading to microtubule instability. Some cytokines have a critical role in the regulation of MAPs and microtubule dynamics, such as controlling centromere localization Cdc2 kinases, mitogen-activated protein kinases ERK, controlling cell migration JNK, and the main serine/threonine phosphatases, type 1 (PP1) and type 2A (PP2A) [7–10].

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The dynamic ability of microtubules to polymerize and depolymerize is essential for cellular division and chromosome segregation during mitosis. Due to their crucial roles in dividing cells, microtubules have been considered a major target for cancer therapy. Microtubule-interacting plant-derived biomolecules, namely, antimitotics, can be classified into two main groups based on their apparent mechanisms of action: microtubule-destabilizing agents act as tubulin polymerization inhibitors, and microtubule-stabilizing agents act as tubulin polymerization promoters [11].

### 2.1. Microtubule-destabilizing agents

Vinca alkaloids and colchicines prevent the polymerization of tubulin and promote the depolymerization of microtubules.

Vinca alkaloids are a series of biologically active agents isolated from *Catharanthus roseus* (*Vinca rosea*) with a potent antitumor activity, related to their ability to inhibit the polymerization of microtubules and preventing cell division [12]. There are approximately 130 vinca alkaloids distributed in different vegetal tissues: vincristine, vinblastine, and yohimbine in the aerial parts; catharanthine and vindoline in leaves; and almalicine and reserpine in roots [13]. They have demonstrated clinical efficacy in a broad spectrum of cancers, both as single agents and in combination. Vincristine, vinblastine, and vindesine are the first vinca alkaloids used as antitumor drugs. Vinorelbine is the first new second-generation vinca alkaloid, while vinflunine, a bis-fluorinated vinorelbine derivative, was synthesized by superacid chemistry and studied in phase I–III clinical trials [14, 15].

The vinca alkaloids are dimeric compounds consisting of two multi-ringed subunits, vindoline and catharanthine, linked by a carbon-carbon bridge [16]. They act by binding specifically to β-tubulin and block its ability to polymerize with $\alpha$-tubulin into microtubules, thus disrupting the mitotic spindle. This blocks mitosis and kills actively dividing cells. The results indicate that vinorelbine and vinflunine affect microtubule dynamics differently from vinblastine and proved to be weak binders [17].

Vincristine is used in the treatment of hematological and lymphatic neoplasms, whereas vinblastine in breast cancer, testicular cancer, choriocarcinoma, and vindesine in non-small cell lung cancer or breast cancer. Vinorelbine is useful for the treatment of non-small-cell lung cancer, and vinflunine has been used in the treatment of bladder, non-small-cell lung, and breast cancers [17].

Similar to Vinca alkaloids, colchicine extracted from plants of the genus *Colchicum* (*autumn crocus*) is a microtubule-destabilizing agent at high concentrations and stabilizes microtubule dynamics at low concentrations [18]. It first binds to soluble tubulin, leading to a complex that copolymerizes into the ends of the microtubules and prevents the elongation of the microtubule polymer. It is severely toxic to normal tissues at high dose, which limits its use in cancer therapies [19]. Colchicine showed different antitumoral effects which include inhibition of metastatic potential [20] and angiogenesis [21], cell blebbing through a Rho/Rho effector kinase (ROCK)/myosin light-chain kinase (MLCK) pathway [22], decrease of ATP influx into mitochondria [23].

Novel microtubule-destabilizing plant-bioactive compounds are summarized in **Table 1**.

| Active substance/herbal formulation | Mechanism of action | Therapeutic use | References |
|---|---|---|---|
| Flavonoids isolated from *Tanacetum gracile* | —Modulate microtubule depolymerization by activating mitotic spindle checkpoint<br>—Bind at α- β interfacial site of tubulin | Breast cancer | [24] |
| Artelastin isolated from the wood bark of *Artocarpus elasticus* | —Radial structure disorganization of the microtubule network<br>—Kinetochores are not affected | Breast cancer | [25] |
| Podoverine A isolated from *Podophyllum versipelle* | —Mitotic arrest and inhibition of microtubule polymerization by targeting the vinca-binding site on tubulin | Renal cancer<br>Breast cancer | [26] |
| Plinabulin chemical probe KPU-244-B3 | —Binds in the boundary region between α- and β-tubulin near the colchicine-binding site<br>—Induce tubulin depolymerization | Fibrosarcoma | [27] |
| 2′-Hydroxy-2,4,6-trimethoxy-5′,6′-naphthochalcone | —Disruption of microtubular networks by inhibition of tubulin polymerization<br>—Failure of mitotic spindle formation and blocking mitosis at the prometaphase or metaphase-anaphase transition | Colon cancer | [28] |
| Aqueous extract of ginger | —Disruption of interphase microtubule network of A549 and HeLa cells<br>—Inhibition of temperature-dependent reassembly of cold-treated depolymerized microtubule of A549 and HeLa cells | Cervical carcinoma<br>Lung carcinoma | [29] |
| Safranal | —Inhibition of tubulin assembly ($IC_{50}$ was obtained at 72.19 μM)<br>—Binds between α- and β-tubulin closer to alpha-tubulin and hydrogen bond with Gly 142<br>—Hydrophobic interactions play critical roles for safranal molecule stabilization in binding site | Cancer therapy | [30] |
| Isochaihulactone | —Inhibition of tubulin polymerization in a concentration-dependent manner in A549 non-small-cell lung cancer cells<br>—Cause G2/M phase arrest and apoptosis in a time- and concentration-dependent manner | Lung cancer | [31] |
| Carnosol | —Modulation of autophagic markers microtubule- associated protein 1A/1B light-chain 3 I (LC3 I) to microtubule-associated protein 1A/1B light-chain 3 II (LC3 II) and p62 in MDA-MB-231 cells | Breast cancer | [32] |

| Active substance/herbal formulation | Mechanism of action | Therapeutic use | References |
|---|---|---|---|
| Angelica shikokiana methanol extract (AME) | —AME and all isolated compounds inhibited tubulin polymerization<br>—Angelicin and kaempferol-3-O-rutinoside were the most active compounds<br>—Phenolic compounds and furanocoumarins showed binding affinity to colchicine-binding site<br>—Quercetin, kaempferol, luteolin, chlorogenic acid, and methyl chlorogenate exhibited the strongest activity against histone deacetylase 8 (HDAC8) and the highest affinity to trichostatin A-binding site. | Human hepatocellular carcinoma, rhabdomyosarcoma (RD), colorectal carcinoma, human epithelioma, and human breast adenocarcinoma | [33] |
| Alkaloids from beach spider Lily (*Hymenocallis littoralis*) | —Interrupt polymerization of microtubules in Hep-G2 cells | Hepatocarcinoma | [34] |
| DYZ-2-90 | —Binds to microtubules and rapidly induces tubulin depolymerization | Colorectal cancer | [35] |
| Indicine N-oxide (INO) | —200 μM induced a mitotic block of about 22% in HeLa cells; 300 μM concentration-induced depolymerization of interphase microtubular network<br>—The effect was similar to the depolymerizing effects of the drugs such as colchicine and vinblastine, although the concentration used here was 1000-fold higher than those drugs<br>—Binds to the tubulin dimer through hydrogen bonds and hydrophobic interactions<br>—INO does not make any interactions with the amino acid residues on the tubulin dimer that were reported to be interacting with the taxol or colchicine, but INO-binding site partially overlaps with the griseofulvin-binding site (docking) | Cervical cancer | [36] |

**Table 1.** Potential plant-bioactive compounds that interact with microtubules as microtubule-destabilizing agents for cancer therapy.

## 2.2. Microtubule-stabilizing agents

Taxanes are the main class of microtubule-stabilizing agents, which prevent the depolymerization of microtubules and promote the polymerization of tubulin to microtubules.

One of the most important plant compounds in the fight against cancer was discovered in the bark of *Taxus brevifolia*—taxol, now named paclitaxel, which has become one of the most

effective drugs against breast and ovarian cancer and has been approved for the clinical treatment of cancer patients. Since the first discovery of paclitaxel in the 1960s, a variety of other microtubule-stabilizing agents have been derived primarily from natural resources [37]. The molecular mechanism includes polymerization of tubulin to stable microtubules and also interacts directly with microtubules, stabilizing them against depolymerization and thereby blocks cells in the G2/M phase of the cell cycle [38]. The binding of taxol to β-tubulin in the polymer results in cold-stable microtubules even in the absence of exogenous GTP. Hydrogen/deuterium exchange (HDX) coupled to liquid chromatography-electrospray ionization MS demonstrated a marked reduction in deuterium incorporation in both β- and α-tubulin in the presence of taxol and contributed to increased rigidity in taxol microtubules and complementary to that due to GTP-induced polymerization [39].

Initially obtained from *Taxus brevifolia* bark, paclitaxel is now a semisynthetic product of 10-deacetylbaccatin III, which is extracted from the needles of the *Taxus baccata*. Similarly, docetaxel, a second-generation taxane, was directly obtained semisynthetically by esterification from the inactive taxane precursor 10-deacetylbaccatin III [40]. Paclitaxel and docetaxel bind to the specific binding sites of tubulin, which is different from the binding site of guanosine triphosphate, vinblastine, colchicine, and podophyllotoxin [41].

Docetaxel has a 1.9-fold higher affinity for the site than paclitaxel and induces tubulin polymerization at a 2.1-fold lower critical tubulin concentration. The effect on the cell cycle is different: paclitaxel inhibits the cell cycle traverse at the G2/M phase junction [42], while docetaxel produces its maximum cell-killing effect against cells in the S phase [43].

To decrease the toxicity and enhance delivery and distribution, new taxane formulations of micelles were investigated, including nanoparticles, emulsions, and liposomes [44]. Compounds such as Abraxane, CT-2103, and docosahexaenoic acid (DHA)-paclitaxel are examples of new taxanes with higher activity than paclitaxel in taxane-resistant cancers, as well as in tumors that have been unresponsive to paclitaxel [16].

Protopine is a benzylisoquinoline alkaloid isolated from *Opium poppy*, *Corydalis tubers*, and *Fumaria officinalis*. It stabilizes tubulin polymerization process but has no affinity to taxol-binding site. It induces a marked increase of tubulin polymerization in a dose-dependent manner in human hormone-refractory prostate cancer (PC-3 cells), similar to paclitaxel. It enhances microtubule assembly and formation of mitotic spindles in PC-3 cells [45].

Taccalonolides are plant steroids possessing a C2–C3 epoxide group and an enol-lactone isolated from *Tacca leontopetaloides*, *Tacca plantaginea*, *Tacca chantrieri*, *Tacca plantaginea*, *Tacca integrifolia*, etc. They act as microtubule stabilizers by binding to another microtubule site than taxol resulting in the formation of microtubule bundles and leading to cell cycle arrest and apoptosis. It is also reported that taccalonolides bind to β-tubulin near the lumen of microtubule, which is different from the taxol-binding site stabilizers which bind to α-tubulin protofilaments [46–49].

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Recent study shows that the dietary flavonoid fisetin binds to tubulin and stabilizes microtubules with binding characteristics far superior than paclitaxel. It induces upregulation of microtubule-associated protein (MAP)-2 and microtubule-associated protein (MAP)-4 and increases $\alpha$-tubulin acetylation, an indicator of microtubule stabilization [50].

## 3. Microfilaments as chemotherapeutic targets of plant-derived bioactives

Actin filaments are composed of globular actin (G-actin) which polymerizes into filamentous (F) actin and participates in many important cellular processes including cell division and cytokinesis, cell signaling, vesicle and organelle movement, cell junction establishment, and maintenance.

Like microtubules, actin microfilaments can change rapidly their structure in response to external stimuli. Actin polymerization is stimulated by nucleating factors such as the Arp2/3 complex, which mimics a G-actin dimer in order to stimulate actin polymerization [51]. Actin binds ATP to stabilize microfilament formation and hydrolysis [52]. The growth of microfilaments is regulated by thymosin, which binds G-actin to lead the polymerizing process, whereas profilin binds G-actin and catalyzes the exchange of ADP to ATP, promoting monomeric addition to the plus end of F-actin [53].

During cytokinesis, disruption of actin polymerization can effect cellular structure. Cytokinesis inhibitors such as cytochalasin B disrupt the actin cytoskeleton, and the cell is unable to divide [54] but is still able to initiate another mitotic event, continuing to form nuclei and eventually becoming enlarged and multinucleated [55, 56]. Cell lines derived from bladder, kidney, and prostate carcinomas become multinucleated when grown in cytochalasin B-supplemented medium, whereas cells from corresponding normal tissue remain mono- or binucleate under comparable conditions [55]. These particular features make tumor cells ideal targets for chemotherapy, as they have reduced cytoskeletal integrity and multiple nucleation and increased mitochondrial activity [57].

Actin filaments are also of substantial importance to cancer cell migration. Cancer cell migration can convert between mesenchymal and amoeboid types. This latter can occur, e.g., when cells are exposed to protease inhibitors [58] and thereby mesenchymal cancer cell invasion is repressed by specific targeting of protease function. Inhibiting RhoA/ROCK signaling promotes the formation of multiple competing microfilament-derived lamellipodia that suppress amoeboid migration of tumor cells [59]. Tumor cells unable to move through amoeboid migration will switch to mesenchymal migration [60]. However, tumor cells exposed to protease inhibitors will move mainly through amoeboid migration. Using microfilament disrupting RhoA/ROCK inhibitors in combination with protease inhibitors would simultaneously block both types of cell migration.

Phytomedicine developed actin-targeted potential drugs, designed for cancer therapy (**Table 2**).

| Active substance/herbal formulation | Mechanism of action | Therapeutic use | References |
|---|---|---|---|
| Resveratrol | —50 μM resveratrol decreases Rac and Cdc42 signaling to the actin cytoskeleton<br>—5 μM resveratrol increases Rac signaling to the actin cytoskeleton | Breast cancer | [61] |
| Oleuropein | —Disrupt actin filaments in a dose-dependent manner | Sarcoma | [62] |
| Alkaloid mixture derived from *Senna spectabilis*—cassine and spectaline | —Altered normal distribution pattern of F-actin filaments | Liver cancer | [63] |
| Deoxyelephantopin (DET) | —Affects the actin cytoskeleton network and downregulates calpain-mediated proteolysis of several actin-associated proteins<br>—Inhibition of proteolysis of actin cytoskeleton-associated proteins identified by differential proteomic profiling | Lung metastasis of mammary adenocarcinoma | [64] |
| Cucurbitacin E | —Disruption of the F-actin cytoskeleton<br>—Increases the filamentous or polymerized actin fraction | Prostate carcinoma cells | [65] |
| Cucurbitacin E | —Damaged F-actin without affecting beta-tubulin | 95D lung cancer cells | [66] |
| Cucurbitacin I | —Induced the co-aggregation of actin with phospho-myosin II by stimulation of the RhoA/ROCK pathway and inhibition of LIM-kinase | HeLa cells | [67] |
| Cucurbitacin B | —Induced rapid and improper polymerization of the F-actin network | Myeloid leukemia cells | [68] |
| Jasplakinolide (JAS) | —Rearranged the actin cytoskeleton<br>—JAS has a phalloidin-like action<br>—Distribution of actin filaments was different from that induced by cytochalasin D | Cancer cells | [69] |
| *Ganoderma lucidum* extracts | —Inhibits growth and induce actin polymerization | Bladder cancer cells | [70] |
| 4-Hydroxycoumarin | —Disorganized the actin cytoskeleton correlated with reductions in cell adhesion to four extracellular matrix proteins and inhibition of random motility | Melanoma cell line B16–F10 | [71] |

**Table 2.** Plant-bioactive compounds which interact with actin for cancer therapy.

## 4. Intermediate filaments as chemotherapeutic targets of plant-derived bioactives

Along with microfilaments and microtubules, intermediate filaments are the other component of the cytoskeleton that can be exploited in the clinical treatment of cancer. All intermediate filaments have a central alpha-helical domain that is composed of four protofibrils separated

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by three linker regions [72]. The N- and C-terminus segments of intermediate filaments are non-alpha-helical regions of polypeptide sequences, associated with head to tail into protofilaments that pair up laterally into protofibrils; four of these protofibrils form an intermediate filament.

Whereas microfilaments and microtubules are actin or tubulin polymers, intermediate filaments are composed of 50 different proteins classified into six types based on similarities in amino acid sequence [72]. In regard to potential chemotherapeutic targets, the most promising intermediate filaments are keratins, nestin, and vimentin.

### 4.1. Anti-keratin agents

Keratin and cytokeratin are intermediate filaments found in the cytoskeleton of epithelial tissue. There are twenty different keratin polypeptides (K1–K20) identified and classified into type I (K9–K20) and type II (K1–K8) intermediate filaments [73]. Keratins of importance to cancer therapy are keratin 8 (K8) and keratin 18 (K18), the most common and characteristic members of intermediate filaments expressed in single-layer epithelial tissues [74, 75]. Oncogenes, which activate Ras signaling, stimulate expression of K18 through transcription factors [76]. However, aberrant K8 and K18 expression has been noticed in particularly invasive carcinomas [77, 78]. K18 was found to be a substrate of the cysteine-aspartic proteases during epithelial apoptosis [77].

Based on aberrant keratin expression found in many cancers, these intermediate filaments present a novel chemotherapeutic target that need to be investigated.

Crude acetone extract of *Bupleurum scorzonerifolium* (AE-BS) showed antiproliferative activity, induced cell arrest in G2/M phase, and apoptosis in A549 human lung cancer cells [79]. In a further study, Chen et al. [73] noticed K8 phosphorylation after AE-BS treatment of A549 cells. The association of ERK1/2 activation with K8 phosphorylation may be related to the apoptotic effect of AE-BS.

### 4.2. Anti-vimentin agents

Vimentin functions as a regulator in cancer cells undergoing epithelial-mesenchymal transition (EMT), an important change during tumor progression where cells detached from their original tissue become highly motile and invasive. Studies have shown that quercetin prevented epidermal growth factor (EGF)-induced EMT, migration, and invasion of prostate cancer cells by suppressing the expression of vimentin and N-cadherin [80]. Genistein, an isoflavone found in soybeans, fava beans, and lupine, has been shown to downregulate mesenchymal markers ZEB1, slug, and vimentin and therefore cause reversal of EMT in gemcitabine-resistant pancreatic cancer cells [81]. Similarly, this flavonoid was able to decrease protein expression of vimentin, cathepsin D, and MMP-2 and thus suppressed epithelial-mesenchymal transition and migration capacity of BG-1 ovarian cancer cells [82]. Other natural compounds, like silibinin, induced the morphological reversal of mesenchymal phenotype to epithelial phenotype through downregulation of vimentin and MMP-2 and upregulation of cytokeratin-18 [83]. Moreover, silibinin meglumine, a water-soluble form

of milk thistle silymarin, impedes the EMT in EGFR-mutant non-small-cell lung carcinoma cells by upregulation of the relative mRNA expression of CDH1 (E-cadherin) accompanied by downregulation of vimentin [84]. Berberine, a plant alkaloid present in various plants like *Berberis*, decreased the expression of the mesenchymal markers vimentin and fibronectin and restored the epithelial marker E-cadherin, thereby contributing to the reversal of EMT [85].

Piplartine, a biologically active component from *Piper* species (Piperaceae), also suppresses tumor progression and migration by disruption of the p120-ctn/vimentin/N-cadherin complex, which plays a critical role in tumor progression and invasion/metastasis [86].

Phenethyl isothiocyanate (PEITC), the main bioactive compound present in cruciferous vegetables, decreases breast and prostate tumor growth inhibition through vimentin suppression [87]. Cucurbitacin E induced disruption of vimentin cytoskeleton in prostate carcinoma cells, while microtubules were unaffected [65]. The natural product withaferin A (WFA) exhibits antitumor activity by binding to vimentin and covalently modifying its cysteine residue, which is present in the highly conserved helical coiled coil 2B domain [88]. Penduletin and casticin, flavonoids from the Brazilian plant *Croton betulaster*, induced changes in the pattern of expression of the cytoskeletal protein vimentin and thereby inhibit the growth of human glioblastoma cells [89].

## 5. Protective effect of plant-bioactive compounds on anthracycline-induced cardiac cytoskeletal toxicity

Cardiotoxicity is the most serious side effect of antitumoral anthracyclines, which include adriamycin, doxorubicin, mitoxantrone, daunorubicin, or epirubicin [90]. The main cause of toxicity is their effect on the cardiac cytoskeleton, consisting of myofibrils disarray [91], including both structural and functional changes: troponin I and troponin C phosphorylation mediated by a doxorubicin-induced protein kinase C activation [92, 93] and decrease of troponin I, and changes of $\alpha$-actin, creatine kinase, and myosin light-chain 2 expression [93]. In other studies, degradation of cardiac cytoskeletal proteins, including titin [94] and dystrophin [95], was observed. Recently, changes in the cardiac distribution of desmin have been detected, with areas of decreased expression in the cytoplasm and protein aggregation after mitoxantrone treatment [96, 97]. The use of plant bioactives might protect against the oxidative stress caused by anthracycline drugs, including cytoskeleton injuries. Our group recently demonstrated that the flavonoid chrysin inhibits mitoxantrone-triggered cardiomyocyte apoptosis via multiple pathways, including decrease of the Bax/Bcl-2 ratio and caspase-3 expression along with preservation of the desmin disarray [96].

## 6. Conclusions

Plant-derived bioactive molecules constitute promising tools for the treatment of cancer. The application of plant bioactives in the treatment of cancer has resulted in increased therapeutic

efficacy through targeting the cytoskeleton and prevention of cytoskeletal injuries due to chemotherapy side effects. Research results testify both the evolution of knowledge coming from pharmacognosy and the great possibilities of future progress by means of a rational approach of natural product-based drug discovery or new pharmaceutical formulations.

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## References

[1] Lodish H., Berk A., Zipursky S.L., et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman. 2000.

[2] Saraf S., Patel D.R., Kaur C.D., Saraf S.. Cytoskeleton analysis as target for bioactives. Trends in Applied Sciences Research. 2001;1–12.

[3] Marzo I., Naval J. Antimitotic drugs in cancer chemotherapy: promises and pitfalls. Biochemical Pharmacology. 2013;8:703–710.

[4] Valiron O., Caudron N., Job D. Microtubule dynamics. Cellular and Molecular Life Sciences CMLS. 2001;58:2069–2084.

[5] Etienne-Manneville S. From signaling pathways to microtubule dynamics: the key players. Current Opinion in Cell Biology. 2010;22:104–111.

[6] Regnard C., Audebert S., Boucher D., Larcher J.C., Edde B., Denoulet P. Microtubules: functional polymorphisms of tubulin and associated proteins (structural and motor MAP's). Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales. 1996;190:255–268.

[7] Nigg E.A. Mitotic kinases as regulators of cell division and its checkpoints. Nature Reviews Molecular Cell Biology. 2001;2:21–32.

[8] Ma H.T., Poon R.Y.C. How protein kinases co-ordinate mitosis in animal cells. Biochemical Journal. 2011;435:17–31.

[9] Johnson G.L., Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science. 2002;298:1911–1912.

[10] Tournebize R., Andersen S.S., Verde F., Doree M., Karsenti E., Hyma A.A. Distinct roles of PP1 and PP2A-like phosphatases in control of microtubule dynamics during mitosis. The EMBO Journal. 1997;16:5537–5549.

[11] Negi A.S., Gautam Y., Alam S., Chanda D., Luqman S., Sarkar J., Khan F., Konwar R. Natural antitubulin agents: importance of 3,4,5-trimethoxyphenyl fragment. Bioorganic & Medicinal Chemistry. 2015;23:373–389.

[12] Himes R.H. Interactions of the catharanthus (Vinca) alkaloids with tubulin and microtubules. Pharmacology & Therapeutics. 1991;51:257–267.

[13] Liu Z., Wu H.L., Li Y., Gu H.W., Yin X.L., Xie L.X., Yu R.L. Rapid and simultaneous determination of five vinca alkaloids in *Catharanthus roseus* and human serum using trilinear component modeling of liquid chromatography–diode array detection data. Journal of Chromatography B. 2016;1026:114–123.

[14] Fahy J. Modifications in the "upper" velbenamine part of the Vinca alkaloids have major implications for tubulin interacting activities. Current Pharmaceutical Design. 2001;7:1181–1197.

[15] Yun-San Yip A., Yuen-Yuen Ong E., Chow L.W. Vinflunine: clinical perspectives of an emerging anticancer agent. Expert Opinion on Investigational Drugs. 2008;17:583–591.

[16] Nobili S., Lippi D., Witort E., Donnini M., Bausi L., Mini E., Capaccioli S. Natural compounds for cancer treatment and prevention. Pharmacological Research. 2009;59:365–378

[17] Ngan V.K., Bellman K., Hill B.T., Wilson L., Jordan M.A. Mechanism of mitotic block and inhibition of cell proliferation by the semisynthetic Vinca alkaloids vinorelbine and its newer derivative vinflunine. Molecular Pharmacology. 2001;60:225–232.

[18] Leung Y.Y., Li L., Hui Y., Kraus V.B. Colchicine—update on mechanisms of action and therapeutic uses. Seminars in Arthritis and Rheumatism. 2015;45:341–350.

[19] Bhattacharyya B., Panda D., Gupta S., Banerjee M. Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin. Medicinal Research Reviews. 2008;28:155–183.

[20] Charpentier M.S., Whipple R.A., Vitolo M.I., Boggs A.E., Slovic J., Thompson K.N., et al. Curcumin targets breast cancer stem-like cells with microtentacles that persist in mammospheres and promote reattachment. Cancer Research. 2014;74:1250–1260.

[21] Ganguly A., Yang H., Zhang H., Cabral F., Patel K.D. Microtubule dynamics control tail retraction in migrating vascular endothelial cells. Molecular Cancer Therapeutics. 2013;12:2837–2846.

[22] Meshki J., Douglas S.D., Hu M., Leeman S.E., Tuluc F. Substance P induces rapid and transient membrane blebbing in U373MG cells in a p21-activated kinase-dependent manner. PLoS One. 2011;6:e25332.

[23] Maldonado E.N., Patnaik J., Mullins M.R., Lemasters J.J. Free tubulin modulates mitochondrial membrane potential in cancer cells. Cancer Research. 2010;70:10192–10201.

[24] Sinha S., Amin H., Nayak D., Bhatnagar M., Kacker P., Chakraborty S., Kitchlu S., Vishwakarma R., Goswami A., Ghosal S. Assessment of microtubule depolymerization property of flavonoids isolated from Tanacetum gracile in breast cancer cells by biochemical and molecular docking approach. Chemico-Biological Interactions. 2015;239:1–11.

[25] Pedro M., Ferreira M.M., Cidadea H., Kijjo A., Bronze-da-Rocha E., Nascimento M. Artelastin is a cytotoxic prenylated flavone that disturbs microtubules and interferes with DNA replication in MCF-7 human breast cancer cells. Life Sciences. 2005;77:293–311.

[26] Tran T.T., Gerding-Reimers C., Schölermann B., Stanitzki B., Henkel T., Waldmann H., Ziegler S. Podoverine A—a novel microtubule destabilizing natural product from the *Podophyllum* species. Bioorganic & Medicinal Chemistry. 2014;22:5110–5116.

[27] Yamazaki Y., Sumikura M., Hidaka K., Yasui H., Kiso Y., Yakushiji F., Hayashi Y. Anti-microtubule 'plinabulin' chemical probe KPU-244-B3 labeled both alpha- and beta-tubulin. Bioorganic & Medicinal Chemistry. 2010;18:3169–3174.

[28] Lee J.M., Lee M.S., Koh D., Lee Y.H., Lim Y., Shin S.Y. A new synthetic 2′-hydroxy-2,4,6-trimethoxy-5′,6′-naphthochalcone induces G2/M cell cycle arrest and apoptosis by disrupting the microtubular network of human colon cancer cells. Cancer Letters. 2014;354:348–354.

[29] Choudhury D., Das A., Bhattacharya A., Chakrabarti G. Aqueous extract of ginger shows antiproliferative activity through disruption of microtubule network of cancer cells. Food and Chemical Toxicology. 2010;48:2872–2880.

[30] Naghshineh A., Dadras A., Ghalandari B., Riazi G.H., Modaresi M.S., Afrasiabi A., Aslani M.K. Safranal as a novel anti-tubulin binding agent with potential use in cancer therapy: an *in vitro* study. Chemico-Biological Interactions. 2015;238:151–160.

[31] Chen Y.L., Lin S.Z., Chang J.Y., Cheng Y.L., Tsai N.M., Chen S.P., Chang W.L., Harn H.J. *In vitro* and *in vivo* studies of a novel potential anticancer agent of isochaihulactone on human lung cancer A549 cells. Biochemical Pharmacology. 2006;72:308–319.

[32] Dhaheri Y.A., Attoub S., Ramadan G., Arafat K., Bajbouj K., Karuvantevida N., et al. Carnosol induces ROS-mediated beclin1-independent autophagy and apoptosis in triple negative breast cancer. PLoS One. 2014;9:e109630.

[33] Mira A., Shimzu K.. *In vitro* cytotoxic activities and molecular mechanisms of angelica shikokiana extract and its isolated compounds. Pharmacognosy Magazine. 2015;11:S564–S569.

[34] Ji Y.B., Chen N., Zhu H.W., Ling N., Li W.L., Song D.X., Gao S.Y., Zhang W.C., Ma N.N. Alkaloids from beach spider lily (*Hymenocallis littoralis*) induce apoptosis of HepG-2 cells by the fas-signaling pathway. Asian Pacific Journal of Cancer Prevention. 2014;15:9319–9325.

[35] Wang L.T., Pan S.L., Chen T.H., Dong Y.Z., Lee K.H., Teng C.M. DYZ-2-90, a novel neotanshinlactone ring-opened compound, induces ERK-mediated mitotic arrest and subsequent apoptosis by activating JNK in human colorectal cancer cells. ChemBioChem. 2012;13:1663–1672.

[36] Appadurai P., Rathinasamy K. Indicine N-oxide binds to tubulin at a distinct site and inhibits the assembly of microtubules: a mechanism for its cytotoxic activity. Toxicology Letters. 2014;225:66–77.

[37] Wani M.C., Horwitz S.B. Nature as a remarkable chemist: a personal story of the discovery and development of taxol. Anti-Cancer Drugs. 2014;25:482–487.

[38] Horwitz S.B.. Taxol (paclitaxel): mechanisms of action. Annals of Oncology. 1994;5 Suppl 6:S3–S6.

[39] Xiao H., Verdier-Pinard P., Fernandez-Fuentes N., Burd B., Angeletti R., Fiser A., Horwitz S.B., Orr G. Insights into the mechanism of microtubule stabilization by Taxol. Proceedings of the National Academy of Sciences of the United States of America. 2006;103:10166–10173.

[40] Ringel I., Horwitz S.B. Studies with RP 56976 (Taxotere). A new semisynthetic analogue of taxol, Journal of the National Cancer Institute. 1989;83:288–291.

[41] Rowinsky E.K. The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. Annual Review of Medicine. 1997;48:353–374.

[42] Dorr R.T. Pharmacology of the taxanes. Pharmacotherapy. 1997;17:96S–104S.

[43] Hennequin C., Giocanti N., Favaudon V. S-phase specificity of cell killing by docetaxel (Taxotere) in synchronized HeLa cells. British Journal of Cancer. 1995;71:1194–1198.

[44] Hemmenfent K.L., Govindan R. Novel formulations of taxanes: a review. Old wine in a new bottle?. Annals of Oncology. 2006;17:735–749.

[45] Chen C.H., Liao C.H., Chang Y.L., Guh J.H., Pan S.L., Teng C.M. Protopine, a novel microtubule-stabilizing agent, causes mitotic arrest and apoptotic cell death in human hormone-refractory prostate cancer cell lines. Cancer Letters. 2012;315:1–11.

[46] Buey, R.M., Barasoain, I., Jackson, E., Meyer, A., Giannakakou, P., Paterson, I., Mooberry, S., Andreu, J.M., Diaz J.F. Microtubule interactions with chemically diverse stabilizing agents: thermodynamics of binding to the paclitaxel site predicts cytotoxicity. Chemistry and Biology. 2005;12:1269–1279.

[47] Tinley T.L., Randall-Hlubek D.A., Leal R.M., Jackson E.M, Cessac J.W., Quada, J.C., Hemscheidt T.K., Mooberry S.L. Taccalonolides E and A: plant-derived steroids with microtubule-stabilizing activity. Cancer Research. 2003;63:3211–3220.

[48] Li J., Risinger A.L., Peng, J., Chen, Z., Hu L., Mooberry S.L.J. Hydrolysis reactions of the taccalonolides reveal structure activity relationships. Journal of Natural Products. 2013;76:1369–1375.

[49] Peng J., Risinger A.L., Fest G.A., Jackson E.M., Helms G., Polin L.A., Mooberry S.L. Identification and biological activities of new taccalonolide microtubule stabilizers. Journal of Medicinal Chemistry. 2011;54:6117–6124.

[50] Mukhtar E., Adhami V.M., Sechi M., Mukhtar H. Dietary flavonoid fisetin binds to β-tubulin and disrupts microtubule dynamics in prostate cancer cells. Cancer Letters. 2015;367:173–183.

[51] Higgs H.N., Pollard T.D. Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. Annual Review of Biochemistry. 2001;70:649–676.

[52] Dominguez R., Holmes K.C. Actin structure and function. Annual Review of Biophysics. 2011;40:169–186.

[53] dos Remedios C.G., Chhabra D., Kekic M., Dedova I.V., Tsubakihara M., Berry D.A., Nosworthy N.J. Actin binding proteins: regulation of cytoskeletal microfilaments. Physiological Reviews. 2003;83:433–473.

[54] Somers K.D., Murphey M.M. Multinucleation in response to cytochalasin B: a common feature in several human tumor cell lines. Cancer Research. 1982;42:2575–2578.

[55] Somers K.D., Murphey M.M. Cytochalasin B-induced multinucleation of human tumor and normal cell cultures. Cell Biology International Reports. 1980;4:487–495.

[56] Medina D., Oborn C.J., Asch B.B. Distinction between preneoplastic and neoplastic mammary cell populations *in vitro* by cytochalasin B-induced multinucleation. Cancer Research. 1980;40:329–333.

[57] Trendowski M., Yu G., Wong V., Acquafondata C., Christen T., Fondy T.P. The real deal: using cytochalasin B in sonodynamic therapy to preferentially damage leukemia cells. Anticancer Research. 2014;34:2195–2202.

[58] Fayard B., Bianchi F., Dey J., Moreno E., Djaffer S., Hynes N.E., Monard D. The serine protease inhibitor protease nexin-1 controls mammary cancer metastasis through LRP-1-mediated MMP-9 expression. Cancer Research. 2009;69:5690–5698.

[59] Worthylake R.A., Burridge K. RhoA and ROCK promote migration by limiting membrane protrusions. Journal of Biological Chemistry. 2003;278:13578–13584.

[60] Yamazaki D., Kurisu S., Takenawa T. Regulation of cancer cell motility through actin reorganization. Cancer Science. 2005;96:379–386.

[61] Azios N.G., Krishnamoorthy L., Harris M., Cubano L.A., Cammer M., Dharmawardhane S.F. Estrogen and resveratrol regulate rac and Cdc42 signaling to the actin cytoskeleton of metastatic breast cancer cells. Neoplasia. 2007;9:147–158.

[62] Hamdi H.K., Castellon R.. Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. Biochemical and Biophysical Research Communications. 2005;334:769–778.

[63] Machado P.R., Alvaro F.S., Marcos P., de Avila S.L., da Silva B.V., et al. Alkaloids derived from flowers of Senna spectabilis, (−)-cassine and (−)-spectaline, have antiproliferative

activity on HepG2 cells for inducing cell cycle arrest in G1/S transition through ERK inactivation and downregulation of cyclin D1 expression. Toxicology *In Vitro*. 2016;31:86–92.

[64] Lee W.L., Shyur L.F. Deoxyelephantopin impedes mammary adenocarcinoma cell motility by inhibiting calpain-mediated adhesion dynamics and inducing reactive oxygen species and aggresome formation. Free Radical Biology & Medicine. 2012;52:1423–1436.

[65] Duncan K., Duncan M., Alley M.C., Edward A., Sausville E.A. Cucurbitacin E-induced disruption of the actin and vimentin cytoskeleton in prostate carcinoma cells. Biochemical Pharmacology. 1996;52:1553–1560.

[66] Ma G., Luo W., Lu J., Ma D., Leung C.H., Wang Y., Chen X. Cucurbitacin E induces caspase-dependent apoptosis and protective autophagy mediated by ROS in lung cancer cells. Chemico-Biological Interactions. 2016;253:1e9.

[67] Sari-Hassoun M., Clement M.J., Hamdi I., Bollot G., Bauvais C., Joshi V., Toma F., Burgo A., Cailleret M., Rosales-Hernández M.C., Macias Pérez M.E., Chabane-Sari D., Curmi P.A. Cucurbitacin I elicits the formation of actin/phospho-myosin II co-aggregates by stimulation of the RhoA/ROCK pathway and inhibition of LIM-kinase. Biochemical Pharmacology. 2016;102:45–63.

[68] Haritunians T., Gueller S., Zhang L., Badr R., Yin D., Xing H., Fung M.C., Koeffler H.P. Cucurbitacin B induces differentiation, cell cycle arrest, and actin cytoskeletal alterations in myeloid leukemia cells. Leukemia Research. 2008;32:1366–1373.

[69] Sawitzky H., Liebe S., Willingale-Theune J., Menzel D. The anti-proliferative agent jasplakinolide rearranges the actin cytoskeleton of plant cells. European Journal of Cell Biology. 1999;78:424–433.

[70] Lu Q.Y., Jin Y.S., Zhang Q., Zhang Z., Heber D., Go V.L., Li F.P., Rao J.Y. *Ganoderma lucidum* extracts inhibit growth and induce actin polymerization in bladder cancer cells *in vitro*. Cancer Letters. 2004;216:9–20.

[71] Velasco-Velázquez M.A., Agramonte-Hevia J., Barrera D., Jiménez-Orozco A., García-Mondragón M.J., Mendoza-Patiño N., Landa A., Mandoki J. 4-Hydroxycoumarin disorganizes the actin cytoskeleton in B16–F10 melanoma cells but not in B82 fibroblasts, decreasing their adhesion to extracellular matrix proteins and motility. Cancer Letters. 2003;198:179–186.

[72] Cooper G.M. The Cell: A Molecular Approach, 2nd ed.. Sinauer Associates. 2000.

[73] Chen Y.L., Lin S.Z., Chang W.L., Cheng Y.L., Harn H.J. Requirement for ERK activation in acetone extract identified from Bupleurrum scorzonerifolium induced A549 tumor cell apoptosis and keratin 8 phosphorylation. Life Sciences. 2005;76:2409–2420.

[74] Oshima R.G., Baribault H., Caulín C. Oncogenic regulation and function of keratins 8 and 18. Cancer and Metastasis Reviews. 1996;15:445–471.

[75] Weng Y.R., Cui Y., Fang J.Y. Biological functions of cytokeratin 18 in cancer. Molecular Cancer Research. 2012;10:485–493.

[76] Pankov R., Umezawa A., Maki R., Der C.J., Hauser C.A., Oshima R.G. Oncogene activation of human keratin 18 transcription via the Ras signal transduction pathway. Proceedings of the National Academy of Sciences of the United States of America. 1994;91:873–877.

[77] Oshima R.G. Apoptosis and keratin intermediate filaments. Cell Death and Differentiation. 2002;9:486–492.

[78] Fortier A.M., Asselin E., Cadrin M. Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through claudin1 upregulation. Journal of Biological Chemistry. 2013;288:11555–11571.

[79] Cheng Y.L., Chang W.L., Lee S.C., Liu Y.G., Lin H.C., Chen C.J., Yen C.Y., Yu D.S., Lin S.Z., Harn H.J. Acetone extract of *Bupleurum scorzonerifolium* inhibits proliferation of A549 human lung cancer cells via inducing apoptosis and suppressing telomerase activity. Life Sciences. 2003;73:2383–2394.

[80] Bhat F.A., Sharmila G., Balakrishnan S., Arunkumar R., Elumalai P., Suganya S., Singh R.P., Srinivasan N., Arunakaran J. Quercetin reverses EGF-induced epithelial to mesenchymal transition and invasiveness in prostate cancer (PC-3) cell line via EGFR/PI3K/Akt pathway. Journal of Nutritional Biochemistry. 2014;25:1132–1139.

[81] Li Y., Vanden Boom T.G., Kong D., Wang Z., Ali S., Philip P.A., et al. Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. Cancer Research. 2009;69:6704–6712.

[82] Kim Y.S., Choi K.C., Hwang K.A. Genistein suppressed epithelial–mesenchymal transition and migration efficacies of BG-1 ovarian cancer cells activated by estrogenic chemicals via estrogen receptor pathway and downregulation of TGF-β signaling pathway. Phytomedicine. 2015;22:993–999.

[83] Polachi N., Bai G., Li T., Chu Y., Wang X., Li S., Gu N., Wu J., Li W., Zhang Y., Zhou S., Sun H., Liu C. Modulatory effects of silibinin in various cell signaling pathways against liver disorders and cancer—a comprehensive review. European Journal of Medicinal Chemistry. 2016;123:577–595.

[84] Cufí S., Bonavia R., Vazquez-Martin A., Corominas-Faja B., Oliveras-Ferraros C., Cuyàs E., Martin-Castillo B., Barrajón-Catalán E., Visa J., Segura-Carretero A., Bosch-Barrera J., Joven J., Micol V., Menendez J.A. Silibinin meglumine, a water-soluble form of milk thistle silymarin, is an orally active anti-cancer agent that impedes the epithelial-to-mesenchymal transition (EMT) in EGFR-mutant non-small-cell lung carcinoma cells. Food and Chemical Toxicology. 2013;60:360–368.

[85] Naveen C.R., Gaikwad S., Agrawal-Rajput R. Berberine induces neuronal differentiation through inhibition of cancer stemness and epithelial-mesenchymal transition in neuroblastoma cells. Phytomedicine. 2016;23:736–744.

[86] Lee S.W., Mandinova A. Patent application title: methods for the treatment of cancer using piperlongumine and piperlongumine. Analogs 2009. WO 20090312373.

[87] Gupta P., Wright S.E., Kim S.H., Srivastava S.K. Phenethyl isothiocyanate: a comprehensive review of anti-cancer mechanisms Biochimica et Biophysica Acta. 2014;1846:405–424.

[88] Bargagna-Mohan P., Hamza A., Kim Y, Ho K.H., Mor-Vaknin N., Wendschlag N., Liu J., Evans R.M., Markovitz, D.M., Zhan C.G., Kim K.B., Wendschlag N., Liu J., Evans R.M., Markovitz D.M., Zhan C.G., Kim K.B., Mohan R. The tumor inhibitor and antiangiogenic agent withaferin A targets the intermediate filament protein vimentin. Chemistry & Biology. 2007;14:623–634.

[89] Cerqueira Coelho P.L., Villas-Boas de Freitas S.R., Seara Pitanga B.P., da Silva V.D.A., Oliveira M.N., Grangeiro M.S., dos Santos Souza C., dos Santos El-Bachá R., Costa M.D., Barbosa P.R., de Oliveira Nascimento I.L., Lima Costa S. Flavonoids from the Brazilian plant croton betulaster inhibit the growth of human glioblastoma cells and induce apoptosis. Revista Brasileira de Farmacognosia. 2016;26:34–43.

[90] Monsuez J.J., Charniot J.C., Vignat N., Artigou J.Y. Cardiac side-effects of cancer chemotherapy. International Journal of Cardiology. 2010;144:3–15.

[91] Hsiu-Chuan Y., Oberley T.D., Vichitbandha S., Ye-Shhi H.O., St Clair D.K. The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. Journal of Clinical Investigation. 1996;98:1253–1260.

[92] Ito H., Miller S.C., Billingham M.E., Akimoto H., Torti S.V., Wade R. Doxorubicin selectively inhibits muscle gene expression in cardiac muscles cells *in vivo* and *in vitro*. Proceedings of the National Academy of Sciences of the United States of America. 1990;87:4275–4279.

[93] Sussman M.A., Hamm-Alvarez S.F., Vilalta P.M., Welch S., Kedes L. Involvement of phosphorylation in doxorubicin-mediated myofibril degeneration. Circulation Research. 1997;80:52–61.

[94] Lim C.C., Zuppinger C., Guo X., Kuster G.M., Helmes M., Eppenberger H.M., Suter T.M. et al. Anthracyclines induce calpain-dependent titin proteolysis and necrosis in cardiomyocytes. Journal of Biological Chemistry. 2004;279:8290–8299.

[95] Chen Y., Daosukho C., Opii W.O., Turner D.M., Pierce W.M., Klein J.B., Vore M. et al. Redox proteomic identification of oxidized cardiac proteins in adriamycin-treated mice. Free Radical Biology and Medicine. 2006;41:1470–1477.

[96] Anghel N., Cotoraci C., Ivan A., Suciu M., Herman H., Balta C., Nicolescu L., Olariu T., Galajda Z., Ardelean A., Hermenean A. Chrysin attenuates cardiomyocyte apoptosis and loss of intermediate filaments in a mouse model of mitoxantrone cardiotoxicity. Histology and Histopathology. 2015;30:1465–1475.

[97] Fisher P.W., Salloum F., Das A., Hyder H., Kukreja R.C. Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity. Circulation Journal. 2005;111:1601–1610.