Chapter

The Use of Plants' Natural Products in Breast Cancer: Have We Already Found the New Anticancer Drug?

Isadora de Fátima Braga Magalhães, Kátia da Silva Calabrese, Ana Letícia Marinho Figueirêdo, Ana Lucia Abreu-Silva and Fernando Almeida-Souza

Abstract

The importance of a new anticancer drug for breast cancer is well established. Natural compounds that can prevent this disease or be used as an adjuvant treatment associated with conventional drugs could be the solution for this. This chapter is an overview of agents extracted from plants with outstand results in the last six years. Green tea, berberine, thymoquinone and cannabidiol are compounds isolated from medicinal plants. These agents showed action through induction of apoptosis, down regulation of inflammation, epigenetics, hormonal modulation, among other. In vitro effect against cancer cells, in vivo experiments mainly with murine model and clinical trials reassured their efficacy against breast cancer. A protective effect against recurrence cases and chemosensitization to standard drugs was also successful. The use of nanotechnology provided a optimize delivery of these therapeutical molecules. Taken together this information led us to acknowledgement that we do probably have the natural agents for a future adjuvant treatment against breast cancer.

Keywords: plants, phytotherapy, breast cancer, green tea, berberine, thymoquinone, cannabidiol, anticancer

1. Introduction

Breast cancer (BC) remains one of the leading causes of death [1] and one of the most common types of malignancies among women worldwide [2]. The conventional treatment includes chemotherapy, hormone therapy, radiotherapy and surgery. Problems such as high recurrence and toxicity to medication are frequent [1]. Due to this, the combination of the conventional treatment with a new approach is the key to a higher degree of success in the therapeutic of this disease.

Complementary therapies are already used among many women who have BC to help dealing with adverse effects or against recurrence. Phytotherapy is one of the most popular adjuvant therapies and a common target for a new BC drug [1]. Plant-derived anticancer therapeutics aim to reduce side effects and increase the sensitivity to chemotherapy and the overall effectiveness of the treatment [3].

Although there is a false believe in the harmlessness of plants, they can cause negative effects and even reduce the therapeutic effects of standard drugs. Therefore, is crucial to understand their correct dosage and potential effects [1].

Over the past decades, many bioactive phytochemicals from medicinal plants have being studied and some of them have remarkable results. Among all these natural compounds, it is natural to ask ourselves: have we found the answer yet? What is the role of plant derived compounds in reducing breast cancer cells and promoting survival and less recurrence among breast cancer patients? This chapter is an overview of agents extracted from plants with outstand results in the last six years. Therefore, can be helpful in trying to answer these questions.

2. Green tea

Tea has become one of the most popular beverages all over the world. The consumption of green tea gained popularity in the last years and is now associated with a different lifestyle. Green tea, *Camellia sinensis* [4], has shown anticancer effect on different types of cancer [5] and apparently possess many chemopreventive qualities in primary breast cancer and recurrence [6].

In the search for different forms that green tea can act as an anticancer agent in BC, hormonal modulation has been considered. Supplementation with decaffeinated green tea extract significantly increases circulating of estradiol in healthy postmenopausal women. The consumption of green tea extract also reduces circulation of cholesterol and LDL-cholesterol [7], and the regular consume seems to facilitate lipid metabolisms in breast cancer survivors [8]. In a study among Chinese women in Hong Kong, drinking green tea was not associated with overall breast cancer risk, which may be masked by the differential effect in pre- and post-menopausal women, due to modified hormone receptor expression [9].

A great potential as a chemo-preventive agent against breast cancer can be attributed to green tea, especially for recurrence [6]. Drinking at least five cups of green tea per week may be associated with decreased breast cancer risk [10].

Tamoxifen is an adjuvant treatment for hormone receptor-positive breast cancer, but drug resistance related to genetic and epigenetic mechanisms is rising to dangerously levels [11]. Tamoxifen has no pharmacokinetic interaction with green tea [12] which can encourage this association.

Green tea high inhibited the proliferation of cell line derived from breast cancer in mouse, named 4TI cells, with upregulation of Casp8, Casp9, Casp3, Casp6, Casp8AP2, Aifm1, Aifm2 and Apopt1 genes [13]. When associated with silicon nanomaterials, this compound has action against breast cancer in vitro and in vivo with inhibition of tumor growth [14].

Catechin, epicatechin, epigallocatechin and epigallocatechin-3-gallate (EGCG) are the four major constituents of green tea [15]. EGCG has demonstrated potential anticancer effects on several preclinical and clinical researches [16].

Epigenetics are non-mutational events that alter the expression of genes [17]. Catechins from green tea, especially EGCG, are be able to modulate epigenetic processes by increasing transcription of tumor suppressor genes through attenuating the effect of DNA methyltransferase 1 (DNMT1) and consequently reversing DNA methylation [18].

The most active anticancer component in green tea is EGCG [19]. EGCC might act on breast cancer cells progression through inhibition of focal adhesion kinase (FAK) signaling pathway [20], by interfering in proteins involved in cell death and survival, DNA replication, recombination and repair [21]. It can also interfere on the expression of genes such as PTEN, CASP3, CASP9 [22] and through inhibition

of protein tyrosine phosphatase 1B (PTP1B) activity [23], a protein that plays a crucial role in the development of breast cancer, with higher levels associated with tumor size and lymph node metastasis in patients [24]. EGCG also decreases BC cells with similar results as tamoxifen [22].

A protective effect of EGCG on BC was reaffirmed on several experimental models and different conditions with promising clinical implications for breast cancer prevention and therapy [25].

A lecithin formulation of a caffeine-free green tea catechin extract named green select phytosome (GSP) increased the bioavailability of EGCG on early breast cancer patients who received GSP in 300 mg dose, daily, for 4 weeks prior to surgery [26].

Capsules of green tea with a high dose (843 mg) of EGCG provided during 12 months reduced the percent of mammographic density in younger women similar to tamoxifen. This indicates a possible chemo preventive effect on breast cancer risk, although no effect was detected on older women [27]. The treatment with EGCG can also act through epigenetic by reduction of the expression and activity of DNA methyltransferase and decreasing of methylation of the domain-containing epidermal growth factor-like 2 (2SCUBE2), a tumor suppressor that inhibits BC cells migration and invasion [28].

EGCG and quercetin isolated from green tea and green tea alone had anticarcinogenic effect on estrogen receptor-positive and -negative breast cancer cells [29]. It also showed in vitro and in vivo effect, by inhibiting the growth of 4 T1 tumor and increasing the proportions of CD4+ and CD8+ T cells at tumor sites in mice. EGCG regulated the canonical and non-canonical pathways in myeloid-derived suppressor cells (MDSCs) [30].

The action of polyphenols from green tea on BC cells was mediated by apoptosis thought mitochondrial pathway with induction of DNA fragmentation and activation of caspase-3 and caspase-9 [31].

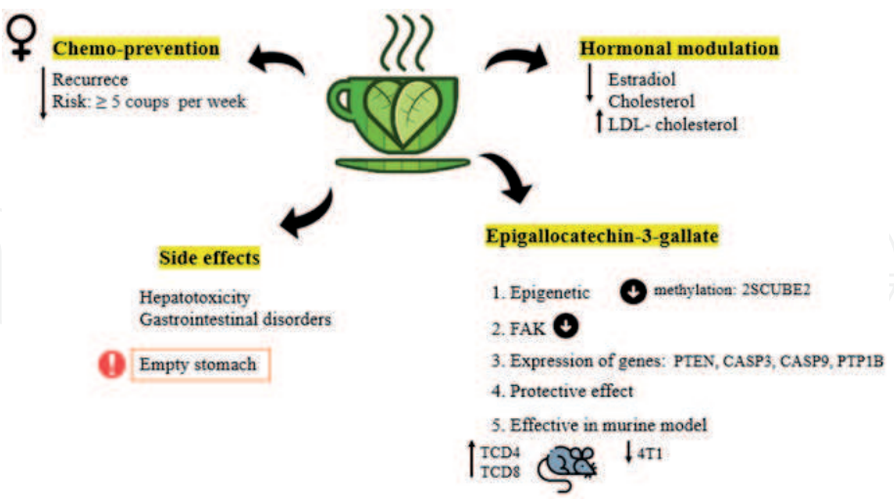

Figure 1.
Green tea and breast cancer. Hormonal modulation. The consumption of green tea causes hormonal modulation through depression of estradiol and cholesterol levels and increase of LDL-cholesterol levels. ***Epigallocatechin-3-gallate****. Epigallocatechin-3-gallate isolated from green tea causes effects in epigenetic by decreasing methylation of tumor suppressors such as epidermal growth factor-like 2 (2SCUBE2); inhibits focal adhesion kinase (FAK) signaling pathway; interferes in the expression of genes such as phosphatase and tensin homolog (PTEN), caspase-3 (CASP3), caspase-9 (CASP9) and protein-tyrosine phosphatase 1B (PTP1B); has protective effect against breast cancer and is effective in murine model by decreasing breast cancer cells 4 T1 and increasing the proportions of CD4+ and CD8+ T cells at tumor sites.* ***Side effects.*** *Hepatotoxicity and gastrointestinal disorders are reported mainly when tea is consumed with an empty stomach.* ***Chemo-prevention.*** *Green tea is a chemo-preventive in women causing less recurrence cases and also lower risk of breast cancer when at least 5 cups of tea are consumed per week.*

Matcha green tea (MGT), a special type of green tea with higher concentrations of catechins due to a different preparation [32] inhibits mTOR, stimulates an antioxidant response and interferes in interleukin signaling in BC cells [33].

Some side effects have been associated with green tea such as hepatotoxicity and gastrointestinal disorders, especially if consumed on an empty stomach (**Figure 1**). Although green tea and its main components are not major teratogen, mutagen or carcinogen substances and have a selective cytotoxicity against cancer cells, some caution needs to be taken in pregnant and breast-feeding women due to the lack of information [4]. Between Japanese women, green tea was the most commonly consumed non-alcoholic beverage, and it had no significant associations with breast cancer risk [34].

3. Berberine

Berberine(5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a] quinolizinium) is an alkaloid with several properties, such as hepatoprotective, immunomodulatory, cardioprotective and antioxidative. Plants containing this compound have been traditionally used in different parts of the world for the treatment of affections in the eyes, inflammatory diseases, dermatitis, wound healing, digestive and respiratory diseases and treatment of neoplasia [35].

A wide variety of different plant species contains berberine (BBR), such as *Coptis chinensis* [36], *Rhizoma coptidis* [37], *Berberis vulgaris* [38], *Arcangelisia flava*, *Berberis aquifolium* and *Berberis aristate*. The genus Berberis contains nearly 550 species [39].

Due to the ability to seize the cell cycle and induce apoptosis of cancer cells, berberine has received considerable research attention [40]. BBR can inhibit tumor growth and metastasis of triple negative breast cancer cells (TNBC) by suppression of transforming growth factor beta 1 (TGF-β1) expression, a multifunctional factor associated with poor prognosis on BC. It also decreased lung metastasis and tumor growth in MDA-MB-231, a cell line originated from an invasive ductal carcinoma, and 4 T1 breast cancer xenograft models [41]. Same effect was detected in tumor growth in MDA-MB-231 nude mouse xenografts, with binding of vasodilator-stimulated phosphoprotein (VASP), related to cell migration and overexpressed in high-motility BC cells [42] and throught caspase-9 pathway [43]. At a 50 mg/kg dose, BBR demonstrated a preventive role in rats with mammary ductal and invasive carcinoma [40].

BBR suppresses breast cancer cells through inhibition of transforming growth factor beta 1 (TGF-β1) expression [41], targeting ephrin-B2 [44], AMPK signaling pathway [45], inhibition of specific activator protein-1 (AP-1) activity [46], triggering to a caspase9-dependent apoptosis [43], affecting mRNA levels of chemokine receptors genes such as C-X-C motif chemokine receptor 1 (CXCR1) and C-X-C motif chemokine receptor 4 (CXCR4) [47] and by inducing nucleolar stress and upregulation of p53, a tumor suppressor gene [48].

The anti-cancer ability of BBR against BC may be partially dependent on the regulation of metadherin [49] and attenuation of inflammation through inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation [50] and reduction of secretion of proinflammatory cytokines such as interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [51]. BBR also reduces interleukin-8 (IL-8) secretion, which is associated with poor outcomes involving metastasis-free survival and relapse-free [52].

In animals, the inhibition of canine mammary gland carcinoma cells, highlights its potential against the most frequent cause of cancer in female dogs [53].

Salt-inducible kinases 3 (SIK3) belong to the AMPK-related family of kinases, and when highly expressed is associated with poor survival among BC patients. This kinase was significantly inhibited by the combination of emodin and BBR [54].

Another successful combination happened between lapatinib and BBR, and reversed the resistance to lapatinib through downregulating of c-Myc, a gene often expressed in cancer [55]. The association between BBR and doxorubicin increased chemosensitivity to this agent [45] and reverted the resistance by inhibiting autophagy [56].

The combination of theophylline and berberine showed a synergistic antiproliferation effect on MDA-MB-231 cells, with a less necrotic effect and increased apoptotic cell death [57]. A sensibilization of BC cells to the chemotherapeutic drugs cisplatin, camptothecin and methyl methanesulfonate was provide by the association with BBR by a deoxyribonucleic acid (DNA) repair pathway involving XRCC1, a protein involved in the efficient repair of DNA [58].

Berberine and tamoxifen together induced cell growth inhibition more effectively than tamoxifen alone [59] and BBR with evodiamine synergistically induced cell cycle arrest and apoptosis of MCF-7 cells [60], an established breast cancer cell line originated from a pleural effusion of a patient with invasive breast ductal carcinoma.

Synergetic effect between poly (lactic-co-glycolic acid) nanoparticles with doxorubicin conjugate for encapsulation and BBR increased rat half-life and antiproliferative action against BC cells [61]. The encapsulation with citrate-capped silver nanoparticles was also efficient [62].

The association of BBR and exercise, consider an immunotherapy treatment, against BC showed a synergistic effect in vitro and in mice, throught the improvement of the immune system, regulation of intestinal microbial metabolite and activation of apoptosis [36].

Although there are many positive outcomes on the therapy with BBR, a low dose of berberine caused attenuation on chemotherapeutic drugs fluorouracil (5-FU) and camptothecin activities [37] (**Figure 2**). Therefore, some caution needs to be taken in regards to its use.

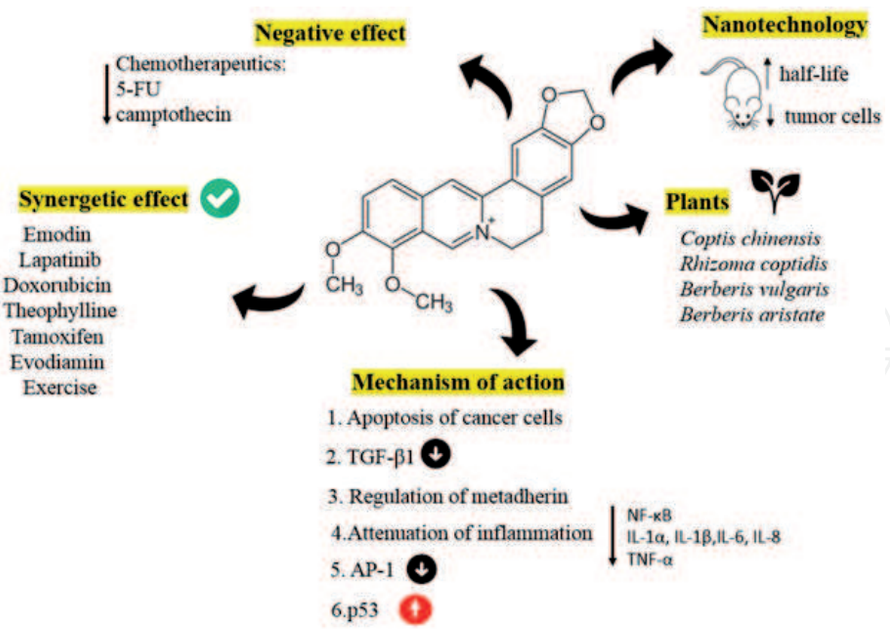

Figure 2.
Berberine and breast cancer. Plants. Many plants can contain berberine, such as Coptis chinensis, Rhizoma coptidis, Berberis vulgaris and Berberis aristate. ***Mechanism of action.*** *Berberine induces apoptosis of cancer cells; reduces transforming growth factor beta 1 (TGF-β1) expression; regulates metadherin; attenuates inflammation by inhibition of nuclear factor kappa B (NF-κB) activation and reduction of interleukins IL-1α, IL-1β, IL-6, IL-8 and tumor necrosis factor-α secretions.* ***Synergetic effect.*** *Between berberine and emodin, lapatinib, doxorubicin, theophylline, tamoxifen, evodiamin, and also with physical exercise.* ***Negative effect.*** *Berberine causes attenuation on chemotherapeutic drugs fluorouracil (5-FU) and camptothecin activities.* ***Nanotechnology.*** *Nanotechnology provides an increased half-life and reduced tumor cells in rats with induced breast cancer.*

4. Thymoquinone

Black cumin seed from *Nigella sativa*, also known as cumin, is used for centuries and it has unsurpassed traditional medicinal value and versatility to treat a wide range of diseases [63]. *N. sativa* is the source of the monoterpene thymoquinone, a compound that can cause anticancer effect in proliferation, migration and invasion in different human cancers including breast cancer lineages [64].

Thymoquinone (TQ) induces apoptosis through death receptors, inhibits TNBC cell line and also can cause in vivo effects on mouse tumor model [65]. TQ inhibits autophagic activity and expression of Beclin-1 and LC3 in TNBC cells and suppresses pathways related to cell invasion and angiogenesis, including integrin-β1, vascular endothelial growth fator (VEGF), matrix metalloproteinase-2 (MMP-2) and MMP-9, suggesting that TQ may be used to control autophagic activity and oncogenic signaling in TNBC [66]. In silico docking studies confirm the action against TNBC, with thymoquinone down-regulating poly (ADP-ribose) polymerase (PARP) gene expression, docking metastatic, apoptotic and cell proliferation targets [67].

TQ-induced ceramide accumulation and endoplasmic reticulum stress, decreased S1P, C1P and NF-κB, triggered apoptosis in BC cells [68] and also changed the cell cycle progression [69].

The combination between different anticancer drugs can be helpful to reduce dose causing less side effects and reducing multidrug resistance. Synergic effect was detected with a combination of TQ with paclitaxel [65] and also with resveratrol, causing a decrease in tumor size, enhanced apoptosis, decreased VEGF expression, elevated levels of interferon-gama (IFN-γ), angiogenesis inhibition with no toxic effect on liver or kidney [70].

TQ and piperine, another bioactive compound of *N. sativa*, together caused an anticancer action in vitro and in vivo in murine model by angiogenesis inhibition, induction of high degrees of apoptosis and shifting the immune response towards a T helper1 response [71], a similar result to the combination with melatonin [72]. When associated with gemcitabine, TQ caused a better anti-cancer activity via modulation of apoptotic and autophagic action [73].

A combined doxorubicin thymoquinone-loaded with aragonite calcium carbonate nanoparticle showed higher efficacy against BC cells at lower dose of doxorubicin and TQ [74]. In a clinical trial, combination between tamoxifen and TQ had a better effect than each of these drugs alone on patients with BC [2].

TQ and black cumin seed oil anticancer effect in BC in female rats induced by 7,12-dimethylbenz[a]anthracene (DMBA), caused alteration on rates of tumor markers such as malondialdehyde (MDA), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and decrease of the expression of Brca1, Brca2, Id-1 and P53 mutations which highlights a protective effective against BC [63]. In xenograft tumors in mice, TQ inhibited metastasis through enhanced promotion of DNA methylation of the TWIST1 gene [75].
A reduced in drug resistance, anti-migratory potency and tumor size in ex-ovo xenograft was possible with TQ associated with Emodin [76].

Using a metastasis breast cancer mouse model, TQ treatment suppressed multiple metastases in bone, brain, lungs. This effect was attributed to down-regulation of NF-κB and chemokine receptor type 4 (CXCR4) expression, an indicator of poor prognosis in patients (**Figure 3**) [77].

Agents than can make a compound more available and deliver a more efficient effect are being searched. The encapsulation of TQ in nanoparticles improved the bioavailability of this compound [78], and a nanostructured lipid carrier enhanced the therapeutic qualities of TQ by increasing the survival rate of mice [79]. Same

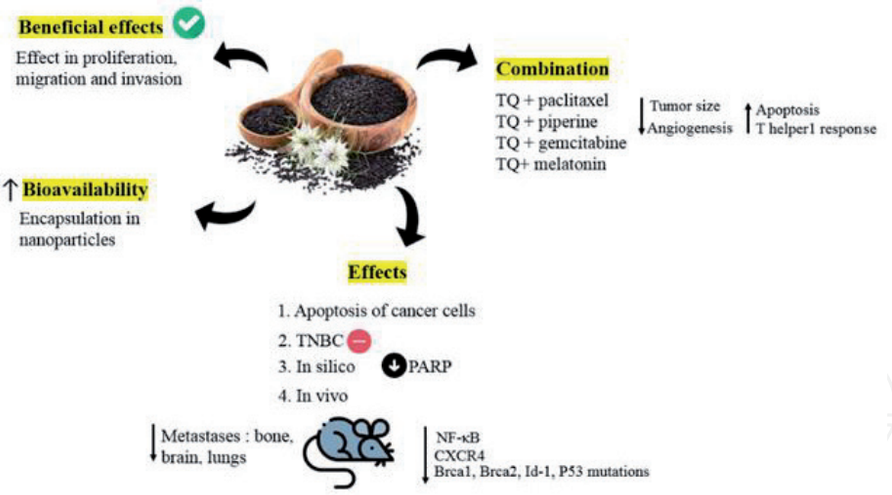

Figure 3.
Thymoquinone and breast cancer. Combination. The combination of thymoquinone (TQ) and paclitaxel, piperine, gemcitabine and melatonin have synergic effects including reduction of tumor size and angiogenesis, increasing of cancer cells apoptosis and shifting of the immune response towards a T helper1 response. ***Effects.*** *The effects in breast cancer includes induction of apoptosis; antiproliferative effect in triple negative breast cancer (TNBC) cells; anticancer effects in silico experiments by downregulation of poly (ADP-ribose) polymerase (PARP) gene expression; suppression of metastases in bone, brain and lungs, down-regulation of nuclear factor kappa B (NF-κB), chemokine receptor type 4 (CXCR4) and Brca1, Brca2, id-1 and P53 mutations expression.* ***Bioavailability.*** *The use of nanothenology increased the bioavailability of thymoquinone.* ***Beneficial effects.*** *Overall action against proliferation, migration and invasion of breast cancer cells was confirmed.*

effects were obtained with low-molecular-weight chitosan-grafted lipid nanocapsules to co-delivery docetaxel and TQ [80], cubosomal nanoparticles used to encapsulate TQ [81] and cabazitaxel and TQ co-loaded lipospheres [82].

5. Cannabidiol

Cannabidiol (CBD) is the main non-psychoactive component of *Cannabis sativa* [83]. Although researches related to cannabis derivates need to face a lot of misunderstanding due to association with psychoactive effects and recreation [84], cannabidiol has proven to stimulate apoptosis pathways and inhibit metastasis, angiogenesis and proliferation of different cancer cells [85].

Cannabidiol can act in cancer cells in a receptor-independent way but also thought CB-receptors such as CB1-R, with moderated expression in BC, and CB2-R with high expression related to tumor aggressiveness. Breast cancer positive for the protein human epidermal growth factor receptor 2 (HER2+), a protein that promotes cancer cells growth, when associated with expression of a cannabinoid receptor (CB2) is associated to poor patient prognosis, therefore can be used as a biomarker with prognostic value [86].

Cannabidiol has an antiproliferative effect against aggressive subtype of BC cells, and also inhibits tumor growth in murine model by interfering in the recruitment of macrophages [83]. The anti-cancer effects of CBD on BC cells are related to regulatory effects on the biogenesis of exosomes and microvesicles released by cells and involved in intercellular communication [87] and by inducing apoptosis with down-regulation of mammalian target of rapamycin (mTOR) and cyclin D1 and up-regulation of peroxisome proliferator-activated receptor gamma (PPARγ) protein expression [88]. CBD also blocks and reverts the effect of IL-1β involved in the change to a malignant phenotype through epithelial-mesenchymal transition [89].

Mice treated with a combination of CBD and doxorubicin had reduced tumor weight and increased apoptosis than the animals treated with CBD or doxorubicin alone [90]. The co-administration of CBD in solution and paclitaxel or doxorubicin showed a synergistic effect, and cannabidiol-loaded microparticles extended release of this compound, causing an optimized action [91]. The cannabinoid combination of tetrahydrocannabinol, cannabigerol, cannabinol and cannabidiol induced apoptosis in BC cell line in a reduced dose with good selectivity, killing BC cells with minimized harmful effects to normal cells [92].

In low dose of 40 mg/day, the treatment with CBD inhibited cytochrome P450 3A4 and P450 2D6, enzymes with important role in cancer treatment, and increased endoxifen levels in a woman with a history of bilateral breast carcinoma in remission [93]. Typical cannabinoids and abnormal cannabidiol had antiproliferative effects on paclitaxel-resistant BC cells and they both reduced tumor growth in zebrafish xenograft model [94].

A botanical drug preparation was more potent than delta-9-tetrahydrocannabinol, a pure compound, as an anticancer agent in cell culture and animal model, which highlights the potential of standardized cannabis drug preparations to manage BC [95]. Synthetic cannabidiol was analyzed in 119 cancer patients over a four-year period and it led to a reduced circulation of tumor cells or reduced tumor size with no side-effects [96].

A precursor of cannabidiol, cannabidiol acid, downregulates the proto-oncogene c-fos and the cyclooxygenase-2 (COX-2) signaling, an anti-inflammatory response that can be linked to the anticancer activity [97].

An effective result in reducing symptoms associated with tumors such as anorexia, nausea and neuropathic pain, and also to decelerate tumor progression in earlier breast cancer cases was attributed to cannabidiol (**Figure 4**) [98].

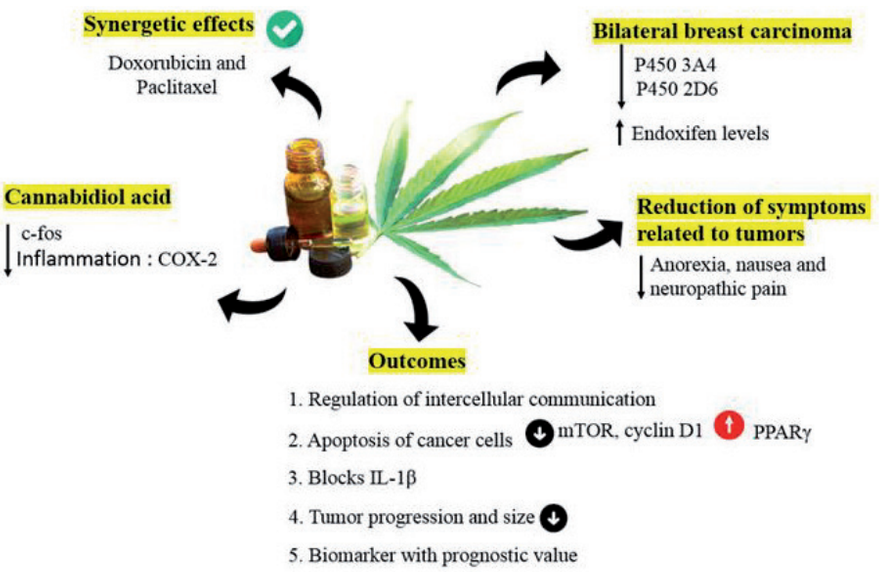

Figure 4.
Cannabidiol and breast cancer. Bilateral breast carcinoma. Treatment with cannabidiol inhibited cytochrome P450 3A4 and P450 2D6 and increased endoxifen levels in a woman with a history of bilateral breast carcinoma. ***Reduction of symptoms related to tumors.*** *Cannabidiol reduces symptoms associated with tumors such as anorexia, nausea and neuropathic pain.* ***Outcomes.*** *Cannabidiol regulates intercellular communication; induces apoptosis of breast cancer cells through down-regulation of mammalian target of rapamycin (mTOR) and cyclin D1 and up-regulation of peroxisome proliferator-activated receptor gamma (PPARγ) protein expression; blocks the effect of IL-1β; reduces tumor size and tumor progression; cannabinoid receptor can be used as biomarker with prognostic value.* ***Cannabidiol acid.*** *Demonstrated anti-inflammatory response by proto-oncogene c-fos and cyclooxygenase-2 (COX-2) signaling downregulation.* ***Synergetic effects.*** *The combination between cannabidiol with doxorubicin or paclitaxel increased the anticancer action.*

6. Conclusions

The need for a more efficient therapeutic to treat breast cancer is imminent, due to side effects and chemoresistance associated with the current treatment. In these chapter we demonstrated the potential of natural compounds to be used as a new anticancer drug against breast cancer.

A natural compound that can protect against one of the most lethal cancers can save thousands of women's lives, and green tea, berberine, thymoquinone and cannabidiol all showed this capability at different experiments.

In vitro action through multiple pathways involving apoptosis and epigenetics, and in vivo experiments, mainly with mouse model, showed decrease of tumor size and angiogenesis. A stimulation of an anti-inflammatory response with cannabidiol, thymoquinone or berberine treatment reveled another link to an anticancer response.

These agents promoted chemo sensitization, making breast cancer cells more sensible to the effect of drugs used in conventional treatment. A synergistic effect with other natural products or standard drugs such as tamoxifen, paclitaxel, doxorubicin and cisplatin were able to reaffirm the possibility of these combination to reduce dose and side effects.

Antiproliferative action against triple-negative breast cancer cells emphasizes the potential of these therapeutic molecules to treat aggressive and difficult cases of breast cancer. Several clinical trials including a large period of time demonstrated a protective and preventive role of these phytocompounds, with almost no side-effects.

Nanotechnology was often used to increase the bioavailability, create a target-oriented delivery and also to provide an effective lower dose of phytomedicine against breast cancer.

Taken together this information led us to acknowledgement that we do probably have the natural agents for a future adjuvant treatment against breast cancer.

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Conflict of interest

The authors declare no conflict of interest.

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