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Role of phytochemicals in cell signaling

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ABSTRACT

Many dietary phytochemicals exhibit health-beneficial effects including prevention of diseases such as cancer, neurological, cardiovascular, inflammatory, and metabolic disorders. Medicinal plants described in Ayurveda and traditional Chinese literatures are the key knowledge resources that inspired molecular research for drug development world over. A precise regulation of redox balance is required for the cellular homeostatic control. Aberrant activation of redox-sensitive transcription factors contributes to carcinogenesis by promoting persistent inflammation, abnormal cell proliferation, evasion from apoptosis, angiogenesis, etc. A wide variety of dietary phytochemicals have been reported to exert cancer chemopreventive properties by suppressing the inappropriate activation of the transcription factors. On the other hand, transcription of genes involved in the activation of cellular antioxidant arsenal and carcinogen detoxification is largely regulated by redox-sensitive transcription factors. Chemoprevention, the use of drugs or natural compounds at pharmacological levels to inhibit the development of cancer, is currently attracting a great deal of interest, particularly in USA, but many of the same mechanisms may be triggered by phytochemicals. Mechanistically, chemoprevention can be achieved by enhancing cellular antioxidant and detoxification capacity, promoting carcinogen detoxification, suppressing abnormally activated pro-inflammatory signaling pathways, down-regulating expression of proteins involved in cell proliferation, inducing apoptosis of precancerous or malignant cells, and inhibiting neovascularization. Therefore, redox-sensitive transcription factors might be potential targets for chemoprevention with dietary phytochemicals. This paper will focus on how a few representative edible phytochemicals viz. curcumin, aswagandha and neem, can exert chemopreventive effects on oxidative stress and inflammation-associated carcinogenesis through modulation of signal transduction mediated by distinct redox-regulated transcription factors.

KEYWORDS

Phytochemicals;
Aswagandha;
Curcumin;
Neem;
Apoptosis.

INTRODUCTION

Studies indicate that the regular consumption of nonnutritive ingredients derived from plant-based diet, collectively termed phytochemicals, can reduce the risk of certain cancers[1,2]. It is now estimated that more than

1,000 different food-derived phytochemicals possess chemopreventive activities. Examples of dietary chemopreventive phytochemicals include resveratrol and proanthocyanidins from grapes, curcumin from turmeric, epigallocatechin gallate (EGCG) from green tea, sulforaphane and isothiocyanates from broccoli, genistein from soybean, indole- 3-carbinol from cabbage, lycopene from tomato, organosulfur compounds from garlic, gingerol from ginger, caffeic acid phenethyl ester (CAPE) from honey bee, propolis, etc.[3].

Recent progress in unraveling the process of carcinogenesis has identified abnormal functioning of the key components of the intracellular signaling network, especially a panel of redox-sensitive transcription factors. These transcription factors regulate the transcription of a wide variety of genes involved in the maintenance of homeostatic cell growth and proliferation, and the protection of cells from oxidative and other noxious insults. Mechanistically, chemoprevention can be achieved by enhancing cellular antioxidant and detoxification capacity, promoting carcinogen detoxification, suppressing abnormally activated pro-inflammatory signaling pathways, down-regulating expression of proteins involved in cell proliferation, inducing apoptosis of precancerous or malignant cells and inhibiting neovascularization[4]. Therefore, redox-sensitive transcription factors might be potential targets for chemoprevention with dietary phytochemicals.

A precise regulation of redox balance is required for the cellular homeostatic control. Aberrant activation of redox-sensitive transcription factors, such as nuclear factor-kappaB (NF-kB), activator protein 1 (AP-1), cyclic adenosine monophosphate response element binding protein (CREB), and hypoxia inducible factor (HIF), contributes to carcinogenesis by promoting persistent inflammation, abnormal cell proliferation, evasion from apoptosis, angiogenesis, etc. A wide variety of dietary phytochemicals have been reported to exert cancer chemopreventive properties by suppressing the inappropriate activation of aforementioned transcription factors. On the other hand, transcription of genes involved in the activation of cellular antioxidant arsenal and carcinogen detoxification is largely regulated by another redox-sensitive transcription factor, i.e. NF-E2 related factor 2 (Nrf2), which plays a role in protecting cells/tissues from oxidative or electrophilic damage. Some food-derived phytochemicals have been shown to activate Nrf2. Therefore, the modulation of cellular signaling mediated by redox-sensitive transcription factors represents a promising approach in achieving molecular target-based chemoprevention with edible phytochemicals.

CURCUMIN

Curcumin [(1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] is an orange-yellow active component from the herb Curcuma longa (usually known as turmeric) commonly used in the Indian and Eastern Asia. It is an orange-yellow crystalline powder with melting point of 183°C, molecular formula of $C_{21}H_{20}O_6$, and molecular weight of 368.37 g/mol. The essential structure of this molecule consists of feruloylmethane skeleton. It is now clear that there are four major curcuminoids namely curcumin, demethoxycurcumin, bis-demethoxycurcumin, and a new identified cyclocurcumin occurring naturally in Curcuma species[5,6].

As a medicine, curcumin is shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities, and thus has a potential to fight against various diseases including diabetes, asthma, allergies, arthritis, atherosclerosis, neurodegenerative diseases and other chronic illnesses like cancers[7].

Curcumin has been extensively studied in modern medicine and Indian systems of medicine for the treatment of various medical conditions, including cystic fibrosis, hemorrhoids, gastric ulcer, colon cancer, breast cancer, atherosclerosis, liver diseases and arthritis. It has been used in various types of treatments for dementia and traumatic brain injury. Curcumin also has a potential role in the prevention and treatment of Alzheimer's disease (AD). Curcumin as an antioxidant, anti-inflammatory and being lipophilic improves the cognitive functions in patients with Alzheimer's disease. A growing body of evidence indicates that oxidative stress, free radicals, beta amyloid, cerebral deregulation caused by bio-metal toxicity and abnormal inflammatory reactions contribute to the key event in Alzheimer's disease

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pathology. Due to various effects of curcumin, such as decreased beta-amyloid plaques, delayed degradation of neurons, metal-chelation, anti-inflammatory, antioxidant and decreased microglia formation, the overall memory in patients with AD has improved[8].

MDM2, the cellular ubiquitin E3 ligase of the tumor suppressor p53, is considered to be an oncoprotein because of its activity in promoting p53 ubiquitination and proteasomal degradation[9,10]. Further, MDM2 binds to the NH_2 terminus of p53 and blocks its transactivational activities[10]. The activation of p53 target genes induces apoptosis, cell cycle arrest, and senescence, which are important to tumor suppression[11,12]. Recently, p53-independent tumorigenic mechanisms for MDM2 have been identified[13]. MDM2 promotes cell cycle progression by binding to and modulating the activities of p21Waf1/CIP1[14,15] and E2F1 proteins[16]. Both animal studies with transgenic mice and clinical observations have established the role of MDM2 in cancer development and the response to treatment, both dependent and independent of p53[17,18].

Curcumin downregulates MDM2 expression in cells with either wild-type (WT) or nonfunctional p53 and that this effect is at the transcriptional level. MDM2 transcription is regulated by the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR)/ erythroblastosis virus transcription factor 2 (ETS2) pathways, which is modulated by curcumin. Curcumin inhibits MDM2 expression in both normal and cancerous human cell lines, independent of p53 activity. In the PC3 human prostate cancer cell line, MDM2 levels were decreased by curcumin in a dose and time-dependent manner. Curcumin inhibited MDM2 in cell lines with either WT p53 (LNCaP and MCF-7) or with p53 knockdown (p53 KD; MCF-7 p53 KD)[19]. In addition, p21Waf1/CIP1 and Bax were induced, whereas E2F1 and Bcl2 were decreased in PC3 cells, likely as a result of MDM2 inhibition. The inhibitory effect of curcumin on MDM2 was also noted in the human normal breast cell line MCF10A[9].

PI3K-mediated signaling is one of the most frequently targeted pathways in human cancers[20]. Activated PI3K promotes cell survival and proliferation through mechanisms that are not fully understood[20]. mTOR, a highly conserved serine/threonine kinase activated by PI3K, is involved in cancer initiation and progression[21].

PI3K/Akt has been implicated in MDM2 protein stabilization and subcellular localization[22-24]. PI3K induces MDM2 transcription through mTOR/ETS2, suggesting that these proteins may govern the mechanisms by which mitogens promote cell proliferation and inactivate p53. Because MDM2 is tumorigenic even in the absence of p53, the presented results provide a mechanism by which mitogens promote cancer progression independent of functional p53[9].

Curcumin inhibits cell proliferation, interrupts the cell cycle, and induces apoptosis in cancer cells[25,26]. Curcumin inhibits proliferation of normal as well as malignant cells, but it induces apoptosis mainly in malignant cells[25].

Reduced thioredoxin is a direct electron donor to peroxiredoxins or thioredoxin peroxidases, which are major hydrogen peroxide- scavenging enzymes normally keep the level of reactive oxygen species in the cell under control. Methionine sulfoxide reductases utilize reduced thioredoxin as an electron donor, and these enzymes serve as specific scavengers of ROS via reversible oxidation of methionine residues[27,28]. The curcumin inhibition of TrxR will lead to an oxidized intracellular environment, and this oxidative stress should stop cell proliferation in normal cells, which do not overproduce cyclins.

Curcumin can irreversibly inhibit TrxR activity by forming covalent adducts and that the inhibition is NADPH-dependent. The modified residues of the enzyme are in the active site, *i.e.* Cys496 and Sec497, which form a selenol/thiol after NADPH reduction[29-31]. In the reduced form generated by NADPH, the active site residues Cys496 and Sec497 are present in the form of free –SH/-SeH groups and exposed at the surface of the enzyme[31], making them easily attacked by alkylating agents[32]. In the oxidized enzyme there is a nonreactive Cys496-Sec497 selenenylsulfide bridge[29].

The α,β-unsaturated ketone structure in curcumin makes it act as a potential alkylator. There is an equilibrium between α,β-unsaturated ketone and its enol form, and the latter is more sensitively attacked by nucleophilic agents. The selenide, with high nucleophilicity and

exposed to the surface of the enzyme, can attack the carbon cation in the enol form of curcumin effectively and produce the covalent adduct.

Obviously, inhibition of TrxR, which will directly affect many redox functions of Trx, should be an important mechanism to explain the antitumor effects of curcumin. Curcumin-modified enzyme had a strongly induced NADPH oxidase activity and produced ROS in the presence of oxygen.

The thioredoxin system, composed of TrxR, Trx, and NADPH, is the major disulfide reducing enzyme system in all cells responsible for maintaining the intracellular redox milieu with a high content of free protein thiols and rare disulfides[33-35]. Separate TrxR and Trx enzymes operate in the cytosol and the mitochondria, and both Trx and TrxR have been described to move into the nucleus in cells consistent with their role in regulation of binding of transcription factors to DNA directly or via the redox activity of ApeI/Ref-1[36]. Trx and TrxR, the major protein disulfide reductase of the cell, have a large range of functions in enzymatic reductions and play multiple roles in intracellular signaling and resistance against oxidative stress. In particular, TrxR levels in tumor cell lines are often 10 times higher than those in normal tissues[37,38] perhaps because tumor cell proliferation is dependent on a constant supply of deoxyribonucleotides and initiation of protein synthesis, both of which require an active thioredoxin system[33,34,39,40]. Another role of the thioredoxin system is to protect against apoptosis, which may be important particularly in tumor cells. Extracellular thioredoxin acts as a growth factor, protecting the tumor cell from natural killer lysin and tumor necrosis factor (TNF) and from respiratory burst of immune cells[41,42]. Considering the tumor-promoting effect of TrxR described above and the much higher levels of the enzyme in tumor cells, with up to 0.5% of total soluble proteins in mammary adenocarcinoma cell lines[37,38], it is clear that this selenoenzyme has major roles in transformed cells.

A specific function of reduced thioredoxin in prevention of apoptosis is via binding with ASK-1. This mitogen-activated protein kinase kinase kinase plays an essential role in apoptosis and is activated by many stress- and cytokine-related stimuli. Saitoh *et al*.[43] found that reduced Trx, but not oxidized Trx, bound directly to the N terminus of ASK-1 and inhibited ASK-1 kinase activity as well as the ASK-1-dependent apoptosis. Curcumin will inactivate TrxR and make it unable to reduce oxidized Trx with loss of the activity to bind ASK-1, causing the signaling cascades ultimately inducing apoptosis.

The most dramatic outcome of the curcumin-modified TrxR was its strongly induced NADPH oxidase activity producing ROS. Thus, the enzyme is converted into a prooxidant rather than an antioxidant. Now the higher levels of TrxR in tumor cells will not act as a protection but on the contrary will produce a lot more ROS. Low concentrations of ROS are involved in proliferation, differentiation, and regulation of transcription factors such as NF-κB[44]. ROS activate transcriptionof NF-κB to the nucleus where ApeI/Ref-1 is involved together with reduced Trx in binding to DNA. Excessive ROS production by modified TrxR will obviously destroy the NF-κB survival mechanism.

Several effects of curcumin on regulation of signaling pathways can be directly explained by inhibition of TrxR and excessive generation of ROS. Curcumin can impair p53 function in colon cancer cells and B cells as reported by Moos *et al*.[45] and Han *et al*.[46], respectively. Also the activity of activator protein 1 (AP-1) was suppressed by curcumin in cultured human promyelocytic leukemia cells[47]. Four of the nine cysteines present in p53 DNA-binding domain are essential for the activity of this factor[50]. This site-specific DNA binding of p53 and transcriptional activity are controlled by thiol redox state, which is regulated by the thioredoxin system. The DNA binding activity of activator protein 1 is facilitated by a nuclear redox protein, ApeI/Ref-1[48]. As discussed above, Trx associates directly with ApeI/Ref-1 in the nucleus and modulates Ref-1 activity. This protein-protein interaction requires the active site cysteine residues in the reduced Trx active site[49]. The thioredoxin system, besides being the main electron donor to peroxiredoxins and directly acting as a ROS scavenger[50], can regenerate vitamin C from ascorbate free radical[51] and dehydroascorbate[51], lipoic acid[52], and ubiquinone[53] to maintain the low molecular weight antioxidant levels in cells. Inhibition of TrxR will impair the antioxidant defense against oxidative stress, ROS will accumulate

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from mitochondrial sources, and most importantly TrxR modified by curcumin will generate ROS. Recent reports by Kang *et al*.[54] showed that high concentrations (higher than 25 _M) of curcumin promoted ROS generation, whereas low concentrations (less than 10 _M) usually diminished ROS in Hep3B cells. The effect was not inhibitable by cycloheximide, consistent with modification of preexisting TrxR.

ASWAGANDHA

Withania somnifera (WS), also known as ashwagandha, has been used as an important herb in the Ayurvedic and indigenous medical systems for over 3000 years[55]. Studies indicate ashwagandha possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary, and central nervous systems.The mechanisms of action for these properties are not fully understood[56].

The chemistry of WS has been extensively studied and over 35 chemical constituents have been identified, extracted, and isolated[19]. The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X)[56]. Two main classes of compounds—steroidal alkaloids and steroidal lactones—may account for its broad range of beneficial effects. Steroidal lactones comprise a class of constituents called withanolides. To date, scientists have identified and studied at least 12 alkaloids and 35 withanolides. Much of ashwagandha's pharmacological activity has been attributed to two primary withanolides, withaferin A and withanolide D[55]. Withanolides are C28-steroidal lactones based on an intact or rearranged ergostane frame through appropriate oxidations at C-22 and C-26 to form a d-lactone ring, and are chemically called 22-hydroxy ergostane-26-oic acid 26, 22-lactone. Withaferin A (WA) is one of the major and most predominant withanolides found in the plant[57]. The pure compound, WA, established itself as antiproliferative[58,59], anti angiogenesis in breast cancer cell lines[60], apoptosis-inducing activity in prostate[61] and HL-60 cells and oxidative stress inducing in cell lines[62].

Several studies support the role of ashwagandha as an effective cancer chemopreventive agent, however limited information is available regarding the mechanism of regulation of these chemopreventive effects[63,64]. Results showed that Ashwagandha treatment modulated several functionally important classes of genes, which are associated with cell cycle regulation, regulation of apoptosis, modulation of stress proteins, cytokine and chemokine regulation, regulation of signal transduction, and oncogene regulation in prostate cancer cells (PC-3)[63]. The three significant pathways that are significantly modulated are JAK-STAT pathway, the apoptosis pathway and the MAPK signaling pathway. Among these the JAK-STAT pathway appears to be the key because it also modulates both the apoptosis process and the MAPK signaling[63].

Ashwagandha treatment significantly downregulated the gene and protein expression of proinflammatory cytokines IL-6, IL-1b, chemokine IL-8, Hsp70 and STAT-2, while a reciprocal upregulation was observed in gene and protein expression of p38 MAPK, PI3K, caspase 6, Cyclin D and c-myc. Among the 249 genes that were significantly modulated by Ashwagandha, several were structural proteins, like filamin A, laminin and tight junction protein-zona occludens 2. The significant antitumor effects of flavonoids are attributed to the modulation of protein tyrosine phosphorylation[65]. Suppression of carcinogenesis by tumor suppressor genes is mediated by constraining tumor cell proliferation and colony formation. The tumor suppressor genes, suppresses tumor growth by modulating the phosphatidylinositol 3-kinase (PI3K) and causes G1 cell cycle arrest and cell death[66-68]. The cyclin D gene encodes a regulatory subunit of the CDK4 and CDK6 holoenzyme complex that phosphorylates and inactivates the tumor suppressor protein, pRB, thereby modulating cell cycle progression into late G1 and S phases[68-70].

Ashwangandha treatment down regulates the expression of the proinflammatory cytokines IL-6, IL-8 and IL-1b hereby acting as an immunomodulator, via the dynamic regulation of these cytokines. These cytokines are crucial to innate and adaptive inflammatory responses, cell growth and differentiation, cell death, angiogenesis

and developmental as well as repair processes. The janus kinase/signal transducer and activator of transduction (JAK/STAT) pathway is a intracellular signal-transducing pathway that is activated by oxygen radicals, various cytokines, and growth factors in various disease states[71,72]. Originally described as the regulator for cytokine signaling, JAK/STAT pathway is now recognized as an important membrane-to-nucleus signaling pathway for a variety of stress responses and oxidative stress. STAT proteins have the dual function of signal transduction and activation of transcription as part of a phosphorylation cascade[71]. Ashwagandha downregulates the expression of STAT2 which is a potent transactivator and forms stable homodimers which complex with adaptor protein p48 and bind to the interferon-stimulated response element (ISRE) thereby modulating cell proliferation and immunity.

Expression of Hsp70 in tumor cells has been proposed to enhance their immunogenicity and prevent tumor cell death[73]. Ashwagandha treatment significantly decreases the gene and protein expression of Hsp70, which may result in the generation of a specific immune response by promoting apoptosis. The oncogene c-myc gene participates in most aspects of cellular function, including replication, growth, metabolism, differentiation, and apoptosis[74,75]. Our studies highlight that treatment of PC-3 cells with Ashwagandha results in the activation of c-Jun N-terminal kinase (JNK) signaling, indicating that it may be the key signaling pathway involved in the process leading to activation of apoptosis in these cancer cells[63].

The p38MAPK activity has been reported to be associated with apoptotic induction in several cell types and in response to a multitude of cellular stress[57,76]. In the p38MAPK signaling cascade, signaling is initiated by phosphorylation of p38MAPK at Thr180, Tyr182 and subsequently HSP27. Such phosphorylation may be responsible for a change in the tertiary structure of HSP27 and increased concentrations that possibly modulate cell viability by actin polymerization and reorganization[77,78]. The active p38MAPK also phosphorylated another important transcription factor ATF-2 in WA-treated cells, which has been implicated in the regulation of wide sets of genes that force the leukemic cells to undergo apoptosis. Taken together, our results indicate that early initiation of the p38MAPK signaling cascade within 2.5 h is a central event for the induction of apoptosis by WA.

In the complex signaling events of apoptosis, the p38MAPK activation reduces the mitochondrial function initiated by alterations in the ratio of pro-(Bax) and antiapoptotic (Bcl-2) members of the mitochondria causing the loss of $\Delta\Psi_m$, release of cytochrome c and activation of caspases leading to apoptosis[79]. Bcl-2 directly or indirectly prevents the release of cytochrome c from mitochondria, and its BH_4 domain can bind to the C terminal part of Apaf-1, thus inhibiting the association of caspase-9 with Apaf-1 and ultimately inhibiting apoptosis[80].

NEEM

Azadirachta indica A. Juss, commonly known as neem, elaborates a vast array of bioactive phytochemicals that exhibit potent medicinal properties[81,82]. Mitochondria, which play a pivotal role in apoptosis, are major sites of ROS generation. Excessive ROS generation can lead to opening of the mitochondrial permeability transition pore with consequent release of cytochrome c from the intermembrane space into the cytosol culminating in activation of the caspase cascade and apoptotic cell death[84]. Bcl-2, the major antiapoptotic protein of the Bcl-2 family inhibits ROS production, cytochrome c release, and caspase-3 activation, whereas Bax, is a pore-forming proapoptotic protein that facilitates cytochrome c release, triggering caspase-mediated apoptotic cell death. Bcl-2 and Bax have become attractive targets for designing new anticancer drugs, and agents that lower the Bcl-2/Bax ratio are regarded as promising chemopreventive and chemotherapeutic agents[84,85]. A decrease in the Bcl-2/Bax ratio seen after exposure of BeWo cells to nimbolide in the present study together with increased expression of Apaf-1 and caspase-3, and cleavage of PARP, a nuclear enzyme involved in DNA repair, and maintenance of genomic integrity, provide compelling evidence that nimbolide induced apoptosis is mediated by the mitochondrial pathway[81].

Bcl-2, a major anti-apoptotic protein inhibits apoptosis by preventing mitochondrial release of cyto-

chrome C eventually resulting in inhibition of caspase activity[86,87]. Bim, a BH3-only proapoptotic member of antiapoptotic proteins such as Bcl-XL and Bcl- w. In addition, Bim has been reported to induce cytochrome C release from the mitochondria[88]. The release of cytochrome C from the mitochondria is also induced by caspase 8, an initiator caspase that links the death receptor and mitochondrial pathways of apoptosis. Caspase 3 is an effector caspase that executes cell death by cleavage of proteins vital for cell survival[89]. Overexpression of antiapoptotic proteins with downregulation of proapoptotic proteins and caspases has been documented in malignancies[90,91]. Enhanced expression of Bcl-2 associated with diminished expression of Bim, caspase 8 and caspase 3 seen in the present study may facilitate evasion of apoptosis and development of the malignant phenotype in HBP carcinomas[86].

There is compelling evidence to show that azadirachtin and nimbolide transduce apoptosis by both the mitochondrial and death receptor pathways. In addition, both the limonoids also activated initiator and effector caspases and enforced nuclear localization of survivin enabling increased susceptibility to intrinsic apoptosis. The intracellular localisation of survivin is crucial for its anti-apoptotic function; while cytosolic localisation enables interaction with caspases and Smac/DIABLO thereby blocking apoptosis, nuclear localisation favours apoptosis by increasing p53 and Bax levels[92,93]. Upregulation of wild type p53 in turn has been reported to ablate the anti-apoptotic activity of survivin, and induce a number of propapoptotic factors including Bax and caspases[94]. Nimbolide induced cell cycle arrest and antiproliferative effects in a wide range of human cancer cell lines[95-97]. Cells treated with nimbolide exhibited apoptotic features characterised by nuclear condensation and fragmentation, progressive vacuolization, membrane blebbing, increased appearance of annexin V positive cells, increase in the number of cells in the sub-G1 fraction, expression of phosphatidylserine in the outer cell membrane, generation of reactive oxygen species, and caspase-dependent apoptosis[96,98,99]. The higher efficacy of nimbolide has been attributed to its α,β-unsaturated ketone element[100].

PCNA, a cofactor for DNA polymerase δ that plays a central role in the cell cycle also blocks apoptosis by inhibition of Gadd45 and MyD118, negative regulators of growth[101,102]. Activation of wild type p53 exerts its antitumoral effects through activation or inactivation of Bcl-2 family proteins[103]. In particular, targeting NF-κB that plays a vital role in cell proliferation, differentiation, apoptosis, inflammation, stress response, and several signal transduction pathways is considered a novel preventive and therapeutic strategy against human cancers[104,105]. NF-κB undergoes activation via the canonical pathway leading to the dissociation of p50-p65 subunits with subsequent nuclear translocation and activation of downstream target genes involved in carcinogenesis. Thus inhibition of PCNA and nuclear translocation of NF-κB with upregulation of IκB and p53 by azadirachtin and nimbolide may be key factors for suppressing the growth of HBP carcinomas and inducing apoptosis[92].

Combination chemoprevention has attracted the focus of research attention due to its high potency and reduced toxicity. Further, medicinal plants in combination are shown to interact synergistically with high efficacy and have a broader spectrum of action. The decrease in Bcl-2/Bax ratio, a reliable indicator of the overall propensity of a cell to undergo apoptosis coupled with the overexpression of cytochrome C and caspase-3, underscores the apoptosis-inducing potential of the AI(*Azadirachta indica)*-OS (*Ocimum sanctum*) combination. The combinatorial chemopreventive potential of Al and OS leaf extract seen in the present study may be ascribed to the rich array of constituent phytochemicals that are known to exhibit potent antiproliferative properties. The use of the AI-OS combination is a strategic approach to administer a cocktail of phytochemical entities that could modulate multiple signal transduction pathways that are aberrant in cancer. Furthermore, synergistic interactions among the phytochemicals can ensure higher efficacy and potency in addition to overcoming problems of toxicity and resistance[106].

CONCLUSION

The health benefits of the three phytochemicals –

curcumin, aswagandha and neem has been discussed in this paper. The regular intake of these photochemicals has been proved to be beneficial. These phytochemicals have some properties in common, like they have anti-oxidant compounds, they are anticancerous and they have antitumorigenic properties and have antibacterial properties.

In the current era of molecular target-based chemoprevention, many food factors, especially phytochemicals present in the regular diet, have been explored as promising cancer chemopreventive agents, which modulate the function of one or more of redox-regulated transcription factors.

While the magnitude of cancer as a global threat is so frightening, there are glimpses of hope flared through the chemoprevention research conducted with the phytochemicals. Numerous food-derived phytochemicals have been shown to be effective in preventing malignant transformation of cells in culture and experimentally induced tumorigenesis in various animal models in vivo.

Extensive studies on synergistic pharmacodynamic interactions of the phytochemicals as well as the effects on signal modulation are essential for the development of multiactive natural drugs for cancer chemoprevention in the future.

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