Chemopreventive prospective of dietary spices against hepatocellular carcinoma

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Hepatocellular carcinoma (HCC), a primary liver cancer, is one of the most fatal cancers having universal prevalence. Developing countries of Asia and Africa are reported with more HCC cases compared to the United States and Europe. Surgical resection and liver transplantation present limited treatment choices for HCC. It is need of the hour to investigate promising alternative chemopreventive and therapeutic strategies to control the disease. In most cases HCC develops and progresses in an environment of inflammation and oxidative stress. Phytochemicals such as dietary spices and their active components gifted with potent anti-inflammatory and antioxidant properties, offer an appropriate alternative mitigation of HCC. Ginger, turmeric and garlic are the commonly used spices. Studies suggest that these have anti-inflammatory, antioxidant and antitumour activities. This article reviews their apoptotic, anti-inflammatory and antioxidant effects as well as involvement of various molecular signalling pathways.

Keywords: Apoptosis, carcinogenesis, chemoprevention, hepatocellular carcinoma, inflammation.

HEPATOCELLULAR carcinoma (HCC) is the fifth leading form of cancer in men and the eighth in women[1,2]. HCC is the third largest reason for cancer-related deaths all over the world with about 700,000 deaths documented annually[3,4]. Although majority of HCC cases is a consequence of illness owing to the hepatitis B and C viruses, major risk factors for this include obesity, alcoholic and non-alcoholic cirrhosis and iron overload, in addition to dietary hepatocarcinogens, for example aflatoxins and nitrosoamines[5–7]. Surgery and liver transplant together offer limited treatment choices for HCC. Numerous factors, including tumour size, multifocality in conjunction with vascular invasion limit the selection of surgical resection[8]. While liver transplantation is functional in the cure of early-phase HCC, it is accessible barely to a minute number of patients mainly because of the shortage of donor organs. The success of the transplant option is also strictly limited by the rapid and repeated recurrence of HCC in the transplanted liver[9].

Plants play a fundamental role for the survival of life on earth. There is no record that people in prehistoric times used synthetic medicines for their ailments; they tried to make use of the things they could easily find in their environment, i.e. plants[10]. This awareness about herbal medications was passed on to generations in the form of folk medicine. The use of herbs, both internally and externally, has constantly been a key feature in the practice of medicine. The healing advantage of medicinal plants is recognized by almost every society today[11]. In recent times, there is greater focus on the studies on complementary and alternative remedies that are in use for the treatment of cancer. There is a long history on the use of plants in the treatment of cancer[12]. Sixty per cent of the presently used anticancer mediators have originated from natural sources[13].

Chemopreventive effect of ginger

Ginger is amongst the most commonly used dietary supplements throughout the world. Ginger oleoresin and its polyphenols, including zingerone, shogaol and 6-gingerol have been examined widely for their antioxidative and chemopreventive properties in carcinogenesis[14–16]. Tumour-infiltrating inflammatory cells produce different types of pro-inflammatory cytokines including IL-6, IL-1, TNF-α and interferon-γ. Pro-inflammatory cytokines contribute to carcinogenesis by affecting the proliferation, mutation, growth, survival and movement of tumour cells. Pro-inflammatory cytokines can activate the transcriptional factor NFκB, while some of the effects of pro-inflammatory cytokines may be mediated through the NFκB pathway. Many studies have linked the NFκB signalling pathway and carcinogenesis. Thus, inhibiting the NFκB signalling pathway might be a very effective therapeutic strategy. Ginger extract has the ability to down-regulate NFκB regulated gene outcomes involved in tumourigenesis and angiogenesis[17–19]. Ginger components (gingerols and zerumbone) possibly act as an anti-inflammatory and anti-cancer agent by blocking TNF-α and then activation of NFκB[17]. Ginger may interrupt the signalling pathway of NFκB at one or more steps, for example, the signals that activate the translocation of NFκB into the nucleus, NFκB signalling cascade or interactions with the transcriptional machinery. NFκB blockage consequently restrains the growth of tumour cells and block metastasis and angiogenesis (Figure 1)[20].

Reactive oxygen species (ROS), for example, hydroxyl radicals, hydrogen peroxide and superoxide anions are

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formed under normal situations in living systems. Conversely, elevated levels of ROS have been associated with carcinogenesis[15,17,21,22]. Hydroxyl radicals or superoxide anions formed cause oxidative damage of RNA and DNA directing to mutagenesis[22]. To scavenge these free radicals, our body not only relies on endogenous protective antioxidants but also needs exogenous antioxidants, including flavonoids, vitamins A, C, E, and polyphenols obtained from different plants. Plant derivatives play an essential role in cancer prevention, with their ability to scavenge free radicals, inhibit cell growth and induce apoptosis[23]. Oxidative stress and tumour promotion are closely related to each other. Antioxidatives are supposed to act against tumour progression. Ginger extract increases superoxide dismutase (SOD), an antioxidant enzyme activities and inhibit phospholipid peroxidation. Enhanced level of superoxide anions and hydroxyl radicals cause oxidative damage to DNA and RNA and leads to carcinogenesis. SOD might have critically important for cancer prevention because of their capacity to scavenge these free radicals[16].

Ginger components also block lipid peroxidation and scavenge ROS[15]. Decreased level of MDA after treatment with ginger extract is an indication of its protective nature[23,24]. It has been reported[16,25] that the pungent ginger components, for example, [6]-paradol and [6]-gingerol have possible *in vitro* antitumour action against diverse cell lines, including hepatoma cell line, HepG2. Recent studies indicate that some of the food elements like phytochemicals, can simply transform the complex multistage process of carcinogenesis by modification of the genes and cell apoptosis[16]. Thus, ginger acts as an antitumour agent by decreasing oxidative stress and by stimulating immune cells, thus blocking the growth of cancer cells.

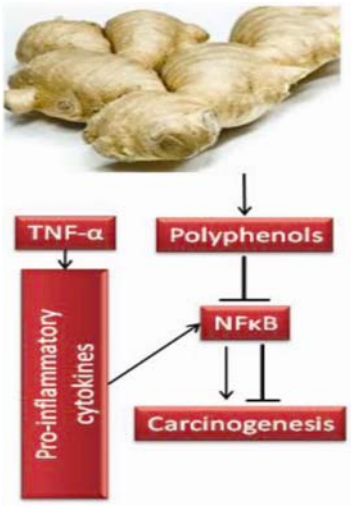

Figure 1. Proposed mechanism for NFκB pathway arrest and anticarcinogenic activity of ginger. NFκB, Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α; Tumour necrosis factor alpha.

Chemopreventive properties of turmeric

Turmeric, also known as Indian saffron, belongs to the ginger family Zingiberaceae, indigenous to the Indian subcontinent and other tropical countries[1]. *Curcuma longa* has a long history of being used as an anti-inflammatory, antiseptic and antibacterial agent for the treatment of infections, swelling, respiratory ailments and rheumatism[20,26,27]. Turmeric has also been featured with anticarcinogenic, antiarthritic, hepatoprotective, cardioprotective and thrombosuppressive properties. Antioxidant, anti-inflamatory and the capability to initiate an array of signalling pathways make curcumin an ideal therapeutic agent[28–30].

Curcumin affects an overabundance of signalling pathways associated with carcinogenesis. It interacts with various cell signalling proteins such as protein kinases, transcriptional factors, cytokine signalling receptors, adhesion molecules and growth factors, in addition to anti-apoptotic proteins. Furanodiene, a curcuma component, stimulates P38 and ERK1/2 MAPK signalling cascades, which are involved in the activation of mitochondrial and caspase-dependent pathways to induce HepG2 cell apoptosis[31]. This includes the stepwise stimulation of depolarization of $\Delta\Psi m$, followed by mitochondrial cytochrome *c* discharge, caspase-3 activation, PARP cleavage, S/G2 cell cycle arrest and finally DNA fragmentation. Caspase-3 activation and proteolytic cleavage of PARP are the most prominent indicators of apoptosis[32]. PARP is basically a nuclear enzyme related to DNA stability and repair[33]. Apoptotic signals are mediated by p38 and JNK, whereas growth, differentiation and proliferation are promoted by ERK. Action of curcumin leads to suppression of several inflammatory cytokines such as chemokines, interleukin (IL)-1, IL-6, IL-8, as well as TNF-α and cyclooxygenases[34,35]. Curcumin treatment also reduces the level of anti-apoptotic protein Bcl-2 with the help of tumour suppressor protein p53 activation. Moreover, a time-dependent activation of caspase-3 and -9 has also been reported in the curcumin-treated HepG2 cells (Figure 2)[36].

The anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax have been documented to control the initiation of apoptosis through the control of mitochondrial function[37–39]. The ion channel perforation action of Bcl-2 and Bax may control apoptosis by changing the permeability of the membranes and cytochrome *c* release from mitochondria. In case of overexpression of Bcl-2, cytochrome *c* release is blocked in reaction to different apoptotic stimuli[37,39–41]. Furthermore, Bcl-2 heterodimerization with Bax exerts dominant negative inhibition of pro-apoptotic Bax activity[42]. Therefore, when the Bcl-2 expression level is low and the Bax expression level is maintained, homodimers of Bax will always be formed and apoptosis will be stimulated[36]. After being liberated from the mitochondria, cytochrome *c* interacts with

Apaf-1 favouring the activation of procaspase-9 (refs 32, 43, 44). Caspase-9 can modulate the activation of caspase-3 and -7 by proteolysis, thereby transmitting the apoptotic indication to the execution phase[45]. Treatment with turmeric results in the activation of procaspase-3, as seen in the formation of a 12 kDa protein which corresponds to the catalytically active subunit[43]. Caspase-7 illustrates[46] the same synthetic substrate specificity as caspase-3, suggesting that caspase-3 and -7 may have overlapping roles in apoptosis[47].

Curcumin is involved in the disturbance of membrane potential of the mitochondria and results in disruption of intracellular-free Ca^{2+} concentration. Intracellular-free calcium [Ca^{2+}] is a universal signalling molecule regulating many cellular functions, including apoptosis[48]. Together the excess and reduction of endoplasmic reticulum Ca^{2+} pool end results are the initiation of ER stress, and further stimulation of apoptotic pathway[49,50].

Oxidative stress has been shown to play a vital part in carcinogenesis. Studies have shown the strong free-radical scavenging capability of curcumin. Free-radical-mediated lipid peroxidation was attenuated by curcumin as well as its curcuminoid analogs in several experimental studies[51–53]. Antioxidant properties of curcuminoids are provided by 1,3 β-diketone moiety, methoxy group and phenolic hydroxyl group[54]. They can provoke intracellular ROS generation, which subsequently induces ERK and JNK activation and increases PUMA, Bax, Fas, and DR4 expression. Upregulation of Fas and DR4 elicits the extrinsic apoptotic pathway by activating caspase-3 and -8; moreover, increasing Bax with decreasing Bcl-2 and Bcl-xL, initiating the intrinsic apoptotic pathway by promoting the release of cytochrome *c* and the subsequent activation of caspase-9 and -3 (ref. 1).

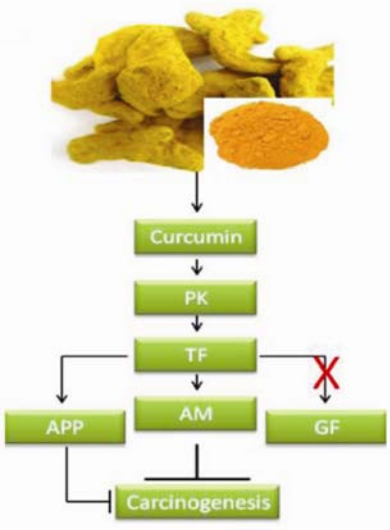

Figure 2. Molecular targets moderated by turmeric. AM, Adhesion molecules; APP, Anti-apoptotic proteins; GF, Growth factors; (Pk) Protein kinase; TF, Transcription factor.

Chemopreventive properties of garlic

Garlic belongs to the family Amaryllidaceae and is a commonly used aromatic plant. Clinical, laboratory and epidemiological investigations have revealed that garlic and its active components, for example, allicin, diallyl sulphide (DAS), diallyl disulphide (DADS) and diallyl trisulphide (DATS), offer a wide range of biological actions, such as antibiotics, blood sugar modulation, antitumorigenesis and antiatherosclerosis[55–57]. Garlic uses several mechanisms to act as antitumorigenic mediator, including a boost in antioxidant defence action and modulation of immune responses and drug metabolizing enzyme activity and reduction in cell proliferation. Immune response directs cytokine secretion and natural killer cell activities[58–60]. DADS antiproliferative activity against human HepG2 was correlated to highly reactive sulfane sulphur. DADS, DAS and DATS having allyl groups were efficient to suppress tumour cell proliferation compared to *S*-ethylcysteine and SAC having one or devoid of allyl group[60]. The antiproliferative activity of garlic against cancer cells is linked to modulations in membrane and cytoplasmic thiols. Thiols are known to be one of the most reactive and ever-present ligands in the biological system. Cells exposure to some disulphides is well-known to alter cellular thiols and important proteins engaged in growth and function of cells. Disturbance of this homeostasis is often pursued by disturbance in intracellular calcium and ATP homeostasis[61,62]. Additionally, DADS can also reduce calcium dependent ATPase action to restrict the calcium transported from cells and to develop an irreversible cell injury[63]. DADS also plays its part in modulation of cyclin-dependent kinases (Cdks). The eukaryotic cell cycle is synchronized by the sequential

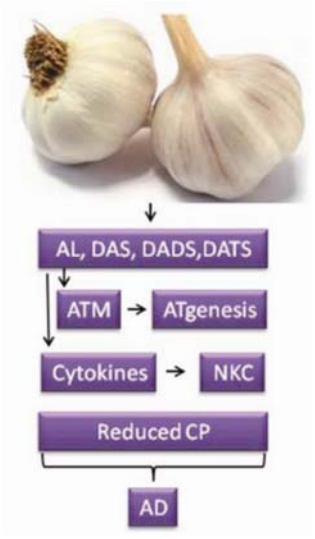

Figure 3. Proposed mechanism for garlic derivatives induced cell cycle arrest and apoptosis. AD, Antioxidant defence; AL, Allicin; ATgenesis, Antitumorigenesis; ATM, Antitum origenic mediator; CP, Cell proliferation; DADS, Diallyl disulphide; DAS, Diallyl sulphide; DATS, Diallyl trisulphide; NKC, Natural killer cell.

activation and inactivation of Cdks that drive cell cycle sequence through phosphorylation and dephosphorylation of numerous regulatory proteins (Figure 3)[64].

Garlic components not only inhibit expression of P450 2E1, P450 1A1, 3A1 and 2B1, but also increase the level of mRNA and proteins in the cell. DAS in rat livers was metabolized into diallyl sulphoxide and diallyl sulphone. Diallyl sulphoxide has been shown to be responsible for the increase in mRNA and protein expression[65]. Diallyl sulphone has been reported to participate in the inhibition of *N*-nitrosodimethylamine demethylase activity and the decrease in P450 level in the hepatic microsomes of rats treated with DAS[66]. Results of different studies show that garlic allyl sulphides simultaneously modulate a couple of P450 genes, however, with changeable efficacy[67]. Thus garlic allyl sulphides differentially modulate the hepatic P450s, mRNA and protein levels, and the effectiveness is associated with the number of sulphur atoms in the molecule.

Conclusion

The essential basis for the potential utilization of dietary spices as chemopreventive and therapeutic mediators in HCC depends on their potent anti-inflammatory and antioxidant activities as well as their ability to modulate signalling mechanisms. It is evident from the *in vitro* and *in vivo* models that ginger, turmeric and garlic hold immense promise as therapeutic agents for HCC.

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