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TARGETING APOPTOSIS WITH COMPOUNDS FROM COMMONLY-USED MEDICINAL PLANTS: A POSSIBLE AID IN THE FIGHT AGAINST CANCER?

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ABSTRACT. THE AIM OF THIS REVIEW is to provide an updated overview of experimental in vitro and in vivo investigations focusing on the anticancer activity of few herbs specially phytochemicals from daily kitchen ingredients. Potential use of these natural agents in cancer therapy and chemoprevention is also discussed. Studies using animal models and cultured human malignant cell lines have demonstrated antitumor and cancer preventive activities of many herbs and their main ingredients. Possible mechanisms of few of these (e.g., thymoquinone from nigella sativa, curcumin from turmeric, and organosulfur compounds from garlic) along with their biological activities are discussed here.

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1. INTRODUCTION

1.1. CANCER – A DREADFUL DISEASE

Cancer is one of the most dreadful diseases of mankind not only due to its disease pattern but also due to failure of treatment and cost involved in the therapy. Researchers throughout the world have contributed on their part by working on different aspects of cancer, like cellular mechanisms involved in cancer development, different treatment options like surgery, chemotherapy, immunotherapy and radiotherapy. But complete cure is still awaited in most of the cases. Chemo-resistance and the recurrence of tumor are the main miseries to scientific world in the pharmacological management of cancer. To overcome these miseries scientists started targeting genes and the apoptosis pathways.

1.2. APOPTOSIS

Apoptosis, also known as a programmed cell death, is an important mechanism in controlling cell numbers and proliferation. It plays an important role in the tissues of developing embryos. It is a major control mechanism consisting of external and internal pathways by which cell dies [1]. Kerr et al. in 1972 were the first to propose the name apoptosis to a cellular mechanism that has been known by biologists as a cell suicide mechanism. They were the first to hypothesize that apoptosis plays a much broader role in life processes and its failure contributes to a variety of diseases, including cancers [2].

The understanding that inhibition of apoptosis is related to cancer has taken many researchers by storm, involved in the field of cancer research, with the hope that by knowing mechanism underlying apoptosis, tackling of cancer cell would be possible.

The link between cancer and apoptosis was well established by several published works that have identified the role of many key regulatory gene products like p53, Bcl-2, caspases and others in apoptosis of cancerous cell [3-5]. p53, widely known as a guardian of human genome, acts as an important tumor suppressor either by blocking the

cell division of a genetically damaged cell, or induces apoptosis by acting on mitochondria to release cytochrome c. Loss of p53 function leads to genomic instability, cell cycle dysregulation, and inhibition of apoptosis [6-7].

Bcl-2 has been characterized as a suicide brake gene as it blocks apoptosis [8]. The Bcl-2 family is the key regulator of apoptosis that includes pro-apoptotic members such as Bax, bak, bad, Bcl-Xs, Bid, Bik, Bim, and Hrk, and anti-apoptotic members such as Bcl-2, Bcl-X_L, Bcl-W, Bfl, and Mcl-1. Anti-apoptotic member of Bcl-2 acts by blocking mitochondrial cytochrome c release, and it is overexpressed in many malignancies [9-10].

The final proteolytic activity in apoptosis is triggered by a family of proteins called caspases. These caspases must be activated in order to activate the final pathway of apoptosis. They form cascade of proteases which are activated in the process of programmed cell death. Caspases like 3, 6, 7, 8 and 9 have been well described [11].

Efforts have been put forward to target these apoptotic pathways or key regulators of apoptosis like p53, Bcl-2 family and caspases with the aim of pharmacological management of cancer. Some of them are already in clinical trials, but complete success is still awaited [1].

2. ROLE OF SOME HERBS AND THEIR PHYTOCHEMICALS IN APOPTOSIS OF CANCER CELLS

Considering the hazards of treatment failure, drug resistance, heavy costs and other problems associated with current cancer therapy, medicinal plants have attracted interest of many researchers in this field. The use of the medicinal herbs for curing disease has been practiced in the history of all civilizations including ancient Egyptian, ancient Chinese, Indian Ayurvedic and Unani medicine [12].

It was proven through research that certain plants contain active principles, which are responsible for therapeutic action of the herbs. Scientific research has proven the utility of formulations based on medicinal herbs of ancient healers, who

themselves were not aware of the chemical composition of plants, and used these traditional remedies purely based on beliefs or experiences.

The plants were initially used in unmodified form, then as extracts, and in 19th century, thanks to the advances in biotechnology which made it possible to isolate the active compounds from some plants of medicinal importance. A large number of pharmaceutical compounds used today contain natural compounds including those with modification to original molecule [13]. In addition, bioactive plant compounds serve as template for several synthetic drugs, and as precursors used in the production of semi-synthetic drugs [14-16].

Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils. Today there is a growing interest in chemical composition of plant-based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity.

There are many distinct chemical substances derived from plants such as digitalis, pilocarpine, quinine that are considered as important drugs currently in clinical use [12]. Several of the drugs used today are simple synthetic modifications or analogs of the naturally obtained substances. For example, *Cephaelis ipecacuanha*, a tropical plant and its chemical emetine was discovered many years ago. A drug was developed from this plant chemical called Ipecac which was used for many years to induce vomiting. Another example of this is the plant chemical named Taxol originally discovered in the plant and later marketed as paclitaxel, which is used in various types of tumors today in the U.S and many other countries [12]. Vinblastine and Vincristine are other well known examples of plant-derived anti-cancer drugs from *Madagascar periwinkle*.

Isolated active constituents from plants are used for applied research. For the last few decades, phytochemistry has been making rapid progress and herbal products are being seriously considered as the alternative form of medicines.

The purpose of this review is not to give a

description of all the herbs presently known to have anti-cancer effect, but to highlight some of the herbs especially those that are used as our daily kitchen ingredients. Natural herbs and products like black cumin, turmeric and garlic to name a few are an integral part of daily food ingredients in many countries especially the Asian kitchen. There has been a plenty of scientific publications available that depict their role in maintaining the health and in diseases including cancer.

2.1. BLACK CUMIN (*Nigella sativa*)

Nigella sativa is the source of a black seed that has a distinctive reputation in Eastern medicine. It is a commonly used ingredient of many recipes in South Asia and elsewhere. The medicinal value of black cumin was known to ancient Middle East population.

Extensive analysis has been done on the active constituents and the pharmacological actions of black cumin. There are two major groups of active ingredients, terpenes (making up the essential oil) and alkaloids. The seeds also contain small amounts of saponins and a significant amount of fixed oil (rich in linolenic and linoleic acids). The terpenes are derivatives of thymol: mainly thymoquinone (TQ) and its polymers (such as dithymoquinone), and p-cymene and α -pinene. These are volatile compounds.

In a study, two purified components of *Nigella sativa* seeds, (TQ) and dithymoquinone were assayed *in vitro* and reported as potent cytotoxic for several human tumor cell lines such as human pancreatic adenocarcinoma cell line CFPAC-1, the human uterine sarcoma cell line MES-SA and its MDR variant, Dx-5, and human leukemic cell line K562 [17].

Promising results of TQ were found in mice bearing Ehrlich ascites carcinoma xenograft [18], in which TQ showed improved therapeutic efficacy of a cyclophosphamide analogue ifosfamide. In another study, TQ was found to inhibit the benzopyrene-induced forestomach carcinogenesis in mice. The possible mode of action was proposed to be through its anti-oxidant and anti-inflammatory activities, coupled with enhancement of

detoxification processes [19].

TQ was also found to inhibit significantly the tumor incidence and tumor burden on fibrosarcoma induced by 20-methylcholanthrene *in vivo* and *in vitro* in male Swiss albino rats [20]. Recently, the immunomodulating and cytotoxic properties of *Nigella sativa* volatile oil was investigated, which showed a potent activity against human stomach cancer cell lines [21]. Chemopreventive effects of volatile oil from the seeds of *Nigella sativa* have also been reported against rat colon carcinogenesis in Fischer 344 rats [22].

Recently TQ was investigated against human colon cancer cells lines (HCT-116) and the possible molecular mechanism of action was explored by using TUNEL staining and flow cytometry analysis. These experiments showed that TQ triggered apoptosis in a dose and time dependant manner and was associated with a 2.5-4.5 fold increase in mRNA expression of p53 and the downstream p53 target gene, p21WAF1. Simultaneously, there was increase in p53 and p21WAF1 protein levels but a significant reduction in anti-apoptotic Bcl-2 protein [23].

α -Hederin, a pentacyclic triterpene saponin, is another recently isolated compound of *nigella sativa* seeds, reported to have a potent *in vivo* anti-tumor activity against Lewing lung carcinoma (LL/2) in BDF 1 mice [24]. Recently, it was also observed to cause a dose and time dependant increase in apoptosis of murine leukemic P388 cells. It was noted that α -hederin caused the release of cytochrom c from the mitochondria to cytosol, leading to caspase-3 activation [25].

2.2. TURMERIC (*Curcumin longa*)

Curcuma longa, commonly called as turmeric is another traditional kitchen ingredient of many countries. Its uses in various health problems have been reported in the history of mankind. Much scientific evidence in support of its various medicinal properties has been documented [26-28]. A vast majority of these studies were carried out with curcumin (diferuloyl methane), a polyphenol derived from this plant. There have been plenty of studies presented to suggest the beneficial effect of

curcumin on tumors [29-30].

The anti-cancer potential of curcumin has been attributed to its ability to suppress proliferation of a wide variety of tumor cells, down-regulation of nuclear factor (NF-kappa B), AP-1 and Egr-1, down-regulation of the expression of cyclooxygenase-2, nitric oxide synthase, matrix metalloproteinase-9, tumor necrosis factor, chemokines, cell surface adhesion molecules, cyclin D1, and inhibition of the activity of c-Jun N-terminal kinase, protein tyrosine kinases and protein serine/threonine kinases [31-36]. Moreover, curcumin has been found to be safe in humans [37]. Recently, the role of Bax in curcumin-induced apoptosis has been explored using isogenic human colon cancer cells. This study demonstrated Bax as a critical regulator of curcumin-induced apoptosis, and implies the Bcl-XL, or overexpression of second mitochondria derived activator caspase (Smac), as potential target to deal with Bax-defecient chemoresistant cancers for curcumin-based therapy [38]. All of these research works suggest that curcumin has a vast potential in the prevention and treatment of cancer.

2.3. GARLIC (*Allium sativum*)

Garlic; another popular spice added to several edible preparations, has been a remedy for a variety of ailments in many different cultures. Epidemiological studies have supported that regular consumers of garlic and garlic products have an inverse relationship to the risk of stomach and colon cancer [39]. Organosulfur compounds present in *Allium* vegetables e.g. garlic and onion are considered to be responsible for the beneficial effects of these herbs [40-41]. Garlic has been shown to be metabolized into N-aceryl-S-allyl cysteine, allyl mercaptan, diallyl disulfide, diallyl sulfide, diallyl sulfoxide, diallyl sulfone, and allyl methyl sulfide [42-43]. A number of mechanisms through which garlic brings about the anti-carcinogenic effect have been shown, such as the scavenging of radicals modulating the levels of glutathione and enzymes such as glutathione S-transferase and catalase [44-45] inhibition of cytochrome P450 2E1 [46].

Several other garlic compounds including allicin

and its corresponding sulfide inhibit the proliferation and induce apoptosis of several malignant cells including breast [47-48], bladder [49], colon [50-51], liver [52], prostate [53-54], blood [55] and skin tumour cell lines [56-57]. Ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide) is a garlic-derived compound produced most efficiently from pure allicin. Ajoene was shown to inhibit proliferation as well as it was found to enhance the chemotherapy-induced apoptosis in CD34-positive resistant human myeloid leukaemia cells [55].

Recently, possible mechanisms of action of garlic-derived compounds in relation to apoptosis in various types of cancer cells has been suggested, such as, modulating Bcl-2, Bax, Bim and caspases [58], a caspase-independent apoptotic pathway mediated by mitochondrial release of AIF and PKA [59], modulation and regulation of the tumor suppressor p53 along with its downstream effective molecule, p21/waf1 [60], promotion of caspase-3 activity, increase in the product of intracellular hydrogen peroxide, cyclin E, and decreased CDK2 gene expression which arrest cells in G₂/M cells [49], depolymerization of microtubule and c-Jun NH₂ terminal Kinase 1 activation [61].

4. CONCLUSION

Targeting cell cycle and apoptosis pathways has emerged as an attractive approach for the treatment of cancer. Apoptosis can be modulated by targeting pro-apoptotic or pro-survival pathways. Several proteins relevant to oncogenic and proliferative processes, such as p53, bcl-2, AKT, ras and epidermal growth factor receptor are also important, and the herbal plants and their chemicals discussed in this review article have shown to be effective on these known oncogenic and proliferative processes. Novel technologies such as genomics and proteomics will be instrumental in designing combinational regimens or purely plant-based medicines. Further research on the modulation of apoptosis and cell cycle machinery by herbal plants and their chemicals for oncology therapy is the need of time, considering the side effects, very high cost and non-compliance associated with prevalent chemotherapy.

From this review, it is obvious that although work has been there in the field of medicinally important plants and their effects on cancer cells but still more extensive and rigorous work is warranted, to target more plants which are still untouched. Moreover, the aim of further studies should be to confirm that whether the data generated from the pre-clinical studies can be applied to humans in clinical settings as well. For this, clinical studies investigating the effect of natural products on the prevention and treatment of cancers need to be done. Usefulness of these herbal products in malignancy, if proven scientifically, may be a big leap forward in the field of therapeutics as familiarity of mankind with these daily kitchen ingredients may improve the compliance of patients.

It is possible that clinical studies may not prove successful but these identified compounds from the herbal sources may prove their worth as pharmacological tools for elucidating the modes of action of other drugs, or investigating for basic pharmacological or physiological mechanisms. Cocaine (a norepinephrine uptake inhibitor) and yohimbine (a specific α_2 adrenergic antagonist) are few well-known examples in this regard.

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