Pharmacological Approaches: A Review of Piperine Anticancer Activities in Oral Cancer

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Abstract

Cancer is one of the major causes of death that is still being eradicated on a global scale. Multi-drug resistance (MDR) is a significant issue with the available therapeutic alternatives. It is now generally accepted that a variety of herbal dietary supplements may act as anti-cancer agents against common cancer types. A variety of biological and pharmacological properties, including anti-pyretic, anti-metastatic, antidepressant, anti-apoptotic, and anti-cancer activity, are shown by the alkaloid piperine. In this review, online literature, including books on phytochemistry and the electronic search engine, was studied to investigate the impact of piperine on anticancer research related to its mechanism of action and its capacity to regulate cancer-related gene action, such as oncogenic and tumour suppressor gene activity in cycle and apoptosis, as well as its therapeutic perspectives on oral cancer (PubMed, the Web of Science, SCI, Scopus, Google Scholar, ResearchGate, etc.). Numerous studies have looked at how piperine affects the apoptosis pathway via caspase signalling, where piperine inhibits cell growth and triggers apoptosis. Piperine may stop the cell cycle in the G2/M phase, activate the caspase-3 and caspase-9 cascades, which have been shown to have selective cytotoxicity, downregulate cyclin B1, and increase the phosphorylation of CDK1 and checkpoint kinase 2 in the cell cycle. Additionally, it prevents P-glycoprotein (P-GP) and CYP3A4 from doing their jobs, which not only changes how drugs are metabolised but also makes MDR cancer cells more susceptible to other drugs. The G-quadruplex structure generated at the c-myc promoter

region is stabilised by piperine's anti-proliferative and pro-apoptotic properties, which also suppress the production of c-myc in cancer cells. The potential to cure oral cancer is based on piperine's utility for the aforementioned molecular process linked to other cancers because there is very little research on the condition. This shows the effectiveness of piperine in the fight against oral cancer. For the development of anticancer drugs for the treatment of oral cancer, it is necessary to further investigate the anticancer efficacy of piperine in vivo and in clinical studies.

Keywords: - *Anticancer Activities, Piperine, Mouth Cancer, Caspases, Apoptosis.*

Introduction

Cancer, the leading cause of morbidity and mortality worldwide, is emerging as a major global problem, characterised by the uncontrolled growth and spread of abnormal cells. Statistics indicate that 18.1 million new cancer cases and 9.6 million cancer deaths occurred globally in 2018[1]. The etiological factors include cigarette smoking, environmental carcinogens, ultraviolet (UV) exposure, inheritance, stress, obesity, and physical inactivity, in which an unhealthy diet plays a huge role in the development of cancer. As in all cancer-related deaths, 30–35% are linked to diet, almost 25–30% are due to tobacco, about 15–20% are due to infections, and only 5–10% are due to genetic defects (mutations) that are inherited from a parent[2]. Therefore, since cancer continues to be a worldwide eradicator, it is essential to search for naturally occurring drugs to minimise health problems. The studies indicate that many herbal dietary products are available as chemoprotective agents against commonly occurring cancer types[3]. The development of novel anticancer agents is contingent on alternation in the cell cycle, regulation of apoptotic cascades at the molecular level, and oncogene regulation. Piperine, an alkaloid and hydrophobic amide, is a major constituent of Piper nigrum (black pepper) and Piper longum of the Piperaceae family[4]. Traditionally, pepper has been used for many ailments, such as stomach upset, bronchitis, malaria, cholera, and cancer. Piperine has a diverse set of biological and pharmacological properties, including anti-pyretic, anti-metastatic, antidepressant, anti-apoptotic efficacy, and potent immunomodulatory and anti-tumor activity[5]. In this review, we mainly focus on the piperine anticancer research related to their mechanism of action and the guidelines in place for their regulations. Piperine's anticancer activity has been linked to its ability to induce apoptosis, inhibit cell proliferation, activate the immune system, and reduce metastasis, as well as its ability to interact with and modulate multiple molecular pathways involved in cancer progression[6]. As a result, piperine has the potential to become a powerful anti-cancer agent, though more research is needed in order to fully understand its anti-cancer properties and how it can be used effectively as part of cancer treatments. Piperine has been linked to anti-apoptotic, anti-metastatic, and immunomodulatory effects, suggesting that it has great potential in helping to treat a variety of cancers[7]. In addition, the exact mechanism of piperine's anti-cancer effects and its potential side effects must be better understood in order to develop more effective and safe therapies. Currently, piperine is being studied for its potential use in the treatment of cancer and other diseases[8]. As more studies are conducted, it is hoped that piperine can be used as an effective component of cancer treatments, improving the survival rate and quality of life for those with cancer as well as having antioxidative, anti-inflammatory, and antiproliferative properties[9]. As piperine has been shown to have several different potential therapeutic applications in the treatment of cancer, more research needs to be done to fully understand its effects and its possible side effects in order to develop safer and more effective treatments[10]. Additionally, a better understanding of the pharmacokinetic profile of piperine is essential to the successful use of piperine in cancer therapies. Further studies should be conducted to understand the exact mechanisms of action that piperine has on the body, as well as explore more uses for piperine in cancer treatments[11]. This research could potentially

lead to piperine becoming an important part of cancer treatments, helping improve the quality of life for those living with the disease. To summarize, piperine has the potential to be an invaluable tool in cancer treatment if the underlying pharmacokinetic properties and mechanisms of action are further studied[12]. As the effects of piperine on cancer cells are promising, more research must be done in order to determine the optimal use of this compound in cancer treatment protocols[13].

Pathogenesis and Pathophysiology of Carcinoma

Cells undergo a number of metabolic and behavioural changes throughout the multi-step process of carcinogenesis, which causes them to proliferate excessively and prematurely, allowing them to invade distant organs and generate metastases. These changes develop throughout time as a result of alterations to the genetic programs that regulate cell division, longevity, interactions with nearby cells, and immunity-evading abilities[14]. Therefore, finding mutations in cancer cells might have a wide range of effects on both research and treatment. The most researched cancer gene is TP53, which produces the p53 protein, a tumor suppressor that accumulates around 24,000 mutations and is mutated in nearly half of all human cancer cases[15]. However, in order for this cell to develop into cancer, a number of alterations in oncogenes and tumor suppressor genes must occur, giving the cell the ability to proliferate much beyond its usual capacity[16]. Growth factors (such as TGFA), growth factor receptors (such as epidermal growth factor (EGF) and its close homolog, ERBB2), receptor-coupled signal transduction molecules (specifically, several small guanosine triphosphate (GTP)-binding proteins located on the inner face of the cell membrane, such as the various members of the RAS family), kinases (SRC, ABL, RAF1), and regulatory subunits Since they are produced by plants and are generally harmless in nature, apoptosis and the genes that control it are the main mechanisms of anticancer medications in cancer treatment and have become an effective target for the discovery and development of new anticancer agents[17]. of protein kinases (such as PI3K and AKT) are all involved in these complex pathways and must be identified and studied in order to effectively treat cancer. These pathways and genes must be thoroughly understood in order to effectively treat cancer and take advantage of the body’s natural defences against it[18]. The various components that make up the complex network of pathways, genes, and proteins that control apoptosis play a crucial role in cancer treatment and must be properly identified and studied in order to maximize the effectiveness of anticancer agents. Thus, apoptosis and its underlying genes are a key focus in cancer research, as they offer insight into the body's natural defenses against the disease[19]. Apoptosis, or programmed cell death, is an integral component of the body's natural defense system against cancer. In order to properly exploit the body's natural defenses against cancer, researchers must thoroughly understand the underlying mechanisms of apoptosis and identify the genes, proteins, and pathways involved in the process[20]. In order to do this, researchers are utilizing the latest technologies to identify the genetic and molecular networks associated with apoptosis and to determine how they can be manipulated in order to effectively combat cancer. This knowledge can be used to create more effective treatments for cancer by targeting genes and proteins that are essential for apoptosis and preventing the tumor cells from escaping apoptosis, as well as by developing drugs that promote apoptosis in cancer cells[21]. These drugs can be

used to kill cancer cells more efficiently as well as reduce the toxicity of traditional chemotherapeutic treatments. In addition, the use of bioinformatics and systems biology techniques can provide insight into how genetic mutations contribute to cancer development and progression, improving our understanding of the underlying biology that drives tumor formation[22]. This improved understanding will enable researchers to design more effective treatments tailored to individual patients, leading to increased survival rates and an improved quality of life for those living with cancer[23]. Furthermore, understanding the underlying mechanisms of tumor formation can also provide insight into potential targets for cancer prevention strategies, providing an additional avenue for reducing the impact of this devastating disease[24].

Piperine's involvement in the caspases-mediated apoptosis pathway

The apoptosis receptor (extrinsic) and mitochondrial-mediated (intrinsic) signalling pathways are the two primary signalling routes that largely induce apoptosis. The deregulation of caspases (a family of proteases specialised for the amino acid cysteine) during apoptosis may result in a number of illnesses in humans, including cancer and inflammatory conditions[25]. Caspase signalling was divided into initiator (caspases-8, -9, and 10) and effector (caspases-3, -6, and -7), where activation of caspases-3 and -7 is necessary for causing downstream DNA cleavage molecules, which are associated with both extrinsic and intrinsic apoptotic cell death[26]. Numerous studies have looked at how piperine affects the caspases' signalling pathway that leads to apoptosis. Through the cleavage of PARP-1, inhibition of phosphorylated STAT-3 (transcription proteins), and inhibition of NF-kB expression, piperine effectively reduced the proliferation of prostate cancer cells and induced apoptosis in these cells[27]. This may be the molecular mechanism by which piperine inhibits cell proliferation and triggers apoptosis in prostate cancer cells. Piperine, which has been discovered to cause apoptosis by mainly targeting the stimulation of caspases 3 and 9, has also been examined by Gnanasekar et al. Piperine has the potential to trigger cell cycle arrest in the G2/M phase and to activate the caspase-3 and caspase-9 cascades, showing preferential cytotoxicity against the lung cancer cell line (A549)[28]. Piperine displayed substantial potential against STAT3/NF-kB in living people with cervical cancer and ovarian cancer, and it aided the cervix cell line in reducing cell viability and triggering apoptosis through the JNK/p38 MAPK-mediated intrinsic apoptotic pathway. Piperine therapy reduced proliferation and induced apoptosis in HER2-overexpressing breast cancer cells by activating caspase-3 and cleaving PARP, according to Do et al[29]. These investigations showed that piperine reduces tumour development by activating caspase signaling, which causes cell death. This suggests that piperine could be a potential therapy for cancer and that its anti-cancer properties are mediated through activating the caspase cascade and inducing apoptosis by stimulating the caspase cascade and causing apoptosis[30]. In conclusion, piperine has shown great promise as a potential therapeutic option for cancer due to its ability to reduce cell viability, trigger apoptosis, and activate caspase signalling in HER2-overexpressing breast cancer cells. Furthermore, the findings from Do et al. also show that piperine may have the potential to be used as an adjuvant therapy to cancer treatments, such as chemotherapy and radiation therapy, due to its ability to increase the effectiveness of these treatments by enhancing the apoptotic process and reducing tumour

growth[31]. As such, more research is needed to investigate the potential of piperine as a viable therapy for cancer. Thus, it is clear that piperine holds promise for future use as a therapy for cancer and has the potential to reduce mortality in cancer patients. In addition, further studies should also focus on elucidating the mechanisms of piperine's effects and exploring potential drug interactions, as well as its pharmacokinetic properties, in order to ensure that it is safe for human use[32]. Moreover, with further research into the effects of piperine, there is hope that it could become a viable therapy option for those suffering from cancer in the near future. With its ability to inhibit tumour cell growth, piperine is a promising candidate for the development of new anticancer drugs[33]. Despite this potential, there are some caveats and additional research that must be conducted in order to fully understand the effects of piperine on the human body before it can be considered a viable therapy option for cancer patients. For instance, the current research has mainly focused on animals and cell lines and has not yet been extended to clinical trials. In order to move forward with piperine as a potential therapy option, further research is needed on the effects of piperine on human cells in both laboratory and clinical settings[34]. Additionally, an analysis of the side effects associated with piperine must be conducted before it can be approved for use in humans. Furthermore, it is important to understand the pharmacokinetics of piperine and how it is metabolised in humans in order to determine safe and effective dosages for clinical applications[35]. Such research is essential in order to ensure that the benefits of piperine outweigh the risks associated with its use in humans. Additionally, researchers must investigate the mechanism of action of piperine in order to understand how it works at the molecular level and identify any potential interactions with other drugs[36].

Effects of piperine on tumour suppressor genes and oncogenes

Gene mutations are cellular or viral (i.e., virus-inserted into the cell) genes, and when they are expressed, neoplasms may form. Protooncogenes are normal cellular genes that may become oncogenes by a variety of processes, including amplification or alteration. When overexpressed, protooncogenes can either turn normal cells into cancer cells or, conversely, act as tumour suppressor genes[37]. Several proteins, including cyclin D, cyclin E1, CDK2, CDK4, CDK6, c-myc, tumour necrosis factor-alpha, interleukin-1 (IL-1), IL-6, IL-8, VEGF, and MMP-940, are induced by transcription factors in cancer cells that control proliferation, inflammation, angiogenesis, invasiveness, and apoptosis resistance. Activated transcription factor 2 (ATF-2) and cAMP response element-binding (CREB), among others, are all potently inhibited by piperine[38]. Through the suppression of PKC/ERK1/2 and decreased activation of NF-B and AP-1, it prevents PMA from increasing MMP-9 production. Surprisingly, piperine also interferes with P-glycoprotein (P-gp) and CYP3A4 activities, which have an impact on drug metabolism and re-sensitise multidrug-resistant (MDR) cancer cells. Piperine supplementation significantly decreased DNA damage and DNA-protein crosslinks in the experimental lung cancer caused by benzo(a)pyrene[39]. With the strongest affinity for the G-quadruplex structure generated in the c-myc promoter region, piperine's chemo preventive selectivity for G-quadruplex DNA seems to outweigh that for double-stranded DNA. When G-quartets are arranged in a square planar layout during DNA metabolism, four-stranded DNA structures called G-quadruplexes are created[40]. These structures are essential for controlling

cellular activities that may promote the growth of cancer. Piperine exhibits concentration-dependent cytotoxicity in HeLa, PC3, HepG2, and MCF-7 cell lines, and the c-myc gene is down-regulated in comparison to a ubiquitously expressed housekeeping gene, -actin[41]. The G-quadruplex structure formed by piperine on the c-myc promoter region DNA sequence (Pu24T) exhibits the maximum affinity. One theory for how piperine exerts its anti-cancer function is by stabilising the G-quadruplex structure produced at the c-myc promoter region and decreasing its expression in cancer cells. Piperine is anti-proliferative and pro-apoptotic[42].

Piperine Activates Checkpoint Kinase-1 to Stimulate G1 Phase Apoptosis and the Cell Cycle Arrest in Carcinoma Cells

The proliferation of SK-MEL-28 and B16-F0 cells was reduced by piperine administration in a dose- and time-dependent manner. The cell cycle arrest of both cell lines in the G1 phase was the mechanism via which piperine's growth-inhibitory effects were mediated. Piperine's G1 arrest was accompanied by the down-regulation of cyclin D1 and the activation of p21[43]. Furthermore, phosphorylation of H2AX at Ser139, activation of ataxia telangiectasia, rad3-related protein (ATR), and checkpoint kinase 1 showed that this growth arrest caused by piperine therapy was linked to DNA damage (Chk1). Pre-treatment with the Chk1 inhibitor AZD 7762 prevented both piperine-mediated G1 arrest and Chk1 activation[44]. Similar to this, cells transfected with Chk1 siRNA were totally shielded against piperine-induced G1 arrest. E2F1 was downregulated, and the retinoblastoma protein was phosphorylated after piperine therapy (Rb). Piperine-induced apoptosis was linked to down-regulation of XIAP, Bid (full length), Caspase-3 cleavage, and PARP[45]. Additionally, our findings demonstrated that piperine administration produced ROS in carcinoma cells. Iron's ability to inhibit ROS shielded the cells from apoptosis and cell cycle arrest caused by piperine. These findings imply that DNA damage, Chk1 activation, G1 cell cycle arrest, and apoptosis were all caused by piperine-mediated ROS[46].

Piperine involvement in cell cycle arrest

The main regulators of cell cycle progression are cyclins, their related kinases, and their inhibitors. Cancer may be seen as the result of progressive genetic instability and a lack of cell cycle regulation. The cell cycle is significantly regulated by proteins that belong to the cyclin family. To regulate cell cycle progression, cyclins bind to and activate enzymes of the cyclin-dependent kinase (Cdk) family[47]. Cyclins D1, D2, and D3 and cyclin E form complexes with cdk4 or cdk6, respectively, to regulate the expression of proliferative genes during the G1 phase. During the S phase, cyclin A connects with cdk2, and at the S-G2 border and throughout the G2 phase, it associates with cdc2 (cdk1)[48]. Further, cdc2 complex formation with cyclins B1 and B2 is necessary for progression through G2, which results in mitosis. In 4T1 cells, piperine administration reduced cyclin B1 expression in a dose-dependent manner. Through the downregulation of cyclin B1 and increased phosphorylation of cyclin-dependent kinase-1 (CDK1) and checkpoint kinase 2, piperine arrests osteosarcoma cells in the G2/M phase of the cell cycle (Chk2). Piperine may stop the development of tumours by causing a halt in the cell cycle[49].

Pharmacology Studies in Piperine as a Prospective Anti-Cancer Agent

Piperine, a potent alkaloid present in black pepper and certain other Piper species, has recently been shown in many studies to have anticancer properties. We made an effort to synthesise the information we had collected to support this natural agent's anticancer potential. It has been noted that piperine possesses potent chemo preventive properties[50]. It has been shown to have an impact on a number of modes of action, namely improving the antioxidant system, raising the amount and activity of detoxifying enzymes, and inhibiting stem cell self-renewal. Additionally, it has been shown that piperine inhibits the growth and survival of many malignant cell lines by altering the cell cycle and showing anti-apoptotic activity, respectively[51]. It has been shown that this substance alters the activity of several enzymes and transcription factors to prevent invasion, metastasis, and angiogenesis. It's interesting to note that piperine possesses antimutagenic properties and inhibits the production and activity of multidrug-resistant transporters, including P-gp and MRP-1. Additionally, almost all of the studies that were examined revealed that piperine had a selective cytotoxic effect on malignant cells as opposed to normal cells[52]. Overall, the investigations highlight piperine's favourable potential for future development. The supported modes of action of piperine include its anti-inflammatory, immunomodulatory, anti-mutagenic, and anti-cancer properties, as well as its capacity to disrupt a number of molecular signalling pathways. Piperine's pro-apoptotic and anti-proliferative properties hold promise as a cancer chemo preventive drug through controlling the apoptotic pathway and cell cycle arrest[53]. Further research should be done, particularly in the form of clinical trials on humans, to better understand its anticancer potential, which was established by its induction of apoptosis in various cancer types. However, such information is scarce[54].

Piperine potential for preventing oral cancer

Approximately 2% to 4% of cancer cases worldwide are oral cancer cases. A type of neoplasm known as oral cancer may affect the salivary glands, oral cavity, or pharynx. Oral squamous cell carcinoma (OSCC) is the most common oral cancer, according to 50 studies. The probability for malignant development varies among the most prevalent premalignant lesions, including leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis[55]. Patients with oral cancer are now receiving targeted molecular treatment, such as gene therapy and monoclonal antibody therapy. Contrary to surgery, chemotherapy, and radiation, this kind of treatment has little or no adverse effects on the body's healthy cells[56]. Epidermal growth factor receptor (EGFR), cyclooxygenase-2 (COX-2), peroxisome proliferator-activated receptor (PPAR), and progesterone receptor are the four molecules that have received the majority of attention in targeted molecular treatment[57]. These chemicals are linked to the OSCC's differentiation and proliferative growth. Although piperine has the potential to cure oral cancer due to its efficacy for the aforementioned molecular process linked to other cancers, there is no data supporting either its prevention or therapy[58]. This serves as a poster about piperine's role in the prevention of mouth cancer. Siddiqui et al. discovered that piperine induces the production of ROS in human oral squamous cells, which in turn causes MMP to dissipate, caspases to activate, and cell cycle arrest. However, there is little data to support this claim. Piperine induced MMP loss and caspase-3 activation, which induced cell death[59].

Piperine lowered the DNA quantity and halted cells in the G2/M phase, according to cell cycle research. The effectiveness of piperine in the investigation of oral cancer is suggested by this study[60].

Conclusion

oral cancer is one of the major causes of death that is still being eradicated on a global scale. Multi-drug resistance (MDR) is a significant issue with the available therapeutic alternatives. Piperine may stop the cell cycle in the G2/M phase, which has selective cytotoxicity and downregulates cyclin B1. For the development of anticancer drugs for the treatment of oral cancer, it is necessary to further investigate the anticancer efficacy of piperine.

Conflict of Interest

None

Reference

[1] R. Mehrotra and R. Kaushik, “A stitch in time saves nine: Answer to the cancer burden in India,” *Indian J. Med. Res.*, vol. 147, no. 2, p. 121, Feb. 2018, doi: 10.4103/IJMR.*IJMR*_388_18.

[2] P. Anand *et al.*, “Cancer is a Preventable Disease that Requires Major Lifestyle Changes,” *Pharm. Res.*, vol. 25, no. 9, p. 2097, 2008, doi: 10.1007/S11095-008-9661-9.

[3] A. G. Desai *et al.*, “Medicinal Plants and Cancer Chemoprevention,” *Curr. Drug Metab.*, vol. 9, no. 7, p. 581, Sep. 2008, doi: 10.2174/138920008785821657.

[4] E. Turrini, P. Sestili, and C. Fimognari, “Overview of the Anticancer Potential of the ‘King of Spices’ Piper nigrum and Its Main Constituent Piperine,” *Toxins (Basel).*, vol. 12, no. 12, Dec. 2020, doi: 10.3390/TOXINS12120747.

[5] B. Salehi *et al.*, “Piper Species: A Comprehensive Review on Their Phytochemistry, Biological Activities and Applications,” *Molecules*, vol. 24, no. 7, Jan. 2019, doi: 10.3390/MOLECULES24071364.

[6] R. A. Rather and M. Bhagat, “Cancer Chemoprevention and Piperine: Molecular Mechanisms and Therapeutic Opportunities,” *Front. Cell Dev. Biol.*, vol. 6, no. FEB, p. 10, Feb. 2018, doi: 10.3389/FCELL.2018.00010.

[7] A. Manayi, S. M. Nabavi, W. N. Setzer, and S. Jafari, “Piperine as a Potential Anti-cancer Agent: A Review on Preclinical Studies,” *Curr. Med. Chem.*, vol. 25, no. 37, pp. 4918–4928, May 2018, doi: 10.2174/0929867324666170523120656.

[8] Z. Stojanović-Radić *et al.*, “Piperine-A Major Principle of Black Pepper: A review of its bioactivity and studies,” *Appl. Sci.*, vol. 9, no. 20, Oct. 2019, doi: 10.3390/APP9204270.

[9] "(PDF) A Comprehensive Review of the Systemic Evidence on the Immunomodulatory Properties of Zingiber officinale (Ginger)." https://www.researchgate.net/publication/366191241_A_Comprehensive_Review_of_the_Systemic_Evidence_on_the_Immunomodulatory_Properties_of_Zingiber_officinale_Ginger (accessed Dec. 21, 2022).

[10] S. Mitra *et al.*, "Anticancer Applications and Pharmacological Properties of Piperidine and Piperine: A Comprehensive Review on Molecular Mechanisms and Therapeutic Perspectives," *Front. Pharmacol.*, vol. 12, Jan. 2021, doi: 10.3389/FPHAR.2021.772418.

[11] A. K. Tripathi, A. K. Ray, and S. K. Mishra, "Molecular and pharmacological aspects of piperine as a potential molecule for disease prevention and management: evidence from clinical trials," *Beni-Suef Univ. J. Basic Appl. Sci.*, vol. 11, no. 1, p. 16, Dec. 2022, doi: 10.1186/S43088-022-00196-1.

[12] A. S. Choudhari, P. C. Mandave, M. Deshpande, P. Ranjekar, and O. Prakash, "Phytochemicals in cancer treatment: From preclinical studies to clinical practice," *Front. Pharmacol.*, vol. 10, p. 1614, Jan. 2020, doi: 10.3389/FPHAR.2019.01614/BIBTEX.

[13] R. Wilken, M. S. Veena, M. B. Wang, and E. S. Srivatsan, "Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma," *Mol. Cancer 2011 101*, vol. 10, no. 1, pp. 1–19, Feb. 2011, doi: 10.1186/1476-4598-10-12.

[14] M. A. Feitelson *et al.*, "Sustained proliferation in cancer: Mechanisms and novel therapeutic targets," *Semin. Cancer Biol.*, vol. 35, pp. S25–S54, Dec. 2015, doi: 10.1016/J.SEMCANCER.2015.02.006.

[15] N. Rivlin, R. Brosh, M. Oren, and V. Rotter, "Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis," *Genes Cancer*, vol. 2, no. 4, p. 466, Apr. 2011, doi: 10.1177/1947601911408889.

[16] C. Osborne, P. Wilson, and D. Tripathy, "Oncogenes and tumor suppressor genes in breast cancer: potential diagnostic and therapeutic applications," *Oncologist*, vol. 9, no. 4, pp. 361–377, Jul. 2004, doi: 10.1634/THEONCOLOGIST.9-4-361.

[17] P. Wee and Z. Wang, "Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways," *Cancers (Basel).*, vol. 9, no. 5, May 2017, doi: 10.3390/CANCERS9050052.

[18] I. A. Mayer and C. L. Arteaga, "The PI3K/AKT Pathway as a Target for Cancer Treatment," *Annu. Rev. Med.*, vol. 67, pp. 11–28, Jan. 2016, doi: 10.1146/ANNUREV-MED-062913-051343.

[19] G. Pistritto, D. Trisciuoglio, C. Ceci, Alessia Garufi, and G. D'Orazi, "Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies," *Aging (Albany NY)*, vol. 8, no. 4, p. 603, 2016, doi:

10.18632/AGING.100934.

[20] L. Galluzzi *et al.*, “Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018,” *Cell Death Differ. 2018 253*, vol. 25, no. 3, pp. 486–541, Jan. 2018, doi: 10.1038/s41418-017-0012-4.

[21] J. Hu *et al.*, “Targeting mutant p53 for cancer therapy: direct and indirect strategies,” *J. Hematol. Oncol. 2021 141*, vol. 14, no. 1, pp. 1–19, Sep. 2021, doi: 10.1186/S13045-021-01169-0.

[22] R. J. Boohaker, M. W. Lee, P. Vishnubhotla, J. L. M. Perez, and A. R. Khaled, “The Use of Therapeutic Peptides to Target and to Kill Cancer Cells,” *Curr. Med. Chem.*, vol. 19, no. 22, p. 3794, Jul. 2012, doi: 10.2174/092986712801661004.

[23] C. Pucci, C. Martinelli, and G. Ciofani, “Innovative approaches for cancer treatment: current perspectives and new challenges,” *Ecancermedicalscience*, vol. 13, Sep. 2019, doi: 10.3332/ECANCER.2019.961.

[24] N. Khan and H. Mukhtar, “Cancer and metastasis: prevention and treatment by green tea,” *Cancer Metastasis Rev.*, vol. 29, no. 3, p. 435, Sep. 2010, doi: 10.1007/S10555-010-9236-1.

[25] M. Hassan, H. Watari, A. Abualmaaty, Y. Ohba, and N. Sakuragi, “Apoptosis and molecular targeting therapy in cancer,” *Biomed Res. Int.*, vol. 2014, 2014, doi: 10.1155/2014/150845.

[26] D. R. McIlwain, T. Berger, and T. W. Mak, “Caspase Functions in Cell Death and Disease,” *Cold Spring Harb. Perspect. Biol.*, vol. 5, no. 4, pp. 1–28, Apr. 2013, doi: 10.1101/CSHPERSPECT.A008656.

[27] A. Samykutty *et al.*, “Piperine, a Bioactive Component of Pepper Spice Exerts Therapeutic Effects on Androgen Dependent and Androgen Independent Prostate Cancer Cells,” *PLoS One*, vol. 8, no. 6, Jun. 2013, doi: 10.1371/JOURNAL.PONE.0065889.

[28] H. Chen, H. Sheng, Y. Zhao, and G. Zhu, “Piperine Inhibits Cell Proliferation and Induces Apoptosis of Human Gastric Cancer Cells by Downregulating Phosphatidylinositol 3-Kinase (PI3K)/Akt Pathway,” *Med. Sci. Monit.*, vol. 26, pp. e928403-1, Nov. 2020, doi: 10.12659/MSM.928403.

[29] A. Jafri *et al.*, “Induction of apoptosis by piperine in human cervical adenocarcinoma via ROS mediated mitochondrial pathway and caspase-3 activation,” *EXCLI J.*, vol. 18, p. 154, 2019, doi: 10.17179/EXCLI2018-1928.

[30] D. Meir, “Piperine: A Savor Inducer and a Cancer Reducer,” *OBM Hepatol. Gastroenterol. 2021, Vol. 5, 061*, vol. 5, no. 3, pp. 1–26, Sep. 2021, doi: 10.21926/OBM.HG.2103061.

[31] M. Z. Aumeeruddy and M. F. Mahomoodally, “Combating breast cancer using combination therapy with 3 phytochemicals: Piperine, sulforaphane, and

thymoquinone," *Cancer*, vol. 125, no. 10, pp. 1600–1611, May 2019, doi: 10.1002/CNCR.32022.

[32] K. I. Block *et al.*, "Designing a broad-spectrum integrative approach for cancer prevention and treatment," *Semin. Cancer Biol.*, vol. 35, pp. S276–S304, Dec. 2015, doi: 10.1016/J.SEMCANCER.2015.09.007.

[33] F. Li, I. Aljahdali, and X. Ling, "Cancer therapeutics using survivin BIRC5 as a target: what can we do after over two decades of study?," *J. Exp. Clin. Cancer Res. 2019 381*, vol. 38, no. 1, pp. 1–22, Aug. 2019, doi: 10.1186/S13046-019-1362-1.

[34] PDQ Integrative, Alternative, and Complementary Therapies Editorial Board, "Curcumin (Curcuma, Turmeric) and Cancer (PDQ®): Health Professional Version," *PDQ Cancer Inf. Summ.*, 2002, Accessed: Dec. 23, 2022. [Online]. Available: https://www.cancer.gov/about-cancer/treatment/cam/hp/curcumin-pdq.

[35] R. Ziegenhagen, K. Heimberg, A. Lampen, and K. I. Hirsch-Ernst, "Safety Aspects of the Use of Isolated Piperine Ingested as a Bolus," *Foods*, vol. 10, no. 9, Sep. 2021, doi: 10.3390/FOODS10092121.

[36] K. M. Nelson, J. L. Dahlin, J. Bisson, J. Graham, G. F. Pauli, and M. A. Walters, "The Essential Medicinal Chemistry of Curcumin," *J. Med. Chem.*, vol. 60, no. 5, pp. 1620–1637, Mar. 2017, doi: 10.1021/ACS.JMEDCHEM.6B00975/ASSET/IMAGES/LARGE/JM-2016-009757_0003.JPEG.

[37] A. Botezatu *et al.*, "Mechanisms of Oncogene Activation," *New Asp. Mol. Cell. Mech. Hum. Carcinog.*, Mar. 2016, doi: 10.5772/61249.

[38] K. Vishnoi, N. Viswakarma, A. Rana, and B. Rana, "Transcription Factors in Cancer Development and Therapy," *Cancers (Basel).*, vol. 12, no. 8, pp. 1–32, Aug. 2020, doi: 10.3390/CANCERS12082296.

[39] M. Elmeliegy, M. Vourvahis, C. Guo, and D. D. Wang, "Effect of P-glycoprotein (P-gp) Inducers on Exposure of P-gp Substrates: Review of Clinical Drug–Drug Interaction Studies," *Clin. Pharmacokinet.*, vol. 59, no. 6, p. 699, Jun. 2020, doi: 10.1007/S40262-020-00867-1.

[40] D. Yang and L. Hurley, "Structure of the biologically relevant G-quadruplex in the c-MYC promoter," *Nucleosides. Nucleotides Nucleic Acids*, vol. 25, no. 8, pp. 951–968, Sep. 2006, doi: 10.1080/15257770600809913.

[41] N. Pandya and A. Kumar, "Piperine analogs arrest c-myc gene leading to downregulation of transcription for targeting cancer," *Sci. Rep.*, vol. 11, no. 1, Dec. 2021, doi: 10.1038/S41598-021-01529-3.

[42] S. Stump, T. C. Mou, S. R. Sprang, N. R. Natale, and H. D. Beall, "Crystal structure of the major quadruplex formed in the promoter region of the human c-MYC oncogene," *PLoS One*, vol. 13, no. 10, Oct. 2018, doi: 10.1371/JOURNAL.PONE.0205584.

[43] N. M. Fofaria, S. H. Kim, and S. K. Srivastava, “Piperine causes G1 phase cell cycle arrest and apoptosis in melanoma cells through checkpoint kinase-1 activation,” *PLoS One*, vol. 9, no. 5, May 2014, doi: 10.1371/JOURNAL.PONE.0094298.

[44] K. Myers, M. E. Gagou, P. Zuazua-Villar, R. Rodriguez, and M. Meuth, “ATR and Chk1 suppress a caspase-3-dependent apoptotic response following DNA replication stress,” *PLoS Genet.*, vol. 5, no. 1, Jan. 2009, doi: 10.1371/JOURNAL.PGEN.1000324.

[45] H. A. Rogoff *et al.*, “Apoptosis Associated with Deregulated E2F Activity Is Dependent on E2F1 and Atm/Nbs1/Chk2,” *Mol. Cell. Biol.*, vol. 24, no. 7, p. 2968, Apr. 2004, doi: 10.1128/MCB.24.7.2968-2977.2004.

[46] G. Carrasco-Torres, R. Baltiérrez-Hoyos, E. Andrade-Jorge, S. Villa-Treviño, J. G. Trujillo-Ferrara, and V. R. Vásquez-Garzón, “Cytotoxicity, Oxidative Stress, Cell Cycle Arrest, and Mitochondrial Apoptosis after Combined Treatment of Hepatocarcinoma Cells with Maleic Anhydride Derivatives and Quercetin,” *Oxid. Med. Cell. Longev.*, vol. 2017, 2017, doi: 10.1155/2017/2734976.

[47] L. Ding *et al.*, “The Roles of Cyclin-Dependent Kinases in Cell-Cycle Progression and Therapeutic Strategies in Human Breast Cancer,” *Int. J. Mol. Sci.*, vol. 21, no. 6, Mar. 2020, doi: 10.3390/IJMS21061960.

[48] S. Qie and J. A. Diehl, “Cyclin D1, Cancer Progression and Opportunities in Cancer Treatment,” *J. Mol. Med. (Berl).*, vol. 94, no. 12, p. 1313, Dec. 2016, doi: 10.1007/S00109-016-1475-3.

[49] H. J. Choi, M. Fukui, and B. T. Zhu, “Role of Cyclin B1/Cdc2 Up-Regulation in the Development of Mitotic Prometaphase Arrest in Human Breast Cancer Cells Treated with Nocodazole,” *PLoS One*, vol. 6, no. 8, 2011, doi: 10.1371/JOURNAL.PONE.0024312.

[50] B. Chopra, A. K. Dhingra, R. P. Kapoor, and D. N. Prasad, “Piperine and Its Various Physicochemical and Biological Aspects: A Review,” *Open Chem. J.*, vol. 3, no. 1, pp. 75–96, Jan. 2017, doi: 10.2174/1874842201603010075.

[51] D. Fong and M. M. Chan, “Dietary phytochemicals target cancer stem cells for cancer chemoprevention,” *Mitochondria as Targets Phytochem. Cancer Prev. Ther.*, pp. 85–125, Jan. 2013, doi: 10.1007/978-1-4614-9326-6_5/TABLES/2.

[52] R. Callaghan, F. Luk, and M. Bebawy, “Inhibition of the Multidrug Resistance P-Glycoprotein: Time for a Change of Strategy?,” *Drug Metab. Dispos.*, vol. 42, no. 4, p. 623, 2014, doi: 10.1124/DMD.113.056176.

[53] K. Smilkov, D. G. Ackova, A. Cvetkovski, T. Ruskovska, B. Vidovic, and M. Atalay, “Piperine: Old Spice and New Nutraceutical?,” *Curr. Pharm. Des.*, vol. 25, no. 15, pp. 1729–1739, Jul. 2019, doi: 10.2174/1381612825666190701150803.

[54] R. S. Y. Wong, “Apoptosis in cancer: from pathogenesis to treatment,” *J. Exp. Clin.*

Cancer Res., vol. 30, no. 1, p. 87, 2011, doi: 10.1186/1756-9966-30-87.

[55] V. Borse, A. N. Konwar, and P. Buragohain, "Oral cancer diagnosis and perspectives in India," *Sensors Int.*, vol. 1, p. 100046, Jan. 2020, doi: 10.1016/J.SINTL.2020.100046.

[56] M. Arruebo *et al.*, "Assessment of the Evolution of Cancer Treatment Therapies," *Cancers (Basel).*, vol. 3, no. 3, p. 3279, Sep. 2011, doi: 10.3390/CANCERS3033279.

[57] A. Zarghi and S. Arfaei, "Selective COX-2 Inhibitors: A Review of Their Structure-Activity Relationships," *Iran. J. Pharm. Res. IJPR*, vol. 10, no. 4, p. 655, 2011, Accessed: Dec. 24, 2022. [Online]. Available: /pmc/articles/PMC3813081/.

[58] H. Ram, J. Sarkar, H. Kumar, R. Konwar, M. L. B. Bhatt, and S. Mohammad, "Oral Cancer: Risk Factors and Molecular Pathogenesis," *J. Maxillofac. Oral Surg.*, vol. 10, no. 2, p. 132, Jun. 2011, doi: 10.1007/S12663-011-0195-Z.

[59] A. Kesarwala, M. Krishna, and J. Mitchell, "Oxidative Stress in Oral Diseases," *Oral Dis.*, vol. 22, no. 1, p. 9, Jan. 2016, doi: 10.1111/ODI.12300.

[60] S. Siddiqui, M. S. Ahamad, A. Jafri, M. Afzal, and M. Arshad, "Piperine Triggers Apoptosis of Human Oral Squamous Carcinoma Through Cell Cycle Arrest and Mitochondrial Oxidative Stress," *Nutr. Cancer*, vol. 69, no. 5, pp. 791–799, Jul. 2017, doi: 10.1080/01635581.2017.1310260.