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REVIEW ARTICLE

ANTICANCER DRUG

Nimbolide: An Anticancer Neem Limonoid

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Abstract

Cancer stands as the second most prevalent cause of significant global mortality annually, leading to a substantial loss of lives. As a result of its remarkable impact, it has become a major illness burden, exacerbated by the shortcomings in current treatment methods. Contemporary cancer treatments within allopathic medicine are associated with high costs, the manifestation of side effects, and the potential for alterations in normal functional genes. Natural compounds derived from medicinal plant have garnered considerable attention for their potential anticancer properties. Nimbolide, which is a bioactive compound derived from the neem tree (*Azadirachta indica*), has emerged as a promising element for cancer treatment because of its diverse pharmacological activities and minimal toxicity. A multitude of *in vitro* and *in vivo* studies have demonstrated compelling evidence of nimbolide supporting anti-cancer activity against various types of cancer, indicating its potential chemopreventive efficacy. This organic compound has exhibited noteworthy capabilities including the suppression of cancer cell proliferation, induction of apoptosis, preventing metastasis and angiogenesis. This review aims to give a comprehensive synopsis of the current discernment regarding the inhibitory effects of nimbolide on cancer cells.

Keywords: *Neem, Nimbolide, Anticancer, Chemotherapy*

1 Introduction

Nimbolide (nim), a major tertranortriterpenoid obtained enormously from the leaves of Neem tree, scientifically known as *Azadirachta indica*, belonging to the Meliceae family, which is widely distributed worldwide, particularly in south and southeast Asia(1). Various organic compounds have been extracted from different part of the neem plant which have gained increasing attention in the recent years due to its diverse potential properties. Celebrated for its versatility and natural goodness, neem, an ancient tree, holds a prominent place in traditional Indian Ayurveda Medicine, renowned for its potent antibacterial, antiviral, antifungal, and anti-inflammatory properties, neem finds significant application in treating skin disorders(2). Neem leaf pastes or extracts are frequently utilized topically to address conditions like acne, eczema, psoriasis and skin healing process. The therapeutic advantages of neem are believed to combat harmful bacterial in mouth, potentially preventing cavities, gum diseases and bad breath. Even the Neem-based products have gained popularity in the market for its miscellaneous benefits in healthcare, reflecting the growing recognition of neem's medicinal properties(3). Neem oil, extracted from the seeds of the neem tree, is a commonly incorporated ingredient in soaps, shampoos, creams, hair oils, face washes, and various personal care products due to its effectiveness against various common concerns. Beyond its topical applications, neem extracts are also extensively utilized in Traditional Ayurveda medicine. In this context, neem is employed in treating diverse conditions such as diabetes, malaria, and digestive issues, reflecting its versatile therapeutic properties(4). Esteemed brands such as NeemAura Naturals, Himalaya Herbal, Ayouthveda, Patanjali, Dabur, Ayush Herb, Ozone, Khadi Natural, Neem Shakti, Aroma, Biotique, Indulekha, Kama Ayurveda, Jovees, Lotus, Garnier, VLCC, Boroplus, Colgate, St. Botanica, and others have harnessed the incredible power of neem. Nimbolide, a well-known active ingredient derived from neem, is recognized for its various free radical scavenging and anti-cancer properties. These properties contribute to its effectiveness in combating cancer development and progression, showcasing neem's significant role in the realm of health and wellness(5). Studies provide impregnable evidence supporting the continued development

of nimbolide as a therapeutic agent against cancer. The anticancer effects of nimbolide have been consistently observed in various animal studies also. This promising research underscores the potential of nimbolide as a valuable component in the ongoing pursuit of effective cancer treatments and prevention strategies(6). According to research, nimbolide has demonstrated the ability to activate the apoptotic pathway in cancer cells, ultimately inducing their programmed cell death. Cumulative findings have consistently highlighted the cytotoxic effects of nimbolide, impeding the accumulation of cancer invasion, metastasis, angiogenesis, and inflammation. This is achieved through the modulation of kinase-driven oncogenic signaling networks. These insights emphasize the potentiality of nimbolide as a propitious candidate for therapeutic interventions targeting multiple facets of cancer progression(7). With its continuously expanding repertoire of molecular targets, nimbolide emerges as a exceeding inclusion in the arsenal of anti-cancer drugs, demonstrating its excellent chemopreventive and therapeutic effects. The versatility of nimbolide in targeting various molecular pathways highlights its potential as a beneficial agent in the ongoing efforts to develop effective strategies for cancer prevention and treatment.

2 Molecular Mechanisms of Nimbolide in Targeting Cancer Cells

Cancer is a disorder characterized by abnormal growth of cell with potential to invade or spread to the other parts of the body(1). The bioactive component, nimbolide, derived from neem, exerts its impact on cancerous cells by inducing cell death through the alteration of several molecular pathways(8).

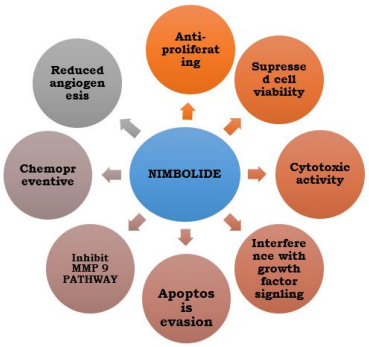

Figure 1: Nimbolide Inhibiting Different Acquisition of Cancer Hallmarks

Recently, there has been a notable focus on the study of this organic product derived from neem, over the past several years across a diverse range of cancer cell lines. Nimbolide has demonstrated its capability to induce apoptosis, a programmed cell death process, in various cancer cell lines. Notably, it inhibits cancer progression by influencing multiple mechanisms, including the prevention of pro carcinogen activation and oxidation(9; 10). Nimbolide, has been shown to exert influence on epigenetic processes in cancer cells, specifically affecting DNA methylation and histone modification. These alterations in epigenetic patterns can have a significant impact on gene expression, subsequently influencing the behavior of cancer cells, including the regulation of Reactive Oxygen Species (ROS). The multifaceted potential mechanisms of cancer prevention by nimbolide, encompassing apoptotic induction, inhibition of cancer progression, and modulation of epigenetic processes, highlight its intricate and promising role in addressing various aspects of cancer development and treatment.

3 Nimbolide as a Potent Apoptosis Inducer

Nimbolide, which have been under investigation for its potential therapeutic applications, has accumulated considerable attention due to its ability to induce apoptosis, a fundamental process, crucial for maintaining tissue homeostasis and eliminating damaged cells. This programmed cell death mechanism is vital in various physiological processes and becomes dysregulated in conditions such as cancer, neurodegenerative disorders, and autoimmune diseases(24). Extensive research on nimbolide's pro-apoptotic effects has revealed a multifaceted mechanism involving both intrinsic and extrinsic apoptotic pathways. In the intrinsic pathway, nimbolide exerts its influence on mitochondria, pivotal regulators of apoptosis. The compound modulates the expression of pro- and anti-apoptotic Bcl-2 family proteins, disrupting the mitochondrial membrane potential. This disruption contributes to the activation of caspase-dependent proteolysis, ultimately triggering cell death(25). Nimbolide's impact on the extrinsic apoptotic pathway is characterized by its interaction with death receptors on the cell surface, such as Fas (CD95) and tumor necrosis factor receptor 1 (TNFR1). These interactions further contribute to the initiation of apoptotic cascades. In human MCF-7 breast cancer cells, nimbolide was found to significantly inhibit cell growth by targeting P13K/AKT and NF-κB pathways, leading to apoptosis and inhibition of metastasis. In prostate cancer, nimbolide inhibits cell proliferation and induces apoptosis through caspase activation and disruption of mitochondrial membrane potential. Its efficacy extends to leukemia, where it induces apoptosis by affecting mitochondrial functions. Nimbolide also demonstrate apoptotic activity in pancreatic cancer by interfering with survival pathways and activating caspases, highlighting its potential as therapeutic agent for hematological malignance and aggressive tumors(26; 27; 28).

4 Nimbolide as an Anti-Metastatic Agent

The process of metastasis involves the detachment of cancer cells from the primary tumor, spreading to other body parts through the bloodstream or lymphatic system, and contributing to heightened morbidity and mortality rates(29). Recognized as a pivotal factor in cancer progression, nimbolide has shown promise as a potential anti-metastatic agent. It exhibits the capability to interfere with various signaling pathways implicated in the metastatic process. Numerous studies have investigated nimbolide's potential to

Table 1: Details of Completed Clinical Trials on PUFAs And/or on the Ratio of Omega-6/omega-3

Sl No.	Type of cancer	Cell lines	IC50	Effects	References
1	Prostrate	DU145, PC-3	5 µM, 2.5 µM	Inhibit cell proliferation, Suppressed cell viability, invasion and migration.	(11)
2	Breast cancer	MCF 7, MDA-MB-231, MDA-MB-468, MCF 10	4.02 µM, 2-5 µM, 2.24 µM, 5 µM	Displayed anti-proliferative activity, Programmed cell death	(12), (13), (14)
3	Oral	SCC 131, SCC 4	6 µM, 6.2 µM	Cell viability was seen decreasing.	(15)
4	Cervical	HeLa	2.08 µM	Showed Inhibitory activity.	(16)
5	Hematologic	U937, THP-1, HL - 60	0.5-5 µM, 0.5-5 µM, 0.5-5 µM	Induced anti-proliferative activity.	(8), (3)
6	Lung	A549, H1650	1.5-6 µM	Decreased cell viability.	(17)
7	Ovary	OVCAR5	1.5-9.2 µM	Showed anti-proliferative activity.	(18)
8	Skin	B16	0.5-5 µM	Exhibit anti-proliferative effect.	(19)
9	Pancreatic	HPAC, MIAPaCa-2, PANC1	5 µM, 3 µM, 5 µM	Showed cytotoxic effect. Suppresses clonogenicity and cell migration.	(20)
10	Glioblastoma	T98G, U87, EGFRVIII	5 µM, 5 µM	Displayed reduced cell viability, Sensitized cancer cell to trail induced apoptosis, Retarded tumor cell growth and migration	(21)
11	Liver	HepG2, Huh7, Hepa 1c1c7	6-10 µM	Cell proliferation, Induces apoptosis	(22)
12	Colon	HCT116, HT29	5 µM, 5 µM	Reduces the growth of cancer cells.	(23)
13	Osteosarcoma	143B-Tk	4.5 µM	Reduces viability of cancer cells.	(24)

mitigate metastasis, offering a promising avenue for the development of novel anti-metastatic therapies(7). Research indicates that nimbolide can diminish cancer cell migration and invasion, thereby hindering their dissemination to distant organs. One primary mechanism of nimbolide involves impeding the movement of cancer cells by modulating the activity of proteins that regulate the cytoskeleton, essential for cell motility(30). Furthermore, studies have confirmed that nimbolide targets various proteins involved in metastasis, including matrix metalloproteinases (MMPs) and epithelial-mesenchymal transition (EMT) markers crucial for the metastatic process(31). Recent studies(32) have demonstrated that nimbolide, a triterpene limonoid compound, can block the growth and metastasis of Pancreatic Ductal Adenocarcinoma (PDAC). This is achieved by suppressing the activity of the SOD2 enzyme, which plays a critical role in the Reactive Oxygen Species (ROS) signaling pathway, leading to the inhibition of tumor growth and metastasis.

5 Nimbolide and Inhibition of Angiogenesis

Angiogenesis, the process of forming new blood vessels from pre-existing ones, is crucial for physiological functions like tissue repair and growth. In cancer, angiogenesis supports tumor growth by supplying nutrients and oxygen, as well as facilitating the spread of cancer cells to distant sites through the bloodstream. Nimbolide, a natural compound under investigation for its potential anti-cancer properties, has exhibited promising results in inhibiting angiogenesis, which could limit the blood supply to tumors and impede their growth(31; 24). This organic compound has been reported to downregulate the expression of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), a key driver of angiogenesis. Nimbolide's potential impact extends to interfering with specific signaling pathways crucial for angiogenesis, such as the VEGF/VEGFR pathway and the PI3K/Akt/mTOR pathway. Studies have demonstrated its anti-cancer effects across various cancer types, including breast cancer (MCF7, MCF10), prostate cancer (PC-3), and pancreatic cancer, suggesting its potential as a therapeutic agent in combating angiogenesis-driven tumor progression. More importantly, nimbolide has demonstrated significant apoptotic effects on various cancer cell lines and exhibits inhibitory properties against both metastasis and angiogenesis(11; 32; 30).

6 Nimbolide Exhibit Anti-inflammatory Properties

Neem, with a rich history in traditional medicine, is renowned for its bioactive compounds. Numerous studies have explored the anti-inflammatory effects of nimbolide, revealing promising outcomes in modulating key pathways (NF-κB, MAPK, STAT) re'levant to inflammatory conditions. Recent research suggests that nimbolide may effectively reduce inflammation by suppressing the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Moreover, nimbolide has been observed to inhibit the activity of inflammation-associated enzymes, including cyclooxygenase (COX) and lipoxygenase (LOX), pivotal in the synthesis of inflammatory mediators like prostaglandins and leukotrienes. These findings extend to diverse diseases, encompassing arthritis, dermatitis, and various inflammatory disorders, underscoring nimbolide's potential as an anti-

inflammatory agent(33; 34; 35; 10).

7 Implication of Nimbolide in P13K/AKT Signaling Pathway

Dysregulation of this pathway is linked to diseases, especially cancer, making it an appealing therapeutic target. Nimbolide has demonstrated inhibitory effects on various cancer cell types in preclinical studies, such as breast, pancreatic, and prostate cancers. It is suggested that nimbolide achieves its anti-cancer effects by inhibiting AKT phosphorylation, leading to cell cycle arrest and apoptosis(32; 26). However, further research is necessary to fully comprehend the molecular mechanisms and implications of nimbolide in the PI3K/AKT signaling pathway(36).

8 Action of Nimbolide in TNF-α/NF-κB Signaling Pathways

Nimbolide, a compound derived from neem, exhibits a crucial application in modulating the TNF-α/NF-κB signaling pathway. This pathway plays a pivotal role in inflammation, immune responses, and cell survival. Upon binding of TNF-α to its receptor, NF-κB, a transcription factor integral to inflammation and immunity, is activated. Nimbolide has been demonstrated to inhibit NF-κB activation, preventing its translocation into the nucleus where it regulates the transcription of pro-inflammatory genes. This results in a reduction in the expression of pro-inflammatory cytokines (such as IL-6, IL-1β) and enzymes (such as COX-2 and iNOS), thereby mitigating inflammation. Additionally, nimbolide induces apoptosis in various cancer cells, inhibiting their pathways associated with cell survival. The antioxidant properties of nimbolide contribute significantly to modulating the TNF-α/NF-κB pathway by reducing oxidative stress, which indirectly influences NF-κB activation, given its sensitivity to cellular redox status(35; 10; 37).

9 Nimbolide Inhibits Various Phases of the Cell Cycle

The cell cycle is a series of events in a cell leading to its growth, division, and duplication. Cyclin-dependent kinases (CDKs), regulated by specific proteins called cyclins, control cell growth and proliferation. CDK-cyclin complexes, formed when CDKs bind to cyclins, initiate different phases of the cell cycle. Uncontrolled growth characterizes tumor cells, and inducing cell cycle arrest is a mechanism to prevent uncontrolled cell proliferation(15). Nimbolide, according to studies, induces cell cycle arrest by affecting regulatory proteins and signaling pathways involved in cell cycle progression. It downregulates the expression of cyclins and CDKs, reducing their activity and leading to cell cycle arrest. Cell cycle checkpoints ensure proper progression, and nimbolide is suggested to activate G1 and G2 checkpoints, allowing time for DNA repair before cell division(8; 38). The tumor suppressor protein p53, crucial for cell cycle regulation and DNA repair, is influenced by nimbolide. Nimbolide increases p53 expression and activity, potentially leading to cell cycle arrest, DNA repair, or apoptosis, depending on DNA damage severity. The mechanism of nimbolide's action on cell cycle arrest varies across cancer cell lines, concentrations used, and other factors.

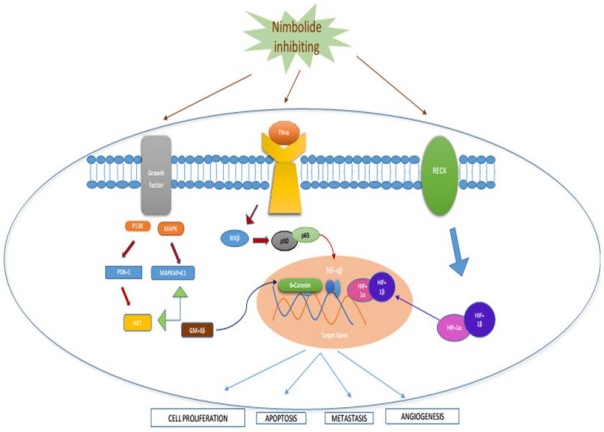

Figure 2: A Schematic Representation Delineating the Molecular Mechanisms Underlying the Anticancer Effects of Nimbolide

10 Preclinical Studies on Nimbolide

Preclinical studies typically involve *in vitro* and *in vivo* experiments to assess the safety and efficacy of a compound before it advances to human clinical trials. Numerous studies have provided evidence supporting the potential therapeutic efficacy of nimbolide in the context of various cancers. Some key findings from preclinical studies on nimbolide as reported in various studies are: Nimbolide treatment has exhibited characteristic apoptotic features in diverse cancer cells (A-549, PC-3, Du-145), manifesting as diminished cell numbers, cellular shrinkage, membrane blebbing, and weakened intercellular connections. In a separate analysis using U937 cells (leukemic cell lines), flow cytometry revealed that nimbolide, administered at concentrations of 1–2.5 μM, induced cell cycle disruption by reducing the number of cells in the G0/G1 phase. The potential of nimbolide to induce apoptosis extends to various cancer cell lines, including breast (MCF7) and liver (HepG2) cancer cells, demonstrating efficacy within the concentration range of 6-10 μM. Nimbolide was seen to reduce the viability of NIE-155 and 143B TK cell lines with the IC50 value 4.75. Furthermore, its anti-proliferative effects were evident, notably yielding a substantial 50% inhibition of the prostate cancer cell line PC-3 at a concentration of 2 μM. Studies *in vivo* have demonstrated substantial reductions in tumor volume with nimbolide treatment. For instance, at a dose of 20 mg/kg, a remarkable 90% reduction in tumor volume was achieved. However, the effectiveness varies with the type and nature of different cell lines. Nimbolide exhibited weak anticancer activity against WM tumors despite high *in vitro* sensitivity (IC50 = 0.2 µM). Similarly, in glioblastoma multiforme, cells were found to be less sensitive to nimbolide, with a relatively high *in vitro* IC50 (3 µM). The efficacy of nimbolide in vivo studies often appears to be dose-dependent. Different doses may lead to varying de-

grees of tumor inhibition or other therapeutic effects. These findings underscore the importance of considering the specific characteristics of cancer cell types in evaluating nimbolide's efficacy(24; 2; 11; 28).

11 Patents on Nimbolide

The authors searched the patent databases and found 2 relevant patents on nimbolide anticancer activity. In one US patent filed by CSIR scientists nimbolide chemical analogs were tested in cell culture and tumor model. It was found nimbolide analog was cytotoxic against several cancer cell lines and caused tumor cell death in pancreatic cancer xenograft. In another World patent filed by Mayo Clinic, USA scientists found out anticancer efficacy of a combination of Nimbolide, Nimbandiol, 2', 3' dihydro Nimbolide, 28 dihydro Nimbolide. They successfully used Prostate cancer xenograft model and prostate cancer cells to observe anticancer effects of nimbolide and other neem limonoids.

12 Conclusions

Phytomedicines are nothing new in cancer chemotherapy. There are ample examples of chemotherapeutic natural products in cancer treatment. For example, Paclitaxel from Pacific Yew, Vincristine and vinblastine from Vinca serve as useful medicines in clinics. However, due to adverse side effects and developing drug resistance there is a demand for new small molecules in oncology. Hence plants are in demand in drug discovery pipeline. Neem, a typical Indian plant has been a storehouse of Ayurvedic medicine. In the past neem has found use in pesticide and as antibacterial in creams and ointments. Neem soap, neem toothpaste and neem mouthwash are in use by several Indian companies. By this review an effort has been made to unearth data on anticancer activity of a neem limonoid Nimbolide. Nimbolide shows promising results in cancer cells and tumour models. Several mechanisms of anticancer efficacy of nimbolide have been brought forward. Nimbolide has been patented for its chemotherapy potential. In future as the small molecules from phytomedicine enters drug discovery pipeline, nimbolide will be a bright candidate for doing so.

Conflict of Interest

The authors declare no competing interest with any person or organization.

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