A REVIEW ON THE PLAUSIBLE EFFECTS OF NIMBOLIDE

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ABSTRACT

Nimbolide is one of the chief components in the leaf extract of Azadirachta indica (A.indica). Vibrant evidence from various in vitro and in vivo studies reveal that nimbolide possesses various biological activities and also shows potential chemopreventive activity in animal models. The major mechanisms of action of nimbolide include anti-proliferation, induction of apoptosis, inhibition of metastasis and angiogenesis, and modulation of carcinogen-metabolizing enzymes. Although numerous pharmacodynamic studies have been carried out, nimbolide is still at the infant stage in the drug development pipeline due to the lack of systematic pharmacokinetic studies and long-term toxicological studies. Therefore this review aims at exploring the various effects of Nimbolide in a short manner.

Keywords: Nimbolide; cancer; arthritis; prostate cancer; apoptosis

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INTRODUCTION

Nimbolide is a major tertranortriterpenoid, isolated from the leaves of Azadirachta indica, commonly known as Neem. Nimbolide is well known for its many uses, such as anti-malarial, antibacterial activity against S.aureus and S.coagulase, anti-feedant, and insecticidal activity [1-3]. In addition, nimbolide has been found to possess antioxidant effect and free radical scavenging activities. In comparison to azadirachtin and ascorbic acid (vitamin C), nimbolide was shown to be a more potent antioxidant [4]. Furthermore, nimbolide has been identified as one of the active ingredients of neem extract, a widely available herbal product used in traditional Indian Ayurvedic medicine to treat acne, wound, gastric ulcer, and infections. In addition, the anticancer and cancer preventive effects of nimbolide were observed in several animal studies [5-10]. Therefore, this review was undertaken to explicate the different effects of Nimbolide in a brief manner.

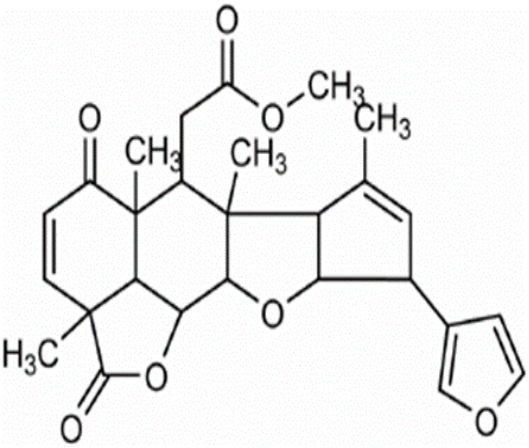

Figure 1: Structure of Nimbolide

2. DIFFERENT EFFECTS OF NIMBOLIDE

2.1 Antiarthritic activity of nimbolide against Freund's adjuvant induced experimental arthritis

Nimbolide (20 mg/kg per day) was given orally to arthritic rats induced with Complete Freund's Adjuvant and changes in paw volume, body weight, organ indices (thymus and spleen), arthritic score, biochemical parameters and proinflammatory cytokines levels were determined. Histopathological analysis and Western blot analysis was also performed. Rats treated with nimbolide displayed marked reduction in arthritic score, organ indices, volume of paw, edema formation, along with substantial enhancement in body weight. Histopathological findings showed significant reduction in destruction of joints and inflammation following nimbolide treatment [11].

2.2 Nimbolide ameliorates fibrosis and inflammation in experimental murine model of bleomycin-induced scleroderma.

Clinical manifestations of skin fibrosis are very variable and ambiguous, making its management quite critical and challenging. The lack of appropriate established pharmacological interventions make its treatment even more complicated. Intricate details of the underlying pathogenesis are thus imperative to further explore different treatment possibilities. Of note, the TGF-β/Smad signaling axis and epithelial to mesenchymal transition (EMT) are the principal offenders in this fibrotic disorder.

The antifibrotic and anti-inflammatory potential of nimbolide, a triterpene derived from Indian traditional plant neem, in a murine model of Bleomycin-induced scleroderma. Male C57BL/6 mice were administered with Bleomycin injections subcutaneously, daily for 28 days, at a constant site on the dorsum of the mice. Treatment with nimbolide lasted from day 1 to day 28. At the time of study termination, the injected sites were collected and stored suitably to conduct further molecular experiments and protein expression studies. The results of this study showed that nimbolide can significantly intervene in the TGF-β/Smad signaling axis and the consequent EMT process, thus attenuating deposition of extracellular matrix. Nimbolide also profoundly caused the regression of established inflammation-driven fibrosis, thus demonstrating both antifibrotic and anti-inflammatory activities. Another commendable finding of this study is that nimbolide was able to decrease the levels of LOXL2, a collagen cross-linker, which is aberrantly expressed in scleroderma. This study showed that nimbolide was a potent antifibrotic agent which can be used as a pharmacological intervention for the treatment of scleroderma [12].

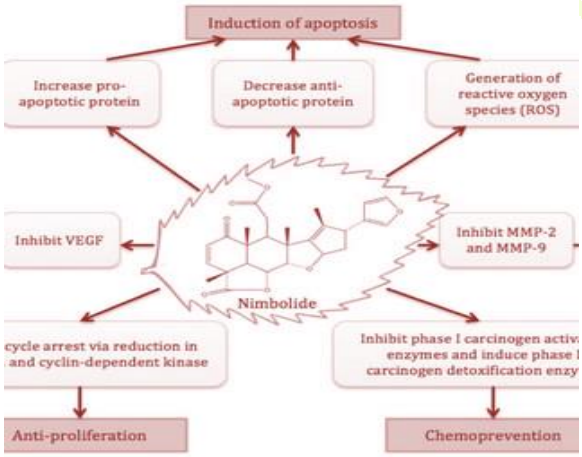

Figure 2: Different effects of Nimbolide [13]

2.3 Nimbolide induced apoptosis by activating ERK-mediated inhibition of c-IAP1 expression in human hepatocellular carcinoma cells.

This study indicated that Nimbolide inhibited cell growth in Huh-7 and PLC/PRF/5 cells. It was also found that nimbolide induced cell death through the induction of G2/M phase arrest and mitochondrial dysfunction, accompanied by the increased expression of cleaved caspase-7, caspase-9, caspase-3, caspase-PARP, and Bax and decreased expression of Mcl-1 and Bcl-2. A human apoptosis antibody array analysis demonstrated that inhibition of the apoptosis family proteins (XIAP, c-IAP1, and c-IAP2) was one of the major targets of nimbolide. Additionally, nimbolide sustained activation of ERK expression. Moreover, pretreatment with U0126 (MEK inhibitor) markedly abolished nimbolide-inhibited cell viability, induced cell apoptosis, ERK phosphorylation, cleaved caspase-9, caspase-3, cleaved-PARP activation, and increased c-IAP1 expression in Huh-7 cells. This study indicated the antitumor potential of nimbolide in human hepatocellular carcinoma cells [14].

2.4 Nimbolide epigenetically regulates autophagy and apoptosis in breast cancer.

Autophagy is a critical regulator of cellular homeostasis and its dysregulation often results in various disease manifestations, including cancer. Nimbolide, an active chemical constituent of neem (Azadirachta indica) exhibits potent anticancer effects. Although, nimbolide mediated apoptosis activation in breast cancer cells is well known. Nevertheless, its role in autophagy induction mechanism and epigenetic alteration is not explored previously. This study intended to bridge the gaps in the existing research by exploring the potential of nimbolide in inducing autophagy, which could counter regulate the transformations in breast cancer. In these studies, nimbolide significantly inhibited the cell proliferation of MDA-MB-231 and MCF-7 cells with IC_{50} values of 1.97 ± 0.24 and 5.04 ± 0.25 μM, respectively. Nimbolide markedly arrested the cell cycle progression and cell survival with loss of mitochondrial membrane potential by reducing Bcl-2 concomitantly inducing Bax and caspases protein expression with modulation of HDAC-2 and H3K27Ac expression. Consequently, characteristic autophagolysosome accumulation was observed by acridine orange, monodansylcadaverine (MDC) and Lysotracker Red staining. Moreover, nimbolide induced autophagy signaling by increasing Beclin 1 and LC3B along with decreased p62 and mTOR protein expression. Thus, these findings imply that nimbolide induces autophagy mediated apoptotic cell death in breast cancer with epigenetic modifications [15].

2.5 Anti-proliferative and apoptosis inducing effect of nimbolide by altering molecules involved in apoptosis and IGF signalling via PI3K/Akt in prostate cancer (PC-3) cell line.

Prostate cancer is responsible for major deaths globally after lung cancer. Nimbolide is an important constituent of neem, and it acts as a potent inhibitor for many cancer cells. This study study was designed to evaluate the effects of nimbolide on apoptosis and insulin-like growth factor (IGF) signalling molecules in androgen-independent prostate cancer (PC-3) cells line. Nimbolide (0.5-2 μM) treatment

resulted in 50% inhibition at a dose of 2 μM in the PC-3 cell line. The mRNA expression of Fas ligand, Fas-associated death domain receptor (FADDR), Bcl-2-associated X protein (Bax), Bcl-2-associated death promoter (Bad), phosphatidylinositide 3-kinases (PI3K), Akt, IGF1, IGF1 receptor (IGF1R) and IGF binding protein 3 were quantified by reverse transcription polymerase chain reaction and protein expression of Bax, cytochrome c, X-linked inhibitor of apoptosis protein (XIAP), B-Cell Lymphoma 2 (Bcl-2), caspases -8, -9, -10 and -3, poly(ADP-ribose) polymerase (PARP), cleaved PARP, IGF1R, PI3K, Akt, p-Akt was determined by western blot analysis, in nimbolide-treated PC-3 cell line. Nimbolide-induced apoptosis by activating DNA fragmentation in PC-3 cells. Nimbolide treatment increased the mRNA of Fas ligand, FADDR, Bax, Bad and IGF binding protein 3, decreased PI3K, Akt, IGF1 and IGF1R, increased protein expression of caspases 8, 3, 10, 9, Bax and cytochrome c and decreased the expression of XIAP, Bcl2, cleaved PARP, p-Akt and IGF1R. The results of this study suggest that nimbolide acts as a potent anti-cancer agent by inducing apoptosis and inhibiting cell proliferation via PI3K/Akt pathway in PC-3 cells [16].

2.6 Nimbolide inhibits growth of human colorectal cancer xenografts by suppressing the proinflammatory microenvironment.

The effect of nimbolide on proliferation of colorectal cancer cell lines was examined by MTT assay, apoptosis by caspase activation and poly-ADP ribose polymerase cleavage, NF-κB activation by DNA-binding assay, and protein expression by Western blotting. The effect of nimbolide on the tumor growth in vivo was examined in colorectal cancer xenografts in a nude mouse model. Nimbolide inhibited proliferation, induced apoptosis, and suppressed NF-κB activation and NF-κB-regulated tumorigenic proteins in colorectal cancer cells. The suppression of NF-κB activation by nimbolide was caused by sequential inhibition of IκB kinase (IKK) activation, IκBα phosphorylation, and p65 nuclear translocation. Furthermore, the effect of nimbolide on IKK activity was found to be direct. In vivo, nimbolide (at 5 and 20 mg/kg body weight), injected intraperitoneally after tumor inoculation, significantly decreased the volume of colorectal cancer xenografts. The limonoid-treated xenografts exhibited significant downregulation in the expression of proteins involved in tumor cell survival (Bcl-2, Bcl-xL, c-IAP-1, survivin, and Mcl-1), proliferation (c-Myc and cyclin D1), invasion (MMP-9, ICAM-1), metastasis (CXCR4), and angiogenesis (VEGF). The limonoid was found to be bioavailable in the blood plasma and tumor tissues of treated mice. This study provide evidence that nimbolide can suppress the growth of human colorectal cancer through modulation of the proinflammatory microenvironment [17].

2.7 Nimbolide abrogates canonical NF-κB and Wnt signaling to induce caspase-dependent apoptosis in human hepatocarcinoma (HepG2) cells.

Nuclear factor kappa B (NF-κB), an oncogenic signaling factor plays a critical role in the development and progression of various cancers. This study was undertaken to investigate the effect of nimbolide, a neem derived tetranortriterpenoid on NF-κB signaling and its downstream events - Wnt/β-catenin activation and apoptosis evasion in

human hepatocarcinoma (HepG2) cells by evaluating NF-κB family members (NF-κB-p50, p65, IκB-α, p-IκB-α, and IKKβ), members of Wnt signaling (GSK-3β and β-catenin), and intrinsic apoptosis (Bcl-2, Bax, cytochrome c, Smac/DIABLO, caspase-3, and caspase-9). Theresults demonstrate that nimbolide concurrently abrogates canonical NF-κB and Wnt signaling and induces intrinsic apoptosis in HepG2 cells. These data suggest that phytochemicals such as nimbolide that can target multiple steps along the NF-κB signaling circuit are promising candidates for future phytochemical-based mechanistic pathway targeted anticancer regimens [18].

2.8 Nimbolide retards tumor cell migration, invasion, and angiogenesis by down regulating MMP-2/9 expression via inhibiting ERK1/2 and reducing DNA-binding activity of NF-κB in colon cancer cells.

This study demonstrate that nimbolide effectively inhibited proliferation of WiDr colon cancer cells through inhibition of cyclin A leading to S phase arrest. It also caused activation of caspase-mediated apoptosis through the inhibition of ERK1/2 and activation of p38 and JNK1/2. Further nimbolide effectively retarded tumor cell migration and invasion through inhibition of metalloproteinase-2/9 (MMP-2/9) expression, both at the mRNA and protein level. It was also a strong inhibitor of VEGF expression, promoter activity, and in vitro angiogenesis. Finally, nimbolide suppressed the nuclear translocation of p65/p50 and DNA binding of NF-κB, which is an important transcription factor for controlling MMP-2/9 and VEGF gene expression [19].

2.9 Nimbolide Induces ROS-Regulated Apoptosis and Inhibits Cell Migration in Osteosarcoma.

Osteosarcoma (OS) is a primary malignant tumor of bone and is most prevalent in children and adolescents. OS is frequently associated with pulmonary metastasis, which is the main cause of OS-related mortality. OS has a poor prognosis and is often unresponsive to conventional chemotherapy. In this study, we determined that Nimbolide, a novel anti-cancer therapy, acts by modulating multiple mechanisms in osteosarcoma cells. Nimbolide induces apoptosis by increasing endoplasmic reticulum (ER) stress, mitochondrial dysfunction, accumulation of reactive oxygen species (ROS), and finally, caspase activation. It was also determined that Nimbolide inhibits cell migration, which is crucial for metastasis, by reducing the expression of integrin αvβ5. In addition, our results demonstrate that integrin αvβ5 expression is modulated by the PI3K/Akt and NF-κB signaling cascade. Nimbolide has potential as an anti-tumor drug given its multifunctional effects in OS. Collectively, these results help us to understand the mechanisms of action of Nimbolide and will aid in the development of effective therapies for OS [20].

CONCLUSION

Due to its diverse functions like anti-oxidant, anti-malarial property, anti-cancer potential etc., Nimbolide can be effectively used as a therapeutic agent for the treatment of several disorders. Thus this review paves way for

further clinical studies of Nimbolide and opens new avenues to further study the diverse functions of Nimbolide in the future.

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