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Review Article

Exploring the therapeutic potential of Neem (*Azadirachta Indica*) for the treatment of prostate cancer: a literature review

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Background and Objective: Multiple studies have demonstrated the medical potency of plant extracts and specific phytochemicals as therapeutics for prostate cancer (PCa) patients. Of note, the Neem plant known for its role as an antibiotic and anti-inflammatory is underexplored with an untapped potential for further development. This review focuses on extracts and phytochemicals derived from the Neem tree (Latin name; *Azadirachta indica*), commonly used throughout Southeast Asia for the prevention and treatment of a wide array of diseases including cancer. To date, there are more than 130 biologically active compounds that have been isolated from the Neem tree including azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, which have demonstrated a wide range of biological activities including anti-microbial, anti-fertility, anti-inflammatory, anti-arthritic, hepatoprotective, anti-diabetic, anti-ulcer, and anti-cancer effects. Very few scientific reports focus on the benefits of Neem in PCa, even though this herb has been used to prevent the disease and its progression for years in complementary and alternative medicine.

Methods: We used the search engines like PubMed, InCommon and Google using the key words: "Neem", "Cancer", "Prostate Cancer" and related words to find the information and data within the time frame from 1980–2022 for our article study.

Key Content and Findings: Here, we provide an overview of Neem extracts and phytochemical derivatives with a focus on their known potential and ability to inhibit specific cellular signaling pathways and processes which drive PCa incidence and progression.

Conclusions: The information presented here indicate that Neem and its derivatives have a therapeutic potential for the treatment of PCa when used as a single agent or in combination with conventional chemotherapeutics.

Keywords: Prostate cancer (PCa); medicinal plant; Neem; *Azadirachta Indica*

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Introduction

Prostate cancer (PCa) is the most commonly diagnosed male malignancy and the fourth leading cause of cancer related male mortalities worldwide (1,2). Although PCa related fatalities have been declining, incidence rates have been on the rise with an increasing number of patients living with the disease (3). A significant number of PCa patients have used or are interested in using natural products to supplement their therapy, a move that is supported by a growing number of epidemiological, clinical, and pre-clinical studies (4), indicating lower toxicities, easier usage and greater availability in many cases, compared to pharmaceuticals (5,6). Multiple pre-clinical studies have reported the anti-cancer properties of Neem and Neem products in PCa cell lines and animal studies (7-14). Key pathways known to drive PCa progression include the androgen receptor (AR) and the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/Akt) pathway, both of which can be modulated by treatment with Neem and its derivatives. Pathways which are linked to chemoresistance (e.g., Bcl-2) are also targets of Neem and its derivatives.

Neem tree extracts and phytochemicals: an overview of general properties and clinical uses

The Neem tree is native to the Indian subcontinent and Burma. For hundreds of years, its various components and derivatives have been widely used in alternate medicine approaches, including Ayurveda, throughout South Asia and beyond (15-17). The Neem leaves, bark, seeds, flowers, and twigs have been reported to have anti-microbial, anti-inflammatory, anti-arthritic, hepatoprotective, anti-diabetic, anti-ulcer, and anti-cancer activities (*Figure 1*) (13,17-29).

Several clinical studies have tested the medicinal properties of Neem. For example, in a pilot study with 14 patients, Bandyopadhyay *et al.* (30) have demonstrated Neem bark extract to have therapeutic potential in controlling gastric hypersecretion and gastroduodenal ulcers. In this study, 30 mg of lyophilised aqueous extract of air-dried Neem bark was encapsulated in the gelatine capsule that was used for oral administration to the selected patients. Treatment with lyophilized Neem bark extract orally for 10 days at 30 mg twice daily resulted in a 77% decrease in gastric acid secretion in 9 patients, while 3 others showed a decrease of 10–13% while 2 exhibited no effect. In addition, the bark extract at the dose of 30–60 mg twice daily for 10 weeks in 6 patients displayed significant reduction of duodenal ulcers (30). A larger study in 80 patients with type 2 diabetes mellitus described how the leaves and twigs of Neem have the potential to significantly reduce glycosylated hemoglobin (HbA1c) levels, insulin resistance (IR)/fasting blood sugar (FBS) and systemic inflammation (31). In this study, subjects received capsules of either 125, 250 or 500 mg of Neem [aqueous extract of *Azadirachta indica* leaves (AIE) and twigs] or placebo twice daily for 12 weeks. At all doses, Neem not only significantly reduced postprandial blood sugar (PPBS) level, HbA1c, FBS and IR but also improved endothelial function, reduced oxidative stress and systemic inflammation when compared to the placebo. The bioactives in these capsules were found to be flavonoids and myo-inositol monophosphate was found to be the predominant bioactive. Neem leaves, seed oil and the bark have also been used against malaria in India, Nigeria, and some other parts of Asia (32-39). The larvicidal properties of Neem seed oil (0.03% azadirachtin) and Neem leaf slurry (over heated Neem leaves dried and minced, extracted using water and water/acetonitrile) was reported to be successful in controlling malaria (36,38-41). The Neem seed oil/leaf slurry has been shown to induce sterility in insects by interrupting sperm production in males and hormone control of oogenesis thereby exerting a cytotoxic effect on both follicular cells and oocytes of the malaria vector. To our knowledge, no clinical studies have formally been conducted to evaluate the effects of Neem in cancer patients, however, pre-clinical studies support the usage of Neem and its various components in the treatment of cancer. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-94/rc).

Methods

We performed a systematic literature search for all relevant articles in English language from the PubMed, InCommon and Google, limiting the publication date from 1980 to April, 2022. The following key words "Neem", "Cancer", "Prostate Cancer" and related terms (*Table 1*) were used for the literature search. The search was performed at the same time by multiple researchers, and disagreements were resolved through discussions with each other on regular basis for consensus.

Table 1 The search strategy summary

Items	Specification
Date of search (specified to date, month and year)	15th September 2020
Databases and other sources searched	PubMed, InCommon and Google
Search terms used (including MeSH and free text search terms and filters)	"Neem" AND "Prostate cancer"
	"Neem, neem oil, neem extract, prostate cancer"
	"Nimbolide, Nimbin, Azadirachtan, flavonoids, androgen receptor in prostate cancer"
Timeframe	1980–2022
Inclusion and exclusion criteria (study type, language restrictions etc.)	All relevant articles in English language
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Selection was conducted independently and discussed routinely for Consensus
Any additional considerations, if applicable	None

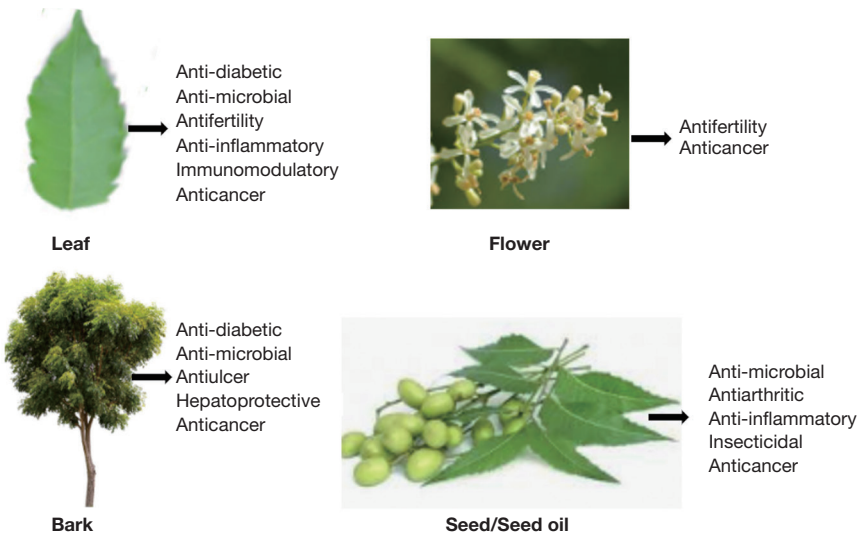

Figure 1 Important parts of the Neem tree (*Azadirachta indica*) and their therapeutic benefits.

Active components of Neem and dose forms

Chemical composition of Neem

Neem is a complex mix of various chemical constituents. Hossain *et al.* (42) have shown that the primary constituents of crude Neem leaf extract (NLE) include hydrocarbons, phenolic compounds, terpenoids, alkaloids, and glycosides [2-ethylhexyl tetradecyl est (13.70%), methyl petroselinate (11.23%), eicosane, 7-hexyl (10.01%), heptacosane (8.10%), hexadecamethylcyclo-octasiloxane (7.46%), octacosane (7.09%), heptacosane, 7-hexyl (6.77%), butyl palmitate (6.69%), isobutyl stearate (4.25%), nonadecane (3.75%), (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol (2.99%), 2,6,10,14-tetramethylheptadecane (2.68%), phytol (2.61%), methyl isoheptadecanoate (2.19%), and gamma-elemene (1.05%)]. Multiple studies have shown that terpenoids (including limonoids) are primarily responsible for Neem's biological activities (*Table 2*). Chemical characterization of Neem extracts via LC-MS by Santos *et al.* (92) demonstrated the following terpenoids in Neem extracts; 2,3-Dihydronimbolide (22.8%), Nimbolide + 3-Deacetylsalannin (19.7%), Nimbandiol (12.8%), Nimonol (10.1%), 6-Deacetylnimbinene (9.3%), 6-Deacetylnimbin (8.4%), Gedunin (4.8%), Nimbanal (4.4%), Salannin

Table 2 Active components of Neem (*Azadirachta indica*) and their potential therapeutic effects

Azadirachta indica plant compound	Class of compound	Part	Potential therapeutic effects	References
2',3'-dihydronimbolide	Terpenoid	Leaf	Anticancer	(13)
2',3'-dehydrosalannol	Triterpenoid	Leaf	Antifeedant, anticancer	(12)
28-deoxonimbolide	Terpenoid	Seed	Anticancer	(13)
6-deacetylnimbinene	Limonoid	Bark	Antiangiogenic, anti-cancer	(12)
Azadirachtin	Limonoid	Seed	Anticancer	(23)
Azadiradione	Limonoid	Fruit	Neuro-protective	(43,44)
Azadiramide	Limonoid	Seed	Anticancer	(28,44)
Azadirone	Limonoid	Seed	Anticancer	(45)
Catechin	Flavonoid	Bark	Antioxidant, anti-inflammatory	(46,47)
Epicatechin	Flavonoid	Bark	Antioxidant, anti-inflammatory	(46,48)
EAD	Limonoid	Fruits, seeds	Anti-inflammatory, anticancer	(49,50)
Gedunin	Limonoid	Leaf, seed	Anticancer, anti-allergic	(44,51)
Isomargolonone	Diterpenoid	Bark	Antibacterial	(46,52)
Margolonone	Diterpenoid	Bark	Antibacterial	(25,46,52-65)
Margolone	Diterpenoid	Bark	Antibacterial	(46,52)
Nimbandiol	Limonoid	Leaf, root	Anti-inflammatory, cytotoxic and antimycobacterial	(13,46,52, 66)
Nimbidin	Triterpenoid	Seed	Anti-inflammatory, antibacterial, antifungal	(13,66,67)
Nimbin	Triterpenoid	Seed	Anti-inflammatory, antibacterial, antihistamine, antipyretic, antiviral	(12,46,67-73)
Nimbolide	Limonoid	Leaf, seed	Anticancer, antibacterial, anti-malarial	(8,9,12,13,19-21,46,67-85)
Nimolinone	Protolimonoid	Leaf	Antiangiogenic, anti-cancer	(8,9,12,13,19-21,67,74-85)
Nimonol	Limonoid	Leaf	Anticancer, antifungal	(12,86-88)
Nimbinene	Limonoid	Seed	Anti-insecticidal, anticancer	(12,86-91)
Quercetin	Flavonoid	Leaf	anti-inflammatory	(12,48,89-91)

EAD, epoxyazadiradione.

(3.9%), Rutin (3.6%). It is noteworthy that four different extraction solvents were used in their study and while the percentage of each compound obtained in each extraction fraction was similar, their concentration varied depending on the polarity and capacity of different solvent used for the extraction.

Active compounds of Neem

Multiple medically active compounds including terpenoids and steroids have been isolated from Neem. The most highly studied compounds are azadirachtin, nimbolinin, nimbin, nimbidin, and nimbidol, however, multiple other bioactive components have also been identified (*Table 2*) (18,29). The well documented flavonoid, quercetin, has also been identified in NLE (48).

The limonoids, azadirachtin and nimbolide were shown to inhibit the DMBA-induced buccal pouch carcinomas through inhibition of tumor invasion, suppression of procarcinogen activation and oxidative DNA damage in addition to upregulation of antioxidant and carcinogen detoxification enzymes (93,94). These limonoids also

displayed inhibition of HeLa cervical cancer cell growth via a cell cycle arrest mechanism in the G0/G1 phase. The study described an upregulation of p21, p53, reactive oxygen species (ROS), Bax, survivin and a downregulation of cyclin B and D, Bcl-2, PCNA and NF-κB upon treatment with these limonoids (95).

Azadiradione extracted from the seeds of Neem were found to be effective in ameliorating symptoms in mammalian and fly models of neurodegenerative diseases making it a potential candidate for consideration against neurodegenerative diseases (43). Additionally, Azadiradione and Gedunin were found to bind and inactivate human pancreatic α-amylase (a well-known anti-diabetic target) and thus are considered as lead drug candidates to control post-prandial hyperglycemia (44). Azadiramide B, isolated from the extracts of *Bacillus subtilis*-fermented Neem seeds was shown to selectively inhibit the growth of MDA-MB-231 a triple-negative breast cancer (TNBC) cell line with an IC_{50} value of 15.73±6.07 μM (28). Azadirone was found to sensitize cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) through a ROS-ERK-CHOP-mediated up-regulation of death receptor DR5 and DR4 signaling in addition to a decrease in cell survival proteins, and an increase in proapoptotic proteins (45).

Another Neem derivative, Catechin, was found to be one of the best phytocompounds against dental plaque forming bacteria and displayed promise as a drug for treating biofilm related acute infections (46,47). Epicatechin was reported as a potent antibacterial agent in addition to its antioxidative activity (46,48). Epoxyazadiradione (EAD) extracted from the Neem seeds was found to induce mitochondrial apoptosis and inhibited NF-κB nuclear translocation in human cervical cancer cells (50). The study demonstrated that EAD is a potent and safe chemotherapeutic agent. Another study reported that EAD suppressed breast tumor growth via mitochondrial depolarization in addition to caspase-dependent apoptosis by attenuating PI3K/Akt-mediated AP-1 activation (49).

Neem is known for its antibacterial and antifungal activity that is mainly attributed to its bioactive compounds such as nimbidin, nimbolide, mahmoodin, margolone, margolonone, and isomargolonone (52). Nimbidin is a mixture of tetranor-triterpenes extracted from Neem oil. It has been reported as a potent anti-inflammatory and anti-arthritic agent. Recent studies reported that oral administration of 5–25 mg/kg nimbidin to rats for 3 consecutive days significantly inhibited the migration of macrophages to their peritoneal cavities in response to inflammatory stimuli. Thus, nimbidin could be useful for treating inflammation/inflammatory diseases (96). Nimbidin was also shown to exhibit significant gastric anti-secretory activity in pylorus ligated rats and cats. At the dose of 40 mg/kg (i.v.) to perfused rats, nimbidin suppressed carbachol stimulated gastric acid output (97). Recent studies have shown that nimbin, an active compound extracted from Neem leaf, has anti-viral effect against dengue virus. Interestingly nimbin was reported to be effective against the envelope protein of different types of the dengue virus (72). A very recent study used molecular docking to show that nimbin exhibits highest interaction with spike glycoprotein of SARS-CoV-2 with a MolDock score of 148.621 kcal/moL and ACE2 receptor with MolDock score—140.108 kcal/moL. This suggests that NLE may be useful as a therapeutic and/or prophylactic agent for SARS-CoV-2 (98).

Nimbolide, extracted from the Neem leaves, is known for its pathogenicity against various cancers by inhibiting cell proliferation, causing apoptosis, and impairing invasion and metastasis (99). Nimbolide was reported to inhibit breast cancer cell proliferation by disrupting RNF114-substrate recognition that results in the inhibition of ubiquitination and degradation of tumor suppressors such as Cyclin Dependent Kinase Inhibitor 1A (p21) (100). Several studies demonstrated potential of nimbolide as an antibacterial agent for the treatment of infections caused by multidrug-resistant strains in addition to its antimalarial potential (16,36,101). Nimonol is known for its antifungal activity against *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria solani* and *Sclerotinia sclerotiorum* (86-88). Nimbinene was reported to have anticancer effect on breast cancer by inducing ROS generation resulting in mitochondria-mediated apoptosis (89,90). Nimbinene was also reported to have anti-insecticidal activity (91). Given the large number of compounds with medicinal properties extracted from this one plant, it is no wonder that a number of patents on various parts of the tree and its extracts (USA, Japan, Australia, India) have been awarded, and are now being commercialized for various treatments (102). Generally Neem is accepted in the ayurvedic medical tradition as a therapy for various diseases as there are still many hurdles that limits large-scale use of Neem. One of the biggest hurdles in the commercialization of Neem is the lack of industrial interest, mainly due to the difficulty in patenting natural products, in addition to lack of enough scientific evidence or documents to support the benefits of these natural substances. These limitations must be overcome by proper scientific evidence and documentations so that the

full potential of Neem can be explored. Unfortunately, due to these limitations many of these products have not been tried in PCa or other diseases.

Neem has various components which contribute to the many dosage forms of *Azadirachta indica*. The most common used parts of Neem are the leaves and seeds. Components of Neem are often extracted with water or ethanol (10,103). The extract can stay in an aqueous fluid or be further dried to make a powder (29,104). Seed oil can be extracted with ethanol and supercritical carbon dioxide (CO_2), among other solutions. Neem plant extracts (NPEs) have many indications. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles composed from Neem leaf-extracted nimbolide have anticancer effects. The PLGA polymer surface can be modified by conjugating polyethylene glycol with receptor binding ligands for targeted delivery of nimbolide in cancer treatments (105).

Other extracts from Neem plant, such as Neem gum collected from incised bark of *Azadirachta indica* trees, has an abundance of functional groups which make it efficient for drug delivery applications. Bioadhesive buccal tablets, made from Neem gum, containing nicorandil were studied for its potential to increase contact between drug and absorbing surface, to prevent first pass effect, lengthen half-life and ultimately reduce frequency of nicorandil administration during treatment of hypertension and angina pectoris (106). Neem gum has also been used to make hydrogel for drug delivery of anticancer drug methotrexate (MTX) under different pH conditions. Neem gum-based hydrogel was determined to be a good carrier for MTX as the gel protects the drug from hostile environmental conditions, releases the drug in optimal quantities, therefore reducing the toxic side effects (107). Carbon dots, derived from Neem gum have biolinkers attached on their surface and stable green fluorescence which make them suitable for drug delivery system and/or biosensors (108).

Natural NPE also has the ability to reduce ions and has been used to reduce silver ions for hydrogel nanocomposite with mechanical toughness, large swelling and deswelling ability and electrical conductivity. Synthetic reducing agents limit potential silver hydrogel nanocomposite biomedical applications due to toxicity from harsh synthesis conditions (109). Reducing and stabilizing properties of aqueous NLE from Neem mistletoe was also tested for its ability to fabricate silver nanoparticles (AgNPs). Neem reduced AgNPs were found to be cytotoxic against human breast carcinoma cell lines and therefore a promising candidate for cancer therapy (110). In addition to plant extracts, Neem fungal endophyte, *Fusarium oxysporum*, has also been studied for its possible anticancer application. Gold nanoparticles derived from *Fusarium oxysporum* is anti-proliferative towards breast cancer, Burkitt lymphoma and human peripheral blood mononuclear cells, while safe toward normal human cells (111). Neem nanoemulsions (NE) have also been utilized for drug delivery purposes (112). NE from Neem seed oil has been used as drug delivery dosage form for poorly aqueous soluble drugs. Neem oil anti-oxidant properties were maintained in NE dosage form (113).

Use of Neem and its components for inhibition of PCa cells and tumor growth

Using natural compounds in PCa

A growing number of natural compounds have been tested in PCa, with varying results. Docetaxel, an FDA approved chemotherapeutic that is now standard-of-care for PCa, is a derivative of paclitaxel, which is derived from the bark of the Pacific yew tree (*Taxus brevifolia*) (114). However, other common foods and dietary supplements (e.g., green tea; pomegranate; lycopene; soy; mistletoe; vitamins C, D, and E; selenium; resveratrol) which are used by patients as treatments for cancer (115), did not receive FDA approval. This is due to various reasons such as variations in content of the trial compound and lack of financial incentives for funders to conduct phase III trials. The success of paclitaxel suggests that natural compounds for specific diseases is moved forward when clearly defined and active molecules from the natural product are used rather than whole extracts. The fact that so many of the Neem ingredients have been patented is therefore encouraging.

To date, only a handful of Neem products have been tested in PCa. Interest in Neem as a treatment for PCa stems from the fact that in India, where Neem is widely available, Neem oil is used as a male contraceptive (116). Later, a study in 2006 demonstrated that Neem ethanol extract induced apoptosis in PC-3 cells, which are of PCa origin (10). Since PC-3 cells do not express the AR, a key regulator of PCa in most cases, the scientific community remained unconvinced until in 2011 it was demonstrated that similar extracts also affected LNCaP cells, and C4-2B tumor xenografts—both of which express a mutant AR(T877A) (11,12). In 2014 Nimbolide, the active ingredient of Neem, was shown to have effects in PC-3 cells similar to that of the ethanol extract (9), while other studies suggested that the active ingredients of the Neem extract affecting LNCaP xenografts included nimbandiol, nimbolide,

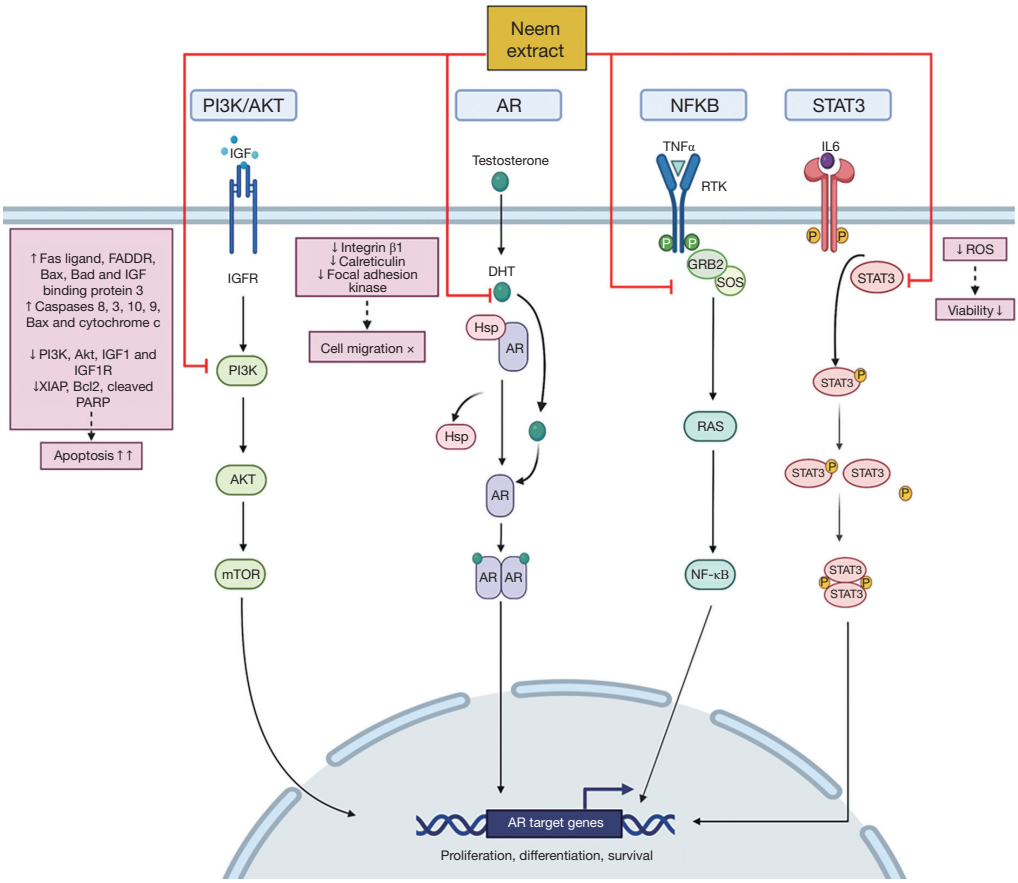

Figure 2 The effect of Neem on different pathways. The figure is created using BioRender (https://biorender.com/). AR, androgen receptor; DHT, dihydrotestosterone; ROS, reactive oxygen species.

2',3'-dihydronimbolide, and 28-deoxonimbolide (13). Importantly, these ingredients appeared to inhibit both the AR and the PI3K/Akt pathway that is important in the growth of PCa cells (9,11,13).

Inhibition of the AR pathway

The AR signaling pathway is important for normal functioning of prostate cells. Binding of androgen ligands such as testosterone and dihydrotestosterone (DHT) to cytoplasmic AR causes a conformational change in the receptor that enables nuclear translocation of AR and subsequent activation of the AR gene that governs growth and viability of prostate cells (117). Aberrant AR signaling has been implicated in primary PCa and in castration-resistant prostate cancer (CRPC) (118). Multiple pre-clinical reports have documented the efficacy of Neem extracts in PCa models (8,12,13). In 2014, Wu *et al.* (13) evaluated the use of supercritical extract of Neem leaves (SENL) as a treatment for *in vitro* and *in vivo* models of PCa. Supercritical extract refers to the use of supercritical fluid grade CO_2—that is, CO_2 that is held at or above its critical temperature (31.1 ℃) and critical pressure (72.9 atm/7.39 MPa), for extracting components from Neem. The AR is a steroid receptor that is highly expressed, not only in hormone sensitive prostate cancer (HSPC), but also in CRPC. The study showed that both the AR-positive HSPC cell line LNCaP and the AR-null CRPC PC-3 cell lines responded to SENL *in vitro*. SENL suppressed integrin β1, calreticulin and focal adhesion kinase activation (*Figure 2*). In addition, the report suggests suppression of cell growth, induction of apoptosis and significant reduction in DHT-induced AR and prostate-specific antigen (PSA) levels. DHT is a powerful AR ligand that binds to the receptor and enables its localization to the nucleus, where the AR binds to target DNA and promotes transcription of target genes including PSA that is used

as a biomarker to track AR activity in the prostate. Also, in mice bearing LNCaP xenograft tumors, oral administration of SENL was found to reduce xenograft tumor volume (13). These results support a tumor suppressive action of Neem in AR associated PCa. It may be noted that ethanolic extract of Neem leaves (EENL) was found to decrease the relative expression of AR after 48 hours of treatment at 1.0 µg/mL in MCF-7 and MDA-MB-231 breast cancer cell lines (119). Thus, the effect of Neem on the AR pathway was not confined to PCa alone.

Inhibition of the PI3K/Akt pathway

The PI3K/Akt signaling pathway is a key transduction cascade involved in promoting survival and growth of malignant cancer cells. PI3Ks are a family of related intracellular enzymes that phosphorylate a hydroxyl group on the inositol ring of a phosphatidylinositol (120). The PI3K family is divided into four different classes: Class IA PI3Ks are composed of a heterodimer between a p110 catalytic subunit and a p85 regulatory subunit (120) while class IB PI3Ks are heterodimers of a p110 catalytic subunit and a p101 regulatory subunit. Class II comprises of three catalytic isoforms, but, unlike Classes I and III, has no regulatory proteins. Class III is primarily involved in the trafficking of proteins and vesicles and has both regulatory and catalytic subunits. The Class IA catalytic subunit p110α is the best understood and is transcribed by the oncogene *PIK3CA*. It catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] into phosphatidylinositol 3,4,5-trisphosphate [$PI(3,4,5)P_3$], an event that is reversed by the tumor suppressor PTEN, a phosphatase. [$PI(3,4,5)P_3$] phosphorylates a number of downstream targets including phosphoinositide-dependent kinase-1 (PDK1) (121), which in turn phosphorylates key survival kinases such as Akt1 at Thr308 (122). Akt has a number of cellular functions that promote the progression of PCa to CRPC (123).

Owing to its strong association with cancer development and progression, multiple studies have aimed to target the PI3K/Akt pathway for anti-cancer therapy (9,11). Inhibiting the PI3K/Akt pathways leads to apoptosis via key players like Bad, caspase 3 and NF-Kβ (124). In 2014, Raja Singh *et al.* (9) demonstrated that nimbolide can cause apoptosis by inhibiting the PI3K/Akt pathway in PC-3 cells. Their results showed that nimbolide induced apoptosis by activating DNA fragmentation in PC-3 cells. In addition, nimbolide treatment increased the expression of pro-apoptotic genes such as Fas ligand, FADDR, Bax, Bad and IGF binding protein 3, while decreasing the expression of PI3K, Akt, IGF1 and IGF1R. Nimbolide also increased protein expression of caspases 8, 3, 10, 9, Bax and cytochrome c while it decreased the expression of XIAP, Bcl2, cleaved PARP, p-Akt and IGF1R (*Figure 2*). Similarly, Gunadharini *et al.* (11) observed an induction of apoptosis and inhibition of cell proliferation through the inhibition of the PI3K/Akt pathway in both LNCaP and PC-3 cell lines. Ethanolic Neem caused 50% inhibition at a dose of 100 µg/mL in both PC-3 and LNCaP cells and had similar effects on the PI3K/Akt pathway. Decrease in p-Akt by Neem caused an increase in the protein level of Bad and p21 while it decreased cyclin D1 levels supporting the notion that Neem affected both cell proliferation and survival via the PI3K/Akt pathway.

The presence of phytochemicals such as nimbolide (a major limonoid) In the Neem extracts may confer major advantages to the use of Neem extracts in comparison to single-molecule targeting pathway inhibitors since nimbolide has been shown to target multiple proteins and non-coding RNAs involved in the PI3K/Akt pathway. Sophia *et al.* (125) showed that in SCC131 and SCC4 oral cancer cells, nimbolide induces apoptosis in cancer cells by modulating the phosphorylation of proteins Akt and GSK-3β and ncRNAs miR-26 and HOTAIR causing the inactivation of the PI3K/Akt/GSK3 signalling pathway. Nimbolide induced targeting of the complex interaction of molecules involved in the PI3K/Akt pathway may therefore be more beneficial as a cancer therapeutic than inhibitors.

Inhibition of alternative pathways driving PCa progression and resistance to therapy

Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine that activates the cytoplasmic transcription factor NF-κB through a proteasome-mediated degradation of I-κB (inhibitor of NF-κB). Activated NF-κB then undergoes nuclear translocation and activates cell survival pathways (126). Singh *et al*. (7) showed that nimbolide suppressed expression of the TNF-α/TNFR1 signaling molecules TNF-α, SODD, Grb2, SOS mRNA which regulate NF-κB and MAPK pathways and thereby inhibited the survival and proliferation of PCa cells (*Figure 2*). Zhang *et al.* (8) also demonstrated that treatment of DU145 and LNCaP cells with nimbolide significantly inhibited cell viability, induced apoptosis, and suppressed tumor cell invasion and migration *in vitro*. Their subsequent *in vivo* studies using the transgenic adenocarcinoma of mouse prostate (TRAMP)

Table 3 *In vitro* anti-cancer effect of Neem (*Azadirachta indica*) on PCa

Type of study	Cell line	Type of extract	Concentration	Effect	Mechanism	References
In vitro	PC-3	Nimbolide	1–2 μM	Inhibited cell survival and proliferation	Inhibition of NF-κB and MAPK pathways	(7)
In vitro	DU145 and LNCaP	Nimbolide	0–20 μM	Reduced invasion and migration, induced apoptosis	Inhibits STAT3 activation	(8)
In vitro	PC-3	Nimbolide	1–2 μM	Reduced cell proliferation and induced apoptosis	Inhibits IGF1/IGF1R-PI3K/Akt pathway	(9)
In vitro	PC-3	ENLE	10–100 μg/mL	Induced apoptosis	Increased Bcl-2 protein and decreased Bax protein	(10)
In vitro	LNCaP and PC-3	ENLE	50–100 μg/mL	Inhibited cell proliferation	Inhibits PI3K/Akt pathway	(11)
In vitro	C4-2B and PC-3M-luc2	ENLE	5–50 μg/mL	Inhibited tumor cell growth	Upregulated HMOX1 and AKR protein	(12)
In vitro	LNCaP-luc2 and PC-3	SENL	5–25 μg/mL	Suppressed tumor growth and induced apoptosis	Suppressed integrin and FAK signaling	(13)
In vitro	LNCaP	Neem oil	300 μg/mL	Induced apoptosis	Induced caspase and AIF mediated apoptosis	(14)

PCa, prostate cancer; ENLE, ethanolic Neem leaf extract; SENL, supercritical extract of Neem leaves.

model demonstrated that nimbolide treatment suppressed tumor growth and decreased metastasis without significant side effects. The tumor suppressive effects were attributed to STAT3 inhibition caused by increased production of ROS due to an imbalance of the glutathione redox system (GSH/GSSG). The authors showed that nimbolide downregulates the activation of STAT3 transcription factor through the induction of oxidative stress (*Figure 2*). They found significant GSH reduction and GSSG increase in nimbolide treated cells compared to untreated cells. Similarly, Kumar *et al.* (10), in 2006, studied the effect of ethanolic Neem extract on the PCa cell line PC-3 *in vitro*. Using immunoblotting they showed a decreased level of anti-apoptotic protein Bcl-2 and an increased level of Bax protein with a subsequent increase in apoptosis with an increasing dosage of Neem extract.

Mahapatra *et al.* (12), in 2011, studied the effect of ethanolic Neem extract *in vitro* and *in vivo* models of PCa using C4-2B and PC-3M-luc2 PCa cells through qPCR and immunoblotting. They reported that ethanolic Neem extract caused an upregulation of cell death and drug metabolism genes, a down regulation of cell cycle, DNA replication, recombination, and repair genes *in vitro*, and tumor growth inhibition in C4-2B and PC-3M-luc2 PCa xenograft models in nude mice. Srivastava *et al.* (14) in 2012 used Neem oil containing multiple limonoids on LNCaP cells and reported that Neem induced caspase dependent and apoptosis inducing factor (AIF) mediated apoptosis and autophagy in PCa cells. They demonstrated that Neem limonoids caused release of mitochondrial cytochrome c and AIF resulting in caspase dependent and caspase independent cell death, respectively. They also reported p53 independent induction of autophagy in cells treated with limonoids.

Tables 3,*4* outline various studies that shows *in vivo* and *in vitro* anticancer effects of Neem on PCa.

Impact of Neem and Neem-derived compounds on some common cancer-related physiological processes. The anti-cancer activity of Neem has been studied using cell line and animal models which are representative of multiple types of cancers, for example skin, cervical, ovarian, breast, colon lung, stomach, liver, bladder cancers and Ehrlich's carcinoma (EC) as well as PCa (*Table 5*). The data from these studies demonstrate that Neem and its derivatives can inhibit the common physiological processes which drive cancer incidence and progression and can thereby be used to help further inform Neem usage for PCa.

Promotion of apoptosis

Killing cancer cells is the goal of most chemotherapy

Table 4 *In vivo* anti-cancer effect of Neem (*Azadirachta indica*) on PCa

Type of study	Cell line	Type of extract	Concentration	Dosage	Effect	Mechanism	References
In vivo	TRAMP	Nimbolide	3 mg/kg	5 times/week for 6–12 weeks	Reduced tumor growth and metastasis	Inhibition of STAT3 phosphorylation, decrease in Ki-67	(8)
In vivo	C4-2B and PC-3M-luc2 in nude mice	Ethanolic Neem leaf extract	100, 200 mg/kg	6 times/week for 8–11 weeks	Inhibited tumor growth	Promotion of hyalinization and apoptosis	(12)
In vivo	LNCaP-luc2 xenograft in nude mice	SENL	100, 200 mg/kg	6 times/week for 9 weeks	Reduced tumor growth	Promotion of hyalinization and apoptosis	(13)

PCa, prostate cancer; SENL, supercritical extract of Neem leaves.

Table 5 Anti-cancer effect of Neem (*Azadirachta indica*) on different cancer types

Cancer type	Mechanism	Neem component	References
Skin cancer	Inhibition of pro cancer inflammatory signals	NLE	(127)
	Cytotoxic activity	Limonoid	(128)
PCa	Apoptosis	NLE	(129)
	Cytotoxicity	Nimbolide	(130)
Cervical cancer	Cell cycle arrest	NLE	(128)
Ovarian cancer	Cytotoxicity	Nimbolide	(75)
Breast cancer	Destabilization of mitochondrial membrane potential. ROS generation and cell cycle arrest	Neem seed oil	(24)
Colon cancer	Apoptosis	Nimbolide	(19)
	Cell cycle arrest	Limonoid	(131)
Lung cancer	Apoptosis	Nimbolide	(130)
Stomach cancer	Elevated antioxidant enzymes and inhibition of lipid peroxidation	NLE	(132)
Liver cancer	Apoptosis	Nimbolide	(133)
EC	Increased production of $CD4^+$ and $CD8^+$ T cells	NLE	(134)

PCa, prostate cancer; NLE, Neem leaf extract; ROS, reactive oxygen species; EC, Ehrlich's carcinoma.

regimens (135,136). Neem and its components have been shown to promote apoptosis in several cancer types (19,20,24,77,78,125,137-139). For example, treatment of HeLa cells with ENLE resulted in cell death associated morphological changes at 175 µg/mL over time (up to 48 hours) (140). Ethanolic solution of Neem seed oil (contains azadirachtin and nimbin) was found to be cytotoxic to MDA-MB-231 and MCF-7 breast cancer cells at an IC_{50} of 20 and 10 µL/mL solution concentration, respectively (24). Efficacy has also been observed *in vivo*; an animal model study of stomach cancer demonstrated that Neem leaf extract (AAILE) could reduce tumor size (141). Several studies indicate apoptosis occurs due to activation of the intrinsic pathway of apoptosis, and that components of the NF-kB pathway also play a role. For example, treatment of hepatocarcinoma cell lines with Neem decreased Bcl-2 expression levels and increased caspase-3 and Bax expression (components of the intrinsic pathway of apoptosis) and that this occurred due to decreased expression of p50 and p65 (key components of the NF-kB pathway) (78). Nimbolide has also been shown to promote cancer cell apoptosis in neuroblastoma and colon cancer through reducing Bcl-2 levels while increasing Bax levels and caspase levels (133,142,143). When used in combination with standard

of care cytotoxic chemotherapy, Neem and Neem-based products can further increase drug-mediated cancer cell apoptosis. For example, treatment of HeLa cells (derived from a cervical cancer patient) with a combination of cisplatin and ENLE resulted in a 50% increase it cytotoxicity when compared with treatment with Neem or cisplatin as single agents (140). Nimbolide induced apoptosis in PC-3 cells by activating DNA fragmentation, and increased levels of the Fas ligand, FADDR, Bax, Bad and IGF binding protein 3, while decreasing PI3K, Akt, IGF1 and IGF1R. Nimbolide also increased the expression of caspases 8, 3, 10, 9, and cytochrome c and decreased the expression of XIAP, Bcl2, cleaved PARP and p-Akt (9). ENLE was also shown to induce DNA fragmentation in PC-3 cells (10), and induced apoptosis in both LNCaP and PC-3 (11). Another study showed that ENLE-suppression of C4-2B and PC-3M-luc2 tumor growth is associated with the formation of hyalinized fibrous tumor tissue and the induction of cell death by apoptosis, as well as an increase in the protein expression levels of HMOX1, AKR1C2, AKR1C3, and AKR1B10 (12). Thus there is significant evidence that Neem extracts can induce apoptosis in PCa cells.

Inhibition of cell proliferation

Many cancer drugs work by inhibiting cancer cell proliferation (144). Neem and its components have been shown to inhibit the proliferation of several types of cancer cells. For example, azadirachtin can inhibit cervical cancer cells (HeLa) by decreasing levels of cyclin B and cyclin D1 and thereby causing cell cycle arrest at the G0/G1 phase (145). Nimbolide has been demonstrated to reduce proliferation of bladder cancer cells at an IC_{50} of 3 μM with cell cycle arrest in the G2/M phase (81), and in oral squamous cell carcinoma can reduce cell proliferation in a dose-dependent manner (127). In swiss albino mice, NLE was used as prophylactic treatment for EC (134,146). In this study, a significant increase in antibody production against B16 melanoma antigen was detected in mice treated with Neem leaf preparation (NLP) once weekly for 4 weeks. An increase in number of splenic T lymphocytes ($CD4^+$ and $CD8^+$) and NK cells were also recorded in treated mice. Nimbolide treatment also suppressed expression of TNF-α, SODD, Grb2, SOS mRNA and modulated TNF-α/TNFR1 regulated NF-κB and MAPK signaling molecules in PC-3 cells (7). This inhibited PCa cell survival and proliferation via NF-κB and MAPK pathways. Nimbolide acts as a potent anti-cancer agent by inhibiting cell proliferation via PI3K/Akt pathway in PC-3 cells (9). Treatment of C4-2B and PC-3M-luc2 cells with ENLE also inhibited cell proliferation (12). Similar effects were also seen in LNCaP and PC-3 cells (11). These evidences point to anti-proliferative effects of Neem derivatives that can be used in preventing PCa proliferation.

Angiogenesis

Angiogenesis promotes cancer metastasis and as such is a key target of several cancer drugs, for example bevacizumab and thalidomide (147). Angiogenesis also plays a key role in driving PCa progression (148). Neem and its components have been shown to inhibit angiogenesis through suppressing levels of vascular endothelial growth factor (VEGF) and thereby inhibiting proliferation, migration, and invasion of human umbilical vein endothelial cells (HUVEC) (90). An *in vivo* study by Gupta *et al.* (149) showed that the Neem component nimbolide (at 5 and 20 mg/kg b.w., i.p.) significantly reduced the growth of colorectal cancer xenografts. They found a significant downregulation in the expression of NF-κB regulated tumorigenic proteins including VEGF, the growth factor which drives angiogenesis. Another *in vivo* study in DMBA-induced hamster buccal pouch (HBP) carcinogenesis model demonstrated inhibition of angiogenesis by NLE when administered at 10 mg/kg body weight (150). After analyzing the mechanism of chemo-prevention they found multitargeted mode of action including angiogenesis and multiple polar phytochemicals responsible for the action (150). A similar study also using the DMBA-induced HBP carcinogenesis model compared the chemopreventive potential of the Neem limonoids azadirachtin and nimbolide. They found that nimbolide was a more potent antioxidant and chemopreventive agent as compared to azadirachtin and results in multitargeted prevention and treatment of cancer including inhibition of tumor invasion and angiogenesis (94). While no published studies focused on the role of Neem in preventing angiogenesis in PCa, the above reports point to the possibility of using Neem extracts and derivatives in an anti-angiogenic role.

Inhibition of inflammation

Inflammation has been shown to contribute to the incidence and progression of several cancer types, including PCa (151). Inflammation primarily occurs through activation of the NF-

kB signaling pathway (152-154). While anti-inflammatory drugs such as prednisone and dexamethasone are often co-administered with chemotherapy to help reduce inflammation and other chemotherapy-related side effects (155), they are not currently used as individual agents for PCa chemoprevention or treatment, however, several pre-clinical studies are on-going to test their effects (156). Preclinical studies in other cancer types indicate that Neem can inhibit inflammation through abrogation of the synthesis and/or activation of cytokines, transcription factors, enzymes, and receptors which drive NF-kB signaling pathway activation (26,48). For example, in chronic myeloid leukemia (CML) K562 and Jurkat cells, it was shown that quercetin, a component of NLE (100 µM), could inhibit TNF-α induced NF-kB signaling (48). In the same study, it was found that this was due to the ability of NLE and quercetin to reduce the catalytic activity of IKKβ. Multiple studies also point to a role for Neem and its derivatives in preventing NF-kB signaling in PCa (7). Thus, PCa inflammation can be suppressed by Neem as well.

Impact of combining Neem with standard of care treatments for cancer

Cancer is a complex disease which needs amalgamation of multiple regimens to enhance efficacy, particularly for late-stage disease. For example, a common treatment regimen for CRPC are the androgen synthesis inhibitor Abiraterone acetate plus prednisone, the AR inhibitors enzalutamide, apalutamide and darolutamide, the chemotherapeutic agents docetaxel and cabazitaxel, and others such as the radiotherapeutic radium-223 and the immunotherapeutic sipuleucel-T (157). Several studies have reported that Neem extracts can induce chemo-sensitization of tumor cells in addition to reducing the adverse effects and toxicity of chemotherapeutic drugs. For example, combination therapy of Neem-derived Gedunin and cisplatin was evaluated on SKOV3, OVCAR4, and OVCAR8 ovarian cancer cell lines proliferation. Their results showed about 50% decrease in proliferation of cancer cells compared to the cells treated with only cisplatin (158). Another study showed the synergistic growth inhibition of breast and cervical cells via combinations of ethanolic Neem leaf extract (ENLE) with cisplatin as compared to the individual drugs, combination index <1 (140). The adriamycin (ADR) resistance in multidrug-resistant MCF-7 human breast cancer cell line is associated with the presence of high-level expression of P-glycoprotein. Quercetin, a Neem-extracted flavonoid was shown to reduce the expression of P-glycoprotein in MCF-7 ADR-resistant cells and thus proved to show additive effect on ADR therapy for breast cancer *in vitro* study (159). Ghosh *et al.* (160) showed in both *in vitro* and *in vivo* conditions that NLE is an effective tool to reduce CYP-induced hematological complications such as the occurrence of leukopenia and neutropenia that are found to be a life-threatening complication of chemotherapy. They found that treatment with NLE alleviates CYP-caused leukopenia and neutropenia in both normal mice and the tumor bearing mice (160). Another study showed that NLE has significant effect in prevention of leukocyte apoptosis that may happen by treatment with cisplatin plus 5-fluorouracil (5-FU) in swiss mice (161). In addition, they report that the efficacy of NLE is comparable to granulocyte colony stimulating factor (GCSF) in its ability to protect against leukocyte apoptosis induced by chemo agents and that it would be a better choice of treatment because GCSF is found to be tumor promoting and expensive. Cisplatin is one of the most valuable and potent chemotherapeutic drug used for the treatment of broad spectrum of malignancies such as testicular, head and neck, ovarian, cervical, and NSC-lung carcinoma. However, its clinical dose-limiting side effect is nephrotoxicity. Abdel Moneim *et al.* (162) showed that methanolic NLE can attenuate cisplatin-induced nephrotoxicity. In their study, they investigated the effects of methanolic NLE (500 mg/kg bwt) given by gastric gavage on cisplatin induced toxicity of kidneys in rats. The injuries of the renal tissue (as histopathological damages and increased serum uric acid, urea, and creatinine) by cisplatin were rescued by oral administration of methanolic NLE for 5 days. In addition, the other major side effect of cisplatin, hepatotoxicity, could also be prevented by administration of Neem leaf supplements (137). A significant decrease of elevated serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, urea, uric acid, and creatinine was observed by before or after administration of Neem supplement. Another study reported that nimbolide, a triterpenoid isolated from Neem, can enhance the sensitization of tumor cells to chemotherapeutic agents by inhibiting the activation of NF-κB (163). While Neem has not been shown to be effective against docetaxel, it has been found to be effective in paclitaxel resistant colon cancer and liver cancer cells (164). It remains to be seen whether Neem can be effective in docetaxel resistant PCa cells as well.

Toxicities

While medicinal plants, including Neem, have been used for several millennia for diverse purposes in the life of mankind particularly as medicines for the treatment of various diseases, it is well known that some of them can have serious toxicities at higher doses. A major concern is that extraction methods can change the concentration of specific compounds and that the concentration of these compounds in the final extract is often unknown. Toxicities have been reported for Neem and Neem products. For example, excessively high doses of Neem oil has been demonstrated to cause poisoning in children in the form of hepatic toxicity, encephalopathy, metabolic acidosis, vomiting and at lower levels in adults (165). A case study on a patient who had accidently consumed 20 mL of Neem Oil reported that the patient suffered seizures, vomiting and encephalopathy. The potential for toxicity of Neem extracts has been investigated in several animal models. When mice were treated with Neem in an aqueous extract form it was found to have an LD_{50} value of 13 g/kg (which is quite high), similar values were obtained when using methanolic extract form in rats (26). However, other groups showed that the aqueous extract of Neem leaf is not toxic to rats and they reported LD_{50} as >2.5 g/kg body weight (166). The latter group demonstrated in their toxicity studies that no mortality observed with 2.5 g/kg dose of AIE in mice and no significant alterations in body or tissues weight, food and water intake, hematological profile and various liver and kidney function in rats when treated for 28 days with 1 g/kg dose of AIE. In addition, Raizada *et al.* (167) found that azadirachtin from Neem when administered orally to rats of both sexes at doses of 500, 1,000 and 1,500 mg/kg/day for 90 days did not produce any signs of toxicity, mortality, changes in tissue weight, pathology and serum and blood parameters. Toxicities may also result from the route of administration. For example, nimbolide and nimbic acid, when given intravenously (i.v.) or intraperitoneally (i.p.) led to causalities in both rats and rabbits with 24 hours LD_{50} values of 14 and 24 mL/kg, respectively whereas it wasn't toxic when given via intragastric (i.g.) route (168).

The combined data indicate that caution should still be taken when consuming Neem and Neem products even though Neem-based products have been consumed for several millennia and the controlled consumption of Neem products can be considered as safe.

Other uses of Neem

Azadirachta indica, which can be found in abundance in the southeast Asian tropics, demonstrates ability to act as efficacious dosage forms for pesticidal agents as well as cytotoxic agents. In a study, Neem powder was revealed to have similar biopesticidal effects on larvae as aqueous NLE. However in equal concentrations, powdered Neem appeared to act faster on mosquitoes than its aqueous counterpart (104). In comparing NLE and Neem oil as fungicidal agents, Neem oil seemed to be more effective at suppressing fungal growth than NLE (169). Many studies find NLE to be efficacious biocidal agents. Methanolic extract of Neem leaves were synthesized into anti-bacterial microspheres and incorporated into a topical gel for the treatment of bacterial infection. The methanolic extract of Neem leaves overcomes poisonous effects of its synthetic gel counterpart (170). ENLE was used to form ethosome for dermal delivery of a biocidal fungicidal agent called luliconazole. The dosage form was successful for sustained release, targeted delivery, and increased drug permeability through lipid vesicle which all aided to enhance the bioavailability of luliconazole (171). Neem leaf powder was combined with alginate to create beads for fungicide thiram delivery and found to potentiate pesticide contents due to inherent pesticidal activity with less toxic effects (172). Neem leaf powder has also been used to make activated charcoal and shown to adsorb heavy metals (Pb, Cu, Cd, Zn, Ni, Cr) in a spontaneous, endothermic and favorable manner (173).

In addition to the above plant extracts, seed oil from the *Azadirachta indica* have also been utilized for its biocidal effects. Neem oil is notable for its biologically active compound azadirachtin which has been promoted as a low cost, eco-friendly and easy to handle insecticide (174). Zein nanoparticles, a precipitated dosage form of Neem oil, employ an environmentally friendly antisolvent. The nanocarrier allows lower doses and numbers of applications of the pesticide in agriculture (175). NE were compared to non-formulated Neem oil. NE was found to increase mortality of tested species *Sitophilus oryzae* and *Tribolium castaneum* with less toxicity than synthetic pesticide (176). Larvicidal properties of Neem oil were studied as urea NE which demonstrated to be an environmentally benign efficacious form of *A. aegypti* and *C. tritaeniorhynchus* mosquito control. Neem oil has also been studied for its ability to disrupt bacterial cell membrane of *Vibrio vulnificus*.

The antibacterial NE was found to be nontoxic to human lymphocytes at lower concentrations, but possibly toxic to human lymphocytes at higher concentrations with depletion of catalase, SOD and GSH (177).

Conclusions and future perspective

Neem and Neem-based products can clearly inhibit the processes which drive PCa incidence, progression, and resistance to chemotherapy in pre-clinical settings, and do so by inhibiting the pathways known to mediate these processes including the AR pathway, PI3K/Akt pathway, and intrinsic pathway of apoptosis amongst others. This, combined with the favorable safety profile of Neem, indicates that clinical studies of Neem for the treatment of PCa are warranted. Which Neem derivative should be used for clinical studies is a major consideration as pre-clinical studies have not directly compared efficacy or mechanism of action and there are likely to be differences between them. With this in mind, consideration should be given to matching known effects of each Neem product with PCa disease stage and/or knowledge of which pathway is driving patient disease progression or resistance to therapy. Further investigation of Neem and Neem products for treatment of PCa is supported by their relatively low cost and low toxicity levels, as long as used at reasonable (physiological) doses, and used in reasonable routes of administration. It may be kept in mind, that taxols, when first isolated from the bark of the Pacific yew tree, was considered to be toxic, but then went on to create therapeutic history by being one of the most widely used chemotherapeutic drugs available.

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Footnote

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References

1. Mattiuzzi C, Lippi G. Current Cancer Epidemiology. J Epidemiol Glob Health 2019;9:217-22.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
3. Jemal A, Fedewa SA, Ma J, et al. Prostate Cancer Incidence and PSA Testing Patterns in Relation to USPSTF Screening Recommendations. JAMA 2015;314:2054-61.
4. Cicero AFG, Allkanjari O, Busetto GM, et al. Nutraceutical treatment and prevention of benign prostatic hyperplasia and prostate cancer. Arch Ital Urol Androl 2019.
5. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim Biophys Acta 2013;1830:3670-95.
6. Katz L, Baltz RH. Natural product discovery: past, present, and future. J Ind Microbiol Biotechnol 2016;43:155-76.
7. Singh PR, Priya ES, Balakrishnan S, et al. Nimbolide inhibits androgen independent prostate cancer cells survival and proliferation by modulating multiple pro-survival signaling pathways. Biomed Pharmacother 2016;84:1623-34.
8. Zhang J, Ahn KS, Kim C, et al. Nimbolide-Induced Oxidative Stress Abrogates STAT3 Signaling Cascade and Inhibits Tumor Growth in Transgenic Adenocarcinoma of Mouse Prostate Model. Antioxid Redox Signal 2016;24:575-89.
9. Raja Singh P, Arunkumar R, Sivakamasundari V, et al. 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. Cell Biochem Funct 2014;32:217-28.
10. Kumar S, Suresh PK, Vijayababu MR, et al. Anticancer effects of ethanolic neem leaf extract on prostate cancer cell line (PC-3). J Ethnopharmacol 2006;105:246-50.
11. Gunadharini DN, Elumalai P, Arunkumar R, et al. Induction of apoptosis and inhibition of PI3K/Akt pathway in PC-3 and LNCaP prostate cancer cells by ethanolic neem leaf extract. J Ethnopharmacol 2011;134:644-50.
12. Mahapatra S, Karnes RJ, Holmes MW, et al. Novel molecular targets of Azadirachta indica associated with inhibition of tumor growth in prostate cancer. AAPS J 2011;13:365-77.
13. Wu Q, Kohli M, Bergen HR 3rd, et al. Preclinical evaluation of the supercritical extract of azadirachta indica (neem) leaves in vitro and in vivo on inhibition of prostate cancer tumor growth. Mol Cancer Ther 2014;13:1067-77.
14. Srivastava P, Yadav N, Lella R, et al. Neem oil limonoids induces p53-independent apoptosis and autophagy. Carcinogenesis 2012;33:2199-207.
15. Rupani R, Chavez A. Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent. Clin Dermatol 2018;36:306-9.
16. Kharwar RN, Sharma VK, Mishra A, et al. Harnessing the Phytotherapeutic Treasure Troves of the Ancient Medicinal Plant Azadirachta indica (Neem) and Associated Endophytic Microorganisms. Planta Med 2020;86:906-40.
17. Hummel HE, Langner SS, Leithold G, et al. NEEM: Unusually Versatile Plant Genus Azadirachta With Many Useful And So Far Insufficiently Exploited Properties For Agriculture, Medicine, And Industry. Commun Agric Appl Biol Sci 2014;79:211-28.
18. Alzohairy MA. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evid Based Complement Alternat Med 2016;2016:7382506.
19. Patel MJ, Tripathy S, Mukhopadhyay KD, et al. A supercritical CO2 extract of neem leaf (A. indica) and its bioactive liminoid, nimbolide, suppresses colon cancer in preclinical models by modulating pro-inflammatory pathways. Mol Carcinog 2018;57:1156-65.
20. Elumalai P, Arunakaran J. Review on molecular and chemopreventive potential of nimbolide in cancer. Genomics Inform 2014;12:156-64.
21. Ahmad MF, Ansari MO, Jameel S, et al. Protective role of nimbolide against chemotherapeutic drug hydroxyurea induced genetic and oxidative damage in an animal model. Environ Toxicol Pharmacol 2018;60:91-9.
22. Zingue S, Kamga Silihe K, Fouba Bourfane I, et al. Potential of Regular Consumption of Cameroonian Neem (Azadirachta indica L.) Oil for Prevention of the 7,12-Dimethylbenz(a)anthracene-Induced Breast Cancer in High-Fat/Sucrose-Fed Wistar Rats. Evid Based Complement Alternat Med 2019;2019:2031460.
23. Patel SM, Nagulapalli Venkata KC, Bhattacharyya P, et al. Potential of neem (Azadirachta indica L.) for prevention and treatment of oncologic diseases. Semin Cancer Biol 2016;40-41:100-15.
24. Sharma R, Kaushik S, Shyam H, et al. Neem Seed Oil Induces Apoptosis in MCF-7 and MDA MB-231 Human Breast Cancer Cells Asian Pac J Cancer Prev 2017;18:2135-40.
25. Goswami KK, Sarkar M, Ghosh S, et al. Neem leaf glycoprotein regulates function of tumor associated M2 macrophages in hypoxic tumor core: Critical role of IL-10/STAT3 signaling. Mol Immunol 2016;80:1-10.
26. Gupta SC, Prasad S, Tyagi AK, et al. Neem (Azadirachta indica): An indian traditional panacea with modern molecular basis. Phytomedicine 2017;34:14-20.
27. Satyanarayana K, Sravanthi K, Shaker IA, et al. Molecular approach to identify antidiabetic potential of Azadirachta indica. J Ayurveda Integr Med 2015;6:165-74.
28. Lin P, Fan X, Lu X, et al. Four limonoids from Bacillus subtilis-fermented neem seeds and their cytotoxic activity. Fitoterapia 2019;135:73-8.
29. Paul R, Prasad M, Sah NK. Anticancer biology of Azadirachta indica L (neem): a mini review. Cancer Biol Ther 2011;12:467-76.
30. Bandyopadhyay U, Biswas K, Sengupta A, et al. Clinical studies on the effect of Neem (Azadirachta indica) bark extract on gastric secretion and gastroduodenal ulcer. Life Sci 2004;75:2867-78.
31. Pingali U, Ali MA, Gundagani S, et al. Evaluation of the Effect of an Aqueous Extract of Azadirachta indica (Neem) Leaves and Twigs on Glycemic Control, Endothelial Dysfunction and Systemic Inflammation in Subjects with Type 2 Diabetes Mellitus - A Randomized, Double-Blind, Placebo-Controlled Clinical Study. Diabetes Metab Syndr Obes 2020;13:4401-12.
32. Lucantoni L, Yerbanga RS, Lupidi G, et al. Transmission blocking activity of a standardized neem (Azadirachta indica) seed extract on the rodent malaria parasite Plasmodium berghei in its vector Anopheles stephensi. Malar J 2010;9:66.
33. Jones IW, Denholm AA, Ley SV, et al. Sexual development of malaria parasites is inhibited in vitro by the neem extract

azadirachtin, and its semi-synthetic analogues. FEMS Microbiol Lett 1994;120:267-73.
34. Abiy E, Gebre-Michael T, Balkew M, et al. Repellent efficacy of DEET, MyggA, neem (Azedirachta indica) oil and chinaberry (Melia azedarach) oil against Anopheles arabiensis, the principal malaria vector in Ethiopia. Malar J 2015;14:187.
35. Luong K, Dunkel FV, Coulibaly K, et al. Potential use of neem leaf slurry as a sustainable dry season management strategy to control the malaria vector Anopheles gambiae (DIPTERA: CULICIDAE) in west African villages. J Med Entomol 2012;49:1361-9.
36. Rochanakij S, Thebtaranonth Y, Yenjai C, et al. Nimbolide, a constituent of Azadirachta indica, inhibits Plasmodium falciparum in culture. Southeast Asian J Trop Med Public Health 1985;16:66-72.
37. Benelli G, Chandramohan B, Murugan K, et al. Neem cake as a promising larvicide and adulticide against the rural malaria vector Anopheles culicifacies (Diptera: Culicidae): a HPTLC fingerprinting approach. Nat Prod Res 2017;31:1185-90.
38. Okumu FO, Knols BG, Fillinger U. Larvicidal effects of a neem (Azadirachta indica) oil formulation on the malaria vector Anopheles gambiae. Malar J 2007;6:63.
39. Gianotti RL, Bomblies A, Dafalla M, et al. Efficacy of local neem extracts for sustainable malaria vector control in an African village. Malar J 2008;7:138.
40. Dhar R, Zhang K, Talwar GP, et al. Inhibition of the growth and development of asexual and sexual stages of drug-sensitive and resistant strains of the human malaria parasite Plasmodium falciparum by Neem (Azadirachta indica) fractions. J Ethnopharmacol 1998;61:31-9.
41. Udeinya IJ. Anti-malaria activity of Nigerian neem leaves. Trans R Soc Trop Med Hyg 1993;87:471.
42. Hossain MA, Al-Toubi WAS, Weli AM, et al. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. Journal of Taibah University for Science 2013;7:181-8.
43. Nelson VK, Ali A, Dutta N, et al. Azadiradione ameliorates polyglutamine expansion disease in Drosophila by potentiating DNA binding activity of heat shock factor 1. Oncotarget 2016;7:78281-96.
44. Ponnusamy S, Haldar S, Mulani F, et al. Gedunin and Azadiradione: Human Pancreatic Alpha-Amylase Inhibiting Limonoids from Neem (Azadirachta indica) as Anti-Diabetic Agents. PLoS One 2015;10:e0140113.
45. Gupta SC, Francis SK, Nair MS, et al. Azadirone, a limonoid tetranortriterpene, induces death receptors and sensitizes human cancer cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) through a p53 protein-independent mechanism: evidence for the role of the ROS-ERK-CHOP-death receptor pathway. J Biol Chem 2013;288:32343-56.
46. Lakshmi T, Krishnan V, Rajendran R, et al. Azadirachta indica: A herbal panacea in dentistry - An update. Pharmacogn Rev 2015;9:41-4.
47. Lahiri D, Nag M, Dutta B, et al. Catechin as the Most Efficient Bioactive Compound from Azadirachta indica with Antibiofilm and Anti-quorum Sensing Activities Against Dental Biofilm: an In Vitro and In Silico Study. Appl Biochem Biotechnol 2021;193:1617-30.
48. Schumacher M, Cerella C, Reuter S, et al. Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (Azadirachta indica) leaf extract are mediated via modulation of the nuclear factor-κB pathway. Genes Nutr 2011;6:149-60.
49. Kumar D, Haldar S, Gorain M, et al. Epoxyazadiradione suppresses breast tumor growth through mitochondrial depolarization and caspase-dependent apoptosis by targeting PI3K/Akt pathway. BMC Cancer 2018;18:52.
50. Shilpa G, Renjitha J, Saranga R, et al. Epoxyazadiradione Purified from the Azadirachta indica Seed Induced Mitochondrial Apoptosis and Inhibition of NFκB Nuclear Translocation in Human Cervical Cancer Cells. Phytother Res 2017;31:1892-902. Erratum in: Phytother Res 2021;35:7064.
51. Tanagala KKK, Baba AB, Kowshik J, et al. Gedunin, A Neem Limonoid in Combination with Epalrestat Inhibits Cancer Hallmarks by Attenuating Aldose Reductase-Driven Oncogenic Signaling in SCC131 Oral Cancer Cells. Anticancer Agents Med Chem 2018;18:2042-52.
52. Bansal V, Gupta M, Bhaduri T, et al. Assessment of Antimicrobial Effectiveness of Neem and Clove Extract Against Streptococcus mutans and Candida albicans: An In vitro Study. Niger Med J 2019;60:285-9.
53. Goswami KK, Barik S, Sarkar M, et al. Targeting STAT3 phosphorylation by neem leaf glycoprotein prevents immune evasion exerted by supraglottic laryngeal tumor induced M2 macrophages. Mol Immunol 2014;59:119-27.
54. Chakraborty T, Bose A, Goswami KK, et al. Neem leaf glycoprotein suppresses regulatory T cell mediated suppression of monocyte/macrophage functions. Int Immunopharmacol 2012;12:326-33.
55. Chakraborty K, Bose A, Pal S, et al. Neem leaf glycoprotein restores the impaired chemotactic activity of peripheral blood mononuclear cells from head and neck

squamous cell carcinoma patients by maintaining CXCR3/CXCL10 balance. Int Immunopharmacol 2008;8:330-40.
56. Roy S, Goswami S, Bose A, et al. Neem leaf glycoprotein partially rectifies suppressed dendritic cell functions and associated T cell efficacy in patients with stage IIIB cervical cancer. Clin Vaccine Immunol 2011;18:571-9.
57. Roy S, Barik S, Banerjee S, et al. Neem leaf glycoprotein overcomes indoleamine 2,3 dioxygenase mediated tolerance in dendritic cells by attenuating hyperactive regulatory T cells in cervical cancer stage IIIB patients. Hum Immunol 2013;74:1015-23.
58. Goswami S, Bose A, Sarkar K, et al. Neem leaf glycoprotein matures myeloid derived dendritic cells and optimizes anti-tumor T cell functions. Vaccine 2010;28:1241-52.
59. Mallick A, Ghosh S, Banerjee S, et al. Neem leaf glycoprotein is nontoxic to physiological functions of Swiss mice and Sprague Dawley rats: histological, biochemical and immunological perspectives. Int Immunopharmacol 2013;15:73-83.
60. Chakraborty T, Bose A, Barik S, et al. Neem leaf glycoprotein inhibits CD4+CD25+Foxp3+ Tregs to restrict murine tumor growth. Immunotherapy 2011;3:949-69.
61. Bose A, Chakraborty K, Sarkar K, et al. Neem leaf glycoprotein induces perforin-mediated tumor cell killing by T and NK cells through differential regulation of IFNgamma signaling. J Immunother 2009;32:42-53.
62. Sarkar K, Bose A, Chakraborty K, et al. Neem leaf glycoprotein helps to generate carcinoembryonic antigen specific anti-tumor immune responses utilizing macrophage-mediated antigen presentation. Vaccine 2008;26:4352-62.
63. Sarkar K, Goswami S, Roy S, et al. Neem leaf glycoprotein enhances carcinoembryonic antigen presentation of dendritic cells to T and B cells for induction of anti-tumor immunity by allowing generation of immune effector/memory response. Int Immunopharmacol 2010;10:865-74.
64. Bose A, Chakraborty K, Sarkar K, et al. Neem leaf glycoprotein directs T-bet-associated type 1 immune commitment. Hum Immunol 2009;70:6-15.
65. Mallick A, Barik S, Goswami KK, et al. Neem leaf glycoprotein activates CD8(+) T cells to promote therapeutic anti-tumor immunity inhibiting the growth of mouse sarcoma. PLoS One 2013;8:e47434.
66. Passos MS, de Carvalho AR, Boeno SI, et al. Terpenoids isolated from Azadirachta indica roots and biological activities. Revista Brasileira de Farmacognosia 2019;29:40-5. doi: https://doi.org/10.1016/j.bjp.2018.12.003.
67. Pillai NR, Santhakumari G, Laping J. Some pharmacological actions of 'nimbidin'- a bitter principle of azadirachta indica-a juss (neem). Anc Sci Life 1984;4:88-95.
68. Ramesh A, Balasubramanian M. Rapid preconcentration method for the determination of azadirachtin-A and -B, nimbin and salannin in neem oil samples by using graphitised carbon solid phase extraction. Analyst 1999;124:19-21.
69. Sharma V, Vijay J, Ganesh MR, et al. Multilayer capsules encapsulating nimbin and doxorubicin for cancer chemo-photothermal therapy. Int J Pharm 2020;582:119350.
70. Yang LJ, Yang B, Chen W, et al. Host-guest system of nimbin and beta-cyclodextrin or its derivatives: preparation, characterization, inclusion mode, and solubilization. J Agric Food Chem 2010;58:8545-52.
71. Mitchell MJ, Smith SL, Johnson S, et al. Effects of the neem tree compounds azadirachtin, salannin, nimbin, and 6-desacetylnimbin on ecdysone 20-monooxygenase activity. Arch Insect Biochem Physiol 1997;35:199-209.
72. Lavanya P, Ramaiah S, Anbarasu A. Computational analysis reveal inhibitory action of nimbin against dengue viral envelope protein. Virusdisease 2015;26:243-54.
73. Simmonds MS, Jarvis AP, Johnson S, et al. Comparison of anti-feedant and insecticidal activity of nimbin and salannin photo-oxidation products with neem (Azadirachta indica) limonoids. Pest Manag Sci 2004;60:459-64.
74. Prashanth Goud M, Bale S, Pulivendala G, et al. Therapeutic effects of Nimbolide, an autophagy regulator, in ameliorating pulmonary fibrosis through attenuation of TGF-β1 driven epithelial-to-mesenchymal transition. Int Immunopharmacol 2019;75:105755.
75. Sastry BS, Suresh Babu K, Hari Babu T, et al. Synthesis and biological activity of amide derivatives of nimbolide. Bioorg Med Chem Lett 2006;16:4391-4.
76. Kigodi PG, Blaskó G, Thebtaranonth Y, et al. Spectroscopic and biological investigation of nimbolide and 28-deoxonimbolide from Azadirachta indica. J Nat Prod 1989;52:1246-51.
77. Sophia J, Kiran Kishore T K, Kowshik J, et al. Nimbolide, a neem limonoid inhibits Phosphatidyl Inositol-3 Kinase to activate Glycogen Synthase Kinase-3β in a hamster model of oral oncogenesis. Sci Rep 2016;6:22192.
78. Kavitha K, Vidya Priyadarsini R, Anitha P, et al. Nimbolide, a neem limonoid abrogates canonical NF-κB and Wnt signaling to induce caspase-dependent apoptosis in human hepatocarcinoma (HepG2) cells. Eur J Pharmacol 2012;681:6-14.
79. Tong B, Spradlin JN, Novaes LFT, et al. A Nimbolide-

Based Kinase Degrader Preferentially Degrades Oncogenic BCR-ABL. ACS Chem Biol 2020;15:1788-94.
80. Kowshik J, Mishra R, Sophia J, et al. Nimbolide upregulates RECK by targeting miR-21 and HIF-1α in cell lines and in a hamster oral carcinogenesis model. Sci Rep 2017;7:2045.
81. Shin SS, Hwang B, Muhammad K, et al. Nimbolide Represses the Proliferation, Migration, and Invasion of Bladder Carcinoma Cells via Chk2-Mediated G2/M Phase Cell Cycle Arrest, Altered Signaling Pathways, and Reduced Transcription Factors-Associated MMP-9 Expression. Evid Based Complement Alternat Med 2019;2019:3753587.
82. Li H, Xia B, Chen W, et al. Nimbolide prevents myocardial damage by regulating cardiac biomarkers, antioxidant level, and apoptosis signaling against doxorubicin-induced cardiotoxicity in rats. J Biochem Mol Toxicol 2020. [Epub ahead of print]. doi: 10.1002/jbt.22543.
83. Annaldas S, Saifi MA, Khurana A, et al. Nimbolide ameliorates unilateral ureteral obstruction-induced renal fibrosis by inhibition of TGF-β and EMT/Slug signalling. Mol Immunol 2019;112:247-55.
84. Diddi S, Bale S, Pulivendala G, et al. Nimbolide ameliorates fibrosis and inflammation in experimental murine model of bleomycin-induced scleroderma. Inflammopharmacology 2019;27:139-49.
85. Bansod S, Aslam Saifi M, Khurana A, et al. Nimbolide abrogates cerulein-induced chronic pancreatitis by modulating β-catenin/Smad in a sirtuin-dependent way. Pharmacol Res 2020;156:104756.
86. Kabaleeswaran V, Malathi R, Rajan SS, et al. Nimonol and 6-oxonimonol. Acta Crystallogr C 2000;56:82-4.
87. Mahmoud DA, Hassanein NM, Youssef KA, et al. Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. Braz J Microbiol 2011;42:1007-16.
88. Takagi M, Tachi Y, Zhang J, et al. Cytotoxic and melanogenesis-inhibitory activities of limonoids from the leaves of Azadirachta indica (Neem). Chem Biodivers 2014;11:451-68.
89. Arumugam A, Subramani R, Nandy S, et al. Desacetyl nimbinene inhibits breast cancer growth and metastasis through reactive oxygen species mediated mechanisms. Tumour Biol 2016;37:6527-37.
90. Mahapatra S, Young CY, Kohli M, et al. Antiangiogenic Effects and Therapeutic Targets of Azadirachta indica Leaf Extract in Endothelial Cells. Evid Based Complement Alternat Med 2012;2012:303019.
91. Koul O, Multani JS, Singh G, et al. 6beta-hydroxygedunin from Azadirachta indica. Its potentiation effects with some non-azadirachtin limonoids in neem against lepidopteran larvae. J Agric Food Chem 2003;51:2937-42.
92. Santos KS, Barbosa AM, Freitas V, et al. Antiproliferative Activity of Neem Leaf Extracts Obtained by a Sequential Pressurized Liquid Extraction. Pharmaceuticals (Basel) 2018;11:76.
93. Harish Kumar G, Vidya Priyadarsini R, Vinothini G, et al. The neem limonoids azadirachtin and nimbolide inhibit cell proliferation and induce apoptosis in an animal model of oral oncogenesis. Invest New Drugs 2010;28:392-401.
94. Priyadarsini RV, Manikandan P, Kumar GH, et al. The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic-metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis. Free Radic Res 2009;43:492-504.
95. Priyadarsini RV, Murugan RS, Sripriya P, et al. The neem limonoids azadirachtin and nimbolide induce cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells. Free Radic Res 2010;44:624-34.
96. Kaur G, Sarwar Alam M, Athar M. Nimbidin suppresses functions of macrophages and neutrophils: relevance to its antiinflammatory mechanisms. Phytother Res 2004;18:419-24.
97. Pillai NR, Santhakumari G. Effect of nimbidin on gastric Acid secretion. Anc Sci Life 1985;5:91-7.
98. Maurya VK, Kumar S, Prasad AK, et al. Structure-based drug designing for potential antiviral activity of selected natural products from Ayurveda against SARS-CoV-2 spike glycoprotein and its cellular receptor. Virusdisease 2020;31:179-93.
99. Jaiswara PK, Gupta VK, Sonker P, et al. Nimbolide induces cell death in T lymphoma cells: Implication of altered apoptosis and glucose metabolism. Environ Toxicol 2021;36:628-41.
100. Spradlin JN, Hu X, Ward CC, et al. Harnessing the anti-cancer natural product nimbolide for targeted protein degradation. Nat Chem Biol 2019;15:747-55.
101. Sarkar P, Acharyya S, Banerjee A, et al. Intracellular, biofilm-inhibitory and membrane-damaging activities of nimbolide isolated from Azadirachta indica A. Juss (Meliaceae) against meticillin-resistant Staphylococcus aureus. J Med Microbiol 2016;65:1205-14.
102. Campos EV, de Oliveira JL, Pascoli M, et al. Neem Oil and Crop Protection: From Now to the Future. Front

Plant Sci 2016;7:1494.
103. Maurya M. Antinociceptive Activity of Ethanolic Neem (Azadirachta Indica) Leaf Extract (Enle) Through Opioid Pathway in Adult Rats. J of Pharmacol & Clin Res 2017;3:555603.
104. Kudom AA, Mensah BA, Botchey MA. Aqueous neem extract versus neem powder on Culex quinquefasciatus: implications for control in anthropogenic habitats. J Insect Sci 2011;11:142.
105. Patra A, Satpathy S, Hussain MD. Nanodelivery and anticancer effect of a limonoid, nimbolide, in breast and pancreatic cancer cells. Int J Nanomedicine 2019;14:8095-104.
106. Kotadiya R, Shah K. Development of Bioadhesive Buccal Tablets of Nicorandil Using a Factorial Approach. Turk J Pharm Sci 2020;17:388-97.
107. Mankotia P, Choudhary S, Sharma K, et al. Neem gum based pH responsive hydrogel matrix: A new pharmaceutical excipient for the sustained release of anticancer drug. Int J Biol Macromol 2020;142:742-55.
108. Phadke C, Mewada A, Dharmatti R, et al. Biogenic Synthesis of Fluorescent Carbon Dots at Ambient Temperature Using Azadirachta indica (Neem) gum. J Fluoresc 2015;25:1103-7.
109. Gulsonbi M, Parthasarathy S, Bharat Raj K, et al. Green synthesis, characterization and drug delivery applications of a novel silver/carboxymethylcellulose - poly(acrylamide) hydrogel nanocomposite. Ecotoxicol Environ Saf 2016;134:421-6.
110. Sathishkumar G, Gobinath C, Wilson A, et al. Dendrophthoe falcata (L.f) Ettingsh (Neem mistletoe): a potent bioresource to fabricate silver nanoparticles for anticancer effect against human breast cancer cells (MCF-7). Spectrochim Acta A Mol Biomol Spectrosc 2014;128:285-90.
111. Siddiqui EA, Ahmad A, Julius A, et al. Biosynthesis of Anti-Proliferative Gold Nanoparticles Using Endophytic Fusarium oxysporum Strain Isolated from Neem (A. indica) Leaves. Curr Top Med Chem 2016;16:2036-42.
112. Mishra P, Samuel MK, Reddy R, et al. Environmentally benign nanometric neem-laced urea emulsion for controlling mosquito population in environment. Environ Sci Pollut Res Int 2018;25:2211-30.
113. Rinaldi F, Hanieh PN, Longhi C, et al. Neem oil nanoemulsions: characterisation and antioxidant activity. J Enzyme Inhib Med Chem 2017;32:1265-73.
114. Paller CJ, Denmeade SR, Carducci MA. Challenges of conducting clinical trials of natural products to combat cancer. Clin Adv Hematol Oncol 2016;14:447-55.
115. Patterson RE, Neuhouser ML, Hedderson MM, et al. Changes in diet, physical activity, and supplement use among adults diagnosed with cancer. J Am Diet Assoc 2003;103:323-8.
116. Progress in male contraceptive research. Popul Briefs 1995;1:3-4.
117. Hoang DT, Iczkowski KA, Kilari D, et al. Androgen receptor-dependent and -independent mechanisms driving prostate cancer progression: Opportunities for therapeutic targeting from multiple angles. Oncotarget 2017;8:3724-45.
118. Lonergan PE, Tindall DJ. Androgen receptor signaling in prostate cancer development and progression. J Carcinog 2011;10:20.
119. Braga DL, Mota STS, Zóia MAP, et al. Ethanolic Extracts from Azadirachta indica Leaves Modulate Transcriptional Levels of Hormone Receptor Variant in Breast Cancer Cell Lines. Int J Mol Sci 2018;19:1879.
120. Wang Y, Kreisberg JI, Ghosh PM. Cross-talk between the androgen receptor and the phosphatidylinositol 3-kinase/Akt pathway in prostate cancer. Curr Cancer Drug Targets 2007;7:591-604.
121. Manna P, Jain SK. Phosphatidylinositol-3,4,5-triphosphate and cellular signaling: implications for obesity and diabetes. Cell Physiol Biochem 2015;35:1253-75.
122. Vincent EE, Elder DJ, Thomas EC, et al. Akt phosphorylation on Thr308 but not on Ser473 correlates with Akt protein kinase activity in human non-small cell lung cancer. Br J Cancer 2011;104:1755-61.
123. Ghosh PM, Malik S, Bedolla R, et al. Akt in prostate cancer: possible role in androgen-independence. Curr Drug Metab 2003;4:487-96.
124. Luo HR, Hattori H, Hossain MA, et al. Akt as a mediator of cell death. Proc Natl Acad Sci U S A 2003;100:11712-7.
125. Sophia J, Kowshik J, Dwivedi A, et al. Nimbolide, a neem limonoid inhibits cytoprotective autophagy to activate apoptosis via modulation of the PI3K/Akt/GSK-3β signalling pathway in oral cancer. Cell Death Dis 2018;9:1087.
126. Chopra DP, Menard RE, Januszewski J, et al. TNF-alpha-mediated apoptosis in normal human prostate epithelial cells and tumor cell lines. Cancer Lett 2004;203:145-54.
127. Morris J, Gonzales CB, De La Chapa JJ, et al. The Highly Pure Neem Leaf Extract, SCNE, Inhibits Tumorigenesis in Oral Squamous Cell Carcinoma via Disruption of Pro-tumor Inflammatory Cytokines and Cell Signaling. Front Oncol 2019;9:890.
128. Chen J, Fan X, Zhu J, et al. Limonoids from seeds of

Azadirachta indica A. Juss. and their cytotoxic activity. Acta Pharm Sin B 2018;8:639-44.
129. Wu SH, Chen YW, Shao SC, et al. Two new solanapyrone analogues from the endophytic fungus Nigrospora sp. YB-141 of Azadirachta indica. Chem Biodivers 2009;6:79-85.
130. Kashif M, Hwang Y, Kim WJ, et al. In-vitro Morphological Assessment of Apoptosis Induced by Nimbolide; A Limonoid from Azadirachta Indica (Neem Tree). Iran J Pharm Res 2019;18:846-59.
131. Roy MK, Kobori M, Takenaka M, et al. Inhibition of colon cancer (HT-29) cell proliferation by a triterpenoid isolated from Azadirachta indica is accompanied by cell cycle arrest and up-regulation of p21. Planta Med 2006;72:917-23.
132. Dasgupta T, Banerjee S, Yadava PK, et al. Chemopreventive potential of Azadirachta indica (Neem) leaf extract in murine carcinogenesis model systems. J Ethnopharmacol 2004;92:23-36.
133. Hao F, Kumar S, Yadav N, et al. Neem components as potential agents for cancer prevention and treatment. Biochim Biophys Acta 2014;1846:247-57.
134. Haque E, Mandal I, Pal S, et al. Prophylactic dose of neem (Azadirachta indica) leaf preparation restricting murine tumor growth is nontoxic, hematostimulatory and immunostimulatory. Immunopharmacol Immunotoxicol 2006;28:33-50.
135. Leal F, García-Perdomo HA. Effectiveness of Platinum-Based Chemotherapy in Patients With Metastatic Prostate Cancer: Systematic Review and Meta-analysis. Clin Genitourin Cancer 2019;17:e627-44.
136. Shore ND, Drake CG, Lin DW, et al. Optimizing the management of castration-resistant prostate cancer patients: A practical guide for clinicians. Prostate 2020;80:1159-76.
137. Moga MA, Bălan A, Anastasiu CV, et al. An Overview on the Anticancer Activity of Azadirachta indica (Neem) in Gynecological Cancers. Int J Mol Sci 2018;19:3898.
138. Chitta K, Paulus A, Caulfield TR, et al. Nimbolide targets BCL2 and induces apoptosis in preclinical models of Waldenströms macroglobulinemia. Blood Cancer J 2014;4:e260.
139. Harish Kumar G, Chandra Mohan KV, Jagannadha Rao A, et al. Nimbolide a limonoid from Azadirachta indica inhibits proliferation and induces apoptosis of human choriocarcinoma (BeWo) cells. Invest New Drugs 2009;27:246-52.
140. Sharma C, Vas AJ, Goala P, et al. Ethanolic Neem (Azadirachta indica) Leaf Extract Prevents Growth of MCF-7 and HeLa Cells and Potentiates the Therapeutic Index of Cisplatin. J Oncol 2014;2014:321754.
141. Ramzanighara A, Ezzatighadi F, Rai DV, et al. Effect of Neem (Azadirchta indica) on serum glycoprotein contents of rats administered 1,2 dimethylhydrazine. Toxicol Mech Methods 2009;19:298-301.
142. Veeraraghavan J, Aravindan S, Natarajan M, et al. Neem leaf extract induces radiosensitization in human neuroblastoma xenograft through modulation of apoptotic pathway. Anticancer Res 2011;31:161-70.
143. Babykutty S, Priya PS, Nandini RJ, et al. Nimbolide retards tumor cell migration, invasion, and angiogenesis by downregulating MMP-2/9 expression via inhibiting ERK1/2 and reducing DNA-binding activity of NF-κB in colon cancer cells. Mol Carcinog 2012;51:475-90.
144. Leslie SW, Soon-Sutton TL, Sajjad H, et al. Prostate Cancer. In: StatPearls. Treasure Island, FL, USA: StatPearls Publishing, 2021.
145. Priyadarshini M, Khan RH, Bano B. Physicochemical properties of thiol proteinase inhibitor isolated from goat pancreas. Biopolymers 2010;93:708-17.
146. Haque E, Baral R. Neem (Azadirachta indica) leaf preparation induces prophylactic growth inhibition of murine Ehrlich carcinoma in Swiss and C57BL/6 mice by activation of NK cells and NK-T cells. Immunobiology 2006;211:721-31.
147. Kluetz PG, Figg WD, Dahut WL. Angiogenesis inhibitors in the treatment of prostate cancer. Expert Opin Pharmacother 2010;11:233-47.
148. Melegh Z, Oltean S. Targeting Angiogenesis in Prostate Cancer. Int J Mol Sci 2019;20:2676.
149. Gupta SC, Prasad S, Sethumadhavan DR, et al. Nimbolide, a limonoid triterpene, inhibits growth of human colorectal cancer xenografts by suppressing the proinflammatory microenvironment. Clin Cancer Res 2013;19:4465-76.
150. Manikandan P, Ramalingam SM, Vinothini G, et al. Investigation of the chemopreventive potential of neem leaf subfractions in the hamster buccal pouch model and phytochemical characterization. Eur J Med Chem 2012;56:271-81.
151. Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. Immunity 2019;51:27-41.
152. Taniguchi K, Karin M. NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol 2018;18:309-24.
153. Liu T, Zhang L, Joo D, et al. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2:17023.
154. Staal J, Beyaert R. Inflammation and NF-κB Signaling in Prostate Cancer: Mechanisms and Clinical Implications.

Cells 2018;7:122.
155. De Santis M, Saad F. Practical Guidance on the Role of Corticosteroids in the Treatment of Metastatic Castration-resistant Prostate Cancer. Urology 2016;96:156-64.
156. Hayashi T, Fujita K, Matsushita M, et al. Main Inflammatory Cells and Potentials of Anti-Inflammatory Agents in Prostate Cancer. Cancers (Basel) 2019;11:1153.
157. Messner EA, Steele TM, Tsamouri MM, et al. The Androgen Receptor in Prostate Cancer: Effect of Structure, Ligands and Spliced Variants on Therapy. Biomedicines 2020;8:422.
158. Kamath SG, Chen N, Xiong Y, et al. Gedunin, a novel natural substance, inhibits ovarian cancer cell proliferation. Int J Gynecol Cancer 2009;19:1564-9.
159. Scambia G, Ranelletti FO, Panici PB, et al. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. Cancer Chemother Pharmacol 1994;34:459-64.
160. Ghosh D, Bose A, Haque E, et al. Pretreatment with neem (Azadirachta indica) leaf preparation in Swiss mice diminishes leukopenia and enhances the antitumor activity of cyclophosphamide. Phytother Res 2006;20:814-8.
161. Ghosh D, Bose A, Haque E, et al. Neem (Azadirachta indica) leaf preparation prevents leukocyte apoptosis mediated by cisplatin plus 5-fluorouracil treatment in Swiss mice. Chemotherapy 2009;55:137-44.
162. Abdel Moneim AE, Othman MS, Aref AM. Azadirachta indica attenuates cisplatin-induced nephrotoxicity and oxidative stress. Biomed Res Int 2014;2014:647131.
163. Gupta SC, Prasad S, Reuter S, et al. Modification of cysteine 179 of IkappaBalpha kinase by nimbolide leads to down-regulation of NF-kappaB-regulated cell survival and proliferative proteins and sensitization of tumor cells to chemotherapeutic agents. J Biol Chem 2010;285:35406-17.
164. Kawami M, Yamada Y, Issarachot O, et al. P-gp modulating effect of Azadirachta indica extract in multidrug-resistant cancer cell lines. Pharmazie 2018;73:104-9.
165. Mishra A, Dave N. Neem oil poisoning: Case report of an adult with toxic encephalopathy. Indian J Crit Care Med 2013;17:321-2.
166. Dorababu M, Joshi MC, Bhawani G, et al. Effect of aqueous extract of neem (Azadirachta indica) leaves on offensive and diffensive gastric mucosal factors in rats. Indian J Physiol Pharmacol 2006;50:241-9.
167. Raizada RB, Srivastava MK, Kaushal RA, et al. Azadirachtin, a neem biopesticide: subchronic toxicity assessment in rats. Food Chem Toxicol 2001;39:477-83.
168. Glinsukon T, Somjaree R, Piyachaturawat P, et al. Acute toxicity of nimbolide and nimbic acid in mice, rats and hamsters. Toxicol Lett 1986;30:159-66.
169. Mossini SA, Arrotéia CC, Kemmelmeier C. Effect of neem leaf extract and neem oil on Penicillium growth, sporulation, morphology and ochratoxin A production. Toxins (Basel) 2009;1:3-13.
170. Raju D, Jose J. Development and evaluation of novel topical gel of neem extract for the treatment of bacterial infections. J Cosmet Dermatol 2019;18:1776-83.
171. Dave V, Bhardwaj N, Gupta N, et al. Herbal ethosomal gel containing luliconazole for productive relevance in the field of biomedicine. 3 Biotech 2020;10:97.
172. Singh B, Sharma DK, Kumar R, et al. Development of a new controlled pesticide delivery system based on neem leaf powder. J Hazard Mater 2010;177:290-9.
173. Patel H. Batch and continuous fixed bed adsorption of heavy metals removal using activated charcoal from neem (Azadirachta indica) leaf powder. Sci Rep 2020;10:16895.
174. Mittal PK, Subbarao SK. Prospects of using herbal products in the control of mosquito vectors. ICMR Bulletin 2003;33:1-10.
175. Pascoli M, Jacques MT, Agarrayua DA, et al. Neem oil based nanopesticide as an environmentally-friendly formulation for applications in sustainable agriculture: An ecotoxicological perspective. Sci Total Environ 2019;677:57-67.
176. Choupanian Choupanian MM, Omar Omar DD, Basri Basri MM, et al. Preparation and characterization of neem oil nanoemulsion formulations against Sitophilus oryzae and Tribolium castaneum adults. J Pestic Sci 2017;42:158-65.
177. Jerobin J, Makwana P, Suresh Kumar RS, et al. Antibacterial activity of neem nanoemulsion and its toxicity assessment on human lymphocytes in vitro. Int J Nanomedicine 2015;10 Suppl 1:77-86.

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