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Review

Potential of neem (*Azadirachta indica* L.) for prevention and treatment of oncologic diseases

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ABSTRACT

Throughout time, plants have often displayed medicinal properties that have been underscored. We often derive medicines involved in treating cancer from components in plants. *Azadirachta indica*, commonly known as "neem", has been used to treat different ailments in many Asian countries. Due to its widespread beneficial uses, *A. indica* has often been referred to as "the wonder tree" or "nature's drug store". Various parts of this plant, including, leaves, flowers, fruits, seeds, roots, bark and oil, produce a large number of phytochemicals with various biological and pharmacological activities. The numerous biological activities of the phytoconstituents of *A. indica* explain its beneficial uses for the prevention and therapy of cancer. The chemopreventive and anticancer therapeutic efficacy of *A. indica* fractions and compounds could be explained by multiple cellular and molecular mechanisms, including free radical scavenging, carcinogen-detoxification, DNA repair, cell cycle alteration, programmed cell death (apoptosis) and autophagy, immune surveillance, anti-inflammatory, anti-angiogenic, anti-invasive and anti-metastatic activities as well as their ability to modulate several dysregulated oncogenic signaling pathways. This article aims to present the collective and critical analysis of multiple phytoconstituents of *A. indica* and their molecular mechanisms implicated in cancer chemopreventive and therapeutic effects based on published preclinical and clinical results. Current limitations and future directions of research on this medicinal plant are also critically discussed.

1. Introduction

Azadirachta indica (family: Meliaceae), known as neem, nimtree and Indian Lilac, was first discovered in India about 4500 years ago. Neem has been given the Latinized name *A. indica*, which is derived from the Persian language and literally means "the free tree of India" [1]. Various parts of neem tree, including leaves, flowers, fruits, seeds and bark, find extensive use in traditional systems of medicine (e.g., Ayurveda, Unani and Siddha) for treating various human diseases, including tumor [2–4]. Due to its tremendous therapeutic potential, neem is also referred to as "Village pharmacy", "Tree of the 21st century" and "A tree for solving global problems" [1,5].

Today, neem tree can be found in at least 30 countries in Asia, Africa, Australia as well as Central and South Americas [1]. *A. indica* can grow in dry and hot climates, allowing it to tolerate a temperature of 50–98 °F [2]. *A. indica* has a low tolerance for rainfall allowing it to grow best in poor soils that are sandy, deep and have a pH of about 6.2–7 [2]. Usually, *A. indica* tree is found in dry, tropical or subtropical locations; however, it can also be found along the sandy riverbanks in Australia [2].

The tree (Fig. 1A) is known to grow to approximately 15–20 m high and can live for about 200 years [1]. Since the tree develops a deep and strong tap root, the tree branches spread out widely and form an oval crown [2]. The oil from *A. indica* is usually from the seed and branches of the tree. The bark is brown and vertically

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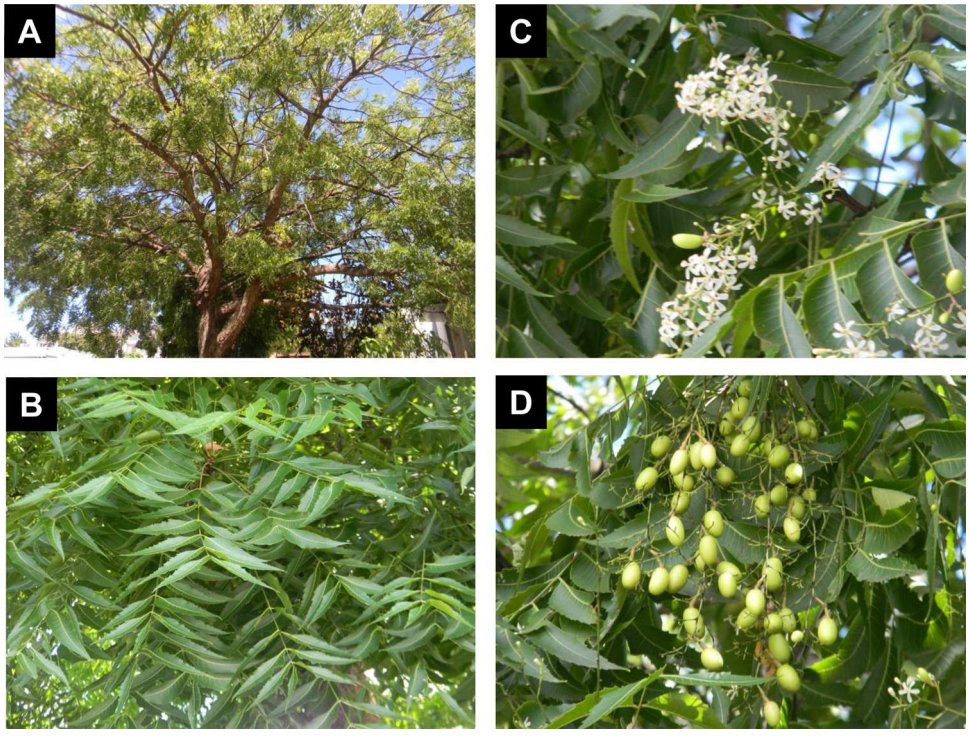

Fig. 1. Various photographs of *A. indica* L. showing a whole tree (A), leaves (B), flowers (C), and fruits (D).

fissure. The leaves (Fig. 1B) are pinnate and green; however, when the plant is younger, the leaves show a purple-red color [6]. *A. indica* has small, drooping and fragrant white flowers (Fig. 1C) that are about 25 cm long each [6]. The fruit (Fig. 1D) is small, yellow and edible [6]. It looks like an olive which is smooth and round with a single brown seed in the middle [6].

In the last several years, neem phytoconstituents have been shown to possess a plethora of biological and pharmacological activities. These activities include and are not limited to anti-inflammatory, anti-pyretic, anti-histamine, anti-fungal, anti-bacterial, anti-ulcer, analgesic, anti-arrhythmic, anti-tubercular, anti-malarial, diuretic, spermicide, anti-arthritic, anti-protozoal, insect repellant, anti-feedant and anti-hormonal properties [1,3,7]. Neem-derived constituents can block cancer growth through diverse biomolecular and cellular mechanisms. Neem phytochemicals suppress proliferation and growth of cancer cells, induce cell cycle arrest and apoptosis, interfere with growth factor signaling, inhibit angiogenesis, and decrease tumor cell invasion and migration. These curative powers of neem tree could be due to presence of numerous chemical constituents, such as azadirachtin, gedunin, nimbidin, nimbidol, nimbin, salannin and quercetin, present in various parts of the plant. Several excellent recent reviews highlight anti-cancer pharmacological properties of *A. indica* [5,8–11]. The purpose of this review is to collectively and critically analyze available and up-to-date literature that presents cancer chemopreventive and therapeutic effects of various extracts, fractions and phytochemicals of *A. indica* based on published preclinical and clinical results.

2. Chemical constituents of *A. indica*

Each anatomical part of neem tree, including the whole tree, stems, branches, leaves, flowers and fruits, produces different phytochemicals. The chemical composition of *A. indica* is very complex as it contains remarkably diverse array of phytochemicals, such as terpenoids, flavonoids, coumarins, carbohydrates, proteins, fatty acids and their esters and hydrocarbons [6]. However the presence of phytochemicals varies in accordance with their differential growth, harvesting, processing and storage conditions.

In 1942, Siddiqui [12] reported, for the first time, the isolation of chemical constituents, namely nimbin, nimbinin and nimbidin, from neem oil. The next two decades witnessed very little success towards identification and isolation of any new phytochemicals. However, the emergence of new isolation methods and improved analytical techniques for structure elucidation led to identification of many important phytochemicals previously unknown [13]. The chemical arsenal of neem is very diverse and there are reports of more than 300 phytochemicals isolated and characterized [14]. The crude extracts prepared with water, chloroform, ethanol, butanol, ethyl acetate and hexane revealed the presence of numerous phytochemicals. These phytochemicals are grouped based on the presence or absence of isoprene units as isoprenoids and non-isoprenoids (Fig. 2) [2,6].

Neem isoprenoids are categorized into three classes, namely diterpenoids, triterpenoids and steroids. However, triterpenoids represent the major class since more than 180 triterpenoids have been isolated from the different parts of neem tree [6,14]. Triterpenoids have 30 carbon atoms arranged in 4-rings (A, B, C and D) with a short side chain of carbon atoms, which possess either acyclic or cyclic structure. The parent triterpenoid apotirucallol (Fig. 3) has 30 carbon atoms [6,15]. The neem triterpenoids are subdivided into classes based on the removal of carbon atom either from the side chain or from the ring skeletal structure of the parent compound apotirucallol [6]. The derived compounds are referred to as “nor compounds” based on IUPAC nomenclature recommendations to indicate the loss of carbon atom from the parent structure [16].

Neem triterpenoids are categorized into protolimonoids, mononortriterpenoids, dinortriterpenoids, trinortriterpenoids, tetranortriterpenoids, pentanortriterpenoids, hexanortriterpenoids, octanortriterpenoids and nonanortriterpenoids. Protolimonoids have C-8 side chain with all the 30 carbons similar to parent triterpenoid structure apotirucallol. Protolimonoids are

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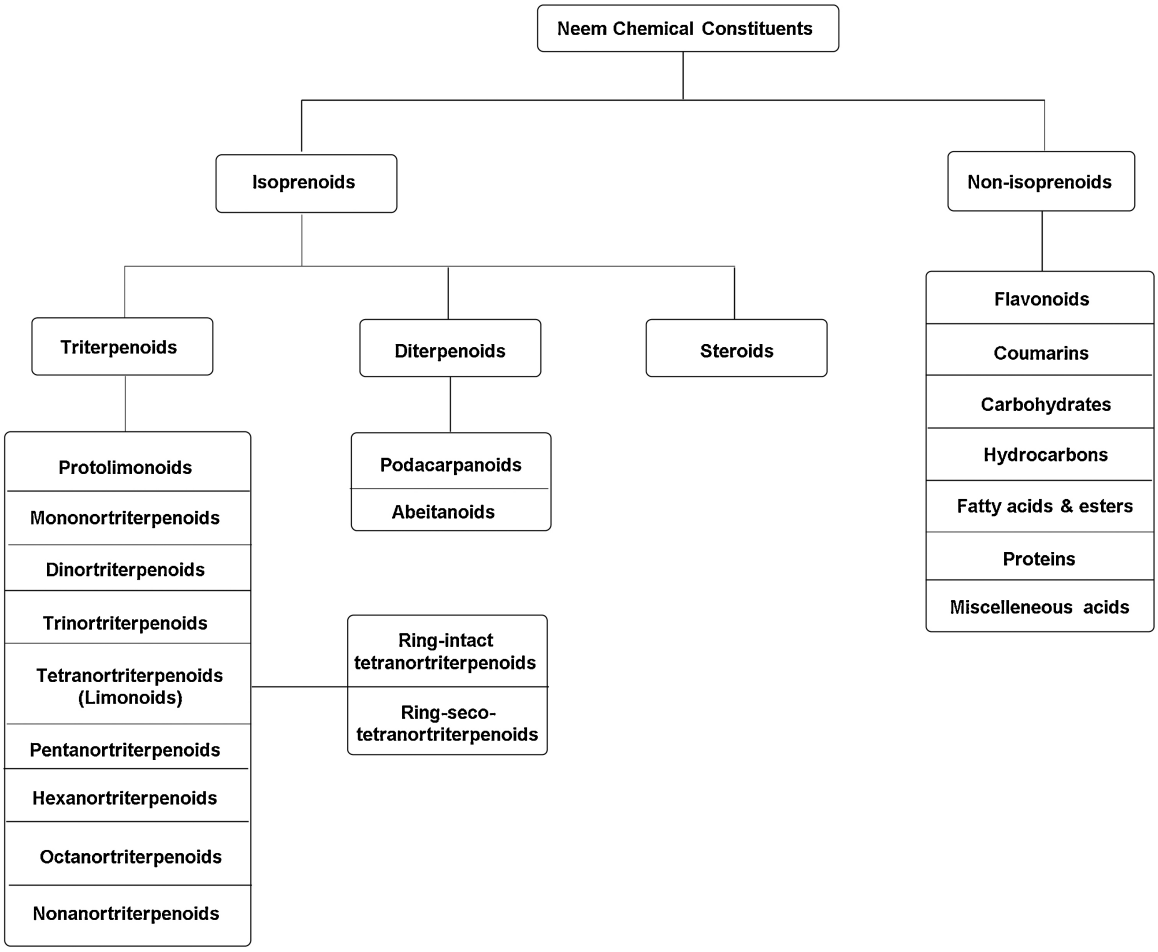

Fig. 2. Classification of neem chemical constituents.

considered as precursors to limonoids. The mononor, dinor, trinor, tetranor, pentanor, hexanor, octanor and nonanor triterpenoids are represented by the loss of 1, 2, 3, 4, 5, 6, 8 and 9 carbons, respectively, from the parent triterpenoid apotirucallol structure. In view of exhaustive literature on chemistry of neem triterpenoids, only a brief review of these compounds is given below. The interested reader can get detailed information on neem triterpenoids from the existing literature [2,6,14,15,17].

Tetranortriterpenoids, also known as limonoids, are the most important and well-studied class of triterpenoids and about one-third of phytochemicals isolated from neem belong to this class [14]. Limonoids are oxygenated triterpenes bearing a furan ring, which is formed by the loss of 4 carbon atoms from the side chain of the protolimonoid [6,15]. Limonoids are categorized into two classes based on their skeletal ring structure, namely ring intact limonoids and ring seco limonoids. As the name suggests in ring intact limonoids, rings A, B, C and D are intact, whereas in ring seco limonoids, there is a cleavage of one or more rings of the steroidal skeleton [15,16]. Azadirone, azadiradione, isonimolide, azadirachtin, salannin, nimbolide, gedunin and 7-deacetyl-7-benzoylepoxyazadiradione are few representative limonoids of both the classes. Azadirachtin, nimolide and gedunin have been extensively studied for their anti-cancer properties [14]. Azadirachtin, a ring-C seco limonoid with 16 stereogenic centers, is the most studied limonoid and due to its structural complexity, it took 18 years to solve the structure. Moreover, due to its unique structure and wide range of biological properties, it was referred to as “scientific gold mine” [18]. Due to their wide range of bioactivities, there is continued interest towards the identification and isolation of limonoids. Gualteri et al. [19] have recently reported the isolation of 8 limonoids from neem leaves.

Diterpenoids represent another important class of neem isoprenoids that are well characterized. These compounds are derived from 4-isoprene units and their biosynthetic precursor is 20-carbon diterpene alcohol geranylgeraniol. However, due to chemical modifications occurring at later stages of biosynthetic pathway, numerous diterpenoids with wide variety of chemical structures are generated. There are more than 20 diterpenoids isolated from different parts of neem tree. Neem diterpenoids mainly belong to two classes, namely podacarpanoids (margolone) and abeitanoids (sugiol) (Fig. 3) [6,20]. Apart from triterpenoids and diterpenoids, several important steroids which are previously known, such as cholesterol, β-sitosterol and stigmasterol, have also been isolated from neem tree [6].

In addition to isoprenoids, neem tree is a rich source for number of non-isoprenoids. Various classes of non-isoprenoids include flavonoids (quercetin, catechin and nimbaflavanone), coumarins (scopoletin), isocoumarins (margocetin), acids and their derivatives (nimbochalcin, nimbocetin, gallic acid and tiglic acid), styryl derivatives (α-hexyl cinnamaldehyde and β-asarone) hydrocarbons (octadecane and nonadecane), fatty acids and their derivatives (arachidic acid, stearic acid and oleic acid), carbohydrates and proteins (D-glucose, L-arabinose and D-glucosamine), thiols (dipropyl sulfide) [2,6]. Structures of few selected compounds are shown in Fig. 4.

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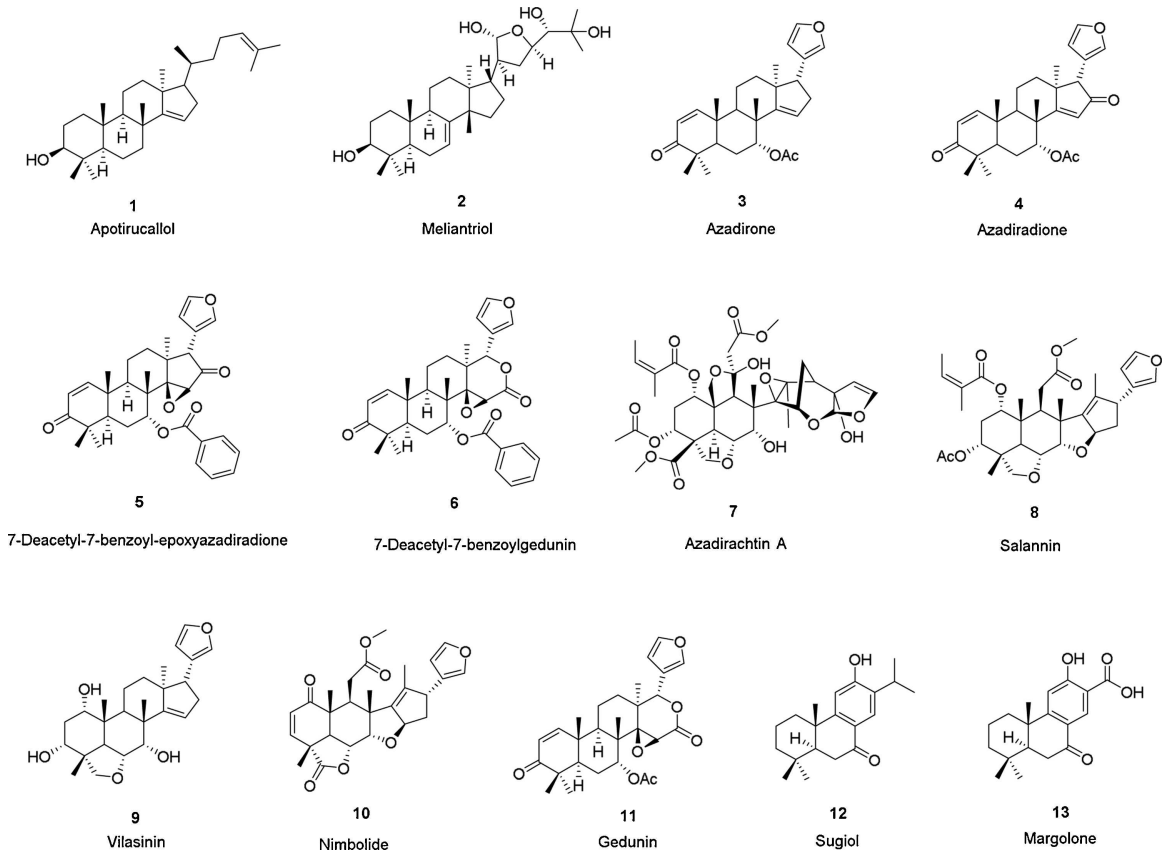

Fig. 3. Structures of isoprenoid neem constituents.

3. *A. indica* and cancer

Research articles presented in this review exemplify the chemopreventive and chemotherapeutic potential of *A. indica* against various cancer models. The following sections showcase in vitro, in vivo and clinical studies executed around the world by many researchers regarding anticancer activities of neem. Several databases, including PubMed, EBOSCOhost, and Google Scholar, were used to find all primary literature. There were no time restraints on articles that were published. The publications considered for this work were all in English language. First, the abstracts of original research papers were reviewed, followed by the retrieval of the full article to analyze anticancer activities of *A. indica*. Various combinations of major keywords included: *A. indica*; neem; chemopreventive; chemotherapeutic; cancer; tumor; prevention; treatment and clinical studies. Additional relevant articles were collected by studying the references of the primary articles. In order to obtain information regarding clinical studies; Clinicaltrials.gov was used in addition to the previously mentioned sources.

3.1. Preclinical studies

The following sections present various anticancer studies conducted using neem extracts and isolated pure compounds as well as established cancer cell lines (Table 1) and animal models (Table 2). The underlying molecular mechanisms are also analyzed.

3.1.1. Breast cancer

Crude aqueous extracts of *A. indica* leaves and seeds inhibited the growth of Ehrlich ascites carcinoma cells, but no specific mechanisms were identified [21]. Elumalai et al. [22] found that ethanolic neem leaf extract (ENLE) induced apoptosis and inhibited the proliferation of estrogen-dependent (MCF-7) and estrogen-independent (MDA-231) breast carcinoma cells. Mechanistic studies showed that ENLE induced cell cycle arrest at G0/G1 phase, decreased B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra-large (Bcl-xL) mRNA expression, increased B-cell associated X protein (Bax) and Bcl-2-associated death promoter (Bad) in both breast cancer cell lines. Additionally, caspase-3 activity was increased which led to cleavage of several substrates, including poly(ADP-ribose) polymerase (PARP), a nuclear enzyme involved in DNA repair and maintenance. The investigators also reported downregulation of insulin-like growth factor-1 receptor (IGF-1R), rat sarcoma (Ras), Raf, p-Akt, p-Erk and cyclin D1 protein expression [22]. In a separate study, the same research group treated MCF-7 and MDA-MB-231 cells with nimbolide, a limonoid present in leaves and flowers of *A. indica*, and observed antiproliferative activity. The pro-apoptotic activity demonstrated by nimbolide was mediated via the upregulation of Bax, Bad, Fas-L, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), Fas-associated death domain receptor (FADDR) and cytochrome *c* (cyt. *c*) with a downregulation of Bcl-2, Bcl-xL, Mcl-1 and XIAP-1 [23]. Sharma et al. [24] confirmed the cytotoxic effects of ENLE in MCF-7 cells. The treated cells exhibited apoptosis possibly through upregulation of Bax and downregulation of cyclin D1 and cytochrome P450 monooxygenases (CYP 1A1 and CYP 1A2). Collectively, these mechanisms contributed to a decrease in cell viability and antiproliferative activity. Several limonoid compounds isolated from *A. indica* ethanolic leaf extract displayed cytotoxic activities against SK-BR-3 breast cancer cells [25]. Desacetyl nimbinene (DAN), an active ingredient of neem, inhibited the growth of MCF-7 as well as MDA-MB-231 cells through induction of reactive oxygen species (ROS) and loss of mitochondrial membrane potential, resulting in mitochondria-dependent apoptotic cell death. Moreover, DAN sig-

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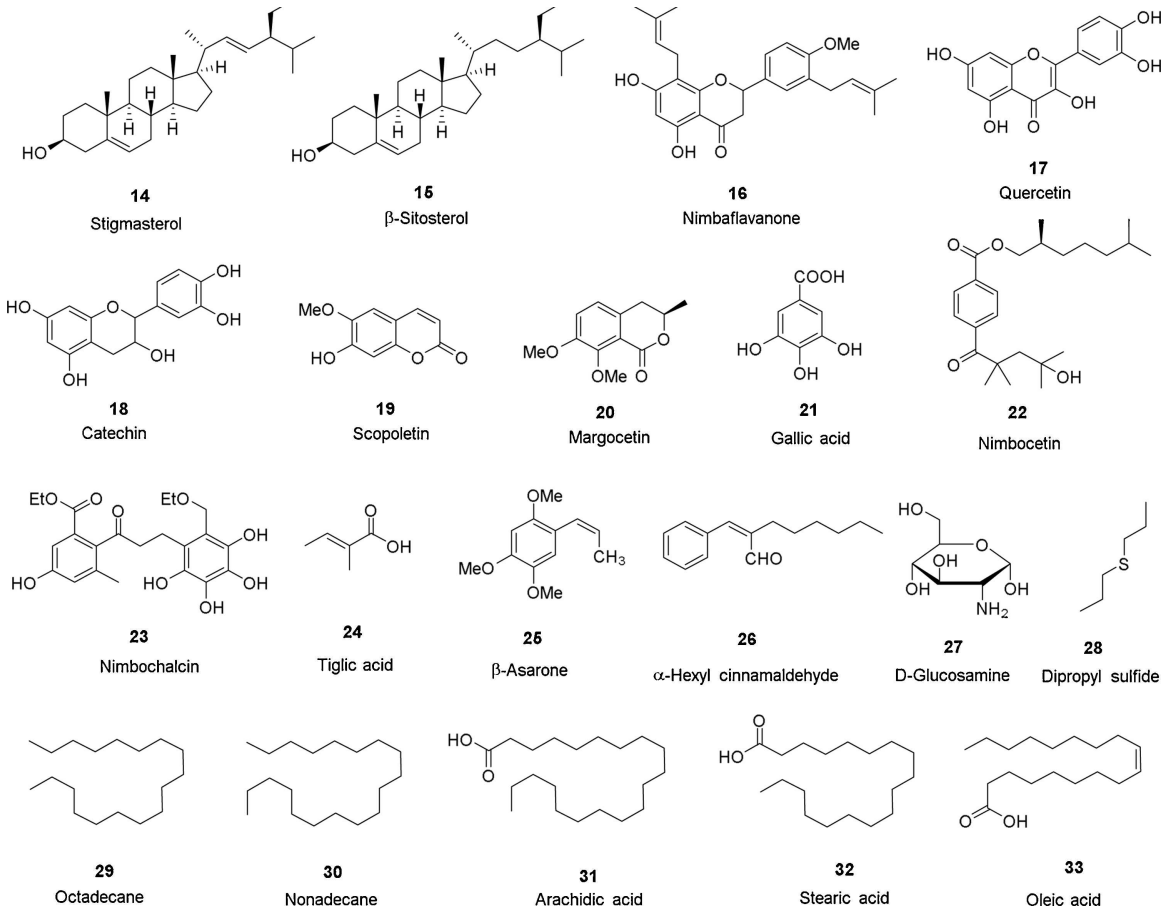

Fig. 4. Structures of steroids and non-isoprenoid neem constituents.

nificantly suppressed the migration and invasion of MDA-MB-231 cells [26].

Several research groups investigated breast cancer preventive and therapeutic effects of neem fractions and components using various preclinical animal models. Tepsuwan et al. [27] completed a study using freeze-dried flowers of *A. indica* in which female Sprague Dawley rats subjected to 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced mammary gland carcinogenesis were fed the test material for 20 weeks. This study showed a marked reduction of the incidence of mammary gland tumors. Vinothini et al. [28] investigated chemopreventive potential of ethyl acetate and methanolic fractions of *A. indica* leaves against DMBA-induced mammary gland carcinogenesis in female Sprague Dawley rats. Intragastric administration of both fractions exerted suppression of tumor incidence through inhibition of cell proliferation, induction of apoptosis, modulation of hormone and receptor status, alterations in xenobiotic-metabolizing enzymes, upregulation of antioxidant status and inhibition of oxidative DNA damage. The ethyl acetate fraction was more effective than ethanolic fraction in modulating multiple molecular targets. Administration of an ethanolic fraction of neem leaf inhibited the growth and multiplicity of mammary tumors induced by *N*-methyl-*N*-nitrosourea (NMU) in female Sprague Dawley rats. There was an upregulation of p53, Bax, Bad, caspases, phosphatase and tensin homolog (PTEN) and c-Jun N-terminal kinase (JNK) with a downregulation of Bcl-2, angioprotein, vascular endothelial growth factor A (VEGF-A), cyclin D1, cyclin-dependent kinase 2 (Cdk2), Cdk4, and mitogen-activated protein kinase 1 (MAPK1) due to treatment with the neem fraction [29].

Baral and Chattopadhyay [30] investigated the effect of neem leaf preparation (NLP, an aqueous extract) on Ehrlich carcinoma, a spontaneous murine mammary adenocarcinoma. Tumor-bearing Swiss mice were treated with NLP at 1 unit/mouse/week which led to tumor growth retardation and increased survival. Flow cytometric analysis suggested an upregulation in CD4+ and CD8+ as well as an increased lymphocyte count. Haque et al. [31] confirmed that NLP restricted Ehrlich carcinoma growth in female Swiss albino mice. The investigators prophylactically treated female Swiss albino mice with NLP at 0.5, 1 and 2 units, showing an increase in the production of splenic T lymphocytes. Ghosh et al. [32] investigated whether NLP could offer protection against cyclophosphamide-induced leukopenia in normal and Ehrlich's carcinoma-bearing mice. Pretreatment of mice with NLP abrogated the extent of leucopenia and neutropenia in normal, tumor-bearing and cyclophosphamide-treated mice. Moreover, NLP pretreatment enhanced cyclophosphamide-mediated tumor growth inhibition and host survival. An ancillary study from the same group provided evidence that NLP pretreatment significantly reduced the immunotoxic effects of cisplatin and 5-fluorouracil in Ehrlich's carcinoma-bearing mice [33]. NLP has been shown to enhance Th1 type immune response and anti-tumor immunity against breast tumor-associated antigen (BTAA) in Swiss and Balb/c mice as well as Sprague Dawley rats. When the intravenous injection of NLP was given, there was a significant increase in anti-BTAA antibody response. Mechanistic studies showed a decrease in Th1 and interleukin-10 (IL-10) with an increase in interferon-γ (IFN-γ) and IgG2a antibody [34]. Othman et al. [35] evaluated the effect of ethanolic neem leaf extract on the expression of c-Myc oncogene in xenografted 4T1 breast tumor in mice. The extract,

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at 500 mg/kg, has been found to downregulate c-Myc expression compared to control animals. A neem leaf glycoprotein present in aqueous extract reduced tumor volume and increased survival mice inoculated with Ehrlich's carcinoma cells [36]. Banerjee et al. [37] also used Ehrlich's carcinoma model in which tumor growth was restricted and tumor angiogenesis was reduced by neem leaf glycoprotein through the downregulation of CD31, VEGF and vascular endothelial growth factor receptor 2 (VEGFR2).

3.1.2. Gastrointestinal tract and associated cancers

Human peripheral blood mononuclear cells stimulated by NLP released IFN-γ and TNF-α which triggered NLP-mediated growth restriction via apoptosis in KB human oral cancer cells [38]. A neem leaf glycoprotein (NLGP) exhibited anti-tumor T cell functions in KB as well as COLO205 (human colon cancer) cell lines [39]. Chakraborty et al. [40] also used KB cells and found that NLGP created an anti-tumor immune environment. NLGP inhibited regulatory T-cell (Tregs)-induced suppression of tumoricidal functions of CD14 + CD68 + monocyte/macrophages and inhibition of perforin/granzyme B expression. Overall, this led to the upregulation of CD80, CD86 and HLA-ABC in monocyte/macrophages [40]. Goswami et al. [41] found that NLGP was successful in preventing stage III supraglottic laryngeal tumor cell lysate (SLTCL)-induced generation and function of pro-tumorigenic M2 tumor-associated macrophages. This promoted the cytotoxic activity and suppressed Tregs through a downregulation of phosphorylation of targeted signal transducer and activator of transcription 3 (STAT3) in oral squamous cell carcinoma cells.

An ethyl acetate fraction of *A. indica* leaf extract showed antiproliferative potential in HT-29 human colon adenocarcinoma cells [42]. Ethanolic and aqueous extracts of neem leaf were effective in exerting antiproliferative effects in HT-29 colon cancer cells through apoptotic cell death driven by elevated production of ROS [43]. Kavitha et al. [44] showed that nimbolide, a neem-derived tetranotriterpenoid, deactivated nuclear factor-κB (NF-κB) and consequently Wnt/β-catenin, a glycoprotein that controls embryonic development and adult homeostasis, leading to reduced cell viability and elevated apoptosis in HepG2 human hepatocarcinoma cells. Gupta et al. [45] showed that nimbolide sensitized human colon cancer cells to TRAIL-mediated apoptosis via three distinct mechanisms, namely ROS- and ERK-induced upregulation of death receptor 5 (DR5) and DR4, downregulation of cell survival proteins, and upregulation of Bax and p53. In another colon cancer cell line, WiDr, nimbolide was found to exert antiproliferative effect through inhibition of cyclin A, resulting in S phase arrest. Nimbolide also retarded migration and invasion of tumor cells through inhibition of matalloproteinase-2 (MMP-2), MMP-9 and VEGF as well as suppression of nuclear translocation of p65/p50 and DNA-binding activity of NF-κB [46]. In HCT116 colon cancer cells, neem oil limonoids induced autophagy and caspase-dependent as well as apoptosis-inducing factor (AIF)-mediated apoptosis [47]. Recently, Yadav et al. [48] investigated the mechanism of neem-induced apoptotic death in HCT116 and HT-29 cells. Neem limonoids were able to target oxidative phosphorylation (OXPHOS) system to trigger cancer cell death which did not require upregulation or activation of proapoptotic Bcl-2 family proteins. Sastry et al. [49] reported that several analogues of nimbolide exhibited stronger cytotoxic activities in colon cancer cell lines (HT-29 and SW-620) than by the parent compound nimbolide.

Balasenthil et al. [50] investigated the effect of an aqueous extract of neem leaves on DMBA-induced buccal pouch carcinogenesis model in male Syrian hamsters. There was a reduced incidence of oral neoplasms when the neem extract was administered at 100 mg/kg thrice a week for 14 weeks. The investigators showed that antioxidants and detoxification systems were modulated due to neem extract treatment as evidenced by a significant decrease in lipid peroxidation and elevation in reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST) and gamma glutamyl transpeptidase (GGT) in the oral mucosa of tumor-bearing animals. Subapriya et al. [51] showed that ENLE suppressed DMBA-induced buccal pouch carcinogenesis (squamous cell carcinoma) with induction of apoptosis-related proteins Bim, caspase-3 and caspase-8 as well as inhibition of Bcl-2. A follow-up study from the same group [52] reported that ENLE significantly inhibited the development of hamster buccal pouch carcinomas with simultaneous decrease in the expression of proliferating cell nuclear antigen (PCNA), mutant p53 and Bcl-2 as well as upregulation of cytokeratin in buccal pouch mucosa. Using the same experimental model, Manikandan et al. [53] found that *A. indica* leaf fractions (ethyl acetate and methanolic) reduced preneoplastic lesions at a lower concentration compared to a crude extract with simultaneous modulation of phase I and phase II xenobiotic metabolizing enzymes, lipid and protein oxidation, upregulation of antioxidant defense systems, inhibition of cell proliferation and angiogenesis as well as induction of apoptosis. Priyadarsini et al. [54] investigated the effects of several neem phytochemicals, namely azadirachtin and nimbolide, on DMBA-induced buccal pouch carcinomas in male Syrian hamsters. Intragastric administration of azadirachtin and nimbolide inhibited the occurrence of DMBA-induced buccal pouch carcinomas through modulation of multiple events, including suppression of procarcinogen activation and oxidative DNA damage, upregulation of antioxidant and carcinogen detoxification enzymes, and inhibition of tumor invasion and neovascularization. Kumar et al. [55] confirmed antitumor effects of azadirachtin and nimbolide in DMBA-induced buccal pouch carcinogenesis in male Syrian hamsters. There was an increase in apoptosis, decrease in cell proliferation and alterations in PCNA, cyclin D1, p53, Apaf-1, caspase-3, GST-P, NF-κB and inhibitor of κB (IκB) following treatment with neem phytochemicals. Another study was conducted to evaluate chemopreventive efficacy of ethyl acetate chloroform insoluble subfraction and methanol ethyl acetate insoluble fraction against hamster buccal pouch carcinogenesis model. The investigators concluded that there was a reduction in preneoplastic lesions and squamous cell carcinomas possibly due to multitargeted molecular mechanisms that involved alterations in xenobiotic metabolism, apoptosis induction and abrogation of NF-κB signaling [56]. Recently, oral administration of gedunin, a neem limonoid, was able to cease the progression of hamster buccal pouch carcinomas by inhibiting phosphoinositide 3-kinase (PI3 K)/Akt and NF-κB pathways via inactivation of Akt and inhibitory κB kinase (IKK), respectively. Immunoblot and molecular docking studies revealed that suppression of the aforementioned signaling pathways may be mediated through inactivation of aldose reductase (AR). Gedunin was also found to block neovascularization by downregulating the expression of miR-21 as well as VEGF and hypoxia inducible factor-1α (HIF-1α) in the same tumor model [57].

Subapriya and Nagini [58] investigated the effects ENLE in *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced gastric carcinogenesis in male Wistar rats. The administration of ENLE led to the reduction of stomach tumor incidences and enhanced antioxidant levels. Arivazhagan et al. [59] investigated the effect of aqueous extract of neem leaf on MNNG-induced gastric carcinogenesis in male Wistar rats. This study demonstrated that the neem leaf extract was able to suppress cancer development and reverse oxidative stress in the liver by elevating GSH-dependent antioxidants. Further chemopreventive effects were reported in benzo(α)pyrene (B(α)P)-induced forestomach tumorigenesis in mice. There was a significant inhibition of tumor incidence as well as tumor burden in B(α)P-induced forestomach papillomagenesis in Swiss albino mice subjected to treatment with oral ethanolic extract of *A. indica* leaves. There was an increase in hepatic GST, DT-diaphorase, glutathione reductase (GR), GPx, superoxide dis-

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Table 1
Antineoplastic effects of *A. indica* L. fractions and phytoconstituents based on *in vitro* studies.

Materials tested	Cell lines	Effects	Mechanisms	Conc.	References
Breast cancer					
Aqueous extract of leaves and seeds	Ehrlich ascites	Inhibited carcinoma cell growth		250–500 μg/mL	Amer et al., 2010 [21]
Ethanolic leaf extract	MCF-7, MDA MB-231	Inhibited proliferation	↑Apoptosis; ⊥G0/G1; ↓Bcl-2; ↑Bax; ↑cyt. *c*; ↓Bcl-xL; ↑caspase-3; ↓IGF-1R; ↓Ras; ↓Raf; ↓p-Akt; ↓p-Erk; ↓cyclin D1	10–50 μg/mL	Elumalai et al., 2012 [22]
Nimbolide	MCF-7, MDA-MB-231	Displayed anti-proliferative activity	↑Apoptosis; ↑Bax, ↑Bad; ↑Fas-L; ↑TRAIL; ↑FADDR; ↑cyt *c*; ↓Bcl-2; ↓Bcl-xL; ↓Mcl-1; ↓XIAP-1	4–6 μM/mL	Elumalai et al., 2012 [23]
Ethanolic leaf extract	MCF-7	Showed antiproliferative activity	↑Apoptosis; ↑G0 phase; ↑Bax; ↓cyclin D1; ↓CYP 1A1; ↓CYP 1A2	10–500 μg/mL	Sharma et al., 2014 [24]
Limonoids	SK-BR-3	Exerted cytotoxicity		1–100 μM	Takagi et al., 2014 [25]
Desacetyl nimbinene	MCF-7, MDA-MB-231	Induced cell growth	↑Apoptosis; ↑ROS; pJNK; ↓p-p38	10–50 μM	Arumugam et al., 2015 [26]
Gastrointestinal tract and associated cancer					
Aqueous leaf extract	KB	Restricted tumor cell proliferation	↑Apoptosis; ↓Bcl-2; ↓cyclin D1; ↑caspase-3	3 μg/200 uL	Bose et al., 2007 [38]
Leaf glycoprotein	KB, COLO25	Optimized anti-tumor T cell functions	↑CD83; ↑CD80; ↑CD86; ↑CD40; ↑MHCs; ↑IL-12; ↓IL-10; ↑transcription factors; ↑ikaros; ↑CD28; ↑CD40L; ↑IFN-γ; ↓IL-4	1.5 μg/mL	Goswami et al., 2010 [39]
Leaf glycoprotein	KB	Created anti-tumor immune environment	↓Perforin/granzyme B; ↓CTLA4; ↑CD80; ↑CD86; ↑HLA-ABC	1.5 μg/mL	Chakraborty et al., 2012 [40]
Leaf glycoprotein	SCC131, SCC084	Sustained anti-tumor effector functions	↓phosphorylation of STAT3	1.5 μg/mL	Goswami et al., 2014 [41]
Ethyl acetate fraction of leaf extract	HT-29	Showed anti-proliferative potential		650–1000 μg/mL	Jafari et al., 2013 [42]
Ethanolic and aqueous leaf extracts	HT-29	Displayed reduced cell viability	↑Apoptosis; ↑ROS	0.1–0.4 mg/mL	Roma et al., 2015 [43]
Nimbolide	HepG2	Reduced cell viability	↑Apoptosis; ↓NF-κB; ↓GSK-3B; ↓β-catenin; ↓Bcl-2; ↑Bax; ↑cyt *c*; ↑Smac; ↑caspase-3	50 ng/mL	Kavitha et al., 2012 [44]
Nimbolide	HCT116, HT-29	Sensitized cancer cells to TRAIL-induced apoptosis	↑ROS; ↑DR5; ↑DR4; ↓I-FLICE, ↓cIAP-1; ↓cIAP-2; ↓Bcl-2; ↓Bcl-xL, ↓survivin; ↑p53; ↑Bax	5 μM	Gupta et al., 2011[45]
Nimbolide	WiDr	Retarded tumor cell growth and migration	↑Apoptosis; ↓ERK1/2; ↑p38; ↑JNK1/2; ↓cyclin D1; ↓cyclin A; ↓MMP-2; ↓MMP-9; ↓VEGF-A; ↓NF-κB	1.25 μM	Babykutty et al., 2012 [46]
Limonoids	HCT116	Induced cell death	↑Apoptosis; ↑caspase activation; ↑AIF; ↑LC3-II; ↑autophagy	300 μg/mL	Srivastava et al., 2012 [47]
Liminoids	HCT116, HT29	Induced cancer cell death	↑Apoptosis; ↑caspase activation; ↓OXPHOS Complex I; ↑ROS	150–300 μg/mL	Yadav et al., 2016 [48]
Nimbolide	HT-29, SW-620	Showed cytotoxic activity		3–103 μM	Sastry et al., 2006 [49]
Gynecological cancers					
Nimbolide and analogs	OVCAR-5	Showed antiproliferative activities		1.5–93.1 μM (IC_{50})	Sastry et al., 2006 [49]
Nimbolide	BeWo	Inhibited proliferation	↑Apoptosis; ↓PCNA; ↓Bcl-2/Bax; ↑ROS; ↑Apaf-1; ↑caspase-3	0.3–3.0 μM	Kumar et al., 2009 [67]
Ethanolic leaf extract	HeLa	Exhibited cytotoxic effects	↑Apoptosis; ↑G0 phase; ↑Bax; ↓cyclin D1; ↓CYP 1A1; ↓CYP 1A2	10–500 μg/mL	Sharma et al., 2014 [24]
Azadirachtin, nimbolide	HeLa	Inhibited growth	↑Apoptosis; ⊥G0/G1; ↑p53; ↑p21; ↑Bax; ↑survivin; ↓cyclin B; ↓cyclin D1; ↓PCNA; ↑ROS; ↓Bcl-2; ↓NF-κB	2.5–200 μM	Priyadarsini et al., 2010 [68]
Neem leaf glycoprotein	DCs	Reduced tolerogenecity of DCs	↑2,3-Dioxygenase; ↑IDO; ↑CTLA4; ↓CD40; ↓CD83; ↓CD80; ↓CD86; ↓MHC II; ↑IL-10; ↓IL-2	1.5 μg/mL	Roy et al., 2013 [69]

Table 1 (*Continued*)

Materials tested	Cell lines	Effects	Mechanisms	Conc.	References
Hematologic cancers					
Ethanolic and aqueous leaf extracts	E6-1	Decreased cell viability	↑Apoptosis; ↑ROS	0.01–0.1 mg/mL	Roma et al., 2015 [43]
Aqueous leaf extract	K562	Restricted tumor cell proliferation		3 μg/200 uL	Bose et al., 2007 [38]
Nimbolide	U937 and HL-60, THP1	Induced anti-proliferative effects		0.5–4.0 μM	Roy et al., 2007 [70]
Nimbolide	KBM-5, U937	Suppressed cell proliferation	↑Apoptosis; ↓Bcl-2; ↓Bcl-xL; ↓IAP-1; ↓IAP-2; ↓cyclin D1; ↓c-Myc; ↓COX-2; ↓mmp-9; ↓VEGF; ↓ICAM-1; ↓IL-6	0.5–2.5 μM	Gupta et al., 2010 [71]
Liminoids	HL-60	Exhibited potent cytotoxic activity	↑Apoptosis; ↑caspase-3; ↑caspase-8; ↑caspase-9	2.7–3.1 μM (IC_{50})	Kikuchi et al., 2011 [72]
Nimonol	HL-60	Showed cytotoxicity	↑Apoptosis; ↑Bax; ↓Bcl-2	2.8 μM (IC_{50})	Takagi et al., 2014 [25]
Lung cancer					
Leaf extract and fraction	A-549	Inhibited proliferation		55–56 μg/mL (IC_{50})	Jafari et al. 2013 [42]
Nimbolide	A-549	Decreased cell viability		15.6 μM (IC_{50})	Sastry et al. 2006 [49]
Limonoids	A-549	Displayed potent cytotoxicity		7.6–19.9 μM	Takagi et al., 2014 [25]
Prostate cancer					
Ethanolic leaf extract	PC-3	Decreased cell viability	↑Apoptosis; ↑Bax; ↓Bcl-2	10–100 μg/mL	Kumar et al., 2006 [73]
Ethanolic leaf extract	PC-3 and LNCaP	Inhibited cell proliferation	↑Apoptosis; ↓Akt 1; ↓Akt 2; ↓p-Akt; ↓Akt; ↓PTEN; ↓PI3 K; ↓cyclin D1; ↑p21; ↑Bad; ↑cyt. *c*; ↑caspase-3	50–100 μg/mL	Gunadharini et al., 2011 [74]
Ethanolic leaf extract	C4-2B and PC-3M-luc2	Inhibited tumor cell growth	↑HMOX1; ↑AKR1C2; ↑AKR1C3; ↑AKR1B10	5–50 μg/mL	Mahapatra et al., 2011 [75]
Supercritical extract of leaves	LNCaO-luc2 and PC-3	Suppressed tumor cell growth	↑Apoptosis; ↓DHT; ↓PSA; ↓AR; ↓integrin β1; ↓calreticulin; ↓FAK	5–25 μg/mL	Wu et al., 2014 [76]
Nimbolide and analogs	PC-3	Inhibited proliferation		2.3–48.2 μM (IC_{50})	Sastry et al., 2006 [49]
Limonoids	LNCaP and PPC1	Induced cell death	↑Apoptosis; ↑caspase activation; ↑AIF; ↑LC3-II; ↑autophagy	300 μg/mL	Srivastava et al., 2012 [47]
Nimbolide	PC-3	Inhibited cell proliferation	↑Apoptosis; ↑Fas; ↑FADDR; ↑ Bax; ↑Bad; ↑IGF-3; ↓PI3 K; ↓ Akt; ↓IGF1; ↓IGF1R; ↑cyt *c*; ↑caspase-8, −9, −10, −3; ↓XIAP; ↓Bcl-2; ↓p-Akt; ↓IGF1R	0.5–2.5 μM	Singh et al., 2014 [77]
Nimbolide	DU145	Suppressed cell viability, invasion and migration	↑Apoptosis; ↓p-STAT3; ↓p-JAK1; p-JAK2; ↑ROS; ↓GSH; ↑GSSG	2.5–20 μM	Zhang et al., 2015 [78]
Skin cancer					
Nimbolide	B16	Exhibited antiproliferative effect		0.5–5.0 μM	Roy et al., 2007 [70]
Liminoids	B16	Inhibited cell viability		25 μg/mL	Akihisa et al., 2009 [79], 2011 [80]
Neem leaf glycoprotein	B16	Displayed cytotoxicity	↑Perforin; ↑granzyme B		Barik et al., 2013 [81]

mutase (SOD) and chloramphenicol acetyltransferase (CAT), with a decrease in GSH content [60]. Administration of *A. indica* aqueous leaf extract also decreased tumor burden and multiplicity in the same tumor model. Additionally, scanning electron microscopic analysis confirmed the chemopreventive action of the leaf extract [61].

Tepsuwan et al. [27] studied potential chemopreventive effect of dietary neem flower in liver carcinogenesis in addition to mammary carcinogenesis as mentioned earlier. Dietary neem flower suppressed the incidence of benign and malignant tumors in livers of rats exposed to aflatoxin B_1 (AFB_1). There was also a substantial reduction in serum GGT activity in rats that received both AFB_1 and neem flower compared to AFB_1 control. According to a study conducted by Manal et al. [62], 5% neem leaf aqueous resulted in complete inhibition of diethylnitrosamine (DEN)-initiated and 2-acetylaminofluorene (2-AAF)-promoted rat hepatocarcinogenesis through downregulation of GST, GPx and α-fetoprotein (AFP). Taha et al. [63] reported that 5% *A. indica* aqueous leaf extract inhibited DEN/2-AAF hepatocarcinogenesis in male Sprague-Dawley rats via induction of apoptosis and downregulation of AFP gene.

Ramzinaghara et al. [64] evaluated cancer preventive effects of aqueous *A. indica* leaf extract in dimethylhydrazine (DMH)-induced colon cancer in male Wistar rats. The extract manifested a 60% reduction in tumor incidence with a simultaneous decrease in total sialic acid (TSA) in the sera of experimental animals. Nimbolide, a type of limonoid triterpene, inhibited tumor growth in athymic nu/nu mice inoculated with HCT116 colorectal cancer cells. Mechanistic studies showed a decrease in the expression of proteins involved in tumor cell proliferation (cyclin D1 and c-Myc), cell survival (Bcl-2, Bcl-xL, c-IAP-1, survivin and Mcl-1), invasion (MMP-9 and ICAM-1), metastasis (CXCR4) and angiogenesis (VEGF) [65]. A polyclonal antibody generated against neem leaf glycoprotein, a novel immunomodulator, was found to restrict the growth of xenografted CT-26 colon tumor in female BALB/c mice [66]. Additionally, aqueous neem leaf extract was effective in lowering the growth of implanted HT-29 colon tumors in CD1 nu/nu mice [43].

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Table 2
In vivo chemopreventive and anticancer effects of *A. indica* L. fractions and pure compounds.

Materials tested	Animal models	Effects	Mechanisms	Dose (route)	Duration	References
Breast cancer						
Freeze-dried neem flowers	DMBA-induced mammary gland carcinogenesis in female Sprague Dawley rats	Reduced tumor incidence and multiplicity		10% (diet)	2 weeks	Tepsuwan et al., 2002 [27]
Ethyl acetate and methanolic leaf fraction	DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats	Suppressed tumor incidence	↑Apoptosis; ↑PCNA; ↑Bcl-2; ↓caspase-3; ↑estradiol; ↑ER; ↓GSH; ↓GPx; ↓SOD; ↓CAT; ↑GST-P; ↑NF-κB;	1–10 mg/kg (p.o.)	3 times/week for 12 weeks	Vinothini et al., 2009 [28]
Ethanol fraction of leaves	NMU-induced mammary carcinogenesis in female Sprague Dawley rats	Reduced tumor burden and suppressed tumor progression	↑p53; ↑Bax; ↑Bad; ↑caspases; ↑PTEN; ↑JNK; ↓Bcl-2; ↓cyclin D1; ↓Cdk2; ↓Cdk4; ↓MAPK1	4 mg/kg (p.o.)	Every day for 4 weeks	Arumugam et al., 2014 [29]
Aqueous leaf extract	Ehrlich carcinoma in female Swiss mice	Exerted conditional tumor growth retardation	Immunomodulation	1 unit/mice/week (p.o.)	4 weeks	Baral & Chattopadhyay, 2004 [30]
Aqueous leaf extract	Ehrlich carcinoma in female Swiss mice	Restricted growth of carcinoma	↑CD4+; ↑CD8+	0.25–1.0 mg (p.o.)	1 time/week for 4 weeks	Haque et al., 2006 [31]
Aqueous leaf extract	Ehrlich's carcinoma in female Swiss mice	Inhibited tumor growth	↑Splenic neutrophil production; ↓leukopenia; ↓neutropenia	1 unit (p.o.)	1 time/week for 4 weeks	Ghosh et al., 2006 [32]
Aqueous leaf extract	Ehrlich carcinoma in female Swiss mice	Restricted tumor growth Prevented	↓Leukocyte apoptosis; ↑T cells; ↑NK cells	0.25 mg (p.o.)	1 time/week for 4 weeks	Ghosh et al., 2009 [33]
Aqueous leaf extract	Swiss mice, Balb/c mice and Sprague Dawley rats exposed to BTAA	Enhanced immune responses to tumor vaccines	↓Th1; ↑IFN-γ; ↓IL-10; ↑IgG2a	1 unit (p.o.)	1 time/week for 4 weeks	Mandal-Ghosh et al., 2007 [34]
Ethanolic leaf extract	4T1 xenograft in female Balb/c mice		↓c-Myc	250, 500 mg/kg (p.o.)	Every 2 days for 4 weeks	Othman et al., 2012 [35]
Leaf glycoprotein	Ehrlich carcinoma growth in female in Swiss mice	Reduced tumor volume		0.25 mg	4 weeks	Kundu et al., 2015 [36]
Leaf glycoprotein	Ehrlich carcinoma growth in female in Swiss mice	Restricted tumor growth and normalized tumor angiogenesis	↓CD31; ↓VEGF; ↓VEGFR2	25 μg/mice (s.c.)	1 time/week for 4 weeks	Banerjee et al., 2014 [37]
Gastrointestinal tract and associated cancers						
Aqueous leaf extract	DMBA-induced buccal pouch carcinogenesis of male Syrian hamsters	Reduced incidence and volume of oral tumors	↓Lipid peroxidation; ↑GSH; ↑GPx; ↑GST; ↑GGT	100 mg/kg (p.o.)	3 times/week for 14 weeks	Balasenthil et al., 1999 [50]
Ethanolic leaf extract	DMBA-induce buccal pouch carcinogenesis in male Syrian hamsters	Reduced tumor incidence and burden	↑Apoptosis; ↑Bim; ↓Bcl-2; ↑caspase-8; ↑caspase-3; ↓PCNA; ↓p53; ↑cytokeratin	200 mg/kg (p.o.)	3 times/week for 14 weeks	Subapriya et al., 2005 [51], 2006 52]
Leaf fractions	DMBA-induced buccal pouch carcinogenesis in male Syrian hamsters	Suppressed pre-neoplastic lesions	↓PCNA; ↓Bcl-2; ↑caspase-3; ↑PARP; ↓VEGF;	1–100 mg/kg (p.o.)	3 times/week for 14 weeks	Manikandan et al., 2008 [53]

Table 2 (*Continued*)

Materials tested	Animal models	Effects	Mechanisms	Dose (route)	Duration	References
Azadirachtin and nimbolide	DMBA-induced buccal pouch carcinomas in male Syrian hamsters	Reduced incidence of pre-neoplastic and neoplastic lesions	↑GST; ↑QR; ↑SOD; ↑CAT; ↑GSH; ↑GPX; ↑GGT; ↑GR; ↓MMP-2; ↓MMP-9; ↓HIF-1α; ↓VEGF	10–100 μg/kg (p.o.)	3 times/week for 14 weeks	Priyadarsini et al., 2009 [54]
Azadirachtin and nimbolide	DMBA-induced buccal pouch carcinogenesis in male Syrian hamsters	Reduced tumor incidence and burden	↓cyclin D1; ↓GST-P; ↓PCNA; ↓NF-κB; ↑IκB; ↑p53; ↓Bcl-2/Bax; ↑Apaf-1; ↑caspase-3; ↑cyt. *c*	10–100 μg/kg (p.o.)	3 times/week for 14 weeks	Kumar et al., 2010 [55]
Leaf subfractions	DMBA-induced buccal pouch carcinogenesis in male Syrian hamsters	Reduced preneoplastic lesions and squamous cell carcinomas	↓PCNA; ↑Bax; ↓Bcl-2; ↓NF-κB p65; ↓NF-κB p50; ↑IκBα; ↓IKKβ; ↓CYP1A1; ↓CYP1B1	1–100 mg/kg (p.o.)	3 times/week for 14 weeks	Manikandan et al., 2012 [56]
Gedunin	DMBA-induced buccal pouch carcinomas in male Syrian hamsters	Prevented progression of tumors	↓PI3 K/Akt; ↓NF-κB; ↓IKK; ↓AR; ↓miR-21; ↓VEGF; ↓HIF-1α	1–10 μg/kg (p.o.)	3 times/week for 14 weeks	Kishore et al., 2015 [57]
Ethanolic leaf extract	MNNG-induced gastric carcinogenesis in male Wistar rats	Reduced incidence of stomach tumors	↓GSH; ↓GPx; ↓GST; ↓lipid peroxidation	200 mg/kg (p.o.)	3 times/week for 26 weeks	Subapriya and Nagini, 2003 [58]
Aqueous leaf extract	MNNG-induced gastric carcinogenesis in male Wistar rats	Suppressed cancer development	↑GSH; ↑GPX; ↑GST; ↑vitamin C	100 mg/kg (p.o.)	3 times/week for 22 weeks	Arivazhagan et al., 2004 [59]
Ethanolic leaf extract	B(a)P-induced forestomach tumorigenesis in Swiss albino mice	Inhibited tumor burden and reduced tumor incidence	↑GST; ↑DT-diaphorase; ↑GR; ↑GPX; ↑SOD; ↑CAT; ↓GSH	250, 500 mg/kg (per os)	Every day for 15 days	Dasgupta et al., 2004 [60]
Aqueous leaf extract	B(a)P-induced forestomach tumorigenesis in female Balb/c mice	Decreased tumor burden and multiplicity		100 mg/kg (p.o.)	3 times/week for 4 weeks	Gangar et al., 2006 [61]
Freeze-dried neem flowers	AFB_1-induced hepatocarcinogenesis in male Wistar rats	Reduced incidence of tumors	↓GGT	12.5% (oral)	20 weeks	Tepsuwan et al., 2002 [27]
Aqueous leaf extract	DEN/2-AAF-mediated hepatocarconogenesis in male Sprague Dawley rats	Repaired carcinogenic damage	↓AFP; ↓GST; ↓GPx	5% (drinking water)	10 weeks	Manal et al., 2007 [62]
Aqueous leaf extract	DEN/2-AAF hepatocarcinogenesis in male Sprague-Dawley rats	Reduced incidence of neoplasms	↑Apoptosis; ↓AFP	5% extract (oral)	4 weeks	Taha et al., 2009 [63]
Aqueous leaf extract	DMH-induced colon carcinogenesis in male Wistar rats	Suppressed tumor incidence	↓TSA	100 mg/kg (oral)	3 times/week for 20 weeks	Ramzanighara et al., 2009 [64]

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Table 2 (*Continued*)

Materials tested	Animal models	Effects	Mechanisms	Dose (route)	Duration	References
Nimbolide	HCT116 xenografts in athymic nu/nu mice	Inhibited tumor growth	↓Bcl-2; ↓Bcl-xL; ↓c-IAP-1; ↓survivin; ↓Mcl-1; ↓cyclin D1; ↓c-Myc; ↓MMP-9; ↓ICAM-1; ↓CXCR4; ↓VEGF	5, 20 mg/kg (i.p.)	Once daily for 10 days	Gupta et al., 2013 [65]
Neem leaf glycoprotein	CT-26 colon carcinoma in athymic nude mice	Restricted tumor growth	Immunomodulation	25 μg (s.c.)	1 time/week for 4 weeks	Das et al., 2014 [66]
Aqueous leaf extracts	HT-29 xenografts in CD-1 nu/nu mice	Inhibited tumor growth		450 mg/kg/day (d.w.)	47 days	Roma et al., 2015 [43]
Prostate cancer						
Ethanolic leaf extract	C4-2B and PC-3M-luc2 xenografts in nu/nu mice	Suppressed tumor growth	↑Apoptosis	100, 200 mg/kg (i.p.)	6 times/week; 8–11 weeks	Mahapatra et al., 2011 [75]
Supracritical leaf extract	LNCaP-luc2 xenografts in nu/nu mice	Retarded tumor growth	↓PSA; ↓AKR1C2	100, 200 mg/kg (p.o.)	6 times/week;9 weeks	Wu et al., 2014 [76]
Nimbolide	TRAMP	Inhibited tumor growth and metastasis	↑Apoptosis; ↓Ki-67; ↓p-STAT	3 mg/kg (p.o.)	5 times/week; 6–12 weeks	Zhang et al., 2015 [78]
Skin cancer						
Ethanolic leaf extract	DMBA-induced skin papillomagenesis in Swiss albino mice	Inhibited tumor burden and reduced tumor incidence	↑GST; ↑DT-diaphorase; ↑GR; ↑GPX; ↑SOD; ↑CAT; ↓GSH	250, 500 mg/kg (p.o.)	Every day for 15 days	Dasgupta et al., 2004 [60]
Aqueous leaf extract	DMBA/TPA-induced skin carcinogenesis in male LACA mice	Reduced mean tumor burden and mean tumor volume	↑Bax; ↓Bcl-2; ↑caspase 3; ↑caspase 9;	300 mg/kg (p.o.)	2–3 times/week for 20 weeks	Arora et al., 2011 [82]
Aqueous leaf extract	DMBA/TPA-induced skin carcinogenesis in male LACA mice	Showed anti-neoplastic activity and regulated cell cycle	↑Lipid peroxidation; ↓PCNA; ↑mdm2; ↑p53; ↑p21	300 mg/kg (p.o.)	2 times/week for 20 weeks	Arora et al., 2013 [83]
Azadirachtin	$ONOO^-$-initiated TPA-promoted skin carcinogenesis in mice	Reduced incidence and multiplicity of papillomas		780 nmol	2 weeks	Akihisa et al., 2009 [79]
Aqueous leaf extract	B16 melanoma tumor in C57BL/6 mice	Exerted tumor growth retardation	Immunomodulation	1 unit/mice/week (p.o.)	4 weeks	Baral and Chattopadhyay, 2004 [30]
Leaf glycoprotein	B16F10 melanoma tumor in C57BL/6 mice	Restricted tumor growth and normalized tumor angiogenesis	↓CD31; ↓VEGF; ↓VEGFR2	25 μg/mice (s.c.)	1 time/week for 4 weeks	Banerjee et al., 2014 [37]
Connective tissue cancers						
Leaf glycoprotein	Sarcoma 180 in female Swiss mice	Restricted sarcoma growth	↓GATA3; ↑IFN-γ	25 μg/mouse (s.c.)	1 time/week for 4 weeks	Mallick et al., 2013 [84]
Leaf glycoprotein	Sarcoma 180 in female Swiss mice	Restricted sarcoma growth	↑IL-2; ↑IL-12; ↑IFN-γ; ↓IL-6; ↓IL-10; ↓TGF-β	25 μg/mouse (s.c.)	1 time/week for 4 weeks	Barik et al., 2013 [85]
Leaf glycoprotein	Sarcoma 180 in female Swiss mice	Reduced tumor volume	↑Perforin; ↑granzyme; ↑IFN-γ; ↑antigen specific T-cell proliferation	2×10^5 cells/100 μl/mouse	1 time/week for 4 weeks	Mallick et al., 2014 [86]

3.1.3. Gynecologic cancers

Nimbolide and its analogs showed cytotoxic activities against OVCAR-5 ovary cancer cells [49]. Kumar et al. [67] found that nimbolide treatment resulted in dose- and time-dependent inhibition of growth of human choricarcinoma (BeWo) cells. Mitochondria-mediated apoptosis in BeWo cells was due to the decrease in Bcl-2/Bax ratio with an increase in Apaf-1 and caspase-3 and the cleavage of PARP. Treatment of HeLa cervical cancer cells with ENLE retarded the growth of these cells in a dose- and time-dependent manner via apoptosis induction. ENLE also differentially modulated the expression of Bax, cyclin D1, CYP 1A1 and CYP 1A2 [24]. Priyadarsini et al. [68] showed that azadirachtin and nimbolide suppressed the viability of HeLa cervical cancer cells. The same researchers also observed that an increase in apoptosis in HeLa cells was accompanied by a blockade in G0/G1 phase, increase in p53, p21, ROS, Bax and survivin as well as decrease in cyclin B, cyclin D, PCNA, Bcl-2 and NF-κB. In another study, Roy et al. [69] showed that neem leaf glycoprotein inhibited 2,3 dioxygenase (IDO) induction in co-culture of dendritic cells (DCs) and regulatory T (Tregs) cells obtained from patients with cervical cancer stage IIIb (CaCx-IIIB). Specifically, the cytotoxic T-lymphocyte antigen 4 (CTLA4) induced optimal amount of DC maturation, decreased CD40, CD83, CD80, CD86, MHC-II and IL-2 with an increased IL-10. The investigators were able to conclude that there was a reduced tolerogenecity of DCs.

3.1.4. Hematological cancer

Both ethanolic and aqueous extracts of neem leaf reduced the viability of E6-1 leukemic cells by induction of apoptosis through destabilization of cancer cell mitochondria without harming normal cells [43]. Supernatant from human peripheral blood mononuclear cells stimulated by a neem leaf preparation restricted the proliferation of K562 erythroleukemic cells [38]. Nimbolide was found to exhibit antiproliferative activities against several leukemic cell lines, such as HL-60, U937 and THP1. In U937 cells, nimbolide treatment also resulted in cell cycle disruption by decreasing number of cells in G0/G1 phase with an initial increase in S and G2/M phases. When the cells were exposed to nimbolide for a longer time, there was an increase in damaged DNA and consequently a substantial increase in the numbers of cells in sub-G1 fraction and decrease of cells in all phases [70]. In a separate study, nimbolide suppressed the proliferation of KBM-5 (human chronic myeloid leukemia) as well as U937 cells through proapoptotic mechanisms. Nimbolide also abrogated the expression of proteins associated with proliferation (cyclin D1), survival (Bcl-2, Bcl-xL, IAP-1 and IAP-2), invasion (MMP-9) and angiogenesis (VEGF) by deactivating NF-κB pathway [71]. Kikuchi and coworkers [72] have reported the cytotoxic activities of several limonoids, namely 7-deacetyl-7-benzoyl-epoxyazadiradione, 7-deacetyl-7-benzoylgedunin and 28-deoxonimbolide, against HL60 leukemia cells via both the mitochondrial- and death receptor-mediated pathways. Takagi et al. [25] also found that nimonol, a neem limonoid, displayed cytotoxic activities against HL-60 cells mainly due to apoptosis induction.

3.1.5. Lung cancer

While crude leaf extract and its ethyl acetate fraction showed modest antiproliferative effects against A-549 lung cancer cells [42], nimbolide exhibited potent cytotoxic activity against the same cancer cell line [49]. Takagi et al. [25] reported that several limonoids (e.g., nimonol. isonimocinolide and 28-deoxonimbolide) isolated from *A. indica* leaf showed potent cytotoxic activity against A-549 cells.

3.1.6. Prostate cancer

Kumar et al. [73] have found that an ethanolic extract of *A. indica* leaves decreased cell viability in PC-3 prostate cancer cells by inducing apoptosis. There was an increase in Bax and decrease in Bcl-2 protein. Gunadharini et al. [74] made similar findings in prostate cancer cell lines, PC-3 and LNCaP, in which ENLE inhibited cell proliferation and induced apoptosis with simultaneous decrease in cyclin D1 and increase in p21, cyt. *c* and caspase-3. In another study, Mahapatra et al. [75] found that ethanol extract of neem leaves (EENL) inhibited the growth of prostate cancer cell lines, namely C4-2B and PC-3M-luc2. Microarray analysis revealed differential regulation of various genes. Most of the genes that were upregulated were associated with cell death and drug metabolism while genes associated with cell cycle regulation, DNA replication, recombination and repair functions were downregulated. Additionally, the quantitative PCR analysis revealed increase in gene expression levels of HMOX1, AKR1C2, AKR1C3 and ARK1B10. Overall, this study showed that the pleiotropic effect of EENL was regulated through multiple cellular pathways in prostate cancer. Wu et al. [76] studied antitumor effect of a supracritical extract of neem leaves (SENL) using LNCaP-luc2 and PC-3 prostate cancer cells. Treatment of both cells lines with SENL suppressed cell growth, induced apoptosis and inhibited dihydrotesterone (DHT)-induced androgen receptor (AR) and prostate-specific antigen (PSA) levels. Moreover, SENL inhibited integrin β1, calreticulin and focal adhesin kinase (FAK) activation. Sastry et al. [49] have found that nimbolide and its analogs possessed cytotoxic activities against PC-3 prostate cancer cells. Neem oil limonoids induced p-53-independent apoptosis and autophagy in LNCap and PPC1 cells [47]. Singh et al. [77] confirmed that nimbolide inhibited the proliferation of PC-3 cells by inducing apoptosis. Mechanistic studies exemplified an increase in mRNA levels of Fas ligand, Fas-associated death domain receptor (FADDR), Bax, Bad and insulin growth factor-binding protein 3 (IGF-3), increased protein expressions of cyt. *c*, caspase-8, caspase-9, caspase-10 and caspase-3 and decreased protein levels of PI3 K, Akt, IGF1, IGF1R, XIAP and Bcl-2. Nimbolide inhibited cell viability, induced apoptosis and suppressed cellular invasion and migration of DU145 and LNCaP cells. Moreover, nimbolide abrogated STAT3 activation in DU145 cells and this effect was found to be mediated via an increased production of ROS due to GSH/oxidized glutathione (GSSG) imbalance [78].

There are only few reports on in vivo anti-prostate cancer effects of neem. In one study, EENL inhibited the growth of xenografted C4-2B and PC-3M-luc2 prostate tumors in mice. The tumor inhibitory effects were associated with the formation of hyalinized fibrous tumor tissue and induction of apoptosis [75]. In another study, oral administration of SENL inhibited LNCaP-luc2 xenograft tumor growth in mice. Accompanying immunohistochemical analyses revealed that there was a simultaneous decrease in PSA and increase in aldo-keto reductase family 1 member C2 (AKR1C2) expression [76]. Recently, oral administration of nimbolide suppressed tumor growth and metastasis (to lungs and liver) in transgenic adenocarcinoma of mouse prostate (TRAMP) model. The investigators also observed that nimbolide decreased the expression of Ki-67, increased cleaved-caspase-3 and downmodulated STAT3 signaling in TRAMP tumor tissues [78].

3.1.7. Skin cancer

The B16 murine melanoma cell culture model was used to investigate anticancer effects of various neem extracts and phytochemicals. Nimbolide treatment (0.5–5.0 μM) resulted in moderate to strong growth inhibition of B16 cells [70]. Several limonoids isolated from the seed extract of *A. indica* exhibited cytotoxicity as well as anti-inflammatory activities [79,80]. $CD8^+$ T cells exposed to neem leaf glycoprotein inflicted cytotoxicity to B16 cells in vitro and

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these tumor cells exhibited high expressions of cytotoxicity-related molecules, such as perforin and granzyme B [81].

Dasgupta et al. [60] evaluated the chemopreventive potential of neem leaf in DMBA-induced skin papillomagenesis in Swiss albino mice. Oral feeding of an ethanolic leaf extract reduced tumor burden and tumor incidence. The extract inhibited chemical carcinogenesis through elevation of antioxidant enzymes as well as modulation of phase II detoxification enzymes. Arora et al. [82] studied DMBA/tetradecanoylphorbol-13-acetate (TPA)-induced skin carcinogenesis in male LACA mice that were administered 300 mg/kg of aqueous leaf extract 2–3 times a week for 20 weeks. The investigators observed that the mean tumor burden and mean tumor volume were reduced with concomitant induction of apoptosis. There was an increase in Bax, caspase-3 and caspase-9, while decrease in Bcl-2. The same group [83] later performed mechanistic studies on animals that were treated in the same manner, as in the previous study, and found low expression of PCNA and cyclin D1 as well as high expression of p53 and p21 in tumors obtained from neem extract-treated mice compared to carcinogen control animals. Azadirachtin B elicited chemopreventive effects against a two stage carcinogenesis model of mouse skin tumor induced by peroxynitrite ($ONOO^-$) as an initiator and TPA as a promoter [79]. An aqueous extract of neem leaf showed significant reduction of B16 melanoma tumor growth in mice with increased survivability possibly due to neem-mediated immune activation [30]. A neem leaf glycoprotein restricted B16F10 melanoma growth in mice through anti-angiogenic effect [37].

3.1.8. Connective tissue cancers

Mallick et al. [84] used therapeutic potential of NLGP against sarcoma growth in female Swiss mice. Tumor-bearing animals were treated with NLGP subcutaneously at 25 μg/mouse/injection once a week for 4 weeks. NLGP was able to restrict sarcoma growth in experimental animals. Mechanistically, this therapy recruited type-1 antitumor $CD8^+$ T cells into the tumor environment and reduced immunologic indices associated with immune suppression. A follow-up study conducted by the same research group revealed NLGP-mediated upregulation of IL-2, IL-12 and IFN-γ with a downregulation of IL-6, IL-10 and TGF-β restricted murine sarcoma growth [85]. An extended study by Mallick et al. [86] witnessed a restriction in murine sarcoma growth with NLGP-matured sarcoma antigen-pulsed dendritic cells (DCNLGPTAg). DCNLGPTag activated CD8+ T cells to induce tumor cell killing and increased perforin, granzyme B and IFN-γ secretion.

3.2. Clinical studies

Vasenwala et al. [87] conducted a clinical study to determine the ability of neem extract to induce apoptosis in cervical cancer cells as well as to estimate caspase activity and TNF-α and IFN-γ levels in monocytes from cervical cancer patients. Neem-treated monocytes from the cervical cancer patients displayed high activity levels of capase-3, caspase-8 and caspase-9. There was an increase in IFN-γ and decrease in TNF-α level in culture supernatant of monocytes. Additionally, cyto- and histo-morphology of neem-exposed cervical cancer cells exhibited increased apoptosis. Franco et al. [88] reported that neem oil (Holoil® RIMOS srl, Mirandola, Italy) reduced acute skin toxicity in head and neck cancer patients who were undergoing radiotherapy or chemo-radiotherapy. The investigators indicated a prophylactic effect of the neem product in the prevention of moist desquamation in this single-arm prospective observational study.

4. Toxicity studies

Given that neem-based preparations have been consumed for several millennia, the normal consumption of neem products can be regarded as absolutely safe [2]. However, the question arises whether this safety extends to extracts or pure compounds that may be used in a more concentrated form for treating or preventing different diseases, including cancer. Toxicological studies suggested that the differences in the solvents and methods used to prepare the extracts could affect the toxicity level [89]. In mice, the LD_{50} value of methanolic leaf extract has been shown to be 13 g/kg body weight [90]. Recently, Kurpadinum et al. [91] have reported similar LD_{50} value (12 g/kg body weight) of methanolic extract of neem flowers in rats. Dorababu and co-workers [92] have found that the aqueous extract of neem leaf is non-toxic to mice and they reported LD_{50} as >2.5 g/kg body weight. The acute oral toxicity in rats fed with technical grade azadirachtin in a single dose was greater than 5 g/kg both in male and female rats [93]. Tarbousch and Ashmaoui [94] have demonstrated that oral doses of azadirachtin have not produced any skeletal or morphological changes on fetuses and pups in mice. Srivastava and Raizada [95] demonstrated that there were no adverse effects on the reproductive function or fetal development in rats fed diets containing the equivalent of 5, 25 and 50 mg/kg of azadirachtin daily. However, no information on the safety on neem leaf during lactation was identified. Azadirachtin is registered in the United States as a general use pesticide with a toxicity classification of IV (relatively non-toxic) [96]. Neem seed oil was found to be non-toxic to rats and rabbits when given via intragastric route. However, when given intravenously or intraperitoneally, it caused death in rats and rabbits with 24 h LD_{50} values of 14 mL/kg and 24 mL/kg, respectively [97]. Gandhi et al. [98] reported similar toxic effects in these two species. In humans, the non-aqueous extracts have shown the presence of allergens in the skin prick test for allergenic activity [99]. Pure azadirachtin at a daily dose of 15 mg/kg body weight showed low toxicity in humans [89].

5. Conclusion and future directions

From the studies highlighted in this review, it is apparent that the ethnomedicinal plant, *A. indica*, has the potential to prevent and treat a wide array of human cancers. The chemopreventive and anticancer therapeutic efficacy of *A. indica* fractions and compounds could be explained by multiple cellular and molecular mechanisms, including free radical scavenging, carcinogen-detoxification, DNA repair, cell cycle alteration, programmed cell death (apoptosis) and autophagy, immune surveillance, anti-inflammatory, anti-angiogenic, anti-invasive and anti-metastatic activities as well as their ability to modulate several dysregulated oncogenic signaling pathways.

A careful examination of in vitro and in vivo studies presented here shows that *A. indica*-derived constituents are effective in preventing or treating cancers of the following organs: breast, cervix, colon, intestines, liver, lung, oral cavity, ovary, prostate, skin, stomach and uterus. However, further studies need to be conducted to specify how the mechanisms work to treat each cancer type. A majority of studies showing potential for cancer prevention and treatment utilized neem leaves. Other parts of the plant, such as the stem, flowers, fruits, and seeds, may also represent valuable sources for anti-cancer drug development. However, a high level of quality control measures and improved standards for production of neem-derived phytochemicals are necessary considering these components could be greatly different due to various factors in cultivation (e.g., soil and climate) and extraction process (e.g., different

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solvents) as well as raw material used (leaves, flowers, fruits, seeds and bark).

It is not possible to precisely identify active compounds responsible for anticancer activities of *A. indica*. Based on studies presented here, nimbolide and azadirachtin are two bioactive neem compounds studied extensively. However, as mentioned earlier, *A. indica* has numerous phytoconstituents which include alkaloids, carbohydrates, tannins, reducing sugars, flavonoids, glycoside, phenolic compounds and saponins along with amino acids aspartic acid, asparagine, glutamine, serine, histidine, alanine, proline, tyrosine, methionine, cysteine, isoleucine, leucine, phenylalanine and lysine. Even though several of these components have been acknowledged for observed beneficial effects, the proficiency of each phytochemical should be further investigated. It is likely that neem uses the pleiotropic effects of various phytoconstituents to exert broad chemopreventive and anti-cancer activities. Emerging evidence strongly suggests that specific mechanisms need to be studied to determine the best neem phytochemical constituent required to prevent and treat cancer. It is also possible that the combination of varying constituents, or phytochemical synergy, may be responsible for suppression of tumor development or inhibition of tumor cell growth.

Although there have been an impressive number of preclinical studies performed on *A. indica* as a therapeutic or preventative plant against cancer, the lack of human clinical trials precludes clinical drug development from this medicinal and dietary plant. Randomized-controlled trials are urgently required so that the preclinical evidence highlighted can relay clinical success in the future. Another area for future research could be the development of drugs using advanced technology such as nanoformulation and liposomal drug delivery to improve the efficacy of neem-based therapeutic treatments. However, possible adverse effects of neem constituents need to be taken into consideration. Currently, very limited information is available on pharmacokinetic profiles (absorption, distribution, metabolism and excretion) of neem components. It is very critical for neem-based anti-cancer drug development and more studies are required in this area. Since at least half of cancer cases and mortality are known to occur in low- and middle-income countries [100], exploring lower-cost alternatives to high-cost therapies, such as development of neem-based cancer preventive and therapeutic drug, would have an enormous global impact.

In conclusion, while *A. indica* shows significant promise for preventing and treating cancer, more studies need to be conducted on *A. indica*-based products as efficacious drugs. The preclinical studies presented and analyzed in this review highly suggest that *A. indica* or the "wonder tree" has a grand ability to prevent and treat human cancers.

6. Conflict of interest

Authors disclose no financial conflicts of interest.

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