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Therapeutics role of neem and its bioactive constituents in disease prevention and treatment

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Abstract

Azadirachta indica, commonly known as Neem, it is a member of the Meliaceae family. It has gained research interest worldwide prominence in recent years due to its wide range of medicinal properties such as it is rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbolin, nimbin, nimbidin, nimbidol, salannin, and quercetin isolated from different plant parts. Neem has been extensively used in Ayurveda, Unani and Homeopathic medicines worldwide especially in Indian Subcontinent in the treatment and prevention of various diseases. Neem plays role as free radical scavenging properties, prevention of disease pathogenesis antibacterial, antifungal and anti-inflammatory. It also play a key role as anticancerous agent, antipyretic, hypoglycemic, antigastric ulcer, and antitumour activities. In the present review we have summarized the role of *Azadirachta indica* in the prevention and treatment of diseases via the regulation of various biological and physiological pathways.

Keywords: *Azadirachta indica*, Neem, antioxidant, azadirachtin, antimicrobial, chemopreventive

Introduction

Neem is a member of the maghogany family, Meliaceae. It is mainly cultivated in the Indian subcontinent. The botanical name of Neem is *Azadirachta indica*, it is a fast growing evergreen tree found commonly in India, Africa and America. The neem tree is a native tree of East India and Burma, it grows in tropical and subtropical regions. Various parts of the Neem tree are well known for their medicinal properties and are prescribed by Ayurvedic, Siddha and Herbal medicine practitioners in India. Neem leaf has various properties such as antibacterial, antiparasitic, anti-inflammatory, antioxidant. Neem has been used extensively by mankind to treat various ailments. It plays an important role in diseases prevention and treatment through the enhancement of antioxidant activity, and modulation of various genetic pathways because it has complex of various pharmacologically active bioconstituents including nimbin, nimbidin, nimbolide, and limonoids. From the studies it is known that Quercetin and Beta-sitosterol were first polyphenolic flavonoids purified from fresh leaves of Neem and were known to have antifungal and antibacterial activities^[1].

Botanical Description of Neem

Neem is a fast growing tropical evergreen tree related to a Maghogany family. It is found abundance in tropical and semitropical regions like India, Bangladesh, Pakistan and Nepal^[2]. It will grow in areas where the rainfall is in the range of 450 to 1200 mm^[3]. It is a typical tropical to subtropical tree and exit annual mean temperature of 21 – 32 °C. Neem tree can grow in many different types of soil, but it thrives best on well drain deep and sandy soils. They are reported to live up to 200 years^[4]. Neem is a large growing tree with 20 - 23 m tall and trunk is straight and has a diameter around 4 - 5 feet. The leaves are compound, alternate, imparipinnate, with each comprising 5 – 15 leaflets^[2]. The petioles of Neem tree are short. The tree is often covered with delicate flowers in the early summer. The flowers are arranged in axillary, normally more or less drooping panicles which are up to 25 cm long. The fruits of Neem tree are green drupes which turn golden yellow on ripening in the months of June – August. Taxonomic position of *Azadirachta indica* is classified in Table 1

Table 1: Taxonomic position of *Azadirachta indica* (Neem).

Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	<i>Azadirachta</i>
Species	<i>indica</i>

Active Compounds of Neem

Azadirachta indica shows therapeutics role in health management due to rich source of various types of ingredients. Different parts of the Neem tree contain numerous types of ingredients such as azadirachtin and the others are nimbolin, nimbin, nimbidin, nimbidol, sodium nimbin, gedunin, salannin and quercetin. Leaves of Neem tree contain ingredients such as nimbin, nimbanene, nimbandiol, ascorbic acid, 6-desaacetylnimbinene, nimbolide, n-hexacosanol and amino acid, 7-desaacetyl-7-benzoylgedunin, 7-desaacetyl-7-benzoylazadiradione, 17-hydroxyazadiradione, and nimbiol [5, 6, 7]. Quercetin

and Beta-sitosterol are polyphenolic flavonoids were purified from Neem fresh leaves and they are known to have antibacterial and antifungal properties [1]. Seeds of Neem tree hold valuable constituents including gedunin and azadirachtin. The active constituent of *Azadirachta indica* show therapeutic implications in the modulation of cell signaling pathway involved in the management of cancer. Neem and its active ingredient play a role in prevention and treatment of tumor due to its broader pharmacological activities. Neem tree extracts have been extensively used in health management since ancient times and have a variety of health promoting properties.

Table 2: Chemical Constituents and Uses of Neem Plant

Sr. No	Source	Chemical Constituents	Uses
1	Seed Oil	Nimbidin	Anti-inflammatory, Anti-arthritis, Hypoglycemic, Antipyretic, Spermicidal, Antifungal, Antibacterial, Diuretic.
2	Seed Oil	Azadirachtin	Antimalarial
3	Seed Oil	Nimbin	Spermicidal
4	Seed Oil	Nimbolide	Antimalarial, Antibacterial
5	Seed Oil	Gedunin	Antimalarial, Antifungal
6	Seed Oil	Mahmoodin	Antibacterial
7	Bark	Gallic Acid and Catechin	Antibacterial
	Bark	Margolone, Margolonone and isomargolonone	Antibacterial
9	Leaf	Cyclic Trisulphide and Cyclic tetrasulphide	Antifungal
10	Leaf	Polysaccharides	Anti-inflammatory
11	Bark	Polysaccharides G1A, G1B	Antitumour
12	Bark	Polysaccharides G2A	Anti-inflammatory
13	Bark	NB-2 Peptidoglucon	Immunomodulatory

Table 3: Parts of Neem with their Uses

Sr. No.	Parts of Plant	Uses
1	Cake	Soil Manure and additives, Animal folder, Fertilizer.
2	Seed, Cake, Oil	Plant Protectant, Commercial Pesticide, Medicine, Animal Care, Oil Extraction.
3	Twigs	Cough, Asthma, Piles, Phantom Tomour, Intestinal Worms, Spermatorrhoea, Obstinate Urinary Disorder, Diabetes, and Dental Hygiene.
4	Wood	Fuel, Furniture, Construction Material.
5	Roots and Fruits	Oil Extraction, Medicine.
6	Leaves	Plant Protectant, Animal Care, Cosmetics

Mechanism of Action of Active Compounds of Neem

Azadirachta indica shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbolin, nimbin, nimbidin, salannin, and quercetin. But the exact molecular mechanism in the prevention is not understood entirely. Different parts of Neem tree shows antimicrobial role through inhibitory effect on microbial growth/potentiality of cell wall breakdown. Azadirachtin is a active Neem constituent, a chemical compound that belongs to the limonoid group, which is a secondary metabolite present in the Neem seeds. Azadirachtin is a highly oxidized tetranortriterpenoid which boasts a plethora of oxygen bearing functional groups. It is the key constituent responsible for both antifeedant and toxic effects in insects [8].

Neem plays a major role as free radical scavenging properties due to rich source of antioxidant. The active constituent Azadirachtin and nimbolide showed concentration-dependent

antiradical scavenging activity and reductive potential in the following order: nimbolide > azadirachtin > ascorbate [9]. Neem constituent shows effective role in the management of cancer through the regulation of cell signaling pathways. Neem modulates the activity of different tumour suppressor genes (e.g., p53, pTEN), angiogenesis (VEGF), transcription factors, and apoptosis (e.g., bcl2, bax). Neem also plays role as anti-inflammatory activities via regulation of proinflammatory enzyme activities including cyclooxygenase (COX), and lipoxygenase (LOX) enzyme.

Therapeutic Implications of Neem and its Different Ingredients In health Management

Neem active constituent play a key role in the diseases cure via activation of antioxidative enzyme, rupture the cell wall of bacteria and play role as chemopreventive through the regulation of cellular pathways. Pharmacological activities of neem are discussed in detail (Figure 1).



Fig 1: Pharmacological activities of *Azadirachta indica* L. neem in disease management through the modulation of various activities

Antioxidant Activity

Antioxidants are compound that protect cell against reactive oxygen cells or free radicals in the body. Although they are created as part of the body's normal metabolic function, free radicals react with other cells and may interfere with their ability to function. One of the main culprit in causing of various diseases is free radical or reactive oxygen species [10]. Free radical are believed to play a role in many health conditions, ranging from cancer and atherosclerosis. So by neutralization of free radical activity is one of the important steps in the diseases prevention. The function of antioxidants is to stabilize/deactivate free radicals, often before they attack targets in biological cells [10]. They also play an important role in the activation of antioxidative enzyme that helps plays role in the control of damage caused by free radicals/reactive oxygen species. Most of the medicinal plants have been reported to have antioxidant activity [11]. Different parts of Neem plants such as fruits, seeds, oil, leaves, bark, and roots show an important role in diseases prevention due to the rich source of antioxidant activity.

A study was performed to evaluate the antioxidant activity of different extracts obtained from various parts of the Neem tree. Leaf and bark extracts of *Azadirachta indica* have been studied for their antioxidant activity. The results of the study clearly indicated that all the tested leaf and bark extracts of Neem tree have significant antioxidant activity [12]. Another important study was performed to assess antioxidant properties based on leaves, fruits, flowers, and stem bark extracts from the Siamese neem tree (*Azadirachta indica* A.Juss Var. *siamensis* Valetton, Meliaceae) to evaluate the antioxidant activity and results suggest that extracts from leaf, flower, and stem bark have strong antioxidant potential [13].

A study was carried out to analyze *in vitro* antioxidant activities in different crude extracts of the leaves of *Azadirachta indica* and antioxidant capacity of different crude extracts was as follows:- chloroform > butanol > ethyl acetate extracts > hexane extracts > methanol extracts. The result indicate that the chloroform crude extracts of neem could be used as a natural antioxidant [9].

From the other results it was revealed that azadirachtin and nimbolide showed concentration-dependent antiradical scavenging activity and reductive potential in the following order: nimbolide > azadirachtin > ascorbate. The Neem constituent azadirachtin and nimbolide inhibited the development of DMBA-induced HBP carcinomas through prevention of procarcinogen activation and oxidative DNA damage and upregulation of antioxidant and carcinogen detoxification enzymes [14]. The experiment was made to evaluate the antioxidant activity of the flowers and seed oil of neem plant *Azadirachta indica* A. Juss. And results revealed that ethanolic extract of flowers and seed oil at 200 $\mu\text{g}/\text{mL}$ produced the highest free radical scavenging activity with $64.17 \pm 0.02\%$ and $66.34 \pm 0.06\%$, respectively [15].

The results of the study have showed that root bark extract of Neem tree exhibited higher free radical scavenging effect with 50% scavenging activity at 27.3 $\mu\text{g}/\text{mL}$ and total antioxidant activity of this root bark extract was found to be 0.58 mM of standard ascorbic acid. Leaves, fruits, flowers, and stem bark extracts from the Siamese neem tree were assessed for antioxidant activity *in vitro* using the 1, 1- diphenyl-2-picryl hydrazyl (DPPH) scavenging assay. Although the result of the study have also revealed that the leaf aqueous extracts, flower and stem bark ethanol extracts showed higher free radical scavenging effect with 50% scavenging activity at 26.5, 27.9,

and 30.6 microg/ml, respectively. The total antioxidant activity of extracts was found to be 0.959, 0.988, and 1.064 mM of standard trolox, respectively ^[16].

Anticancerous Activity

Cancer is a multifactorial disease which is a major health problem worldwide. The alteration of molecular or genetic pathway can play important role in development of cancer. The treatment module is based on the allopathic, which is effective but on the other hand shows adverse effect on the normal cells. Earlier studies have reported that plant and their constituents have shown inhibitory effects on the growth of malignant cells via modification of cellular proliferation, apoptosis, tumour suppressor gene and various other molecular pathways ^[17]. Carcinogenesis represents a complex multifactorial process, that characterized by multiple steps from precancerous lesions which modifies normal cell into a cancer cell increased invasive potential. Cancer cells cannot be controlled angiogenesis, resistance to apoptosis. Tumour cells have a special activity to modulate their environment. The important role of neem in cancer prevention consist of the capacity to modulates the tumour environment, including decreasing angiogenesis and increasing the toxicity of the cell. The majority of neem studies based on efficiency of neem extracts on cancer used different mixtures of neem compounds.

Effect of Neem and its constituents on Tumour Suppressor Genes

p53 is the most important tumour suppressor gene, and play an important role in inhibition of the proliferation of abnormal cells, in that way only inhibit the development and progression of cancer. Although studies have revealed that ethanolic fraction of neem leaf that is used for treating effectively upregulated the proapoptotic genes and proteins including p53, Bcl-2-associated X protein (Bax), Bcl-2 which is associated with death promoter protein (Bad) caspases, phosphatase, tensin homolog gene (pTEN), and c-Jun N-terminal kinase (JNK) ^[18]. A finding showing that ethanolic neem leaf extract and enhanced the expression of proapoptotic genes, such as caspase 8, 3 and also suppressed the expression of Bcl-2 and mutant p53 in 7,12 dimethylbenz anthracene induced cancer cell ^[19-20]. Nimbolide downregulated cell survival proteins, including 1-FLICE, CIAP-1, CIAP-2, Bcl-2 and X-linked inhibitor of apoptosis protein that upregulated the proapoptotic proteins p53. Nimbolide, a tetranortriterpenoid limonoid, which is one of the most important contributors to the cytotoxicity of neem extracts ^[21]. Nimbolide downregulated the cell survival proteins, that includes I-FLICE, cIAP-1, cIAP-2, Bcl-2, Bcl-xL, survivin, and X-linked inhibitor of apoptosis protein, and upregulated the proapoptotic proteins p53 and Bax ^[22]. P10 activity is lost via a mutation, deletions and promoter methylation silencing in various primary and metastatic cancer ^[23, 24]. Inactivation of pTEN is noticed on various types of cancer ^[18].

Effect of Neem and its constituents in Apoptosis

bcl2 and bax is very important in the regulation of apoptotic process. Any alteration in bcl2 and bax causes the development and progression of tumours ^[25]. A study was performed to find the effect of extract in an *in vivo* 4T1 breast cancer model in mice and results was confirmed that CN 250 and CN 500 groups had a higher incidence of apoptosis in comparison with the cancer controls ^[26]. Another study reported that the extract has been shown to cause cell death of

prostate cancerous cells (PC-3) via inducing apoptosis ^[27]. A study revealed that the leaf extract had downregulated Bcl-2 expression of the gene and upregulated Bim, caspase-8, and caspase-3 expression of the gene in the buccal pouch which also indicates that it has apoptosis inducing effect in the target organ ^[20]. The results of this study confirmed that leaf extract induced a dose-dependent reduction in chronic lymphocytic leukemia (CLL) cells viability with significant apoptosis observed at 0.06% (w/v) by 24 h. Isolated compounds and chief constituents from neem shows a wide range of activities affecting multiple targets and also plays an important role in the induction of apoptotic cell death in cancer ^[28, 29].

Effects of Neem and Its Constituents on Angiogenesis

Angiogenesis is a complex process that supplies blood to the tissue which is essential for growth and metastasis of tumour. Angiogenesis is regulated and controlled by activators as well as inhibitors. The development of antiangiogenic agents to block new blood vessel growth is crucial step in the inhibition or prevention of tumour growth. Medicinal plants and their ingredients plays very important role in prevention of tumour growth due to their antiangiogenic activities.

A important study reveals that ethanolic fraction of neem leaf (EFNL) treatment effectively inhibited the expression of proangiogenic genes, vascular endothelial growth factor A, and angiopoietin, indicates the antiangiogenic potential of EFNL. Furthermore, inhibition of angiogenesis by ethanolic fraction of neem leaf (EFNL) could be reason for reduction in mammary tumour volume and for block development of new tumours as observed in current studies ^[18]. Another important study was performed to assess the antiangiogenic activities of extract of neem leaves in human umbilical vein endothelial cells (HUVECs) and result shows the treatment of HUVECs with EENL inhibited VEGF induced angiogenic response *in vitro* and *in vivo* and also EENL suppressed the *in vitro* proliferation, invasion, and migration of HUVECs ^[30]. A study was made on zebra fish embryos via treatment of various concentrations of water soluble fraction of crude methanolic extract of neem root, imatinib, and results of the study concludes that the water soluble fractions of methanolic extract of neem root were found to have the ability to inhibit angiogenesis ^[31].

Effect of Neem on Oncogene

An oncogene is a mutated gene that plays very important role in the development and progression of tumours. Experiment was performed to investigate effect of leaf extract on c-Myc oncogene expression in 4T1 breast cancer BALB/c mice and results revealed that 500 mg/kg neem leaf extracts (C500) group showed significant suppression of c-Myc oncogene expression as compared to the cancer control groups ^[32].

Effect of Neem on PI3K/Akt Pathways

PI3K/Akt pathways show pivotal effect in the promotion of tumours. However, the inhibition of PI3K/Akt pathways is one of the most important step towards regulation of tumour development. Effect of leaf extract on PI3K/Akt and apoptotic pathway in prostate cancer cell lines (PC-3 and LNCaP) was investigated and results suggests that effect of leaf extract induces apoptosis and inhibits cell proliferation through inhibiting PI3K/Akt pathway in both PC-3 and LNCaP cells ^[33].

Another important study was conducted to analyze the mechanism which is involved in the induction of apoptosis and the antiproliferative activity which is exerted by the leaf

extracts on the human breast cancer cell lines. The results have confirmed that leaf extract treated cells significantly decreased the protein expressions such as IGF signaling molecules such as:- IGF-1R, Ras, Raf, p-Erk, p-Akt, and cyclin D1 [34].

Another study was carried out to analyse the effects of nimbolide on apoptosis and insulin-like growth factor (IGF) signalling molecules in androgen-independent prostate cancer (PC-3) cell lines and results of the study conducted suggests that nimbolide acts as a potent anticancer agent by inducing apoptosis and inhibiting cell proliferation via PI3K/Akt pathways in PC-3 cells [35].

Effect of Neem on NF- κ B Factor

The NF- κ B transcription factor plays very important role in

cancer and related diseases [36]. However, the inhibition of NF- κ B activities is a vital step in the prevention of cancer development and progression. An important study was performed to investigate the efficacy of bioactive phytochemicals in inhibiting radiotherapy (RT) induced NF- κ B activities, signalling, and NF- κ B-dependent regulation of cell death and results showed that curcumin, leaf extract, and black raspberry extract (RSE) significantly inhibited both constitutive and RT-induced NF- κ B [37]. Another important study was performed which results demonstrate that nimbolide, a neem derived tetranortriterpenoid, concurrently abrogates canonical NF- κ B and Wnt signalling and induces intrinsic apoptosis in human hepatocarcinoma (HepG2) cells [38].

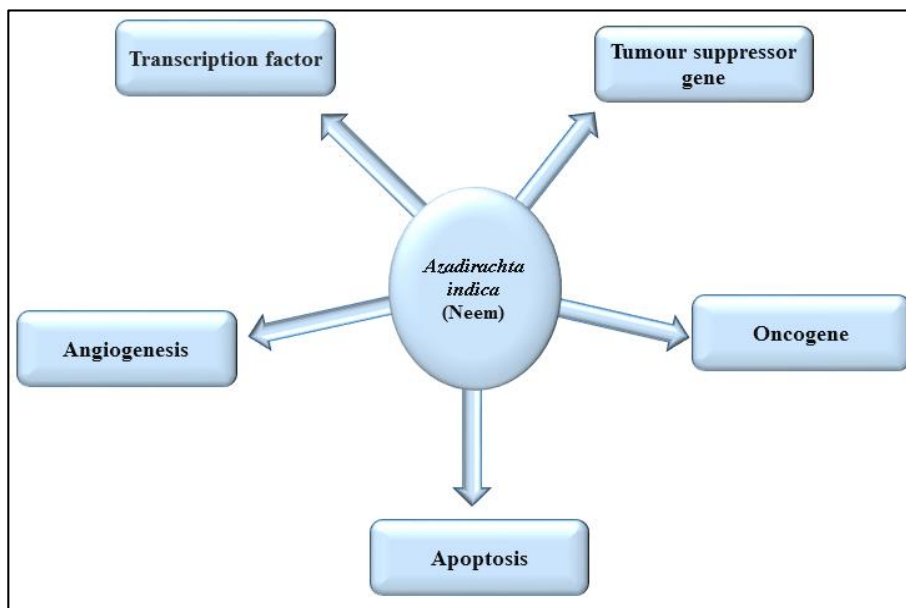


Fig 2: Anticancerous activities of *Azadirachta indica* L. Neem through the modification of various cell signalling pathways.

Effect of Neem as Anti Inflammatory

Plants or their isolated derivatives are in the practice to treat as anti-inflammatory agents. The result of this study has confirmed that extract of *Azadirachta indica* leaves at a dose of 200 mg/kg, showed significant anti-inflammatory activity in cotton pellet granuloma assay in rats [39]. Another study showed that neem leaf extract has significant anti-inflammatory effect but it is also less efficacious than that of dexamethasone [40]. The results of this suggest that nimbidin suppresses the function of macrophages and neutrophils relevant to inflammation [41]. Earlier findings showed immunomodulator and anti-inflammatory effect of bark and leave extracts and antipyretic and anti-inflammatory activities of oil seeds [42, 43]. Experiment was made to evaluate the analgesic activity of neem seed oil on albino rats and results of the study showed that neem seed oil showed significant analgesic effect in the dose of 1 or 2 mL/kg and oil has dose-dependent analgesic activity [44]. Another study was made to investigate the anti-inflammatory effect of neem seed oil on albino rats using carrageenan-induced hind paw edema. The results revealed that neem seed oil showed increase inhibition of paw edema with the increase in dose from 0.25 mL to 2 mL/kg body weight. At the dose of 2 mL/kg body weight, neem seed oil showed maximum (53.14%) inhibition of edema at 4th hour of carrageenan injection [45]. Results of the study concludes that the treated animals with 100 mg kg⁻¹ dose of carbon tetrachloride extract (CTCE) of *Azadirachta*

indica fruit skin and isolated ingredient azadiradione showed significant antinociceptive and anti-inflammatory activities respectively [46].

Hepatoprotective Effect

Neem tree and their active constituents play a pivotal role as hepatoprotective without any adverse complications. An important study was performed to investigate the hepatoprotective role of azadirachtin. An in carbon tetrachloride (CCl₄) induced hepatotoxicity in rat. Histology and ultrastructure study comprised that pretreatment with azadirachtin. A dose dependently reduced hepatocellular necrosis and therefore it protect the liver against toxicity caused by CCl₄ [49]. Furthermore, the results from this study show that pretreatment with azadirachtin-A at the higher dose levels moderately restores the rat liver to normal [47].

Another important study was performed to investigate the active constituents of neem such as nimbolide for the evidence of acute toxicity and its protective effects against carbon tetrachloride (CCl₄) induced liver toxicity in the rats. The results of their study suggest that nimbolide possess hepatoprotective effect against CCl₄ induced liver damage in rats with efficiency similar to that of Silymarin standard [48]. Another study was performed on neem plants it reveals that aqueous leaf extract of *A. indica* was found to have protection against paracetamol induced liver necrosis in rats [49].

A study assess the hepatoprotective activity of *A. indica* leaf extract on antitubercular drugs induced hepatotoxicity in albino rats. The results from this study confirmed that aqueous leaf extract significantly prevented changes in the serum levels of bilirubin, protein, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Similarly significantly prevented the histological changes as compared to the group receiving antitubercular drugs. It significantly reversed the biochemical and histological changes [50].

Additionally, other study was carried out to evaluate the hepatoprotective role of leaf extracts of *A. indica*. Hepatoprotective activities of ethanolic and aqueous extracts of *Azadirachta indica* was examined against carbon tetrachloride induced liver damage in mice using silymarin as control. Phytochemical leaf extracts of neem exhibited significant hepatoprotective activity. The results showed that ethanolic and aqueous leaf extracts of *A. indica* exhibited moderate activity over carbon tetrachloride treated animal [51]. It also confirm that the traditional ethnomedicinal use of *A. indica* as a potential source of hepatoprotective agent [52].

Wound Healing Effect

Numerous plants and their constituent plays very important role in the wound healing effect. A study was made to analyse the wound healing activity of the extract of leaves of *A. indica* using excision and incision wound models in Sprague Dawley rats and results showed that extract of the plant significantly promoted the wound healing activity in both excision and incision wound models [53]. Moreover, in incision wound, tensile strength of the healing tissue of the plant treated groups was found to be significantly higher as compared to the control group [54]. Other results showed that leave extracts of *Azadirachta indica* promote the wound healing activity through increased inflammatory response and neovascularization [55].

Antidiabetic Activity

A study was undertaken to analyse the 70% alcoholic neem root bark extract (NRE) in diabetes and results showed that the neem root bark extract showed that the statistically significant results in 800 mg/kg dose [56]. Another experiment was performed to analyse the pharmacological hypoglycemic action of *Azadirachta indica* in diabetic rats. The results has shown that in a glucose tolerance test with neem extract 250 mg/kg demonstrated glucose levels were significantly reduced as compared to the control group and *Azadirachta indica* significantly reduced glucose levels at 15th day in diabetic rats [57].

Studies using *in vivo* diabetic murine model, *A. indica*, chloroform, methanolic, and aqueous extracts were investigated and results showed that *A. indica* chloroform extracts showed a good oral glucose tolerance and significantly reduced the intestinal glucosidase activity [58]. Another important study suggested that leaves extracts of *Azadirachta indica* have significant antidiabetic activity and could be a potential source for treatment of diabetes mellitus [59].

Antimicrobial Effect

Neem and its ingredients plays a very important role in the inhibition of growth of numerous microbes such as viruses, bacteria, and pathogenic fungi. The role of neem in the prevention of the microbial growth is described individually as following-

Antibacterial Activity

A study was performed to analyse antimicrobial efficacy of herbal alternatives as endodontic irrigants which is compared with the standard irrigant sodium hypochlorite and findings confirmed that leaf extracts showed zones of inhibition suggesting that they had antimicrobial properties [60]. Furthermore, leaf extracts showed significantly the greater zones of inhibition than 3% sodium hypochlorite [60]. The antibacterial activity of neem extracts against 21 strains of foodborne pathogens was analysed and the results of the study suggested that the neem extracts possess compounds containing antibacterial properties that can potentially be useful to control foodborne pathogens and the spoilage organisms [61].

Another experiment was made to analyse the antibacterial activities of the bark, leaf, seed, and fruit extracts of *Azadirachta indica* (neem) on bacterial isolation from adult mouth and results revealed that bark and leaf extracts showed the antibacterial activity against all the test bacteria used [62]. Furthermore, seed and fruit extracts showed antibacterial activity only at the higher concentrations [62].

Antiviral Activity

Results had shown that neem bark (NBE) extract significantly block the entry of HSV-1 into the cells at concentration ranging from 50 to 100 µg/mL [62]. Furthermore, blocking activities of NBE was noticed when the extract was preincubated with virus but not with the target cells suggesting a direct anti-HSV-1 property of the neem bark [63]. Leaves extract of neem (NCL-11) has shown virucidal activity against coxsackievirus virus B-4 as suggested via virus inactivation and the yield reduction assay besides the interfering at an early event of its replication cycle [64].

Antifungal Activity

Experiment was made to analyse the efficacy of various extracts of the neem leaf on seed borne fungi *Aspergillus* and *Rhizopus* and results confirmed the growth of both the fungal species was significantly inhibited and controlled with the help of both alcoholic and water extract. Furthermore, alcoholic extract of neem leaf was most effective in comparison to aqueous extract for retarding the growth of both fungal species [65]. Another finding showed that the antimicrobial role of aqueous extracts of neem cake in the inhibition of the spore germination against three sporulating fungi i.e., *C. lunata*, *H. pennisetti*, and *C. gloeosporioides* f. sp. *mangiferae* [66] and results of the study concluded that the methanol and ethanol extract of *Azadirachta indica* showed growth inhibition against *Aspergillus flavus*, *Alternaria solani*, and *Cladosporium* [67].

Aqueous extracts of various parts of the neem such as neem oil and its chief principles have antifungal activities and have been reported by the investigators earlier [68, 69, 70]. A study was undertaken to analyse the antifungal activities of *Azadirachta indica* against *Alternaria solani* Sorauer and results confirmed that ethyl acetate fraction was found to be most effective in retarding fungal growth with MIC of 0.19 mg and this fraction was also effective than fungicide (metalaxyl + mancozeb) as fungicide has MIC of 0.78 mg [71].

Antimalarial Activity

Experiment was made to analyse the antimalarial activity of the extracts using *Plasmodium berghei* infected albino mice and results showed that neem leaf and stem bark extracts

reduce the level of parasitemia in infected mice by about 51–80% and 56–87%, respectively, [72] and other studies revealed that azadirachtin and the other limonoids available in neem extracts are active on malaria vectors [73, 74, 75]. Another findings based on crude acetone and water (50/50) extract of leaves (IRAB) was performed to analyse the activities against the asexual and the sexual forms of the malarial parasite, *Plasmodium falciparum*, *in vitro* and results revealed that, in separate 72-hour cultures of both asexual parasites and mature gametocytes treated with IRAB (0.5 microg/mL), parasite numbers were less than 50% of the numbers in controlled cultures, which had 8.0% and 8.5% parasitemia, respectively [76].

Role of Neem in Dentistry

Neem bark is used as the active ingredient in a number of toothpastes and toothpowders. *Neem* bark has an anti-bacterial properties, it is quite useful in dentistry for curing gingival problems and maintaining oral health in a natural way. *Neem* twigs are used as oral deodorant, toothache reliever and for cleaning of teeth's.

Neem bark possesses antibacterial and deodorant activity [77, 78]. *Neem* has been found to be safe and effective against periodontal pathogens, and oral acidogenic bacteria responsible for dental caries and dental plagues. *Neem* is a natural antibacterial agent [79]. The antimicrobial effect of *Neem* have been reported against *S. mutans* and *S. faecalis* [80]. Ethanolic extracts of *Neem* leaves and sticks and bark exhibited significant antibacterial activities [81, 82]. Dried chewing sticks of *Neem* showed maximum antibacterial activities against *S. mutans* compared to other dental caries-causing organisms, *S. salivarius*, *S. mitis*, and *S. sanguis* [83]. *Neem* extract showed antimicrobial activity against many microorganisms *S. mutans*, *S. salivarius*, *S. sanguis* and *S. mitis*. The chewing sticks of neem is found to be very important and beneficial in eradicating the dental caries-causing organism [84]. Chloroform extract of *Neem* leaf inhibited *Streptococcus mutans* and *Streptococcus salivarius* and provides an aid for treating the dental caries [85]. Antimicrobial activity of commercially available Himalaya herbal dental cream containing neem and fluoride-containing cheerio gel toothpaste have been assessed in school children. The study showed that the toothpastes have a good antimicrobial effect on caries producing salivary *streptococcus mutans* [86]. The toothpaste containing *Neem* as well as fluoridated toothpaste was equally efficacious against caries-producing bacteria. A study was made to evaluate the efficacy of neem based on mouth rinse regarding its antingivitis effect and study reported that *A. indica* mouth rinse is equally effective in reducing periodontal indices as chlorhexidine [87].

Another important study was carried out to evaluate the antimicrobial properties of organic extracts of neem against three bacterial strains causing dental caries. The results showed that petroleum ether and chloroform extract showed strong antimicrobial activity against *S. mutans*. Chloroform extracts have shown strong activity against *Streptococcus salivarius* and *Fusobacterium nucleatum* was highly sensitive to both ethanol and water extracts [88]. On the earlier finding confirmed that dried chewing sticks of neem showed maximum antibacterial activity against *S. mutans* as compared to *S. salivarius*, *S. mitis*, and *S. sanguis* [89].

Antinephrotoxicity Effect

A study was performed to investigate the effect of methanolic

leaves extracts of *A. indica* (MLEN) on cisplatin (CP) induced nephrotoxicity and oxidative stress rate, the histological observations evidenced the kidney from CP-mediated oxidative damage. Moreover, PCR results for caspase-3 and caspase-9 and Bax genes showed down regulation in MLEN treated groups. Therefore, *A. indica* can be considered as a potential candidate for protection of nephrotoxicity induced by cisplatin [90].

Neuroprotective Effects

A important study was performed to investigate the neuroprotective effects of *Azadirachta indica* leaves against cisplatin- (CP-) induced neurotoxicity [93]. The results showed that CP injection increased lipid peroxidation (LPO), nitric oxide (NO) levels and decreased glutathione (GSH) levels, whereas neem reversed these effects. The morphological brain damage and apoptosis induction whereas apparent in the CP group. It also showed that in the collagenous protein nitrogen (CPN) group, the histological damage and apoptosis induction caused by CP was improved, whereas morphological findings of neem before and after CP injection implied a well-preserved brain tissue. The study also suggests that methanolic extract of neem leaves may be of therapeutic benefit when used with CP [91].

Immunomodulatory and Growth Promoting Effect

A study was conducted to investigate growth promoting and immunomodulatory effects of neem leaves infusion on broiler chicks. The results of this study showed that neem infusion successfully improved antibody titre, growth performance, and gross return at the level of 50 mL/liter of fresh drinking water [92].

Another important study investigated the effects of feeding of powdered dry leaves of *A. indica* (AI) on humoral and cell mediated immune responses, in broilers naturally infected with Infectious Bursal Disease (IBD) virus. The results showed that *A. indica* (2 g/kg) treatment significantly enhanced the antibody titres against new castle disease virus (NCDV) antigen and also potentiated the inflammatory reactions to 1 chloro-2, 4-dinitrobenzene (DNCB) in skin contact test. The results also indicate that *A. indica* could be beneficial in immunosuppressed condition like IBD, in poultry [93].

Safety, Toxicities, and LD₅₀ Values of Neem

The measurement of toxicities of natural compounds is crucial before their applications in the health management. Various studies based on animal model and clinical trials confirmed that the neem is safe at certain dose and on the other side neem and its ingredients showed the toxic/adverse effect.

Several studies reported that, in children, neem oil poisoning causes vomiting, hepatic toxicity, metabolic acidosis, and encephalopathy [94, 95, 96] and another study based on rat model showed that administration of the leaf sap caused an antianxiety effect at the low doses, whereas high doses didn't show such types of effect [97]. An important study based on rats model revealed that the azadirachtin did not show toxicity even at 5 g/kg bw [98]. A study based on rabbits was performed to check toxicological analysis and results of the study revealed that there was a progressive increasing in body weight in both the test and control animals, and during the entire duration of administration of the neem extract, there was no sign of toxicity was observed in both the groups [99].

A study result revealed that, in the acute toxicity test, the LD₅₀ values of neem oil was found to be 31.95 g/kg [100].

Another study was performed to analyze the toxicity in chicken and findings showed that the acute toxicity study of neem leaf aqueous extract revealed an intraperitoneal LD₅₀ of 4800 mg/kg, and the clinical signs were dose dependent^[101].

A study reported that lethal medium doses (LD₅₀) recorded for neem leaf and stem bark extracts were 31.62 and 489.90 mg/kg according to body weight, respectively^[102]. The LD₅₀ of water extract of *A. indica* leaves and seeds were 6.2, 9.4 mL kg⁻¹, respectively^[102]. Lethal dose values was calculated with probit analysis and LD₅₀ and LD₉₀ values was found to be 8.4 and 169.8 µg/fly of neem extract, respectively^[103]. A test for acute oral toxicity in mice revealed that LD₅₀ value of approximately 13 g/kg body weight^[104].

Clinical Studies Based on Neem

Various clinical trials based studies confirmed that herbal products or derivatives from the natural products play vital role in diseases prevention and treatment through the enhancement of antioxidant activity, and modulation of various genetic pathways because it has complex of various pharmacologically active bioconstituents. A studies based on active compounds such as nimbidin were made to check the efficacy in the health management. A important study was performed to investigate the role of neem bark extract as antisecretory and antiulcer effects in human subjects. When lyophilised powder of the extract administered for 10 days at the dose of 30 mg twice daily showed significant decrease (77%) of gastric acid secretion. Also the volume of gastric secretion and its pepsin activity were also inhibited by 63% and 50% respectively. When bark extract was taken at the dose of 30–60 mg twice daily for 10 weeks almost completely healed the duodenal ulcers. Also one case of esophageal ulcer and one case of gastric ulcer healed completely when administrated at the dose of 30 mg twice daily for 6 weeks. Thus the neem bark extract has therapeutic potential for controlling gastric hypersecretion and gastroesophageal and gastroduodenal ulcers.

A clinical study was performed to investigate the evaluation of antiplaque activity of *A. indica* leaf extract gel. A six weeks clinical study was conducted to assess the efficacy of neem extract dental gel with chlorhexidine gluconate (0.2% w/v) mouthwash as positive control. The results of the study showed that the dental gel containing neem extract has significantly reduced the plaque index and bacterial count compared to that of the control group^[105].

A study was performed to investigate the effects of nimbidin on acute and chronic gastroduodenal ulcer models in experimental animals. It was found that *A. indica* possess a significant anti-ulcer potential in various experimental models. Neem possess anti-ulcer potential revealed very significant protective effect of the test drug in doses of 20 to 40 mg/kg in acetylsalicylic acid, stress, indomethacin induced gastric lesions and serotonin in rats. The results of the study showed that in ulcer healing tests, nimbidin significantly enhanced the healing process in acetic acid induced chronic gastric lesions in albino rats and dogs^[106].

Conclusion

Neem and its derivatives show an important role in disease prevention and treatment. It is done by the enhancement of antioxidant activity, inhibition of bacterial growth and by modulate the numerous biological processes without any adverse effect.

Neem and its active bioconstituents have therapeutics implications and have been traditionally used worldwide

especially in Indian subcontinent since ancient times. Clinical based studies confirmed that neem plays a vital role in prevention of various disease and treatment. The role of active ingredients of neem as chemopreventive effect has been noticed in various tumours via modulation of numerous cell signalling pathways. The detailed clinical trials study should be made based on animal to know the exact mechanism of action in the disease management.

Conflict of Interest

There is no conflict of interests regarding the publication of this paper.

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