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Evolution and Exploration of *Azadirachta indica* in Dentistry: An Update

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AG and UPV designed the study. Author AG wrote the protocol, and wrote the first draft of the manuscript with literature searches. Authors UPV, NL and SKO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Despite great achievements globally in oral health of populations, dental caries and periodontal diseases are the most important oral health burdens particularly among under-privileged groups in developed and developing countries. Nonetheless, oral cancer is the sixth most common cancer reported globally with an annual incidence of over 300,000 cases, of which 62% arise in developing countries. Apart from the traditional therapeutic modalities available so far that are usually synthetic drugs, researchers are presently discovering unexplored horizons of herbs for curing these ailments. One such beneficial herb is *Azadirachta indica* (Neem) that has been widely accepted throughout the world for its innumerable medicinal properties. The limitless benefits of neem has been documented in Indian traditional medicinal books like 'Charak – Samhita' & 'Sushruta – Samhita'. Neem tree has been valued long back, since centuries by the Indian citizens for cleaning the teeth, skin diseases, consumption as a tonic & eradicating worms etc & many more. The present review discusses the *in-vivo, in-vitro* and animal studies utilizing the electronic

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databases Pubmed, Embase and Google Scholar till 31st December 2016 that highlights the medicinal properties of this wonder herb from oral health point of view suggesting its role as anti-gingivitis, anti-microbial, antiplaque, anticandidiasis, anti-periodontitis, effective endodontic irrigant, dental erosion therapy, anticaries & oral cancer therapy. Although the results are encouraging, but more scientific validation is required so that the incorporation of this ayurvedic herb into modern dentistry could be justified.

Keywords: Azadirachta indica; antigingivitis; oral health; oral cancer therapy; anticaries; endodontic irrigant.

1. INTRODUCTION

Azadirachta indica of Indian origin is one of the most versatile multifarious trees of the tropics with immense potentiality. It is remedial of the most of the medical problems since ancient times. Dr. Majumdar first proved its curative qualities for mankind and revealed its preparation as a tincture by macerating the powered bark in alcohol. Derived from Persian language, *Azadirachta indica* is a latin term that signifies as "Azad" for "free", "dirakht" for "tree" & "I" for "Hind" meaning belonging to Indian origin. In Sanskrit language, "Neem" is called "arista" meaning "complete & imperishable" [1].

US National Academy of Sciences in 1992 recognised its importance and published a report entitled "Neem – a tree for solving global problems" [2]. Neem tree has been valued long back since centuries by the Indian citizens for cleaning the teeth, skin diseases, consumption as a tonic & eradicating bed bugs, book worms etc & many more. The limitless benefits of neem has been documented in Indian traditional medicinal books like 'Charak – Samhita' & 'Sushruta – Smahita'. Being commonly called as 'Indian Lilic' or 'Margosa', Neem belongs to the family of Meliaceae, sub-family Meloideae & tribe Melieae & possesses maximum useful non-wood products (leaves, bark, flowers, fruits , seeds etc) than any other species (Fig. 1). It is known to have enumerable biological properties being antiallergic, antidermatic, antifungal, anti-inflammatory, antiscabic, antifeedent, cardiac diuretic, insecticidal, larvicidal, nematicidal, spermicidal, antipyorrhoeic etc. Owing to its enormous activities benefitting mankind, 'neem' has been rightly called as 'green treasure' [1].

Azadirachta indica popularly called as 'Neem' is regarded 'innocuous' to humans, animals, birds and beneficial insects. Further United States Environmental Protection Agency has approved its use on food crops [3]. Uptill now two of the species have been reported *Azadirachta indica A. Juss*, indigenous to Indian and Indian

Fig. 1. Various useful non-wood products of Neem *(Azadirachta indica)* with medicinal properties. A) Bark, B) Leaves, C) Flower, D) Fruit and E) Seeds

subcontinents including Nepal, Bangladesh, Pakistan & Sri Lanka and the other species named *Azadirachta excels* commonly called as Phillipines Neem which is native to Phillipines and Indonesia. In Indian subcontinent it can be seen growing densely in the states of Uttar Pradesh, Tamil Nadu & Karnataka in descending order of density [4]. The present literature reviews the studies done on various constituents of Neem from dental point of view focusing on how this ancient miraculous tree can be utilized for improving dental health.

1.1 Search Strategy for the Identifcation of Studies

The PubMed (MEDLINE) database of the U.S. National Library of Medicine, EMBASE (Excerpta Medical Database by Elsevier) and Google Scholar database were utilized as the electronic databases, and a literature search was accomplished on articles using combination of various MeSH and free text words ("Neem mouthrinse", "Neem toothpaste", "Azadirachta indica extract", "Oral rinse", "Plant extracts", "Herbal", "Gingvitis", "Periodontitis", "Antimicrobial", "Antifungal", "Anticandidiasis", "Dental erosion therapy", "Endodontic irrigant", " Oral cancer", "Cancer therapy", "Dental plaque", "Dental biofilm", "Oral hygiene", "Plaque control", "Human gingival fibroblast", "Anticarious", "Plaque index", "Gingival bleeding", "Local drug delivery", "Antiinflammatory" till 31st December 2016). No restriction on the language and publication status of the articles was imposed. Further additional studies were sought by searching the reference lists of identified trials and reviews.

1.2 *Azadirachta indica* (Neem) Phyto-chemicals: Spectrum of Activity

Azadirachta indica (neem) shows numerous applications in health management since it is a rich source of various active phytochemicals namely azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin & quercetin. Compounds essentially found in leaves are nimbin, nimbanene, 6-desacetlynimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol & amino acid, 7-desacetly-7 benzoylazadiradione, 7-desacetly-7-benzoylgedunin, 17-hydroxyazadiradione and nimbiol [5-7].

Extracts from fresh neem leaves contain quercetin & beta sitosterol, polyphenolic flavanoids which are known to have both antibacterial & antifungal properties [8]. Even the seeds of this tree contain useful constituents like Gedunin & Azadirachtin. Some of the active ingredients with their biological activity highlighting general health benefits are here with discussed in detail.

1.3 Essential Active Ingredients in Detail Highlighting Overall Importance

1.3.1 Azadirachtin

In 1968 Butterworth & Morgan gave the name "Azadirachtin" for a substance which they isolated from the neem seeds. As a test of its purity they utilized its feeding inhibiting property against *Shistocerca gregaria*, a desert locust [9]. Further it was found that the seeds contains a complex tetranortriterpenoid limonoid that was responsible for antifeedant & toxic effects against insects [10]. Concentration dependent antiradical scavenging activity & reductive potential is seen in the following order nimbolide > azadirachtin> ascorbate [11]. On larval insects also, azadirachtin have shown to have growth regulatory effects like disruption of moulting, growth inhibition, malformation that may lead to mortality.

1.3.2 Nimbidin

From the oil of seed kernels of *Azadirachta indica*, a crude bitter ingredient is extracted called as "Nimbidin". Following the same principle, other important tetranortriterpenes have been isolated namely nimbin, nimbinin, nimbolide & nimbidic acid. Significant dose dependent anti-inflammatory activity is shown by nimbidin & sodium nimbidate against carrageenin induces acute paw oedema in rats and formalin induced arthiritis [12,13].

Nimbidin has shown significant antiulcer activity against acetyl salicylic acid & indimethacin, stress or serotonin induced gastric lesions followed by histamine induced duodenal ulcers. [14,15] Its antiulcer activity can be explained by its tendency to block H_2 receptors thereby acting as antihistaminic agent. In vitro studies have shown that it acts as an antifungal agent & bactericidal agent by inhibiting the growth of Tinea rubrum & Mycobacterium tuberculosis respectively. Further it has been found to be effective against many skin diseases such as eczema, furunculosis, arsenical dermatitis, seborrhevic dermatitis & scabies [16]. Animal

studies have shown it to have significant hypoglycaemic effect in fasting rabbits. Not but the least it has also demonstrated spermicidal activity.

1.3.3 Neem oil limonoids as bioactive cosmetic component

Neem oil limonoids are naturally extracted from cold pressed neem seed oil with standardization of not less than 50% total limonoids & 1000 ppm of Azadirachtin. Important cosmoceutical properties include antibacterial, antifungal, antiparasitic, antipeduculosis formulations for topical use in skin & hair care, insect repellant etc. On the other hand neemoids obtained from cold pressed neem seed oil is a free flowing pale brown to yellowish brown powder which is utilized in formulations including lotions, creams, hand/body washes, oils & related products [17].

1.3.4 Nimbolide

A major chemical component of neem seed oil which demonstrates both antimalarial & antibacterial activity by inhibiting the growth of Plasmodium falciparum [18,19] and Streptococcus aureus & coagulase respectively [20].

1.3.5 Gedunin

Another active component isolated from neem seed oil and leaves that has shown to possess both antimalarial [21] & antifungal activities [22]. Accumulating evidence through both in vitro and in vivo studies demonstrate it to have antineoplastic properties also [23].

1.3.6 Mahmoodin

A derivative from seed oil, it has been reported to contain moderate antimalarial action against some strains of human pathogenic parasite [21].

1.3.7 Tannins

Condensed from the neem tree bark, it contains gallic acid, epicatechin, gallocatechin, catechin & epigallocatechin of which gallic acid, epicatechin & catechin are mainly responsible for inhibiting the oxidative burst phenomenon of polymorphonuclear leucocytes during inflammatory conditions.

1.3.8 Diterpenoids

Three of these tricyclic diterpenoids namely margolone [24], margolonone [25] & isomargolonone [26] derived from neem stem bark are active against Staphylococcus, Klebsiella & Serratia species [27]. Cyclic trisulphide [28] & tetrasulphide [29], the sulphur containing compounds derived from the fresh matured neem leaves have demonstrated antifungal activity against Trichophyton mentagrophytes [20]. The phytosterol fraction isolated from lipid part of neem fruits demonstrates antiulcer activity in stress induced gastric lesions [30].

2. NEEM AT DENTISTRY

Nowadays dental diseases are considered as a major public health problem throughout the world. Various studies have shown a high correlation between poor oral hygiene status, dental plaque and severity of periodontal diseases. Therefore, the treatment of localized oral infections has been prioritized in the modern world and herbalism has given us a new ray of light. In view of the uncertainty regarding the usage of Neem based products for curing dental ailments, this review has attempted to ascertain the efficacy and safety of this herbal product that have been evaluated in various *in-vitro, in-vivo* and animal based studies till date. The dental benefits of Neem (*Azadirachta indica)* are summarized in the Fig. 2.

2.1 Antiplaque and Antiinflammatory

Periodontal disease as we all know is a multifactorial microbial infection caused by the interplay between the dental plaque and host immune responses. Dental plaque is composed of micorcolonies of gram positive and gram negative micro-organisms randomly distributed in the matrix composed of proteoglycans, gycoproteins and glycosoaminoglycans. It can be eliminated by various mechanical and chemical methods to prevent periodontal diseases. Although, various chemically based antiplaque agents available (chlorhexidine gluconate, essential oils, povidone iodine, antibiotics, chelating agents, metal salts, quaternary ammonium compounds etc) have been utilized in the form of mouthrinses and subgingival irrigating solutions [31]. But they have their own limitations like toxic effects on neutrophils, [32] human epithelial cells [33] and cause delay in wound healing [34]. Therefore researchers are more focusing on exploring phytochemicals based oral formulations (Sanguinarine 31 and Neem [35]) which have been proved to be effective in reducing plaque and gingivitis [36]. This article

specially reviews the current literature on Neem in dentistry incorporating updated glimpses of the related latest clinical studies and in-vitro clinical trials.

Neem, as a tree in whole has versatile properties to be used for various medical and dental ailments. Oil derived from the leaves, seeds and bark of this tree possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms. Antimicrobial effects of aqueous extract of Neem chewing sticks have been demonstrated against *Streptococcus mutans* and *S. faecalis* [37].

On the other hand anti-inflammatory action has been demonstrated by extracts of neem leaf, bark and oil seeds [38,39,40] Nimbidin, a photochemical suppresses the macrophage and neutrophil functions relative to inflammation [41]. Other components shown to have anti-inflammatory activities are sodium nimbidate [42,43], gallic acid [44], epicatechin [45], catechin [46], polysaccharide GIIa [47]. Epicatechin [7] and Nb-II peptidoglycan [48,49] causes immunomodulation which has a pivotal role in inflammatory conditions. Aqueous extract of Neem bark possess anticomplement activity acting both on the alternative as well as the classical pathway of complement system [48]. Whereas aqueous extract of neem leaves affect cell mediated and humoral responses affecting immunostimulation [50,51]. Further neem oil has shown to act by immunostimulation in response to mitogenic or antigenic challenge [52]. These properties of neem have been utilized in dentistry to inhibit plaque formation and reduce inflammatory changes in gingival tissues through its antibacterial and anti-inflammatory actions.

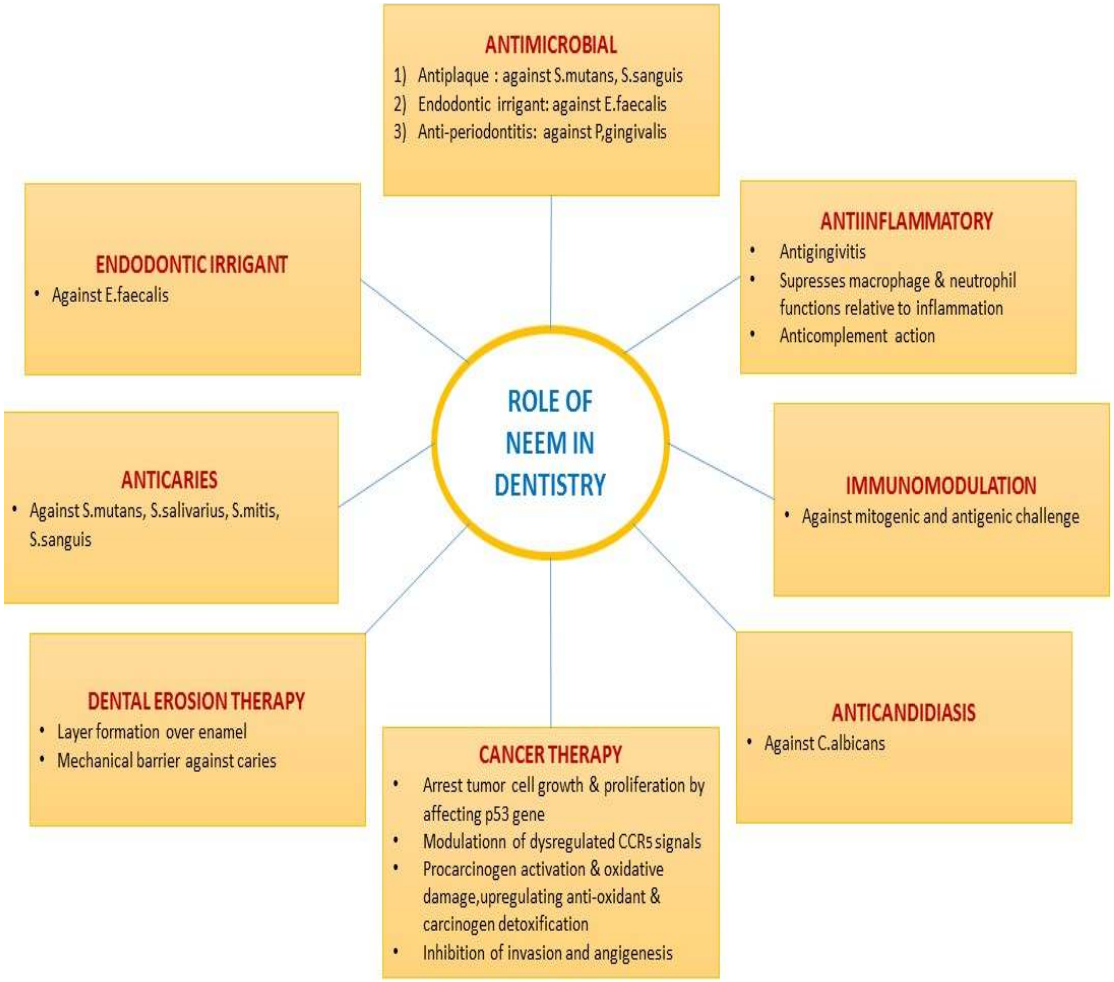

Fig. 2. Depiction of role of neem *(Azadirachta indica)* in dentistry

Since majority of studies related to dentistry have evaluated both of its antiplaque and anti-inflammatory properties together therefore these studies have been clubbed together for the ease of understanding. Various studies with useful conclusions are herewith discussed.

A systematic review was done using the literature search (electronic databases pubmed, Cochrane, central register of controlled trials & embase) upto February 2015 on antiplaque & antigingivitis activity of neem mouthrinses used either alone or as an adjunct to mechanical oral hygiene in comparison to chlorhexidine mouthrinses in gingivitis patients. These studies concluded that neem mouthrinses was as efficacious as an antiplaque & antigingivitis agent when used as an adjunct to toothbrushing in gingivitis patients [53].

A study showed that Neem stick extract can reduce the ability of some Streptococci to colonize tooth surfaces and since these are the primary colonizers to dental plaque formation they can further prevent the colonization of secondary colonizers [54].

In a RCT evaluating the comparative effectiveness of 0.5% tea, 2% neem & 0.2% chlorhexidine mouthwashes on oral health it was observed that all the groups showed antiplaque effectiveness but it was highest in the tea group, although neem & tea showed comparative effectiveness on gingiva better than chlorhexidine [55].

Another study was done on children and it was concluded that neem and mango chewing sticks mouthwashes mouthwashes were effective antiplaque & antigingivitis agents with neem & CHX possessing equivalent antiplaque efficacy while CHX having superior antigingivitis properties when used for twice daily for 21 days.[56]Similar results with neem extract mouthrinse were also seen in other study [57].

The levels of gingival interlukins 2 and interferon gamma were seen in a comparative study using various mouthwashes like chlorhexidine, essential oil, *Azadirachta indica* (neem) extract & povidone iodine in chronic gingivitis. Further, it was observed that although neem mouthwash reduced the plaque & gingival indices but reduction of IL-2 & IF gamma was not statistically significant [58].

Researchers have studied Neem extracts not only been studied in the form of mouthwash formulations but also as toothpastes aiming at improving the oral health. In a study the effect of neem containing toothpaste was seen on plaque & gingivitis using Plaque index & Gingival index and it was thereby suggested that it could be used as an adjunct for maintenance of oral hygiene [59].

An exclusive study utilizing a preparation of 10 natural herbs with *Azadirachta indica* in comparison with 0.2% chlorhexidine gluconate (CHX) showed that although it was a potent plaque inhibitor but it was less effective than CHX. Therefore being a natural derivative it could be regarded as a better alternative in diabetic, xerostomia & other special care patients [60].

Clinical efficacy of CHX and Neem gel was also evaluated and the results showed CHX gel to be more efficacious than chlorhexidine mouthwash and Neem extract gel also showing significant reduction in plaque & gingival scores. Therefore, it was suggested that gels are therapeutically more efficacious for treating oral infections than the conventionally used oral mouthwashes [61]. Further Neem extract has shown to reduce matrix metalloproteinases (MMP-2 & MMP-9) levels but not more than that of doxycycline [62].

These studies emphasize on the role of *Azadirachta indica* based herbal preparations (mouthrinse, gel & toothpaste) as an effective antiplaque and anti-inflammatory agent. Being a natural derivative, components of *A.indica* can prove to be a better alternative to gold standard chemical counterpart chlorhexidine gluconate. It can thus exert an important adjunctive role to mechanical plaque removal methods like scaling and root planing which are aimed at improving the periodontal health by lowering the microbial load either by mechanical removal of plaque or by radical alteration of the subgingival habitats. Despite promising results in existing RCT's, the need of the hour is conduction of good quality RCT's.

2.2 Anticandidiasis

Candida species are opportunistic yeast like fungus that usually infect immune-compromised, immunosuppressed, diabetes mellitus, cancer, HIV infected, AID, intravenous catheters, long term corticosteroids or antimicrobial therapy patients causing a wide range of infections like oral thrush, intestinal candidiasis, vaginal thrush etc. The known approaches like the systemic and locally administered antifungal agents do provide

remarkable benefits but due to their side effects like local itching, burning sensation and redness after local use and diarrhea, indigestion, flatulence etc have resulted in the deployment of natural derivatives for use in dentistry. The current literature have shown that *A. indica* components have hydrophobic, anti-adhesive action against dental biofilm [63] and second highest antimicrobial activity against *C. albicans* after sodium hypochlorite & propolis [64].

2.3 As Endodontic Irrigant

The success of endodontic treatment depends upon effective disinfection and complete sealing of root canals. The most commonly found microorganism in failed/ infected root canals of both primary and permanent teeth is *Enterococcus faecalis* [65,66]. The following are some of the very few studies highlighting the efficacy of extracts of Neem as an efficient root canal disinfecting agent.

In-vitro studies showing antimicrobial activity of Neem extract against *E. faecalis, S. mutans* [67,68,69] *& S. aureus* [37] have been documented in literature. Further in a study five irrigants were formulated from different parts of the tree *Azadirachta indica* (Neem) and compared with 2.5% sodium hypochlorite & 0.2% chlorhexidine gluconate through an agar diffusion test against *Candida abicans & Enterococcus faecalis*. The leaf extract of the tree and a mixture of seed bark powder were active against both organisms [70]. Using quantitative polymerase chain reaction Neem was shown to be highly efficient to 5.25% sodium hypochlorite in reducing both *E. faecalis and C. albicans* organisms within the canals [71].

In a preliminary study the results documented maximum reduction in the terms of microbial loads was seen with a combination of Sodium Hypochlorite & experimental neem irrigant. Hence, neem irrigant has antimicrobial efficacy and can be used for endodontic purposes.

Neem has been shown to be very effective against *S. mutans & S. aureus* with minimum inhibitory concentration value 125 µg. Thus, Neem can be safely used as an endodontic irrigant owing to its biocompatibility and antioxidant properties. Bitter taste associated with this medicinal plant can be overcome by addition of sweeteners and flavors to increase the patient compliance and acceptability.

2.4 Antimicrobial

Following clinical studies were conducted using *Azadirachta indica* highlighting its antimicrobial activity against dental plaque microorganisms. A study evaluated the effect of aqueous extract of neem & arak chewing stick's at various concentrations and concluded that both of them are effective at 50% concentration on *Streptococcus mutans & Sreptococcus faecalis*. Therefore in developing countries for health promotion chewing sticks can be proposed as oral hygiene tools. [72,73]

Also through an *in-vitro* model antibacterial effect of Neem extracts along with mimusops elengi & 2% chlorhexidine was seen on multispecies dentinal biofilm. It was concluded that the most efficacious was 2% chlorhexidine followed by herbal methanolic extracts of Neem & M*imusops elengi* that also showed considerable antimicrobial activity against multispecies dentinal biofilm comprising of *Streptococcus mutans, E. faecalis, S. aureus & C. albicans* [74,75].

An *vitro-study* comparing antibacterial efficiency of neem leaf extracts, grape seeds extracts & 3% sodium hypochlorite against *E. faecalis*. It was hence concluded that neem extract demonstrates significant antimicrobial effect against *E. faecalis* thereby it opens the perspective for its usage as intracanal medicament [76].

It has been shown that the prior treatment of *S. sanguis* with neem stick extract or gallotannin enriched extract from *Melaphis chinensis* at 250 microorganism/ml resulted in a significant inhibition of bacterial adhesion to saliva-conditioned hydroxyapatite [54]. Studies using plaque samples proved the antimicrobial activity of both neem chewing sticks [77-78] and neem oil [79].

Thus the above studies clearly indicate that extracts of *Azadirachta indica* has shown to have antimicrobial effects on plethora of microorganisms like *S. mutans, S. aureus, C. albicans, E. faecalis & S. sanguis* and those associated with diabetes mellitus. Owing to its remarkable antibacterial property, it can thus be used as an efficient antiplaque agent against multispecies dentinal biofilms, intracanal irrigant & antifungal agent.

2.5 Anticaries

The extract of Neem has been found to be specifically active against the caries causing organisms with the capability of not only inhibiting their growth but also reversing incipient caries. The following clinical and in-vitro studies give the glimpses of its beneficial anticarious effect.

Effect of *Azadirachta indica* mouthwash was seen on the salivary levels of *Streptococcus mutans* and *Lactobaccilli* along with its effects in reversal of incipient carious lesion. While *S. mutans* was inhibited by Neem mouthwash with or without alcohol as well as Chlorhexidine, lactobacillus growth was inhibited by chlorhexidine alone. Hence the results appear to prove its capability of reversing incipient carious lesion, although longer term clinical trials are warranted [80].

In vitro study analyzed the effect of mango & neem extract on 4 dental caries causing microorganisms, *Streptococcus mutans, Streptococcus salivarius, Streptcoccus mitis & Streptococcus sanguis*. Neem extract produced maximum zone of inhibition on S. mutans at a 50% concentration. Some inhibition of growth for all the four species of organisms was shown by neem even at 5% concentration [81]. Another *in-vitro* study has shown the similar results when analyzed on afore mentioned 4 caries causing microorganisms with Neem chewing sticks [82]. Other study with neem and babool extracts showed antimicrobial activity against *S. mutans* with neem showing superior activity than babool [83].

In India the prevalence rate of caries is 60-65% which is high enough to cause tooth loss at a very early age. Since the clinical and in-vitro based studies have shown that *A.indica* ingredients can be very useful in caries reversal and prevention and being a natural product necessary weight age should be given to it so that it can be marketed for betterment of humankind.

2.6 Dental Erosion Therapy

Dental erosion is the wearing of dental hard tissues by means of acid etch which is non-bacterial in origin [84] leading to its demineralization and dissolution. Since there is increasing incidence and prevalence [85] of such an event therefore it is important to find out agents that might prevent demineralization of tooth surface.

Around 100 bovine dentine blocks were analyzed for wear (profilometry) and the results showed that although sole application of Neem gel demonstrated lower efficiency than that shown in fluoride gel group but Neem-fluoride gel showed better efficiency in the prevention of dental erosion that was statistically similar to that of fluoride gel. Therefore, these compounds might be consider to act synergistically or additively in respect to inhibition of demineralization. It might be due to presence of phytochemicals namely tannins and resins that have an astringent effect on mucous membrane. They form a layer over enamel thereby providing mechanical barrier against dental caries [84-86].

Hence, this property can be of help in preventing tooth wear because biofilm formation protects the dental hard tissues against acids, abrasion and attrition.

2.7 For Treatment of Periodontitis

Antimicrobial therapy is essential along with conventional therapy (scaling and root planing) in the management of periodontal diseases. In dentistry the usage of herbal substances is just gaining momentum. Local drug delivery system of herbal origin can be an effective alternative for systemic therapy in managing chronic periodontal disease patients.

A clinico-microbiological study was conducted utilizing 10% neem oil chip as a local drug delivery system to evaluate its efficacy in the management of periodontitis. Clinical parameters showed statistical improvement and *P. gingivalis* strains load was significantly reduced in the periodontal pockets treated with the neem chip sites.

Hence 10% neem oil chip can be used for the treatment of periodontal disease patients although multi-centric trials with larger sample size and further research is needed to validate its effect on other periodontal pathogens [87].

2.8 Cancer Therapy

Azadirachta indica (Neem), better called as “wonder tree” has over 60 different types of biologically active phytochemicals including terpenoids and steroids that have been purified from this plant. Although a lot of research has been conducted on Neem but the studies on

molecular mechanisms causing apoptosis, tumor suppression, immune-modulation and perturbation in cell cycle are still lacking. Cancer still remains to be an enigmatic challenge for the oncologists and medical and dental practitioners. One of the primary reasons for the failure in cancer therapy is the unique property of cancer cells which utilize multiple pathways for its survival thereby preventing its targeted elimination. Therefore nowadays research oncologists are aiming at targeting cancer cells via all possible pathways with minimum possible side-effects and discomfort to patients. Certain ethmomedicines and herbal products have attracted the attention during the last decade, especially *Azadirachta indica* (Neem) due to their anticancer properties.

It has been long known to the people of Asia, particularly in India that the neem extract possess potent ability to remove cancerous phenotype. Convincing data suggests that extracts obtained from various parts of neem tree have the ability to suppress the mutagens and pro-carcinogens initiated onset of cancer phenotype [88]. Nimbolide, a triterpenoid present in edible parts of *A. indica* has been shown to arrest tumor cell growth and proliferation by affecting p53 gene and tumorigenesis [89]. Neem also induces apoptosis in cancer cells by suppressing the expression of bcl-2 protein levels (strong pro-survival factor in cancer cells) and at the same point enhances the level of expression of pro-apoptotic Bax protein. Neem is known to modulate active specific immunotherapy intended to potentiate the host's antitumor immune response by proper tumor-associated antigen presentation (TAA) or its derivatives to antigen presenting B and T cells. Hence, by gearing up the immune system against targeted attack, its role in bioimmunotherapy of cancer is appreciable [88].

Only a few studies on oral cancer are present utilizing Neem for anticancer therapy. Limonoids from neem tree (*Azadirachta indica*) have caught the attention of researches these days owing to their cytotoxicity against human cancer cell lines. But unfortunately the antiproliferative & apoptosis inducing effects of neem limonoids have yet not been tested in animal tumor models. An animal based study was conducted to evaluate the relative chemopreventive potential of the neem limonoids, azadirachtin & nimbolide in hamster buccal pouch (HBP) carcinogenesis model. The results showed that comparatively nimolide is more potent as an antiproliferative & apoptotic agent thereby acting as a promising candidate in cancer immunotherapy [92].

Number of studies has shown downregulation of CC chemokine receptor CCR_5 on monocyte/ macrophages surfaces in head and neck squamous cell carcinoma (HNSCC) patients (Stage III B). In addition to this, in vitro studies have shown Neem leaf glycoprotein (NLGP) treated monocyte/macrophages primed T cells can cause the lyses of tumor cells. Therefore, modulation of dysregulated CCR_5 signals from monocyte/macrophage can thus provide a new approach in cancer immunothyerapy [90]. It was also shown that neem leaf glycoprotein (NLGP) causes TCR alpha beta associated cytotoxicity T lymphocyte (CTL) reaction for killing cells of oral cancer [91].

Studies on azadirachtin & nimbolide have highlighted the antiradical scavenging reductive potential in the following order nimbolide > azadirachtin >ascorbate. It was shown that both azadirachtin & nimbolide inhibited the development of dimethylbenz (a) antracene (DMBA) induced hamster buccal pouch carcinoma by affecting the mechanisms involved in procarcinogen activation & oxidative DNA damage, upregulating antioxidant & carcinogen detoxification enzymes, inhibition of tumor invasion & angiogenesis [92].

An ultrastructural study was done to investigate the effects of neem extract (*Azadirachta indica A Juss)* on rat epithelium. Scanning electron microscopic images had shown irregular cell surfaces whereas transmission electron microscopy demonstrated wider intercellular spaces in the treated epithelial spinous cellular layer. It was thus proposed these structural differences may not be regarded as the side effects of neem extract [93].

There are compelling literature suggesting that Neem products e.g., Azadirachtin, Nimbolide, Nimbidin etc., possess anticancer properties. Although molecular mechanisms of their action have been investigated for various carcinomas of human body but oral carcinoma still requires attention. Since Indian subcontinent accounts for one-third of the world's burden as far as prevalence of oral cancer is concerned therefore well conducted clinical trials are the need of the hour which can pave the way to bring these products into the market as anticancer drugs.

2.9 Effect at Cellular and Ultrastructural Level

Azadirachta indica is one of most studied herbal tree because of its limitless beneficial properties to humankind. Its role in reducing gingivitis is outstanding and well known to us but whether is it efficacious for periodontitis as well need to be explored. We have just a handful of in vitro studies that cannot give a proper insight of its action at molecular level and its mechanism of interaction with subgingival microbes and host cells like gingival connective cells that play a pivot role during healing and repair. Exemplary effort was made for conduction of such trials to understand the role of Neem extract on human gingival fibroblasts discussed herewith. Further research in this area is needed so that periodontists can utilize this wonder herb for periodontal regeneration.

A comparative study was attempted to analyse the effect of Chlorhexidine (CHX) & Neem extract (NE) on cultured human gingival fibroblasts (hGF) utilizing morphological and biochemical assays. The results indicated alteration in morphological features when the CHX concentration is more than 1%. However NE showed similar alternations at higher concentrations only. NE demonstrates remarkable safety in comparison to CHX with less than 32% cytotoxicity even at 100% concentration via cytotoxicity and antioxidant analysis.

It was also shown that CHX beyond 1% concentration exhibits toxic effects on hGF at 1 minute time exposure whereas NE even upto 50% concentration does not adversely affect the fibroblasts. This highlights that NE exhibits less toxic effects in comparison with CHX on these cells there by emphasizing on its cytoprotective oral friendly quality and superiority over CHX [94].

3. CONCLUSION

Azadirachta indica, a traditional medicinal plant known since ancient times has incredible therapeutic & medicinal values for humanity. It is being utilized in Ayurveda, Unani, Homeopathic medicine and therefore regarded as cynosure of modern medicinal system. A large number of clinical and in vitro studies have been conducted worldwide focusing on its utilization for a wide range of medical and dental ailments. The present paper specifically reviews the literature presenting the versatile biological properties of *Azadirachta indica* on dental health at clinical, microbiological, cellular and genetic levels.

In the light of the literature available it can be inferred that neem mouth rinse is an efficacious antiplaque and antigingivitis agent and can be used as an adjunct to tooth brushing in gingivitis patients. *A. indica* mouth wash was shown to decrease the salivary levels of *Streptococcus mutans* and *Lactobacill*i with the capability of reversing incipient carious lesions. Also in comparison to 0.2% chlorhexidine mouthwash, 2% neem showed greater oral hygiene improvement and a better effectiveness on gingival with significant reduction in Plaque index and Gingival index. Although inflammatory markers like interlukin-2 and interferon-γ in gingival tissues were reduced but not up to significant levels. It also shows anti-inflammatory action by causing reduction in MMP-2 and MMP-9, although it was found to be inferior to doxycycline. Perhaps one study has also emphasized it to have an equivalent antiplaque efficacy to 0.2% Chlorhexidine mouthwash with Chlorhexidine mouthwash showing superior antigingivitis property over it.

Not only mouthwashes but also Neem based toothpastes have shown reduction in Plaque and Gingival index and therefore can be used as an adjunct for maintaining oral hygiene. Being phytochemical based it can be regarded as a better alternative in diabetic, xerostomia and special care patients. Even its gel has been proved to be more efficacious for treating oral infections than conventional mouthwashes. Its remarkable antimicrobial activity makes extract of neem, a very effective root canal irrigant fo endodontic purposes. Certain *in – vitro* studies have highlighted its anti-adhesive mechanism against *Candida albicans* biofilm formation on composite resin.

Effect of Neem extract on cultured human gingival fibroblasts has shown remarkable safety and superiority over chlorhexidine even at higher concentrations. Phytochemicals of *A.indica* has been shown to arrest tumor cell growth and proliferation by affecting p53 gene and its protein product that regulates large number of genes involved in tumorigenesis. Not only this it has also shown to affect mechanisms involved in procarcinogen activation & oxidative DNA damage, upregulating antioxidant & carcinogen detoxification enzymes, inhibition of tumor invasion & angiogenesis.

But despite of promising results in actions like antiplaque, anti-inflammatory, antimicrobial, antifungal, anticaries, endodontic disinfectant, dental erosion and oral cancer therapy, good quality randomized control trials are lacking to prove the clinical evidence of *Azadirachta indica* in the field of dentistry. Further attention must be paid on the evolution of new drugs based on derivatives of this wonder plant so that it can be utilized in better manner for improving oral health in future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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