

Original Research Article

## Effect of *Azadirachta indica* Stem Bark Extract on Carious Lesions in Deciduous Teeth

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**Abstract:** Caries are the localized destruction of the tissue s of the tooth by bacterial action; either enamel or cementum is demineralized by microbial acids. Traditionally the stem bark of *Azadirachta indica* was used to clean the teeth due to its antibacterial activity which reduced the incidence of dental caries. In modern life style, the use of *Azadirachta indica* was minimized and the incidence of dental caries was significantly increased. The study was to aim evaluate the effect of *Azadirachta indica* stem bark tooth paste (Neem Tooth Paste) on carious lesions in deciduous teeth. 24 deciduous teeth were selected for the study and divided in to 2 groups. Acid-resistant varnish was applied on the teeth, leaving only one area of 5 mm x 1 mm of dental enamel exposed. Group I served as control applied with tooth paste base, and group II was served as test applied with Neem Tooth Paste. The tested products were applied on the teeth and the teeth were stored in a moist environment for 24 hours. Each group of teeth was then subjected to a pH cycling model for 14 days, after which the teeth were cut through the center for an analysis of the depth of the carious lesion by polarized light microscopy. Comparisons were made between the control and Neem Tooth Paste group. There was significant (P<0.001) decrease in the lesion with the Neem Tooth Paste applied group compared to control group. The anti-caries activity may be due to its, antimicrobial activity of *Azadirachta indica*.

**Keywords:** *Azadirachta indica*, Anti-Caries and Deciduous Teeth

### INTRODUCTION

Dental caries is the technical term used by healthcare and dental professionals to describe the disease more commonly known as tooth decay. Dental caries is the localized destruction of a susceptible tooth surface by the action over a long time period of acidic by-products from the breakdown of sugars in a person's diet [1]. In south India, the prevalence of dental caries was 32.9%. It was observed that highest dental caries prevalence was in the age group of 6–10 years, that is, 49.7%. The prevalence of dental caries among females was 34% and among males was 31.8% [2]. The first clinical sign of this demineralization is a slight color change in the enamel surface – the initial caries lesion; if oral pH is restored to a more neutral level by the removal of bacterial plaque and/or carbohydrates, then available calcium, phosphate, and hydroxyl ions in the saliva and plaque participate in re-uptake into the enamel crystalline structure [3]. Thus the dental caries is thought of a chronic and on-going disease, with the

pendulum swinging between demineralization of tooth structure during periods of low pH and remineralization during periods of neutral/higher pH. When the teeth are allowed to demineralize for extended periods of low pH, the eventual total mineral loss is too much to overcome by remineralization and the enamel sub-structure collapses, forming a cavity [4].

Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases owing to a rich source of antimicrobial agents. With the knowledge of curative properties of the medicinal plants against oral microorganisms and their incorporation in clinical practice we can aim to reduce if not remove this disease entity. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over the drugs. Medicinal plants though produce slow recovery but their therapeutic effect is miraculous [5].

*Azadirachta indica*, popularly known as Indian Neem, Margosa tree or Indian lilac, is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. Neem finds immense use in a number of products ranging from cosmetics to agriculture and from pharmaceuticals to Ayurveda. Although literature search reveals that Neem tree has multiple potential uses in dentistry, its application is limited in routine dental practice. Literatures suggest that, neem extracts inhibited the colonies of Streptococcus species which are involved in the development of dental caries such as *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*. A considerable antibacterial activity of Neem aqueous extract on *Lactobacillus sp* has also been noticed at higher concentrations [6]. Several *in vitro* studies have shown that Neem leaf extract is a viable medicament against *C. albicans*, *E. faecalis* and even their mixed state [7]. Various studies have demonstrated that *Azadirachta indica* based mouth rinses are highly efficacious and may be used as an alternative therapy in the treatment of periodontal diseases [8]. The aim of the present study was to investigate the effect of *Azadirachta indica* stem extract in the development of caries in the enamel of deciduous teeth.

## MATERIALS AND METHODS

### Plant Collection

The stem bark of *Azadirachta indica* was collected from the outskirts of Chidambaram. The plant samples were identified and authenticated by the Scientist D, Botanical Survey of India, Southern Regional Center, Agricultural University, and Coimbatore, India. The voucher specimen (BSI/SRC/15/145/15-16/Sci - 122) was deposited in Herbarium for further reference.

### Preparation of Extract

The collected stem bark of *Azadirachta indica* were washed and shade dried. The dried barks were ground to coarse powder using mechanical blender. The powdered *Azadirachta indica* bark was extracted using distilled water by cold maceration method. The extract was concentrated in rotary vacuum evaporator and stored at 4°C.

### Preparation of Neem Tooth Paste [9]

The standard tooth paste was formulated as per the method of Sharma, 1998. The following ingredients were properly mixed to get tooth paste base. Calcium Carbonate 3.5 gm (Abrasive), Sodium Lauryl Sulphate 0.15 gm (Surfactant), Glycerin 3.0 gm (Anti crusting agent), Methyl cellulose 0.1 gm (Gelling agent), Sodium Saccharine 0.03 gm (Sweetener), Methyl paraben 0.01 gm (Preservative), Propyl paraben 0.002 gm (Preservative), Titanium dioxide 0.05 gm (Opacifier), Menthol 0.015 gm (Flavoring agent) and sufficient Purified water (Vehicle). 1.00 gm of *Azadirachta indica* stem bark extract.

### *In vitro* Anti-Caries Study [10]

Twenty four caries-free deciduous teeth were selected, devoid of stains or any other defects visible under a stereoscopic magnifying glass. The teeth were supplied by the tooth bank of Dentistry department, Rajah Muthiah Dental College and Hospital, Annamalai University. After their selection, the teeth were immersed in 0.1% thymol and stored in a refrigerator until their use. Before using the teeth, they were cleaned with pumice and water, after which they were distributed into two groups of 12 each. Group I served as control applied with toothpaste base and group II as test applied with Neem tooth paste. A layer of acid resistant varnish was applied on all the faces of the teeth, leaving only one 5 x 1 mm area of enamel exposed. The control and test tooth paste formulation were applied and stored for 24 hours in a moist environment.

This storage period was followed by prophylaxis using pumice stones and deionized water, after which the groups were subjected to 10 pH cycles for 14 days. To this end, the teeth were immersed for 3 hours in a demineralizing solution (2.0 mM of calcium and phosphate in 75 mM acetate buffer at pH 4.3) at a temperature of 37°C. The teeth were then washed in deionized water, dried with paper towels and placed in a container with a remineralizing solution (calcium 1.5 mM/L, phosphate 0.9 mM/L, potassium chloride 150 mM/L, and cacodylate buffer 20 mM/L, pH 7.0), where they were left for 21 hours. Before and after immersion in the demineralizing and remineralizing solutions, both groups were treated with the control and herbal formulations. The de- and remineralizing solutions were changed daily to prevent depletion or saturation of the solution and accumulation of enamel dissolution products. After the pH cycles, the teeth were cut with a double-faced diamond disk coupled to a cutting machine and prepared for analysis by polarized light microscopy to determine the presence or absence of carious lesions and the depth of the lesions.

### Statistical Analysis

The results were expressed as Mean±S.E.M. Comparisons were made between the control and test groups. The data were analyzed statistically by unpaired 't' test with a P<0.05 level of significance.

## RESULT

The effect of *Azadirachta indica* stem bark extract loaded tooth paste (Neem Tooth Paste) on *in vitro* development of carious lesions in deciduous teeth was shown on the table 1. The average depth of the lesion in the teeth applied with the tooth paste base and Neem tooth paste were 307.44 ± 12.43 and 157.62 ± 8.41 respectively. There was significant (P<0.001) decrease in the lesion with the Neem tooth paste loaded tooth paste applied group. The percentage reduction in the

lesion depth of Neem tooth paste applied group was 48.73%.

**Table 1: Effect of Neem Tooth Paste on *in vitro* development of carious lesions in deciduous teeth**

Group	Average Depth (µm) of the Lesion	% Reduction of Lesion Depth
Control (Tooth Paste Base)	307.44 ± 12.43	-
Neem Tooth Paste	157.62 ± 8.41 ***	48.73

Data's were expressed as Mean ± SEM (n=6)

\*P<0.05, \*\* P<0.01 and \*\*\* P<0.001 Vs Control

## CONCLUSION

From the result, it was concluded that the *Azadirachta indica* stem bark extract loaded tooth paste (Neem Tooth Paste) exhibits anti caries activity, and it may be due to its antimicrobial potential. Further studies may be required to prove the additional mechanism for cure the dental caries.

## REFERENCES

- Selwitz RH, Ismail AI, Pitts NB. Dental caries. The Lancet. 2007 Jan 12; 369(9555):51-9.
- Rajesh SS, Venkatesh P. Prevalence of dental caries among school-going children in South India. International Journal of Medical Science and Public Health. 2016; 5(4):700-4.
- González-Cabezas C. The chemistry of caries: remineralization and demineralization events with direct clinical relevance. Dental Clinics of North America. 2010 Jul 31; 54(3):469-78.
- Peters MC. Strategies for noninvasive demineralized tissue repair. Dental Clinics of North America. 2010 Jul 31; 54(3):507-25.
- Grosso FC, Bergamaschi Cde C, Cogo K, Franz-Montan M, Motta RH, de Andrade ED. Use of phytotherapy in dentistry. Phytotherapy Research. 2008; 22: 993 -998.
- Prashant GM, Chandu GN, Muralikrishnan KS, Saifullah MD. The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Streptococcus sanguis*: An *in vitro* study. Indian Journal of Dental Research. 2007 Oct 1; 18(4):148.
- Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). Current Science, 2002 Jun 10; 82(11):1336-45.
- Botelho MA, Santos RC, Jose Galberto Martins JG, Cintia Oliveira Carvalho Co, Mabel Calina Paz Mc, Cláudio Azenha, Ronaldo Sousa Ruela, Dinalva Brito Queiroz, Wagner Sousa Ruela, Gloria Marinho, Francisca Isabel Ruela. Efficacy of a mouthrinse based on leaves of the neem tree (*Azadirachta indica*) in the treatment of patients with chronic gingivitis: A double-blind, randomized, controlled trial. Journal of Medicinal Plant Research. 2008; 2: 341–346.
- Sharma PP. Cosmetics: Formulation, Manufacture and Quality Control, Vandana Publication, New Delhi, 1998; 500.
- Santos LD, Reis JI, Medeiros MP, Ramos SM, Araújo JM. *In vitro* evaluation of fluoride products in the development of carious lesions in deciduous teeth. Brazilian Oral Research. 2009 Sep; 23(3):296-301.