



The Efficacy of Neem Extract on Four Microorganisms Responsible for causing Dental Caries viz *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: An *in vitro* Study

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ABSTRACT

History and objectives: From the ancient time, neem used to be the traditional medicine for many diseases and was mainly used for cleaning the oral cavity. The incidence of dental caries was less a few decades ago but now the incidence of caries is very aggressive. This might be due to change in dietary habits, life style and more tendency toward processed food. The objective of this study is to find out the truth that if the neem is really efficacious against caries-inducing microorganisms, mainly *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*.

Materials and methods: The dried neem sticks ground into a coarse powder and weighed into 5, 10 and 50 gm were added to 100 ml of deionized double distilled water. After soaking for 2 days, the water was filtered at 4°C and the fine filtrate was inoculated onto blood agar plates contains individual species of microorganisms and incubated at 37°C for 2 days.

Results: At maximum concentrations, neem extract has shown the maximum zone of inhibition on *Streptococcus mutans*. At less concentration, the efficacy of neem has shown some inhibition of growth for all the four species of microorganisms.

Conclusion: Neem chewing provides the maximum benefits. Hence, the use of chewing sticks of neem can be recommended.

Keywords: Inhibition zones, Dried neem sticks, Agar plates, Freezed dried microorganisms.

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INTRODUCTION

Based on change in the dietary habits and socioeconomic status and lack of proper education, the incidence of dental

caries is drastically increasing in rural and semiurban population. It is an affordable oral hygiene device and additional advantages are derived from its functional aspect of chewing as jaw exerciser as well as reflex induction of saliva which is beneficial to oral hygiene. Being lack of knowledge and unavailability of dental clinics, and most of the dental surgeons prefer to settle down in urban areas, the dental treatment is unrealistic in rural areas. Moreover, the option for the treatment measures is expensive and is not cost-effective in case of most of the rural population. Therefore, the emergency required to promote for the purpose should be easily available, cost-effective, traditional and preventive measures are needed.

From ancient times, in our country, and some parts of east China and South Africa, neem sticks were good alternative for toothbrushes and neem sticks have good medicinal properties like antibacterial, anti-inflammatory and analgesic actions similar to commonly used dentifrices. Neem sticks are easily available and cost-effective, and efficacy is equally as good as common dentifrice. Neem sticks maintain good oral hygiene.¹

The present study is aimed to examine and identify the easily available, cost-effective and effective anticariogenic agent.

OBJECTIVE

To evaluate anticariogenic effect of neem sticks against microorganisms like *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis*, which are mainly responsible for development of dental caries.

MATERIALS AND METHODS

The following materials were used in this study:

1. Dried chewing sticks of neem (*Azadirachta indica*)
2. Freeze-dried microorganisms are as follows:
 - a. *Streptococcus mutans*
 - b. *Streptococcus salivarius*
 - c. *Streptococcus mitis*
 - d. *Streptococcus sanguis*
3. Blood agar plates
4. Vernier calipers

Preparation of Neem Sticks

Approximately 10 to 15 cm of cut neem sticks were collected. These were approved by the Department of Pharmacology, College of Pharmacy, Kanpur Central University, Kanpur, Uttar Pradesh, India. The sticks of neem were kept in sunlight for 2 days.

Preparation of the Extract

The dried neem sticks were grinded into effervescent powder. The powder weighed into 5, 10 and 50 gm. These were transferred into bottles to which were added 100 ml of sterile deionized double distilled water. The mixture was soaked for 48 hours at 4°C. It was then filtered to get neem extracts of 5, 10 and 50% respectively.

Collection of Microorganisms

Freezed-dried forms of microorganisms, *Streptococcus mutans*, *Streptococcus sanguis*, were obtained from microbial type collection from KGMC (Chhatrapati Shahuji Medical University), Lucknow, and *Streptococcus mitis* and *Streptococcus salivarius* were obtained from Rama Medical College and Research Centre, Mandana, Kanpur.

Preparation of Culture Media

The ampules containing freezed, dried forms of the microorganisms were added to the nutrient broth, which

was inoculated at 37°C for 24 hours. A sterile cotton swab was dipped into the nutrient broth and then inoculated at 37°C for 12 hours, the growth obtained on the agar plate was transferred onto a blood agar plate to test the antimicrobial activity of the herbal extract.²

Ditch Plate Method

Ditches were prepared on the agar plates at three individual quadrants streaked. The ditches were filled with one drop of the extract, repeating the same procedure for the three different concentrations of neem extracts. Sterile, double distilled deionized water was taken as control. The plates were then incubated at 37°C for 48 hours, after which they were examined for the size of the inhibition zones. The inhibition zones were measured on the underside of the plate by using vernier calipers.^{3,4}

STATISTICAL ANALYSIS

The collected data was analyzed by using the Student t-test.⁵

RESULTS

The effect of various concentrations of neem extract on *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis* is tabulated in Tables 1 to 4.

DISCUSSION

In the present study, neem extracts shown the maximum zone of inhibition with 4.6 mm against *Streptococcus mutans*. Even at 5% concentration, this extract showed some anti-microbial activity with inhibition zone of 3.1 mm. More than thousand years ago, neem has been used in South Asia for teeth cleaning and maintaining gum health. Teeth brushing with neem sticks and chewing of its leaves and

Table 1: Effect of various concentrations of neem extract on *Streptococcus mutans*

Concentrations (%)	Duration (hours)	Mean diameter of zone of inhibition (mm)	Duration (hours)	Mean diameter of zone of inhibition (mm)
C	24	0	48	0
5	24	0.8	48	3.1
10	24	1.1	48	3.7
50	24	1.3	48	4.6

Table 2: Effect of various concentrations of neem extract on *Streptococcus salivarius*

Concentrations (%)	Duration (hours)	Mean diameter of zone of inhibition (mm)	Duration (hours)	Mean diameter of zone of inhibition (mm)
C	24	0	48	0
5	24	0.2	48	1.3
10	24	0.6	48	1.9
50	24	1.2	48	2.3

Table 3: Effect of various concentrations of neem extract on *Streptococcus mitis*

Concentrations (%)	Duration (hours)	Mean diameter of zone of inhibition (mm)	Duration (hours)	Mean diameter of zone of inhibition (mm)
C	24	00	48	00
5	24	0.3	48	1.2
10	24	0.9	48	2.0
50	24	1.3	48	3.1

Table 4: Effect of various concentrations of neem extract on *Streptococcus sanguis*

Concentrations (%)	Duration (hours)	Mean diameter of zone of inhibition (mm)	Duration (hours)	Mean diameter of zone of inhibition (mm)
C	24	00	48	00
5	24	0.4	48	1.7
10	24	0.6	48	2.4
50	24	0.7	48	3.2

seeds after meals became a habit in oral hygiene.⁶ Almas stated that chewing neem sticks maintain oral hygiene and they can be used as oral hygiene tools in developing countries.⁷ Fibrous nature of neem sticks may act as anti-plaque agents. This may cause mechanical removal of plaque and also contains chemotherapeutic agents which removes dental plaque.⁸ The presence of fluoride which is having an anticariogenic action inhibits tooth decay, and silica acting as abrasive and preventing accumulation plaque. Neem is rich of astringents and salts like fluoride, chloride, calcium and sulphur. Neem contains oils, resins, sterols, flavonoids, silica, gum and alkaloids. Calcium which is present in neem, acts as an abrasive agent which is responsible for tooth polishing. Tannins as an astringent, it has analgesic property.⁹ Neem has shown the maximal antimicrobial property even in minimal concentration. Shiela et al stated that neem have antiadhesive effects which minimizes the plaque formation. The antiadhesive effect is may be due to the presence of photochemicals.¹⁰ It has the maximum inhibition against *Streptococcus mutans*, at high concentration. The maximal inhibition of *Streptococcus mutans* might be due to presence of salts like calcium and fluoride. Generally, fluoride has anticariogenic property due to affinity toward hydroxyapatite crystals. The study done by Vanka et al concluded the antimicrobial effect of neem mouthwash against the salivary levels of *Streptococcus mutans* and reversing the incipient carious lesions.¹¹ Salts, like calcium and silica which act as abrasive agents, inhibit the plaque formation. Sulphur and saponins have the antiseptic and carminative properties, which inhibit microbial growth. A study conducted by Khalid, in 1999¹² at Saudi Arabia, reported that the efficacy of neem extracts have the maximal antimicrobial activity at different concentrations. At 50% concentration, maximum inhibition occurs against *Streptococcus mutans*. Basawa et al described the antimicrobial effect of neem oil as bactericidal and

independent of temperature and energy. The bactericidal activity of neem is mainly due to inhibition of cell membrane synthesis in bacteria.¹³ Another study which was conducted by Wolinsky et al stated that bark sticks of neem inhibits the aggregation, growth and adhesion to hydroxyapatite, which affects as inhibitor of plaque formation.¹⁴ Enwonw et al, EI-Siad et al, Akpata and Akinrimisi E described the lower incidence of dental caries among users of chewing sticks compare to nonusers. This attributes to the superior mechanical cleansing action on the teeth and to the antimicrobial properties of neem sticks.¹⁵⁻¹⁷ Packia Lekshmi et al stated different extract of neem, out of that petroleum ether and chloroform extract showed strong antimicrobial activity against *Streptococcus mutans*. Chloroform extract of neem showed maximum inhibition against *Streptococcus salivarius*.¹⁸

CONCLUSION

As the oral cavity is the mirror of the whole body, oral hygiene plays a vital role in general health. The use of neem stick, as a toothbrush for the oral and dental hygiene, is very much prevailing in Indian rural and semiurban population. From the observations of this study, it appears that the chewing of neem stick increases the efficacy of antimicrobial property. Chewing sticks are used not only for hygiene but also for religious and social reasons. The use of natural toothbrush can be made more beneficial by proper technique and handling. The disadvantage of using the neem stick is that sometimes it causes gum injury with improper handling and chewing of the stick. So, the texture of the neem stick should be soft and properly handled so that it does not damage the enamel and gums.

The present study is not clear in the *in vivo* studies about the duration of the contact. Further studies have to be done *in vivo* to give us the clear picture. Certainly, the use of

natural products should be encouraged, as the undesirable effects are less compared to the synthetic products, after proper *in vivo* studies.

CLINICAL SIGNIFICANCE

Even in this tooth brush era also, countries like India still chewing of neem stick is prevalent in rural and semi urban population for the control of dental and periodontal problems. Apart from anti cariogenic activity, neem also has antifungal, antiviral, anti bacterial and anti-plaque activities and helps in the maintenance of oral health. If not in the form of stick some other form of neem or neem extract can be useful. It also possesses astringent property and promotes oral immunity.

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