

Effect of Azadirachta Indica (Neem) Leaf Extract & Twig Extract in Comparison with Chlorhexidine Gluconate on Streptococcus Mutans Induced Bacterial Gingivitis.

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ABSTRACT

Objective: To observe the effects of Azadirachta indica (Neem) leaf extract and twig extract in comparison with chlorhexidine gluconate 0.2% on Streptococcus Mutans induced bacterial gingivitis.

Study Design: Comparative, observational study.

Place and Duration: Baqai dental college department of periodontology and department of microbiology, Baqai Medical University, during December 2011- June 2012.

Material and methods: Sixty consecutive patients aged 15-40 years of either gender, suffering from gingivitis containing plaque were included in the study. The Streptococcus mutans was confirmed by using Dentocult® SM Strip mutans. Orion Diagnostica's Dentocult SM Strip. Cat. No. 67647, which is specific for Streptococcus mutans. Bacteria were grown on agar medium and Streptococcus mutans isolates by lawn preparation. 60 petri plates were divided into four groups, each comprising 15 plates, containing Tryptic soya agar were taken and a plaque bacterial lawn was made in these plates from the bacteria inoculated by the plaque samples. A well was then made in each petri plate and active ingredient namely A. indica leaf extract 5%, twig extract 5%, chlorhexidine gluconate 0.2% and placebo was administered in each well separately through micropipette. The petri plates were then placed in the incubator at 37°C for 20- 24 hrs. The bacterial sensitivity was then noticed in three active salts around the margins of the well except the placebo that did not show any sensitivity.

Results : The plaque from each patient was taken and checked the antimicrobial effect. Antibacterial activity of the active herbal agents was also checked showing zone of inhibition against Streptococcus Mutans.

Conclusion: The twig extract has comparatively better antibacterial effect against the microbe than chlorhexidine gluconate 0.2% gel.

Key words. Azadirachta indica, Chlorhexidine gluconate, Gingivitis, S. Mutans

INTRODUCTION:

Azadirachta indica (Neem) belongs to the family Meliaceae. Every part of this tree has been used as traditional medicine for household remedy against various human ailments¹⁻².

In dentistry, Azadirachta indica (Neem) has been investigated due to its antimicrobial potential against oral microorganisms especially those associated with gingivitis and periodontitis

and is therefore considered to be highly effective and alternative to chlorhexidine (CHX) especially in case of periodontal disorders³⁻⁴.

Aerobic and anaerobic bacteria in a complex biofilm are the primary etiological factor of causing various dental problems like dental caries, gingivitis, periodontitis and peri-implantitis⁵⁻⁷. Bacterial colonization on tooth surfaces is a critical factor in the development of both gingivitis and periodontitis¹⁰⁻¹¹.

Oral cavity contains numerous microflora including Gram positive and negative bacteria along with viruses and fungi. Dental diseases such as dental caries and periodontal diseases are both the result of bacterial infections, particularly S. mutans and lactobacillus acidophilus are mainly responsible for dental caries¹². Bacterial plaque and its bi-products are considered as the primary cause of chronic gingival inflammation¹³.

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Chlorhexidine is a cationic chlorphenyl biguanide with outstanding bacteriostatic properties in dentistry for pre-surgical oral disinfection and endodontics. Chlorhexidine (CHX) has also proved as an effective plaque inhibitor when used as an adjunct for mechanical cleaning procedure as well as when used alone¹⁴⁻¹⁵.

MATERIAL & METHODS:

This was in-vitro experimental study carried out in Baqai dental college department of periodontology and department of microbiology, Baqai Medical University, during December 2011- June 2012. The ethanol- based extracts of both leaf and twig were prepared in the analytical laboratory of HEJ Institute of chemistry, University of Karachi and preparation of gels and the viscosity of both gels were checked at the pharmacology laboratory of Baqai Medical University.

60 consecutive patients (aged 15 to 40 years) were selected into this study suffering with Streptococcus mutans induced Bacterial gingivitis, containing plaque to evaluate the antibacterial activity of 5% Azadirachta indica leaves and twig extracts in comparison with chlorhexidine gluconate 0.2% against Streptococcus mutans. The Streptococcus mutans was confirmed by using Dentocult® SM Strip mutans. Orion Diagnostica's Dentocult SM Strip. Cat. No. 67647, which is specific for Streptococcus mutans. Bacteria were grown on agar medium and Streptococcus mutans isolates by lawn preparation. 60 petri plates were divided into four groups, each comprising 15 plates, containing Tryptic soya agar. A plaque bacterial lawn was made in these plates from the bacteria inoculated by the plaque samples. A well was then made in each petri plate and active ingredient namely A. indica leaf extract 5%, twig extract 5%, chlorhexidine gluconate 0.2% and placebo was administered in each well separately through micropipette. The petri plates were then placed in the incubator at 37°C for 20-24 hrs. The bacterial sensitivity was then noticed in three active salts around the margins of the well except the placebo that did not show any sensitivity.

RESULTS:

The size of zone of inhibition (mm) of the microbes was also determined to check the efficacy of the drugs. Chlorhexidine 0.2% showed 14mm zone of inhibition in the average mean of 15 patients, but in A. indica twig and A. indica leaf has comparatively little smaller zone of inhibition of 12mm and 10.5mm respectively in the average mean of 15 patients The antibacterial activity of placebo showed no zone inhibition, so there is no effect of placebo on gingival plaque bacteria (Table. 1, Fig 1).

Table 1: Antibacterial activity showing zone of Inhibition (mm) against S. Mutans

Name of Drugs	No. of Patients	Average size of zone of mutans inhibition (mm) for oral microbes
Placebo & S. mutans	15	0 mm
Chlorhexidine gluconate 0.2% & S. mutans	15	14 mm
A.indica leaf extract 5% & S. mutans	15	10.5 mm
A.indica twig extract 5% & S. mutans	15	12 mm

Average size of Zone of Inhibition mm

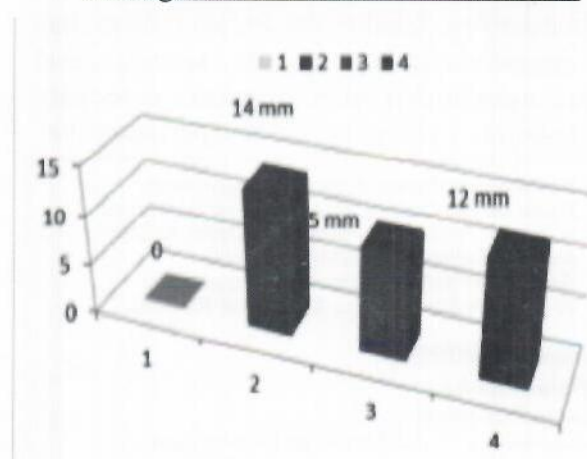


Fig 1: The size of zone of inhibition (mm) of the microbes

DISCUSSION:

The antibacterial activity of the herbal agents Azadirachta indica leaf extract and twig extract was assessed in our study where different materials used were checked by the presence of well defined zone of inhibition in the tryptic soy agar and blood agar medium. The efficacy of the drug was determined by the presence of the size of the zone of inhibition. Chlorhexidine 0.2% showed 14mm zone of inhibition, less inhibition of 10.5mm in A. Indica leaf and 12mm zone of inhibition in A. Indica twig extract while no zone of inhibition has been observed in placebo group, thus showing no bacterial effect in the average mean of 15 patients against S. mutans microbes (Table. 1, Fig 1). The findings of our results are almost in similar with the previous studies^{4,16-19}, who also observed the different of antibacterial effects of A. Indica leaf and A. Indica twig extracts against S. mutans microbes in particular. The similar findings were also noted by other researchers^{4,20}, who also showed significant reduction in gingivitis when treated with A. Indica extract in comparison with commercially available mouth wash Chlorhexidine.

CONCLUSION:

Results of the present study concluded that extracts of Azadirachta indica herbal plants contain active compounds with anti-microbial and medicinal properties to combat pathogenic microorganisms and in the reduction of gingival inflammation. It is found that Neem leaf and twig extract plays as antibacterial medicine in bacterial gingivitis as compare with chlorhexidine gluconate 0.2% and can be use long term because of having less side effects and more effective.

REFERENCES:

1. Chopra RN, Nayar SL, Chopra IC, Asolkar V, Kakkar KK, Chakre OJ, Verma BS. Glossory of Indian medicinal plants. 1956;1(1):113-4.
2. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). J Current sci. 2002;82(11):1336-45.
3. Behal R, Mali AM, Gilda SS, Paradkar AR. Evaluation of local drug-delivery system containing 2% whole turmeric gel used as an adjunct to scaling and root planing in chronic periodontitis: A clinical and microbiological study. J Indian Soc Periodontol. 2011;15(1):35-8.
4. Botelho MA, Santos RC, Martins JG, Carvalho CO, Paz MC, Azenha C. 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. J Med Plants Res. 2008;2(11):341-6.
5. Rosan B and Lamont RJ. Dental plaque formation. Microbes and Infection. 2000;2(13):1599-607.
6. Hall-Stoodley L, Costerton WJ, Stoodley P. Bacterial biofilms: from the Natural environment to infectious diseases. Nature Reviews Microbiol. 2004;2: 95-108.
7. Seki M, Yamashita Y, Shibata Y, Torigoe H, Tsuda H, Maeno M. Effect of mixed mutans streptococci colonization on caries development. Oral Microbiol Immunol. 2006;21(1):47-52.
8. Egelberg J. Local effect of diet on plaque formation and development of gingivitis in dogs. Effect of hard and soft diets. Odontologisk Revy. 1965;16:31-41.
9. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent Jr RL. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
10. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol. 2005;38(1):135-87.
11. Haffajee AD, Socransky SS, Patel MR, Song X. Microbial complexes in supragingival plaque. Oral Microbiol Immunol. 2008;23(3):196-205.
12. Mcdougall HA. Studies on dental plaque. The histology of dental plaque and its attachment. Aust Dent J. 1963;8:261-5.

13. Theilade E. The Experimental Gingivitis Studies: The Microbiological Perspective. *J. Dent. Res.* 1996;75:1434-8.
14. Clarke JR and Blacklock Z. The use of chlorhexidine and "savlon" for decontamination of "medihaler" mouthpieces. *Med J Aust.* 1965;132(20): 827-8.
15. Hull R. Structure of the cauliflower mosaic virus genome. III. Restriction endonuclease mapping of thirty-three isolates. *Virology.* 1980;100:76-90.
16. Siswomihardjo W, Badawi SS, Nishimura M, Hamda T. The diffrence of antibacterial effects of neem leaves and stick extract. *Int chin J dent.* 2007;7:27-9.
17. Botelho MA, Filho JGB, Correa LL, Fonseca SGC, Montenegro D, Gapski R. Effect of a novel essential oil mouthrinse without alcohol on gingivitis: a double-blinded randomized controlled trial. *J Appl Oral Sci.* 2007;15(3):175-80.
18. Aarati N, Ranganath N, Soumya BG, Kishore B, Mithun K. Evaluation Of Antibacterial And Anticandidial Efficacy Of Aqueous And Alcoholic Extract Of Neem (Azadirachta Indica) An In Vitro Study. *Inter J Res Ayurveda Pharmacy.* 2011;2(1):230-5.
19. Lekshmi NCJP, Sowmia N, Viveka S, Brindha JR, Jeeva S. The inhibiting effect of Azadirachta indica against dental pathogens. *Asian J Plant Sci Res.* 2012;2(1):6-10.
20. Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of Azadirachta indica leaf extract gel—a 6-week clinical study. *J Ethnopharmacol.* 2004;90(1):99-103.