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Evaluation of Effect of Neem Oil as a Deliver Vehicle on Antimicrobial Property of Triple Antibiotics Paste: An *In Vitro* Study

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ABSTRACT

Introduction: Probably microbial plaque is the main etiology for periodontal tissue inflammation. Various chemical agents have been evaluated over the years with respect to their antimicrobial effects in the oral cavity. However, all are associated with side effects that prohibit regular long-term use. Hence, in the present study weevaluated the effect of neem oil as a deliver vehicle on antimicrobial property of triple antibiotics paste.

Materials and Methods: Culture was prepared using brain heart infusion broth reagent. Recently extracted intact single rooted human single rooted single canal teeth were selected and sterilized. Three types of microorganisms isolated were used. A mixed suspension of equal volumes of the three organisms was prepared in sterile containers. Neem oil was kept in the agar plate with culture and the diameter of inhibition zones was calculated.

Results: After 48 h, inhibition zones were formed on the agar plates impregnated with neem oil. The inhibition zone signifies the reduction of microorganisms on the agar plate. These inhibition zones on the agar plates looked circular in shape and the zones were measured with the help of a millimeter scale.

Conclusion: This in vitro study concludes that the commonly used natural herbs among the rural people, such as neem, have beneficial antimicrobial activity. Hence, further studies are required in formulating the anti-plaque agents based upon herbs like A. indica.

KEY WORDS: Antimicrobial, anti-plaque agents, herbal, neem.

INTRODUCTION

Periodontal diseases are the most prevalent oral health problems caused by dental plaque. Dental plaque is a complex microbial community with greater than 10^8 bacteria per mg. It is estimated that as many as 400 distinct bacterial species may be found in plaque. Recently, specific microorganisms in plaque have been implicated in chronic periodontal diseases.[1]

A dynamic equilibrium between the periodontal microbiota and host generally results in a clinical state of periodontal health, characterized by minimal inflammatory changes in marginal gingival tissues. Maintenance of health is mostly achieved by controlling the residual mass of microorganisms.[1] The use of Azadirachta indica (Indian neem) is considered as the plants have a wide spectrum of bioactivity. They are used as antibacterial, antifungal, and anticancerous agents. [2] They had shown good broad range of antibacterial activity in vitro (Rae et al. 1986). Hence, in the present study weevaluated the effect of neem

oil as a deliver vehicle on antimicrobial property of triple antibiotics paste.

MATERIALS AND METHODS

SAMPLE PEPARATION

Recently extracted intact single rooted human single rooted single canal teeth were selected and sterilized. Access opening was done, working length determined and All canals sequentially prepared using step-back technique up to size # 35K master apical file under irrigation with saline.

Irrigation protocol included NaOCl and EDTA wash with intermediate saline flush .nail varnish was applied on the root of all the teeth. Teeth placed in a closed container containing 4 ml of brain heart infusion (BHI) broth, sterilized by autoclaving at 121 _C for 20 min and incubated for 24 h at 37°C to confirm sterility by absence of turbidity.

SAMPLE CONTAMINATION

Three types of microorganisms isolated were used. A mixed suspension of equal volumes of the three organisms was prepared in sterile containers. 2 ml of the sterile BHI broth from each tube were replaced by 2 ml of the prepared mixed microbial suspension, and then the test tubes were closed and incubated at 37°C for 24 hours.

After contamination period, each specimen was removed from its test tube under aseptic conditions in the laminar air flow chamber and irrigated with 5 ml of sterile saline and dried with sterile paper points # 35.

The specimens were divided randomly into equal groups according to the intra-canal medications used. Intra canal medicament was introduced in the root canal system using lentulo spirals and hand file .the orifice was sealed with cavit not less than 3 mm in thickness.

TRIPLE ANTIBIOTIC POWDER PREPARATION

Standard preparation:

To prepare 1 g/mL MTAP, 1 g of USP-grade antibiotic powders comprising Ciprofloxacin 14%, Metronidazole 43% and Clindamycin 43% (Skywalk Pharmacy, Wauwatosa, WI, USA) were mixed with 1 mL of sterile water. To prepare a 1 mg/mL solution of TAP or MTAP, 100 mg of each compounded powder mentioned above was dissolved in 100 mL of sterile water

TRIPLE ANTIBIOTIC PASTE PREPARATION :

A fine powder of antibiotic powder was mixed with herbal and homeopathic agents until a workable smooth paste was achieved.

After incubation period, the intra-canal medications were removed. The root canals were irrigated using sterile saline solution and then dried with sterile paper points # 35K left in the root canal for 1 min to absorb the canal fluid and placed in sterile Eppendorf test tube containing 0.5 ml of sterile saline, vortexed for 30 s, and this suspension was represent the specimen taken from the main canal lumen.

Sterile loopes were standardized to carry 1uL of the microbial suspension to be seeded on the three media specific for the growth of the tested microorganisms.

BHI blood agar for counting of E. Faecalis colonies

The plates were incubated at 37 _C for 7 days . Growing colonies were counted and recorded as colony forming units CFU All the collected, tabulated and statistically analyzed. Analysis of variance ANOVA was performed according to the computer program SPSS Version 17for Windows.

RESULTS

After 48 h, inhibition zones were formed on the agar plates impregnated with neem oil. The inhibition zone signifies the reduction of microorganisms on the agar plate. These inhibition zones on the agar plates looked circular in shape and the zones were measured with the help of a millimeter scale [Table 1].

TABLE 1: SIZE OF THE INHIBITION ZONES

S.N.	Study Sample	Number of Colonies (Mean ± S.D.)
1.	Group I F (Triple antibiotic Paste and saline)	36.3±2.66
2.	Group I J (Triple antibiotic and Neem Oil)	63.0±3.36

DISCUSSION

Periodontal disease is a general term which encompasses several pathological conditions affecting the tooth supporting structure. The clinical signs of periodontitis are changes in the morphology of gingival tissues, bleeding upon probing, as well as periodontal pocket formation. This periodontal pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria.[1]Dental plaque is a highly specific variable structural entity formed by sequential colonization of microorganisms on the tooth surface. It is a structured, resilient, yellow-grayish substance that adheres tenaciously to intraoral hard substance. One gram of plaque contains nearly 100,000,000,000 bacteria. Anti-plaque agents prevent the accumulation of plaque on the tooth surface. The efforts of dental researchers have resulted in the availability of wide range of anti-plaque agents, thus preventing the accumulation of plaque. The available antimicrobial agents produce resistant strains. Chlorhexidine mouthwash may stain the teeth. The use of herbs as antimicrobial produces no side effects, and they are easily available to the rural population at a low cost.[4]Neem tree (A. indica) was described as early as in 1830 by De Jussieu.[5] It belongs to Meliaceae family. Neem is an evergreen tree cultivated in Indian subcontinent and is popularly known as “Indian neem” or “Indian lilac.” Every part of the tree has been used in traditional medicine for household remedy.[6,7] Bioactive compounds in neem include: nimbidin, nimbolide, gedunin, and mahmoodin. Nimbidin has anti-inflammatory, anti- arthritic, antipyretic, hypoglycemic, and anti-bacterial property.[8,9] It is active against Klebsiella, Staphylococcus, and Serratia species. It is also active against Streptococcus mutans and Streptococcus faecalis.[10]Reduction of microorganisms is confirmed by the formation of inhibition zones on Mueller-Hinton agar plates. The microorganisms to be tested are obtained from the plaque samples cultured in the brain heart infusion broth. Colonies of microorganisms are formed in the media when incubated for 48 h. When the culture is inoculated onto the Mueller-Hinton agar and neem oil is diffused, it shows formation of inhibition zones. The formation of inhibition zones takes place after 48 h. Henceforth, neem oil can be used to control periodontal diseases and limit the progression of periodontitis.

CONCLUSION

This in vitro study concludes that the commonly used natural herbs among the rural people, such as neem, have beneficial antimicrobial activity. Hence, further studies are required in

formulating the anti-plaque agents based upon herbs like A. indica.

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