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An In-Vitro Evaluation of the Efficacy of GUMTONE[™]As an Antimicrobial Agent on Periodontal Pathogens: A Microbiological Study.

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ABSTRACT:

GumtoneTM(Acacia arabica), a polyherbal gel, has found to be effective as an antiplaque and antibacterial agent for gingivitis however its effect against periodontal pathogens is not known which causes periodontal diseases. The study was conducted to determine the antimicrobial activity of GumtoneTM(Acacia arabica) against the periodontal pathogens.

The inhibitory effect and the time-kill curve of Acacia Arabica gel (GumtoneTM)on Aggregatib - acteractinomycetemcomitans (Aa), Porphyromonasgingivalis(Pg) and Prevotellainter media (Pi) was evaluated. The inhibitory and growth time kill curve were determined using serial dilution methods at 0, 2, 4, 6 and 24hrs. The GumtoneTM showed bacteriostatic and bactericidal activity against the Aaand Pg with minimum inhibitory concentration (MIC) at 0.8 μ l/ml, 0.4 μ l/ml respectively, better bactericidal against Pg. The study shows that GumtoneTM has antimicrobial activity against periodontal pathogens like P. gingivalis, A. actinomycetemcomitans, and this raises the possibility that GumtoneTM may have therapeutic use for periodontitis in clinical practice.

Keywords: Aggregatibacteractinomycetemcomitans, antimicrobial, gum tone, Acacia arabica, Porphyromonasgingivalis.

INTRODUCTION

Bacterial plaque is the primary etiological agent and accumulation is associated with gingivitis and periodontitis indicating the rationale for reducing plaque to lower the likelihood of periodontal destruction. Scaling and root planing, is the firstrecommended step in the management of gingivitis and periodontitis but there are factors, such as inaccessibility of plaque retentive areas, whichcan limit the clinical and microbiological response. [1]

Besides, control of supragingival plaque is vital in order to counteract recolonization of the subgingival area by periodontal pathogens.^[2]

Porphyromonasgingivalis, Prevotellaintermedia, Aggregatibacteractinomycetemcomitans, Capnocytophaga spp. And Treponemadenticola are commonly associated with destructive periodontitis. According to the

¹Priyanka Shivanand, International journal of ayurvedic & herbal medicine 6(3) May.-June..2016(2229-2232)

World Health Organization (WHO), 80% of the world's people rely on traditional herbal medicine for their prime healthcare needs.^[3] Herbal drugs provide alternative formulations for safe and extendeduse. ^[1]

GumtoneTM gel (CharakPharmaPvt. Ltd, India) is polyherbal formulation with Acacia arabica as its principal constituent. Acacia arabica gum is an oral hygiene material used by many communities in the Middle East and North Africa, containing arabica with a complex mixture of the calcium, magnesium and potassium salts of arabic acid. Other elements such as tannins, cyanogenic glycosides, oxidases, peroxidases and pectinases present to individually exhibit antimicrobial properties. ^[11]Clark et al, reported the antibacterial and antiprotease activities of Acacia arabica. Most of these bacteria induce proteolytic enzymes are capable of deteriorating host tissue components and thus constitute potential virulence factors. ^[41]

Hence the purpose of this in vitro study was to investigate the action of GUMTONETM, a routinely prescribed astringent post mechanical therapy if effective against the growth of these periopathogenic organisms.

MATERIAL AND METHODS

GumtoneTM gel (CharakPharmaPvt. Ltd, India) 40mg. The main ingredient of the polyherbal gel was Acacia arabica, and the others being Barleriaprionitis, Emblicaofficinalis, Terminaliachebula, Terminaliabelerica, Vitexnegundo, Quercusinfectoria, Meliaazadirachta, Acacia catechu, Messuaferrea and Embeliaribes.

Each 40 gm of the gel contained 0.8 w/ w%Acaciaarabica, 0.4 w/ w%Barleriaprionitis, 0.24 w/ w% Emblicaofficinalis,0.24 w/ w%Terminaliachebula, 0.24 w/ w%Terminaliabelerica, 0.2 w/ w%Vitexnegundo, 0.08 w/ w%Quercusinfectoria, 0.04 w/ w% Meliaazadirachta, 0.24 w/ w%Acacia catechu, 0.02 w/ w%Messuaferrea and 0.02 w/ w%Embeliaribes. Similarly, each 40 gm of powder contained 8 gm Acacia arabica,4 gm Barleria prionitis, 2.4 gm Emblicaofficinalis, 2.4 gm Terminaliachebula, 2.4 gm Terminaliabelerica, 2gmVitex negundo, 0.8 gmQuercusinfectoria, 0.4 gm Meliaazadirachta, 2.4 gm Acacia catechu, 0.2 gm Messuaferrea and 0.2 gm Embeliaribes.

Stock culture of periodontal pathogens (*P. gingivalis*, *P. intermedia and A. actinomycetemcomitans*) used in this study were obtained from the Department of Microbiology, Maratha Mandal Dental College, Belgaum.

Minimum inhibitory concentration:

The minimum inhibitory concentration (MIC) of gumtone was determined by macrobroth dilution method. ^[5] 9 dilutions of each drug have to be done with BHI for MIC.In the initial tube 20microliter of drug was added into the 380microliter of BHI broth. For dilutions 200microliter of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10⁻¹ dilution. From 10⁻¹ diluted tube 200microliter was transferred to second tube to make 10⁻² dilution. The serial dilution was repeated up to 10⁻⁹ dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity

The lowest concentration of gumtoneTM that completely inhibited the growth of the organisms was considered as MIC.

Minimum bactericidal concentration procedure:

From the MIC dilutions tubes, first 3 or 5 tubes were plated and incubated for 24 hrs then next day the colony count was taken. MBC is done to see whether there was bacteriostatic or bactericidal effect of the GumtoneTM against the organism. If there is no growth then - it's bactericidal effect If there is growth then - it's bacteriostatic effect.

¹Priyanka Shivanand, International journal of ayurvedic & herbal medicine 6(3) May.-June..2016(2229-2232)

MBC was carried out to observe the bactericidal effect of the GumtoneTM against the organism. If there is no growth of microorganisms, then the GumtoneTM is known to have bactericidal effect and if there is growth of microorganisms, then the GumtoneTM is known to have no bacteriostatic effect.

Growth kill curve:

Dilution were done same as MIC and then immediately it was plated for first 3 or 5 dilution this was noted as 0 hrs. Whole of the dilution tubes were kept in CO_2 jar for 2 hrs, at the end of 2hrs again the first 3 or 5 dilution were plated. Same procedure was repeated after every two hrs i.e. after 4 hrs, 6 hrs and 24 hrs. Then plates were incubated in according to the requirement placed in CO_2 jar and anaerobic jar. After 48 hrs of incubation the plates were removed and the colony count was noted.

Proteolytic activity gumtoneTM- Gelatinliquificationtest:

Composition of media- thioglycollate with 5% gelatin in distilled water. This broth was inoculated with the test culture along with positive and negetive controls. This was incubated for 5 days at 37°C all the tubes were in liquid form then it was kept at 4°C for 2 to 3 hrs and checked for liquification. Interpretation:-+ve control-whole the tube was liquidfied even after stored at 4°C. -ve control- whole the tube was solidified at 4°C.

RESULTS:

MIC showed P.gingivalis and A. actinomycetescomitans only sensitive towards GumtoneTM at as low as $0.2\mu g/ml$ and $0.4\mu g/ml$ respectively with Pi being resistant through out.

<u>Proteolytic activity gumtoneTM</u>-Gelatinliquification test. Test- there was liquification around the innoculum after storing that 4° C. (Table 1, Table 2, Table 3)

DISCUSSION

Periodontal disease is an infectious process ranging in severity from mild gingivitis to advanced loss of connective tissue attachment and supporting bone. The successful treatment of periodontitis requires suppression or elimination of the subgingival periodontopathogens. [6]

Acacia gum is known to have antimicrobial, and antiproteolytic activities. ^[4]The results of this study demonstrate that acacia gum possesses inhibitory potential against the growth of suspected periodontal pathogens.

Gumtone® showed bacteriocidal activity on P. gingivalis and A. actinomycetemcomitans. MIC determined 0.2μ l/ml concentration for P. gingivalis, whereas determined 0.4μ l/ml for A. actinomycetemcomitans. But P. intermedia showed no sensitivity throughout.Gumtone® might assist in the prevention of periodontal diseases by restricting their growth.Acacia gum may have the advantage over such agents in that it is a natural product which has been used for many centuries.

No studies, using Medline database are reported, using Gumtone® in an in vitro study against periodontal pathogens, although comparative study of Gumtone® as a gel and powder used in the treatment of gingivitis was done to evaluate clinical and microbiologic effects. ^[1]

CONCLUSION

The study shows that Gum tone [®]has antimicrobial activity against periodontal pathogens like *P. gingivalis*, *A.actinomycetemcomitans*, and this raises the possibility that Gum tone [®] may have therapeutic use for periodontitis in clinical practice.

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