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Evaluation of the clinical efficacy of Achyranthes aspera gel and Ornidazole gel along with scaling and root planing in patients with chronic periodontitis - A Clinico-Microbiological study

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Abstract

Aim: The purpose of the present study to evaluate the efficacy of local drug delivery of Achyranthes aspera gel and ornidazole gel along with scaling and root planing in patients with chronic periodontitis.

Method: Thirty patients with chronic periodontitis were considered in the study and assigned randomly into site I (SRP with Achyranthes aspera gel) and site II (SRP with Oridazole gel) according to the split-mouth design. clinical parameters (gingival index, probing pocket depth, and clinical attachment level) were recorded. Subgingival plaque samples were collected and red complex micro-organism (P. gingival is) were studied using PCR. Clinical and microbiological parameters were recorded at baseline,3 months after treatment.

Result: The results showed a statistically significant difference in GI, PPD and CAL and significant reduction of P. gingival is baseline to 3 months in both sites (P<0.001). Intergroup comparison between site I and site II was statistically insignificant for PPD, CAL. A significantly greater reduction in P. gingival is at 1st month was observed when compared to site II.

Conclusion: Both gels showed improvement in clinical parameters and reduction in the microbial count. Achyranthes aspera gel can be used as an effective local drug delivery along with scaling and root planing in the treatment of chronic periodontitis.

Keywords: Chronic Periodontitis, Achyranthes aspera gel, Ornidazole gel, Polymerase chain reaction

Introduction

Periodontal diseases are chronic inflammatory in nature, characterized by loss of connective tissue attachment of the tooth and pathological migration of the junctional epithelium leading to pocket formation, tooth mobility and tooth loss. Dental plaque is considered the primary etiologic agent and exists in a state of biofilm.[1,2]

Scaling and root planing are critical components of the first phase of periodontal therapy, resulting in significant

improvements in clinical parameters. However, these procedures have some limitations, these include difficult-to-reach areas such as root concavities and narrow furcations, which may act as bacteria reservoirs. Thus, in addition to mechanical instrumentation, adjunctive use of chemotherapeutic agents in the form of local drug delivery effect on bacteria has been advocated.[3]

The concept of local drug delivery was first proposed by Max Goodson et al, in the treatment of chronic periodontitis. The goal is to maintain adequate concentrations of therapeutic agents at the site of action for an extended period. Drug delivery systems can be grouped into synthetic and natural products, and they come in the form of gels, fibers, stripes.[4]

Gel formulations of local drug delivery systems have some advantages, higher biocompatibility, bio adhesive nature, and adhesion to the mucosa in the pocket.[5]

One of the agents that inhibit DNA synthesis is the nitroimidazole compound. It operates on the premise that the inactive form passively diffuses into the cell after it has been activated by chemical reduction. Ornidazole is effective against gram-negative anaerobic, facultative bacteria, which are responsible for periodontal diseases. To suppress the growth of periodontal infections, ornidazole requires a very low minimum inhibitory concentration.[6,7]

Nowadays, herbal plants are gaining their place back by substituting modern drugs all over the world. This changeover is due to the irreversible damage caused by current drugs. Achyranthes aspera, also known as Uttaraene (Telugu), Latjeera (Hindi), and rough chaff tree (English).

Achyranthes aspera has a number of pharmacological characteristics, including antimicrobial, analgesic, antipyretic, anti-inflammatory, immunostimulant, antioxidant, hypoglycemic, antihypertensive properties.[8,9]

So, far there are no studies in the literature evaluating the clinical and microbiological benefits of adjunctive use of Achyranthes aspera root extract gel and Ornidazole gel in addition to scaling and root planing in chronic periodontitis patients. So, the present study focuses on evaluating the clinical and microbiological efficacy of these two gels in conjunction with scaling and root planing in patients with chronic periodontitis.

Material and methods

A double-blinded, simple, randomized, split-mouth clinical study was designed. A total of 60 sites from 30 patients were selected amongst the patients who visited the Department of Periodontology, Lenora Institute of Dental Sciences for the study and were randomly divided by using the lottery method. Site I was treated with scaling and root planing along with Achyranthes aspera gel. Site II. The nature of the study was explained to all the patients, was treated with scaling and root planing along with Ornidazole gel (Ornigreat®-Mankind Pharma Limited), and a written informed consent form was obtained.

Subject selection

Inclusion criteria

- Patients with age between 30-55 years.
- Patients with chronic periodontitis having probing pocket depth≥5mm.
- Patients who can maintain good oral hygiene after the initial treatment were included in this study.
- Patients with more than 20 teeth

Exclusion criteria

- Pregnant and lactating women.
- Patients with a history of smoking and tobacco in any form.

- Patients with any systemic disorders.
- Patients who had a history of antibiotics in the last six months.
- Patients who underwent periodontal therapy in the last six months.

Clinical parameters used for assessment

- Gingival Index(GI)
- Probing Pocket Depth(PPD)
- Clinical Attachment Level(CAL)

Microbial analysis

Multiplex PCR method for quantification of P. gingival is counted as ($\times 10^4$).

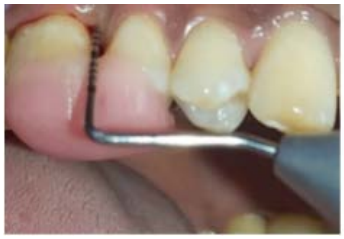
Materials

Fig1& 2: Achyranthes aspera gel, Ornigreat® gel

Methods

Participants were included in the study after thorough case history and clinical examination using a sterile mouth mirror and UNC-15 periodontal probe. At baseline, after recording the clinical parameters in selected patients, subgingival plaque samples were collected from pre-selected sites belonging to both sites. In each patient, on the completion of SRP, selected sites with probing depths ≥5mm were randomly divided into site I and site II in different quadrants (Fig- 3&4). Site I Was treated by Achyranthes aspera gel with the help of a syringe with a blunt cannula into the pocket. Site II Was treated by Ornidazole gel with the help of a syringe with a blunt cannula into the pocket (Fig- 5&6). After placement of the local drug both the sites were covered with a periodontal dressing (COE PAK®). Patients were advised not to chew hard or sticky foods, to gently clean treated areas and not to use any interdental aids for 7 days. At each visit (Baseline, 90(Fig-7&8) days clinical parameters were assessed and microbiological samples were obtained from both sites of the patients.

Fig-3&4: Pocket depth at baseline for (site, site II)



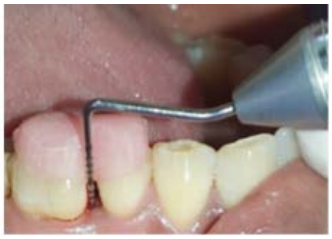
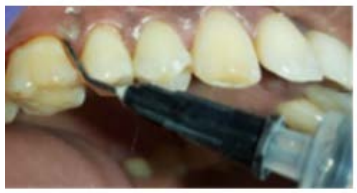


Fig 5&6: Achyranthes aspera gel delivered (site I), Ornigreat® gel delivered (site II)



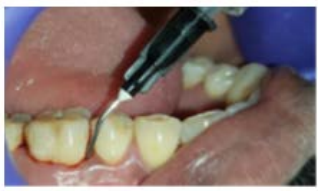
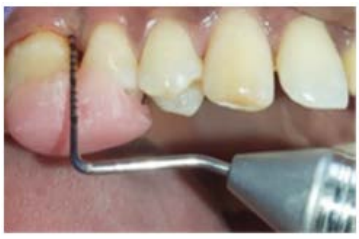
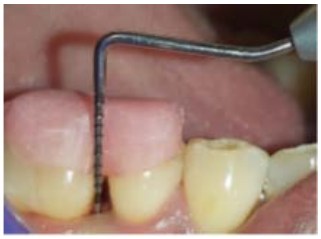


Fig 7&8: Pocket depth at 3 months for (site I, site II)





Microbiological analysis

For the microbial analysis, subgingival plaque samples were obtained by inserting sterile paper points into the sulcus or pocket for thirty seconds and these paper points were removed and dispensed in separate vials containing transport media, the vial was closed and labeled. Eppendorf vials were sent to the microbiological laboratory, where they were subjected to the Multiplex PCR method for quantification of P. gingival is, Counted

as ($\times 10^4$). DNA Extraction Procedure was done by the Modified Proteinase-K method.

Statistical analysis

Statistical analysis of the data was performed by using Statistical Package for the Social Sciences (SPSS) software version 23 (IBM, Chicago, USA). All the values were subjected for statistical analysis by using the ANOVA Test. One-way Analysis of Variance is a way to test the equality of three or more means at one time by using variances. An independent sample t-test was used to test the significant difference between the two means.

Results

Table1: Comparative analysis of clinical parameters at different time intervals

Clinical parameters	Sites	Observation Period	Mean ±SD	p value
Gingival index	Site I	Baseline	2.10±0.28	0.00001
		3months	1.42± 0.29	
	Site II	Baseline	2.04±0.18	0.01
		3months	1.20± 0.20	
Probing depth	Site I	Baseline	6.38±0.94	0.00001
		3months	4.15 ±0.57	
	Site II	Baseline	6.34 ±0.95	0.00001
		3 months	3.97± 0.63	
Clinical attachment	Site I	Baseline	6.52±0.95	0.00001
		3 months	4.28± 0.69	
	Site II	Baseline	6.5± 0.95	0.00001
		3 months	4.07±0.70	

Table 2: Intergroup analysis of clinical parameters at different time intervals

Clinical parameters	Sites	Observation Period	p value
Gingival index	Site I,SiteII	Baseline	0.18
		3months	0.004
Probing depth	Site I ,SiteII	Baseline	0.047
		3months	1.22
Clinical attachment	Site I,SiteII	Baseline	0.5
		3 months	0.17

Table 3: Intragroup comparison of Mean and Standard deviation values of P. gingival is count ($\times 10^4$) in site I and site II at different time intervals

Microbial organism	Sites	Observation Period	Mean±SD	pvalue
Porphyromonas gingivalis	Site I	Baseline	5.59 ± 0.35	
	Site II	3 months	3.84 ± 0.34	0.0001
	Site I	Baseline	5.63 ± 0.44	0.0001
	Site II	3 months	3.48 ± 0.39	

Table 4: Intergroup comparison of Mean and Standard deviation values of P. gingival is count ($\times 10^4$) in site I and site II at different time intervals

Microbial organism	Sites	Observation Period	pvalue
Porphyromonas gingivalis	Site I , Site II	Baseline	
		3 months	0.37
	ite I, Site II	Baseline	0.0014
		3 months	

Gingival Index

Intragroup comparison (Table 1)

The mean and standard deviation of Gingival Index (GI) at baseline was 2.10 ± 0.28, which was reduced to 1.42 ± 0.29 at the end of 3 rd month respectively for site I. It was found to be 2.04 ± 0.18, 1.20 ± 0.20 respectively for site II.

Intergroup comparison (Table 2)

On comparison of a mean gingival index between the two sites at baseline, there was no statistically significant difference observed (p<0.18). At the end of 3rd month, which was statistically significant (p<0.00004).

Probing Pocket Depth

Intra group comparison (Table 1)

The mean and standard deviation of Probing Pocket Depth at baseline was 6.38 ± 0.94mm, which was reduced to 4.15 ± 0.57 mm at the end of 3rd month respectively for site I. It was found to be 6.34±0.95mm, 3.97±0.63mm respectively for site II.

Intergroup comparison (Table 2)

On comparison of mean Probing Pocket Depth between the two sites from baseline (p=0.47) to the end of 3rd month (p=1.22), which was no statistical difference.

Clinical Attachment Level

Intra group comparison (Table 1)

The mean and standard deviation of Probing Pocket Depth at baseline was 6.52±0.95mm, which was reduced to 4.28±0.69 mm at the end of 3rd month respectively for site I. It was found to be 6.5 ± 0.95mm, 4.07 ± 0.70 mm, respectively for site II (Table 1).

Intergroup comparison (Table 2)

On comparison of mean Clinical attachment level between the two sites from baseline (p=0.5) to the end of 3rd month (p=0.17), which was not statistically significant (Table 2).

P. gingivitis count (×10^4)

Intragroup comparison (Table 3)

The mean and standard deviation of P. gingival is count at baseline was 5.59 ± 0.35, which was reduced to 3.84 ± 0.34 at the end of 3rd month, which was statistically significant ai site I. at baseline was 5.63 ± 0.44, which was reduced to 3.48±0.39 at the end of 3rd month, which was statistically significant respectively for site II.

Intergroup comparison (Table 4)

On comparison of mean p. gingival is count between the two sites, at baseline (p=0.37) and at the end of 3rd month (p<0.00014) there were statistically significant.

Discussion

Periodontitis is a multifactorial polymicrobial infection characterized by a destructive inflammatory process resulting in the loss of tooth-supporting structures. The combination of microorganisms and inflammatory response were the main cause of periodontitis. Local administration of the antimicrobial agent to periodontal pockets provided additional benefits in pocket depth reduction and clinical attachment level gain, as compared to SRP alone. PCR was used to evaluate the prevalence of microorganism, P. gingival is at baseline,3 months follow-up.

The gingival index was considered as a true reflection of gingival status in health and disease. GI helps to assess the severity of gingivitis by examining the qualitative changes of gingival tissues. The intragroup comparison showed a statistically significant reduction in mean gingival index score at site I from baseline to 3 months. At baseline, the mean of GI was 2.10 ± 0.28 which was reduced to 1.42 ± 0.29 at the end of the 3 months follow-up at site I. These results were in accordance with a study done by Boyapati R et al.[10] In respect to site II there was a statistically significant mean reduction of GI from baseline to 3 months observed. At baseline, the mean of GI was 2.04 ± 0.18 which was reduced to 1.20 ± 0.20 at the end of the 3 months at site II. These results were in accordance with the previous study.[11]

Comparison of mean GI between the two sites, statistically significant difference was observed between site I and site II at 1 and 3 months follow up. Whereas the baseline did not show any significance. The reduction in GI in both sites may be attributed to the elimination of local etiologic factors like plaque and calculus, clinical resolution of gingival inflammation and antimicrobial effects of Achyranthes aspera gel and ornidazole gel.

Periodontal pocket is considered as the pathognomonic sign of periodontal disease, and reduction of probing pocket depth is one of the determinants for successful periodontal therapy. At baseline, the mean PPD was 6.38 ± 0.94 mm which was reduced to 4.15 ± 0.57mm at the end of the 3 months at site I. These results were in accordance with the previous study.[12] At baseline the mean value of PPD was 6.34 ± 0.95 mm, which was reduced to 3.97±0.63 mm at the end of 3 months at site II. These results were in accordance with the previous study.[11] On inter-group comparison, there was no statistically significant difference observed from baseline

to 3 months. These results were in accordance with the previous studies.[7,13] This significant reduction at both the sites may be attributed to the property of antimicrobial activity of Achyranthes aspera and Ornidazole gel and also the effectiveness of SRP.

CAL, gain in at site I from baseline to 3 months was significant. At baseline, the mean CAL was 6.52 ± 0.95 mm, which was reduced to 4.28 ± 0.69 mm at the end of the 3 months. These results were in accordance with previous studies.[14,15] This gain in CAL may be attributed to the antimicrobial property of Achyranthes aspera gel, terminating the radical chain reduction by inhibiting free radical quenching and high levels of phenolic compounds in the ethyl acetate extracts. A significant gain in CAL at site II from baseline to 3 months was observed. At baseline, the mean CAL was 6.5 ± 0.95 mm which was reduced to 4.07 ± 0.70 mm at the end of the 3 months. These results were in accordance with the study done by Penmetsa G S et al.[16] The gain in CAL is due to the antimicrobial effects of ornidazole gel against gram-negative species.

On intergroup comparison, no statistically significant difference was observed at baseline to 3 months follow up. These results were in accordance with the previous study.[17] This gain in CAL is due to a large number of microorganisms being removed from the subgingival area at the time of instrumentation. The anti-inflammatory activity of Acharanthes aspera, and antimicrobial effects of ornidazole gel may have contributed to it. The added advantages of Acharanthes aspera were wound healing properties and antioxidant properties.

The use of PCR (polymerase chain reaction) for microbiological examination has introduced a new dimension to this study. According to Nayak A et al.[18] Multiplex polymerase chain reaction (PCR) was a sensitive and specific identification method, with the additional possibility to perform quantification of specific bacteria in the subgingival plaque samples.

P. gingival is was a highly virulent organism, implicated as a major pathogen in destructive periodontal disease with the ability to adhere and invade oral epithelium and also implicated to be involved in the development of systemic diseases due to systemic inflammation. With increased circulating cytokines and mediators due to systemic inflammation, direct infection and cross-reactivity molecular mimicry occurred between bacterial antigens and self-antigens.

Multiplex PCR has the ability to amplify a single copy of DNA template millions of times, and it can detect several periodontal pathogens in a single patient sample by mixing many primers in a single reaction mixture. This high sensitivity could be especially valuable for detecting periodontal pathogens in subgingival plaque samples, as identification in low numbers was more significant than those detected by conventional approaches.

To the best of our knowledge, this was the first study to compare the antimicrobial efficacy of Achyranthes aspera and ornidazole gel by studying the count of P. gingival is. On intragroup comparison, there was a statistically significant reduction in the mean microbial count of P .gingival is at site I from baseline to 3months. These results were in accordance with the previous study.[19] Significant reduction could be due to ethanolic extracts of the roots of Achyranthes aspera isolating a new aliphatic acid which was identified as n-hexacos-14-enoic acid. This compound has shown antimicrobial properties which highly inhibit the growth of the isolated microorganism. The mean microbial count reduction at site II from baseline to 3 months follow-up was

statistically significant. These results were in accordance with the previous study.[12] This significant reduction in microbial count could be attributed to the bactericidal activity and mutagenic effect of ornidazole as it specifically acts on gram-negative anaerobic, facultative bacteria which are responsible for periodontal disease.

In intergroup comparison, statistically, significant results were observed at 3 months follow up. These results were in accordance with the previous study.[20] Microbial count reduction was slightly higher at site II, which could be due to the fact that the Ornidazole gel has the added benefit of requiring a very low minimum inhibitory concentration to prevent the growth of periodontal pathogens.

Conclusion

From the results of this study it can be concluded that both Achyranthes aspera gel gel and Ornidazole gel displayed similar periodontally beneficial results at the end of 3 months. But Ornidazole gel edged over Achyranthes aspera gel in terms of GI, PPD and CAL scores. Thus, Achyranthes aspera gel can be used as an excellent alternative to Orndazole gel whenever required.

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