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Original Article

Dentistry Section

Comparison of Plaque Inhibiting Efficacies of Aloe Vera and Propolis Tooth Gels: A Randomized PCR Study

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ABSTRACT

Backgound and Aim: Allopathic medications used for periodontal disease are known to be associated with various side effects. Hence a search for naturotherapies are on the rise. Among the natural pharmacons available aloevera and propolis are considered to be effective and free from adverse effects. Taking this into account, the present study was done to compare the plaque inhibiting efficacies of Aloe vera and Propolis tooth gels in patients with chronic periodontitis.

Materials and Methods: Forty patients diagnosed with chronic periodontitis were randomly allocated to groups A and B containing 20 patients each. Patients in group A were advised to use Aloe vera tooth gel while those in group B were advised to use Propolis tooth gel. Clinical and microbiologic parameters using Polymerase chain reaction (PCR) were recorded at baseline and after 3 months.

Results: Student t-test was performed for all the obtained results. In the Aloe vera group, comparison of baseline PCR and after 3 month results showed reduction only in *P. gingivalis* (p=0.001), where as statistically significant reduction in all the three red complex microorganisms was seen in propolis group. All the clinical parameters (Plaque Index, Gingival Index, Bleeding on Probing, Probing pocket Depth, and Clinical Attachment Level) in both the groups showed statistically significant reductions after 3 months.

Conclusion: Propolis showed a statistically significant reduction in plaque, microbiologic and clinical parameters. However, clinical trials of longer durations with larger sample sizes are required to evaluate the efficacy.

Keywords: Bioflavonoids, Red complex, Vitamin B2

INTRODUCTION

Periodontitis is an infectious inflammatory disease. The microbes present here modulate the inflammatory response and alter the diversity of periodontal disease. In recent years, various host-response modulation therapies and local drug therapies have been developed to block the pathways responsible for periodontal tissue breakdown; however these modalities are known to be associated with various side effects hence its considered that the natural pharmacons such as aloe vera and propolis can be a better alternative.

Aloe vera plant is a member of Liliaceae family. The most beneficial species of the genus Aloe vera is Aloe vera barbadensis. The name Aloe vera or "True Aloe" probably stems from the Arabic word "Alloeh" meaning "shining bitter substance" [1]. It has stiff green, lance-shaped leaves consisting of two different parts, each of which produces substances with completely different compositions and therapeutic properties [2]. The parenchymal tissue makes up the inner portion of Aloe leaves and produces a clear, thin, tasteless, jelly-like material called Aloe vera gel (or mucilage). The gel contains approximately 98.5% water, with the remaining 0.5–1% solid material consisting of a wide range of compounds including vitamins A, C and E [3]. Vitamin B1 (thiamine), niacin, vitamin B2 (riboflavin), choline, folic acid, traces of vitamin B12 and enzymes such as acid phosphatase, alkaline phosphatase, amylase, lactic dehydrogenase and lipase [4].

Likewise, Propolis is a naturally occurring bee product. It is a hard, resinous substance consisting chiefly of wax and plant extracts. In the bee colony, it offers protection against invasion and infection, thus providing the bees with an 'immune system' besides being used to seal the hive. The chemical composition of propolis varies depending on the diversity of plants and the geographic locations from which bees collect it [5]. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other constituents which are amino acids, minerals, vitamins A, B complex, E and a highly active bio-chemical substance known as bioflavonoid (Vitamin P), phenols, aromatic compounds and various other organic substances including debris [6]. Flavonoids are biologically active ingredients which account for most of the well-known plant compounds and have been reported to have antibacterial, antifungal, antiviral, anti-oxidant and anti-inflammatory properties [7]. Studies done independently on aloe vera and propolis have shown promising improvement in periodontal disease; however studies comparing their efficacy on clinical situations have not been reported. Considering this the present study was undertaken to compare the plaque inhibiting efficacies of Aloe vera and Propolis tooth gels in patients with chronic periodontitis.

MATERIALS AND METHODS

Forty out-patients clinically diagnosed with chronic periodontitis reported to our institution were enrolled for study. All the subjects were enrolled by simple random sampling, on voluntary basis and prior to initiation of the study clearance from the institutional ethical committee was obtained. An informed consent was obtained for the samples to be part of the study. The study subjects included patients with chronic periodontitis belonging to the age group of 35-55 years. Patients with history of known systemic diseases, allergic to drugs or chemicals present in the products used for study and patients under antimicrobial therapy, pregnant/lactating women and smokers were excluded from the study.

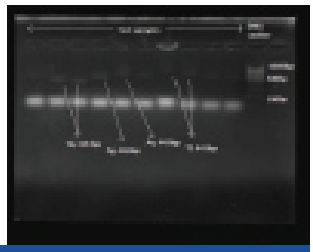
The selected individuals were divided into two experimental groups (20 each) A and B by coin toss method so that they were similar in age, oral hygiene, and periodontal status. Clinical parameters such as plaque score, gingival score, bleeding score, probing pocket depth and clinical attachment level were carried out. Subgingival plaque samples were collected using Gracey curette employing Loe & Sillness, Sillness & Loe indices method respectively. These microbial samples were stored in Tris- EDTA medium (1M tris buffer 0.5ml, 0.5M EDTA 100 µl, Distilled water 50 ml) and sent for DNA extraction and PCR detection of red complex microorganisms

[Table/Fig-1]: DNA Extraction

[Table/Fig-2]: PCR reagent kit

[Table/Fig-3]: Primers

[Table/Fig-4]: Palmtop on thermocycler



[Table/Fig-5]: UV transilluminator with Gel documentation
[Table/Fig-6]: Visualization in UV transilluminator

(*Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td)).

DNA extraction and PCR detection was done in the following manner: First nucleic acids were extracted within 24 to 48 hours from specimens collected [Table/Fig-1,2]. The samples were vortex-mixed and centrifuged to collect the cells. The pellet was suspended in 300 µl of lysis buffer (50 mM Tris, 10 mM EDTA and 10% SDS): plus lysozyme (5 mg/ml) and incubated at 37°C for 1 hour. Then proteinase-K was added and after 1-hour incubation at 65°C the DNA was extracted with phenol and chloroform-isoamyl alcohol treatment. Nucleic acids were precipitated in alcohol, washed with 70% (vol/vol) alcohol and resuspended in sterile water. The DNA extracted from each sample was assayed by multiplex PCR, for the detection of *Porphyromonas gingivalis, Tannerella forsythensis and Treponema denticola.* The multiplex PCR was performed by using specific primers for the 16S RNA gene of each bacterium [Table/Fig-3]. PCR amplification reactions were carried out in a reaction mixture in a final volume of 100 µl consisting of 10 µl of DNA sample, and 90 µl of reaction mixture containing 30 pmol of each primer, 200 µM of a mixture of deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, 1 x PCR buffer (10 mM Tris-HCl, pH 8.0), 50 mM KCl, 2.5 U Hot Start Taq™ DNA Polymerase (Quiagen SpA, Milan, Italy). The PCR protocol was as follows: 98°C for 15 min followed by 40 cycles of 95°C for 30 s, 60°C for 1 min, 72°C for 1 min and a final step of 72°C for 10 min. PCR amplification was performed in an iCycler System (Bio-Rad Laboratories Srl, Segrate, Milan, Italy) [Table/Fig-4]. Amplicons were detected by electrophoresis of 20 µl of samples from each PCR tube in a 2 % agarose gel in TAE (Tris-Acetate- EDTA buffer) for 2 h at 80 V [Table/Fig-5]. The amplification products were visualized and photographed under a UV light transilluminator (Gel Doc 2000-Bio-Rad) after 30 min of ethidium bromide (1 µg/ml) staining [Table/Fig-6]. The molecular sizes of the amplicons were determined by comparison to a commercial DNA molecular weight marker (number VIII, Roche Diagnostics SpA, Milan, Italy) [Table/Fig-7].

After baseline clinical and PCR evaluation of microbes, oral prophylaxis (scaling and root planning) was performed for all the samples. Then Group A subjects were asked to brush their teeth with a commercially available Aloe vera tooth gel while Group B subjects were asked to brush their teeth with a commercially available Propolis tooth gel. Patients were asked to take a peanut sized tooth paste and brush as per modified bass method of brushing (demonstration given at the time of enrollment). Three months after the usage of Aloe vera and Propolis tooth gels, patients were recalled for clinical and PCR follow up evaluation. All the patients were available for recall and the data obtained were subjected to paired and unpaired t-test for assessment and inter comparison of groups.

RESULTS

Twenty patients each in two groups attended the study, clinical and microbiologic parameters were recorded at baseline and after follow-up visits at the end of 3 months. In the Aloe vera group, after comparing the baseline and 3 month PCR results there was a statistically significant reduction seen with *P. gingivalis* (p=0.001). Reductions in the numbers of *Tanneralla forsythia* (Tf) and *Treponema denticola* (Td) were not statistically significant [Table/Fig-8].

In the Propolis group, after comparing the baseline and 3 month PCR results [Table/Fig-9] there was a statistically significant reduction in all the three red complex microorganisms. Comparison

Primer pairs 5'-3'	Amplification length
Porphyromonas gingivalis AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	404 bp
Tannerella forsythensis (Bacteroides forsythus) GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	641 bp
Treponema denticola TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	316 bp
Ubiquitous primer GAT TAG ATA CCC TGG TAG TCC AC CCC GGG AAC GTA TTC ACC G	602 bp

[Table/Fig-7]: Sequences and expected product size for PCR primers

PCR Organisms	PRE OP MEAN±SD	POST OP MEAN±SD	Difference from Pre Op MEAN±SD	p-value
Porphyromonas gingivalis	1.80±0.35	1.23±0.43	0.57±0.08	0.001
Tannerella forsythensis	1.63±0.51	1.50±0.51	0.13±0.00	0.813
Treponema denticola	1.77±0.47	1.47±0.51	0.30±0.04	0.070

[Table/Fig-8]: PCR values of Aloe vera Group

PCR Organisms	PRE OP MEAN±SD	POST OP MEAN±SD	Difference from Pre Op MEAN±SD	p-value
Porphyromonas gingivalis	1.88±0.38	1.17±0.38	0.71±0.00	0.001
Tannerella forsythensis	1.63±0.51	1.27±0.45	0.36±0.06	0.043
Treponema denticola	1.57±0.50	1.10±0.31	0.47±0.19	0.001

[Table/Fig-9]: PCR values of propolis Group

	PRE OP MEAN±SD	POST OP MEAN±SD	Difference from Pre Op MEAN±SD	p-value
Plaque Index	1.77±0.56	1.32±0.49	0.45±0.07	0.001
Gingival Index	2.98±0.93	1.82±0.43	1.16±0.50	0.001
Bleeding On Probing	2.60±0.48	1.31±0.22	1.29±0.26	0.001
Probing depth	5.72±1.01	4.20±0.92	1.50±0.09	0.001
Clinical attachment level	5.23±1.14	3.8±1.19	1.43±1.19	0.001

[Table/Fig-10]: Clinical parameters In Aloe vera group

	PRE OP	POST OP	Difference from Pre Op	
	MEAN±SD	MEAN±SD	MEAN±SD	p-value
Plaque Index	1.87±0.48	1.29±0.27	0.58±0.21	0.001
Gingival Index	3.08±0.63	1.95±0.23	1.13±0.40	0.001
Bleeding on Probing	2.83±0.35	1.26±0.25	1.57±0.10	0.001
Probing depth	5.57±0.82	3.63±0.67	1.94±0.15	0.001
Clinical attachment level	4.57±0.94	3.00±0.95	1.57±0.01	0.001

[Table/Fig-11]: Clinical parameters in propolis group

of three month and baseline clinical parameters in Aloe vera [Table/Fig-10] and Propolis [Table/Fig-11] groups showed statistically significant reductions. On inter-comparison of Group A and Group B microbiologic and clinical parameters, there was a significant reduction in Propolis group (group B) when compared to Aloe vera group (group A).

DISCUSSION

The pharmacological actions of Aloe vera and its usage in periodontal disease have been recorded in the past. Studies have quoted that the subgingival administration of Aloe vera gel resulted in improvement of periodontal condition and Aloe vera mouthwashes showed promising results as an effective anti-plaque agent [8-11]. Aloe vera plant produces at least six antiseptic agents such as lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulphur, three anti-inflammatory fatty acids, cholesterol, campersterol and β- sitosterol, and about 23 polypeptides in Aloe juice which help to control broad spectrum of immune system diseases and disorders [12]. The antimicrobial effects of Aloe vera have been attributed to the plant's natural anthraquinones: aloe emodin, aloetic acid, alonin, anthracine, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamonic acid, isobarbaloin, and resistannol [11,13].

Similarly, certain studies suggest that propolis mouth rinses and irrigants can be used effectively to improve periodontal health [14]. The antibacterial activity of propolis is due to flavonoids, aromatic acids and esters present in the resin. The most effective flavonoids that act against bacteria are galangin, pinocembrin, pinostrobin. Other components that contribute to antibacterial action are ferulic and caffeic acids [15,16].

Although many studies were conducted individually to assess the plaque inhibiting efficacies of aloe vera and propolis, till date no studies have been conducted to assess and compare the plaque inhibiting efficacies using PCR. Considering this in the present study clinical and microbiological analysis utilizing PCR was made.

In this study, the clinical outcome was proportionally similar in both the groups. However, the PCR results among red complex microorganisms in group A (Aloe vera group) showed pronounced reduction in *P. gingivalis* numbers which was statistically significant whereas in group B (propolis group) showed statistically significant reduction in all three micro organisms.

CONCLUSION

Propolis and Aloe vera are new herbal products in medicine and dentistry which gained a lot of attention through recent years. Propolis and Aloe vera are used in various forms such as mouthwashes, local irrigants and local drug delivery agents. In literature, Propolis and Aloe vera have been considered effective remedies against gingival and periodontal diseases. In the present study, with the clinical visual inspection alone the results were same for both the groups; however in terms of statistical numbers the outcome is considered to be better with propolis. Further studies with larger sample studies and longer durations are needed to confirm the reported findings.

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