EFFICACY OF 2% AND 5% GRAPE SEED EXTRACT GEL AS AN ADJUNCT TO CONVENTIONAL SCALING AND ROOT PLANING IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CLINICO-MICROBIOLOGIC STUDY

Periodontology

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ABSTRACT

BACKGROUND: The aim of present study is to assess the efficacy of 2% and 5% grape seed extract (GSE) gel as local drug delivery agent as an adjunct to conventional Scaling and Root Planing (S.R.P) in the treatment of chronic periodontitis.

METHOD: 30 patients with Chronic Periodontitis with pocket depth >5 mm were selected and randomized into 3 groups (10 patients per group). Group 1 will be treated with 2% GSE, group 2 will be treated with 5% GSE and group 3 will be treated with placebo gel.

Scaling and root planing will be performed and oral instructions will be given. Gingival index (GI), plaque index (PI), Probing Pocket Depth (PPD) and clinical attachment level (CAL) will be recorded at baseline and 1 month.

Plaque samples shall be collected at baseline and 1 month and anaerobic culture shall be done to evaluate the antimicrobial efficacy of the Grape seed extract.

RESULTS AND CONCLUSION: The results were calculated statistically and analytically for the same after 1 month.

KEYWORDS

INTRODUCTION:

In the oral cavity chronic periodontitis is considered to be the common disease which consist of inflammation of the periodontal tissues caused by the accumulation of profuse amounts of dental plaque. It is found to be ubiquitous in adults worldwide. Gram-negative microorganisms, which include *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* and *Treponema denticola* were responsible for causing periodontitis.¹

The foremost treatment modality is phase I therapy i.e Scaling and Root Planing followed by other procedures such as Local Drug Delivery, Surgical methods etc. In spite of these various methods, the interest is now been shifting towards newer methods which can be incorporated in general practice as a supplement to the already existing methods. In due course, benefits of nature's gift such as tulsi, clove, mint, Chivauva seeds are well known to all. A relatively newer addition to this is the use of Grape Seed Extract which can be used as an adjunct in the treatment of chronic periodontitis.²

Grape seed extract or *Vitis vinifera* is an industrial derivative of whole grape seeds. The extract contains Proanthocyanidins which has the ability to reduce collagen degradation by inhibiting interstitial and extracellular collagenase, and may have the potential to reduce the progression of periodontitis. Also, GSE has antioxidant property that makes it effective against *P. gingivalis* and *Fusobacterium nucleatum*. Other uses of GSE can be reduce blood pressure, improve blood flow, reduce oxidative damage, improve collagen levels and bone strength, improve kidney function, inhibit infectious growth, reduce cancer risk, enhances wound healing and appearance etc.³ The aim of present study was to assess the efficacy of 2% and 5% grape seed extract (GSE) gel as local drug delivery agent as an adjunct to conventional SRP in the treatment of chronic periodontitis.

MATERIALS AND METHOD:

Study design:

A random sample of 24 patients were screened and examined in the Department of Periodontology and Oral Implantology. The study was approved by the institutional ethical committee and written informed consent was taken from every patient.

smoker, a clinical diagnosis of chronic periodontitis with pockets depth ≥ 5 mm, and willing to provide informed consent. Exclusion criteria for the study were pregnant women, breastfeeding mothers, and patients taking dietary supplements, patients with a GSE allergy, patients with hypertension or bleeding disorders, patients under systemic or topical antibiotic treatments in the past 3 months, smokers, and patients taking any medications that could interact with GSE.

The current clinical trial was single blinded, randomized parallel, longitudinal study. Thirty sites with pocket depth >5 mm were selected from patients with chronic periodontitis. The patients were enrolled in a 1 month clinical study. The gels were labelled as A- 2% grape seed extract, B- 5% grape seed extract and C- Placebo Gel by the examiner (Fig 1).

FIGURE 1: CONSORT FLOWCHART OF STUDY DESIGN

30 sites were randomly divided into 3 groups:

GROUP A: 10 Sites were treated with Scaling and Root Planing followed by local drug delivery of 2% Grape Seed Extract

GROUP B: 10 Sites were treated with Scaling and Root Planing followed by local drug delivery of 5% Grape Seed Extract

GROUP C: 10 Sites were treated with Scaling and Root Planing followed by Placebo Gel.

PREPARATION OF GRAPE SEED EXTRACT AND CONTROL MUCOADHESIVE GELS:

The following materials were used to prepare GSE mucocompressive gel:

- Pure GSE (Bulksupplements, Henderson, NV, USA) containing natural ingredients, including >180 mg proanthocyanidins for every 200 mg serving size of the powder. GSE has been considered safe by the National Center for Complimentary and Integrative Health if used orally for up to 8 weeks
- Carbapol or CB 934
- Sodium-carboxymethyl cellulose, or Na-CMC
- Sodium phosphate monobasic and sodium phosphate dibasic
- Parabens

Two percent and five percent GSE mucoadhesive gels were prepared based on the minimum inhibitory concentration (MIC) and minimum toxic concentration as described by Furiga et al, and depending on the concentration of adhesive polymers that were added to the formula.

For Preparation of 2% GSE: 100mg of GSE and 50mg of Carbapol or CB 934 was dissolved in 50 mL phosphate buffer (pH 6.6) with vigorous mixing until it dissolved completely. Then, GSE and preservatives were dissolved in 25 mL phosphate buffer (pH 6.6). After that, the GSE solution was slowly added into the CB 934 solution with continuous magnetic stirring at a speed of 100 rpm, until a homogenous mixture was obtained. The gelling agent (Na-CMC) was added slowly under continuous magnetic stirring. The volume was then increased to 100 mL with the addition of phosphate buffer. Finally, the prepared gel was kept for 24 hours at room temperature (25°C) for a complete polymer dissolution.⁴

For Preparation of 5% GSE: 250mg of GSE and 50mg of Carbapol or CB 934 was dissolved in 50 mL phosphate buffer (pH 6.6) with vigorous mixing until it dissolved completely. Then, GSE and preservatives were dissolved in 25 mL phosphate buffer (pH 6.6). After that, the GSE solution was slowly added into the CB 934 solution with continuous magnetic stirring at a speed of 100 rpm, until a homogenous mixture was obtained. The gelling agent (Na-CMC) was added slowly under continuous magnetic stirring. The volume was then increased to 100 mL with the addition of phosphate buffer. Finally, the prepared gel was kept for 24 hours at room temperature (25°C) for a complete polymer dissolution.

For Preparation of Placebo Gel: Placebo Mucoadhesive gel was prepared in the same way, but without the addition of the GSE solution.

BASELINE MEASUREMENTS AND APPLICATION OF GEL:

- Clinical parameters of the patient's i.e Plaque Index & Gingival Index,⁵ Pocket Probing depth using UNC-15 probe with acrylic stents were recorded. Bleeding on probing after 30 seconds of probing was noted at first appointment. Subgingival Plaque Sample was collected at baseline and 30 days using Curette and was transferred to an Eppendorf containing 1ml phosphate buffered saline (pH- 6.6) solution.
- In Group A patients Scaling and Root Planing was done along with local drug delivery of 2% GSE using 1 ml 23 gauge disposable syringe.
- In group B patient's Scaling and Root Planing was done along with local drug delivery of 5% GSE using 1 ml 23 gauge disposable syringe.
- In group C Scaling and Root Planing was done along with Placebo gel using 1 ml 23 gauge disposable syringe.

After this COE pack was applied and oral hygiene instructions were given to all the patients.

MICROBIAL CULTURE:

Sub gingival plaque sample collection and microbiological

culture: To prevent contamination by blood, sub gingival plaque was always collected before taking clinical measurements at each of the two specified time periods (start of the clinical trial and at 30 days). Pooled plaque samples were obtained from the sulcus of patients with the deepest probing depth in each quadrant. After collection of the samples with the help of gracey curette (Hu-Friedy) the samples were placed in 1ml of phosphate buffered saline (PBS) in ependroff and labelling was done according to the patient. Samples were preserved at -20 degree Celsius for future evaluation of load of anaerobic bacteria by using trypticase soya agar broth.

Anaerobic Bacterial culture:

Samples were vortexed for 10s and a series of two 10-fold dilutions of each sample were prepared. Ten milliliters of each dilution was seeded on trypticase soya agar plates and labelled. Plaque samples were collected at baseline and 30 days. Bacterial culture was done under anaerobic conditions with an anaerobic system (N2 85%, H2 5%, CO2 10%) using anaero gas jar and gas pack for anaerobe obligate and facultative bacteria. Following the incubation period, numbers of colony forming units (CFUs) were counted using colony counter. The number of pigmented CFUs were counted on plates from anaerobic conditions. CFU were calculated as the number of colonies per 10µl at the minimum dilution with clear, separate and countable colonies.

Statistical Analysis:

All the collected data was entered into Microsoft excel and analysed by the statistical software SPSS version 16. The statistical means and standard deviation were evaluated. The normality of the data was tested by Shapiro's wilk's test and was found to be normal data. The significant difference of the parameters between three groups were tested by one way ANOVA followed by Post HOC Tukey HSD test, and intragroup comparison at different time intervals was done by paired t-test. The level of significance and confidence interval were 5% and 95% respectively

Results:

The preventive treatments applied in the present work showed significant difference in mean Plaque Index, mean Gingival Index and Pocket probing depth (Graph 3) at baseline and at 30 days within the groups which was statistically significant (p <0.05), but the probing depth at 30 days was not statistically significant (p>0.05) Table 1.

Microbiological analysis:

There was statistical significant reduction in the microbiological count from baseline to 30 days (p<0.05).

The graph shows the percentage reduction of all the three groups. On intergroup comparison group A showed more reduction as compared to the other two groups. (Fig 2)

Table 1: Intragroup comparison of clinical parameters from baseline to 30 days *Statistically significant (P<0.05), NS- Statistically non-significant (P>0.05). Group 1 Supragingival scaling+2% grape seed extract, Group 2 Supragingival scaling+5 % grape seed extract, Group 3 placebo gel; CFU/ml – Colony-forming units/ml, GI – Gingival index; PI – Plaque index; PPD – Probing pocket dep

Clinical Parameters		Group 1	Group 2	Group 3	P-value
PI	Baseline	1.56±0.51	1.15±0.12	1.40±0.21	0.00
	30 days	0.36±0.11	0.85±0.10	1.10±0.10	0.00
GI	Baseline	1.41±0.14	0.43±0.11	1.00±0.10	0.00
	30 days	0.44±0.03	0.67±0.07	0.75±0.05	0.00
PPD	Baseline	5.60±0.69	5.00±0.81	4.80±0.78	0.07
	30 days	5.00±0.81	4.50±0.52	4.50±0.97	0.28
MICRO	Baseline	72.20±51.61	44.60±22.22	37.90±12.98	0.05
	30 days	51.20±52.05	31.90±18.22	35.40±11.08	0.04

FIG 2:Percentage reduction graph. Group 1 Supragingival scaling+2% grape seed extract , Group 2 Supragingival scaling+5 % grape seed extract , Group 3 placebo gel; CFU/ml – Colony-forming units/ml, GI – Gingival index; PI – Plaque index; PPD – Probing pocket depth

DISCUSSION:

GSE has antioxidant properties that could make it effective against periodontitis-associated microorganisms. Moreover, it has been demonstrated to reduce collagen degradation, and thus could have the potential to slow down or prevent the progression of periodontitis. The results of the current study revealed that none of the patients had any side-effects related to the application of the gels.

GSE may be beneficial for the treatment of inflammation associated with bone destruction as it may strongly inhibit osteoclast differentiation, reduce osteoclast activity, and stimulate bone formation through its positive action on osteoblast differentiation. The anti-inflammatory effect is modulated by calibrating the delicate balance between pro-inflammatory and anti-inflammatory cytokines through regulating their release and gene expression.

After experimental intervention it was found that gingival index, plaque index, and microbiological count when compared within the groups showed statistically significant results from baseline to 30 days. This was in accordance with the study done by Rudrakshi et al (2017)⁶ in which they concluded that antioxidants have shown to have beneficial outcomes when used as adjuncts to traditional periodontal therapy. Also Rayyan et al in 2018,⁷ conducted a study and found that GSE reduces gingival index and plaque index in chronic periodontitis patients. It was noted by Furiga et al⁸ that the bactericidal effect of GSE is concentration-dependent. This suggests that the different formulae prepared in the present study might have had a different degree of inhibitory effect against different types of bacteria present in the pockets.

The non-significant results of the probing depth of GSE might be due to the dilution with saliva. Study done by Furiga et al have quoted this might be due to the low concentration of the extract used even though it was prepared at different percentages. Also it is worth noting that as the depth of the pockets increases, difficulty in reaching the bottom of the pockets to perform effective scaling and root planing increases.

Our study presents for the first time a useful comparative clinical and microbiological recording of different percentages of grape seed extract with placebo gel, which can be used as an adjunct to scaling and root planing in treating patients with chronic periodontitis.

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