



Comparative Evaluation of Subgingivally Delivered 3% Saussurea Lappa Root Extract Gel with 0.2% Chlorhexidine Gel as an Adjunct to Scaling and Root Planing in the Management of Periodontitis: A Clinical & Microbiological Study.

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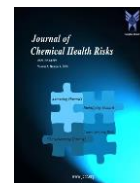
ABSTRACT:

Introduction: Periodontitis is characterized by microbially-associated, host-mediated inflammation that results in loss of periodontal attachment. Mechanical therapy which includes Scaling and Root Planing (SRP) disrupts plaque biofilm effectively for the majority of patients with mild to moderate periodontitis. But mechanical therapy itself may not always reduce or eliminate the anaerobic infection at the base of pocket, within the gingival tissues, and in both structures inaccessible to periodontal instruments. To overcome this, systemic and local drug delivery of antimicrobials were initiated to enhance nonsurgical therapy by serving as an adjunct to SRP. *Saussurea lappa* C. B Clark, (*Saussureacostus*) belonging to the family Asteraceae, commonly known as Costus exhibits anti-inflammatory, anti-tumor, anti-oxidant, anti-fungal, anti-viral and anti-hepatotoxic activity. However, there is a lack of data regarding the use of *Saussurea lappa* as a gel for local drug delivery agents for the treatment of periodontitis.

Objectives: This study aimed to evaluate and compare the efficacy of subgingivally delivered 3% *Saussurea Lappa* gel and 0.2% chlorhexidine gel as an adjunct to scaling and root planing in patients with periodontitis.

Methods: In total, 19 systemically healthy individuals with age group 18-60 years diagnosed with Localized /Generalized Stage I and II, Grade -A, Periodontitis having Interdental CAL which is detectable at ≥ 2 non- adjacent teeth, or Buccal or oral CAL ≥ 3 mm with pocketing > 3 mm detectable at ≥ 2 teeth were included in the study. The study was a double-blind split-mouth randomized control clinical trial. Two sites were selected in each patient and were randomly allocated to control and test sites. At baseline, measurements of site-specific periodontal parameters and collection of subgingival plaque sample were done. After full-mouth SRP, subgingival delivery of 0.2% chlorhexidine gel in control sites and 3% *Saussurea lappa* root extract gel in test sites was done. At 1 and 3 months, subgingival plaque samples were collected again and site-specific periodontal parameters were measured.

Results: The test group (3% *Saussurea lappa* root extract gel) showed statistically significant improvements in periodontal i.e., modified sulcus bleeding index (mSBI) at 1-month, probing pocket depth (PPD), and relative attachment level (RAL) and microbiologic parameters in the form of colony forming units (CFUs) in comparison with control group (0.2% chlorhexidine gel) at 1 month and 3 months.



Conclusions: Subgingival delivery of 3% *Saussurea lappa* root extract gel has exhibited anti-inflammatory, antibacterial, and antimicrobial effects. Because it is biologically accepted by patients and is delivered into the periodontal pockets, it can be recommended as an adjuvant to SRP therapy for the treatment of patients with Localized/Generalized Stage I and II, Grade-A Periodontitis in the periodontal maintenance phase.

1. Introduction

Periodontitis is characterized by microbially-associated, host-mediated inflammation that results in loss of periodontal attachment. This is detected as Clinical Attachment Loss (CAL) by circumferential assessment of the erupted dentition with a standardized periodontal probe with reference to the cemento-enamel junction.¹ Dental plaque which is a primary etiological factor for periodontal disease is composed of bacterial aggregates that are adherent to one another and to surfaces and interfaces². These bacteria form a highly resistant biofilm with an exopolysaccharide matrix protection which prevents drug penetration³. The aim of periodontal therapy is to remove the bacterial plaque and all the factors that favour its accumulation⁴. Mechanical therapy which includes Scaling and Root Planing (SRP) disrupts plaque biofilm effectively for the majority of patients with mild to moderate periodontitis.⁵ It involves the removal of supra and subgingival plaque and calculus there by returning the tissues to a state of health.⁶ However, mechanical therapy itself may not always reduce or eliminate the anaerobic infection at the base of pocket, within the gingival tissues, and in both structures inaccessible to periodontal instruments. To overcome this, systemic and local drug delivery of antimicrobials were initiated to enhance nonsurgical therapy by serving as an adjunct to SRP as putative pathogens associated with periodontal disease are susceptible to a variety of antiseptics and antibiotics.⁷ However, Systemic administration of drugs despite being useful in treating periodontal pockets, has various disadvantages such as the development of resistant bacteria, drug toxicity and requires higher dosage to attain required gingival crevicular fluid concentration at the target site.^{8,9}

Therefore to revoke these shortcomings Dr Max Goodson in 1979 of Forsyth Dental Research Centre developed the concept of Local Drug Delivery (LDD).^{10,11} Methods employed to convey antimicrobial agents into periodontal pockets include rinsing, irrigation, systemic administration and local

application using sustained and controlled delivery devices and the common agents used as local drug delivery include subgingival chlorhexidine, tetracycline fibers, minocycline, doxycycline and metronidazole.^{12,13} Chlorhexidine is considered as a “gold standard” antiplaque and anti-gingivitis agent that has been effectively used in the treatment of periodontal disease which results in decrease in the microbial load.¹⁴ However, it has various side effects like discoloration of teeth, dulling of taste sensation and oral mucosa erosion.¹⁵

Saussurea lappa C. B Clark, (*Saussureacostus*) belonging to the family Asteraceae, commonly known as Costus which is a tall perennial herb that grows to a height of 1-2m, stem is upright, stout and fibrous. It has long lyrate leaves, with flowers purple to black in colour. It exhibits anti-inflammatory, anti-tumor, anti-oxidant, anti-fungal, anti-viral and anti-hepatotoxic activity due to the presence of costunolides, lactones, anthraquinone compounds, favinoids, Shikokiols and chlorogenic acid.¹⁶ Ahsan A, Miana G et al (2019) have shown that *Saussurea lappa* has effective wound healing, anti-inflammatory and anti-microbial properties and formulation of extract in to in-situ gels can enhance the wound healing potential because it provides controlled drug release pattern and greater stability to the extract.¹⁷ Trivedi S, Dixit J (2015) in a pilot randomized trial have shown that subgingival irrigation with *Saussurea lappa* might be a promising adjunct to conventional treatment options in the management of periodontal infections¹⁸. Among various drug delivery systems, gel formulations have the advantages of faster drug release, ease of preparation and administration. They possess higher biocompatibility and bio adhesiveness by allowing adhesion to mucosa in the pocket, hence decreasing the risk of dilution of the material by saliva.¹⁹

However, there is a lack of data regarding the use of *Saussurea lappa* as a gel for local drug delivery agents for the treatment of periodontitis. Hence, the present study was undertaken to compare and evaluate the



clinical and microbiological effect of sub gingivally delivered 3% *Saussurea Lappa* root extract gel and 0.2% Chlorhexidine gel as an adjunct to scaling and root planing in the management of periodontitis.

2. Objectives

To compare and evaluate the clinical and microbiological effect of sub gingivally delivered 3% *Saussurea Lappa* root extract gel and 0.2% Chlorhexidine gel as an adjunct to scaling and root planing in the management of periodontitis.

3. Methods

3% *Saussurea Lappa* root extract gel: Preparation of 1gm of Carbopol 940 and 5gm of carboxymethyl cellulose were dissolved in 20 ml of distilled water and heated for around 50°C in a two different glass beaker to obtain a clear solution and left for 24 hours to make 2% Carbopol 940 and 15% of carboxymethyl cellulose. Then again 2% Carbopol 940, 15 % carboxymethyl cellulose, 15% and poly vinyl alcohol solutions were prepared in a phosphate buffer and left for 24 hours. After 24 hours all the polymers were mixed with 3 gm of *Saussurea lappa* root extract powder to get the final gel preparation & Benzyl alcohol was used as a preservative. The prepared gel was tested for microbial growth by incubation for 48 hours at 37°C and no microbial growth was observed. The gels were packed and sealed in glass containers and stored at room temperature i.e 20- 24°C.

The patients were recruited from the outpatient department, Department of Periodontology, School of Dental Sciences, Sharda University, Greater Noida. The nature and outcome of the study were explained to the patients following which a written consent form was obtained. The study was approved by the Institutional Ethics Committee. A total of 19 sites of each group from both male and female in the age group of 18-60 years with periodontitis were included in the study who had localized /generalized Stage I and II, Periodontitis having Interdental CAL which is detectable at ≥ 2 non- adjacent teeth, or buccal or oral CAL ≥ 3 mm with pocketing > 3 mm detectable at ≥ 2 teeth. Patients who had ≥ 20 natural teeth & patients with no known history of allergy to Chlorhexidine gel and *Saussurea lappa* gel. Patients who had undergone periodontal therapy in the last 6 months or with history of antibiotic therapy within 6 months prior to study were excluded. Pregnant, lactating women,

users of tobacco in any form, and patients not willing to give a written consent form were also excluded from the study. The study was carried out between August 2021 to November 2022.

2 sites were selected in each patient and contralateral sites were randomly allocated to control and test sites by the method of flip of a coin method. Each patient was presented in 4 visits. The study design is described as a split-mouth randomized control clinical trial. Each patient was presented in four visits. At baseline, measurements of periodontal parameters such as Gingival Index (GI) Loe and Silness in 1963²⁰, Plaque Index (PI) Silness & Loe 1964²⁰, modified Sulcus Bleeding Index (mSBI)Mombelli et al.,1987²¹, Probing Pocket Depth (PPD), and Relative Attachment Level (RAL) were done by a UNC 15 probe. Subgingival plaque samples were collected using sterilized curette from the deepest part of the periodontal pocket in both test and control sites to assess total viable anaerobic count (TVAC; CFUs).

After SRP, 0.2% Chlorhexidine gel using tuberculin syringe and periodontal pack was subgingivally delivered in control sites & 3% *Saussurea lappa* gel using tuberculin syringe and periodontal pack was subgingivally delivered in test sites. Routine oral hygiene instructions were given.

At the second visit (seventh day), removal of periodontal pack and assessment for adverse effects (if any) was done. Routine oral hygiene instructions were reinforced. At 1 and 3 months, site-specific periodontal parameters were measured again. Subgingival plaque samples were also collected again to assess the microbiological parameter in the form of CFUs.

The subgingival plaque samples were inoculated on to the brain heart infusion agar plates supplemented with hemin and vitamin K. The four-quadrant streak method was used to spread the inoculums across the plate. A sterile inoculation loop was used for the purpose with repeated flaming time and again to maintain its sterility. A gap of 5 seconds was given for the loop to cool down after each flaming before streaking. The inoculated brain heart infusion agar plates supplemented with hemin and vitamin K were then placed in a cylindrical container (anaerobic jar) along with one AnaeroGas Pack Sachet. The AnaeroGas Pack sachet is a disposable oxygen-absorbing and carbon dioxide-generating agent used in



anaerobic jars for the preparation of anaerobic media. The container was then sealed and placed in the incubator. After 7 days of incubation at 37°C, the TVAC was determined. All the microbiology data were transformed into CFUs/plate using a manual colony counter.

4. Results

The study included 19 patients with mean age of 18 to 60 years with 38 sites, diagnosed with Localized /Generalized Stage I and II, periodontitis, fulfilling the inclusion and exclusion criteria. Intragroup comparisons of the experimental sites (3% *Saussurea Lappa* root extract gel) and control sites (0.2% chlorhexidine gel) showed a statistically significant reduction in all the periodontal parameters and microbiological counts from baseline to 1 month and 3 months intervals. The intergroup comparison of experimental sites (3% *Saussurea Lappa* root extract gel and control sites (0.2% chlorhexidine gel) showed a higher statistically significant reduction in periodontal parameters such as mSBI ($p < 0.003$) at 1 month, PPD ($p < 0.000$), RAL ($p < 0.004$) in the experimental sites (3% *Saussurea Lappa* root extract gel) at 1 month and 3 months intervals when compared with control sites (0.2% chlorhexidine gel). However, the other periodontal parameters such as PI and GI did not show a statistically significant reduction difference between both the groups.

The intergroup comparison of the experimental sites (3% *Saussurea Lappa* root extract gel) and control sites (0.2% chlorhexidine gel) showed a statistically significant reduction in microbiological parameters in the form of CFUs in the experimental sites at 3 months interval when compared with control sites ($p < 0.001$).

5. Discussion

Local drug delivery is used as an adjunct to scaling and root planing aids in the control of growth of pathogenic bacteria²². The principle requirement for a local drug delivery agent is that it should reach the base of the periodontal pocket and its concentration must be maintained at the site for a sufficient amount of time for its effective antimicrobial action to occur²³.

Saussurea lappa has been used as a medicinal plant since Vedic times. *S. lappa* root extract contains essential oil as well as the alkaloid "saussurine," which has analgesic, anti-inflammatory, and anti-tubercular activities, as well

as efficacy against *Streptococcus* and *Staphylococcus* infections²⁴. Its antimicrobial activity has also been established against multidrug-resistant bacteria such as *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia*, extended spectrum beta-lactamase and *Acinetobacter baumannii*, which all serve a role in development of periodontal infections²⁵. A lactone from *S. lappa* has been shown to reduce the formation of inflammatory mediators, tumour necrosis factor, nitric oxide release, and lymphocyte proliferation²⁶.

Trivedi S, Dixit J (2015) conducted a study to assess and compare the clinical effects of irrigation with chlorhexidine and *Saussurea lappa* extract as an adjunct to scaling and root planing in the treatment of chronic periodontitis. The results of this study showed significant reduction in mSBI and PPD in *Saussurea lappa* treated group in 4-5mm pockets. The results of our study are in accordance with the above study where significant reduction was seen in PPD and mSBI in *Saussurea lappa* group.¹⁷ In addition to periodontal parameters our study also showed the effect of *Saussurea lappa* root extract gel on microbiological parameters in the form of CFU. There was a significant reduction in colony forming units in the group in which *Saussurea lappa* root extract gel was placed.

Gel formulations have the advantage of being biodegradable and water-soluble polymers. *Saussurea lappa* root extract gel is released in controlled manner because they have better stability and release profile upon gelation. Before administration, the in situ forming system is in the form of a sol, and when administered gradually, it becomes a gel and because of this it has the potential to maintain high levels of the drug in the gingival crevicular fluid for a longer period²⁷.

An in-vitro study was conducted by Binshabaib M, Harthi S, Helaby B et al (2022) to compare the antibacterial effectiveness of two herbal extracts *Saussurea-costus* (*S. costus*) and *Melaleuca alternifolia* against *Porphyromonas gingivalis*, *Streptococcus mutans* and *Enterococcus faecalis*. The results showed no significant difference in MIC for strains of and *P. gingivalis*, *S. mutans*, *E. faecalis* treated with *S.costus*.²⁸

Herbal therapy /phytotherapy is currently gaining a lot of attention from the medical and dentistry communities because of its natural and non chemical properties²⁹.It



has been proven to provide a wide range of biological competencies, including antibacterial, antioxidant, and anti-inflammatory properties³⁰. The growing popularity of herbal treatments is due to the relatively safe nature of herbal extracts, superior patient tolerance, and public acceptance³¹. Curcumin, aloe vera, neem, lemon grass, babul, tea tree oil, and other herbal products and their components are used in the form of local drug delivery agents for the treatment of periodontitis²³.

To the best of our knowledge there are no published studies conducted to evaluate the efficacy of 3% *Saussurea lappa* root extract gel and 0.2% chlorhexidine gel subgingivally delivered as an adjunct to scaling and root planing on the clinical parameters such as Plaque Index (PI), Gingival Index (GI), Modified Sulcus Bleeding Index (mSBI), Probing Pocket Depth (PPD) and Relative Attachment Level (RAL), and microbiological parameters, the total viable cell (TVAC) count in form of CFU's at baseline, 1 month and 3 months in periodontitis patients.

Although the aim and objectives of the present study were met, there are few limitations. Our study had a small sample size. The study was assessed at 1 month and 3 months only. In the present study, the effect of 3% *Saussurea lappa* root extract gel and 0.2% chlorhexidine gel was not assessed on specific periodontopathogens. The bioavailability of the gels used (3% *Saussurea lappa* root extract gel and 0.2% chlorhexidine gel) may be limited due to use in the gel form.

For longer bioavailability of *Saussurea lappa* in the periodontal pocket, they may be incorporated into a biodegradable matrix of cross-linked hydrolyzed gelatin and made into a chip. *Saussurea lappa* could be compared to other herbal agents such as aloe vera, neem, tulsi, propolis, cocoa husk, pomegranate, and cranberry to assess clinical advantage and side effect of the agent used as an adjunct for conventional scaling and root planing. Further studies can be performed to evaluate the efficacy of 3% *Saussurea lappa* root extract gel on different periodontopathogens using polymerase chain reaction (PCR) in periodontitis patients.

Further perspectives could be aimed at conducting studies with larger sample size including studies of 6-month follow-ups or more to assess the long-term effectiveness of subgingivally delivered *Saussurea lappa* gel as an adjunct in non-surgical periodontal therapy.

Multicentred research trials should be conducted to evaluate the effects of subgingivally delivered 3% *Saussurea lappa* root extract gel in patients with gingivitis, its effects on bone loss, advanced chronic periodontitis, aggressive periodontitis, in smokers with periodontitis, peri-implant mucositis, peri-implantitis and periodontitis with medically compromised individuals.

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**Table 1:-**Intragroup and Intergroup comparison of PI, GI, mSBI, PPD, RAL, CFU

Index	sites	N	Mean± Std. Deviation (BASELINE)	Mean± Std.Deviation (ONE MONTH)	Mean± Std.Deviation (THIRD MONTH)	p-value of Intragroup comparison	Post hoc pairwise comparison
Plaque Index	Control	19	2.00±0.29	1.23±0.24	.6±0.12	0.000**,S	a>b>c
	Test	19	1.95±0.34	1.28±0.24	.58±0.12	0.012**,S	a>b>c
p-value of Intergroup comparison			0.312	0.589	0.892		
Gingival Index	Control	19	1.73±0.24	1.13±0.17	.88±0.10	0.010**,S	a>b>c
	Test	19	1.87±0.15	1.11±0.14	.71±0.07	0.022**,S	a>b>c
p-value of Intergroup comparison			0.011**	0.236	0.071		
Modified Sulcus Bleeding Index	Control	19	1.68±0.47	1.00±0.0	.68±0.47	0.010**, S	a>b>c
	Test	19	1.73±0.45	0.97±0.0	.47±0.51	0.022**, S	a>b>c
p-value of Intergroup comparison			0.485	0.003**	0.112		
Probing Pocket Depth	Control	19	5.68±0.47	3.84±0.50	2.73±0.45	0.000**, S	a>b>c
	Test	19	5.63±0.49	3.63±0.59	2.36±0.49	0.031**, S	a>b>c
p-value of Intergroup comparison			0.002**	0.012**	0.000**		
Relative Attachment level	Control	19	6.84±0.68	5.47±0.51	4.63±0.49	0.001**, S	a>b>c



	Test	19	6.63+0.59	4.84+0.76	3.26+0.45	0.016**, S	a>b>c
p-value of Intergroup comparison			0.021**	0.000**	0.004**		
CFU	Control	19	55.68+8.6	37.36+2.08	26.78+1.2	0.021**, S	a>b>c
	Test	19	56.36+8.1	31.57+1.57	22.84+1.1	0.001**, S	a>b>c
p-value of intergroup comparison			0.002**	0.003**	0.001**		

TABLE 2:- Intergroup comparison of Mean absolute reduction in PI, GI, mSBI, PPD, RAL, CFU of control sites and test sites from baseline to 1 month, baseline.

Index	sites	N	Mean± Std. Deviation (BASELINE TO 1 MONTH)	Mean± Std. Deviation (BASELINE TO 3 MONTHS)	Mean± Std. Deviation (1 MONTH TO 3 MONTHS)
Plaque Index	Control	19	0.76±0.30	1.32±0.77	0.6±0.01
	Test	19	0.67±0.11	1.37±0.81	0.7±0.03
p-value			0.1.00	0.068	0.100
Result			Non-significant	Non-significant	Non-significant
Gingival Index	Control	19	0.6±0.2	0.84±0.24	0.2±0.1
	Test	19	0.76±0.27	1.16±0.03	0.4±0.1
p-value			0.749	0.110	0.411
Result			Non-significant	Non-significant	Non-significant



Modified Sulcus Bleeding Index	Control	19	0.68±0.12	1±0.44	0.3±0.0
	Test	19	0.74±0.14	1.26±0.57	0.5±0.0
p-value			0.000	0.000	0.000
Result			Non-significant	Non-significant	Non-significant
Probing Pocket Depth	Control	19	1.84±0.88	2.95±1.02	1.1±0.31
	Test	19	2.00±1.09	3.36±1.21	1.3±0.40
p-value			0.043	0.013	0.010
Result			Significant	Significant	Significant
Relative Attachment level	Control	19	1.37±0.91	2.21±1.12	0.8±0.01
	Test	19	1.79±0.32	3.37±1.41	1.6±0.51
p-value			0.021	0.041	0.028
Result			Significant	Significant	Significant
CFU	Control	19	18.32±3.83	28.89±4.21	10.6±1.12
	Test	19	24.79±3.02	33.53±4.33	12.7±1.01
p-value			0.000	0.000	0.000
Result			Significant	Significant	Significant