Section A-Research paper
ISSN 2063-5346

Comparative effectiveness of intra-alveolar Stevia extract powder versus amoxicillin trihydrate powder in healing of mandibular third molar extraction sockets among 18-45 year-old Indian adults - a split mouth randomized controlled trial.

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DOI: 10.48047/ecb/2023.12.si4.1703

Abstract

Background: Plant based compounds are gaining popularity in healthcare. Stevia, a plant based sweetener, has antihyperglycemic, antihypertensive, anti-inflammatory, antitumor, diuretic, anti-microbial and immunomodulatory effects. Its use as an antimicrobial agent in the extraction sockets of mandibular third molars has never been reported in the literature.

Aim and objectives: This study aims to find an answer to avert adverse effects related to post-operative oral antimicrobials and thus evaluates and compares the effectiveness of stevia with amoxicillin trihydrate used in sockets of surgically extracted impacted mandibular third molars.

Materials and methods: A split mouth randomised controlled trial was undertaken on 18-45 years old 40 healthy subjects with bilaterally impacted mandibular third molars ie, 80 surgical sites. After extractions, Group 1(n=40) received stevia extract powder and Group 2 received amoxicillin trihydrate powder in the sockets before suturing. Facial measurements, inter-incisal mouth opening, WBC counts, rise in axillary temperature, presence of suppuration, lymph node palpability, intra-oral erythema, wound dehiscence and pain were noted pre and post-operatively on days 1,3,7.

Results: There was no statistically significant difference ($p>0.05$) on inter-group comparisons in all variables except pain and facial measurements ($p<0.05$). There was no incidence of infection/overt inflammation in either group.

Conclusion: Intra-alveolar Stevia powder showed comparable antimicrobial effect as amoxicillin trihydrate powder and can be used to improve overall patient's comfort, avoiding systemic adverse effects of oral antimicrobials, following impacted mandibular third molar surgery.

Keywords: Stevia rebaudiana, impacted mandibular third molars, Intra-alveolar antibiotic, local antimicrobial agent.

Introduction

Although surgical extraction of impacted mandibular third molars is routinely undertaken procedure in Oral and Maxillofacial surgical practice, its possible intra and post-operative sequelae include (but are not limited to), bleeding, damage to adjacent tooth, pain, trismus, swelling, dry socket and infection of the surgical site. Given the incidence of post-operative infection in literature, use of routine antibiotic prophylaxis in patients undergoing such surgeries is considered unsubstantiated.[1] Use of antibiotic prophylaxis for reduction of infection related postsurgical complications, such as pain, trismus, delayed wound healing, and swelling have been discussed and debated.[2] Supporters of systemic antibiotics for such cases, consider oral route as the gold standard. Since amoxicillin is proven to be effective against common oral infection isolates, it has been used in most of the regimens.[3] However, systemic administration of an antimicrobial agent might not yield desired availability of the drug at the target site (due to poor bone penetrance) hence greater dosage or a drug with broader spectrum may be required in certain cases. Adverse effects of the drug per se, microbial resistance towards the drug, destruction of eco- niches e.g., intestinal microflora and their sequelae, makes local application of such agents, a better alternative especially in accessible areas such as mandibular third molar region.[4]

There seems to be a perpetual need for discovering new antimicrobial compounds with varying chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and re-emerging infectious diseases and development of microbial resistance to the antibiotics in current clinical use.[5]

A global shift towards discovery and application of plant based products for therapeutic use in humans cannot be understated. Plant extracts and phytochemicals with known antimicrobial properties can individually or as an adjunct, significantly aid therapeutic treatment regimens. Minimum side effects, ease of availability and cost-effectiveness are their major advantages .[5]

Stevia is one such natural product, extracts of which are widely used as sweetening agent. Recently, it has been proven to have antimicrobial activity and beneficial effects on human health.[6.7.8] Commercially available Stevia is an extract from the leaves of the plant Stevia rebaudiana. Dr. Moises Santiago Bertoni discovered Stevia in the 19th century. This plant belongs to Chrysanthemum plant species and is related to Lettuce, Marigold and Chicory. Stevia was used by Guarani Indians for sweetening tea, as well as making sweet treats. The chemical compounds that produce its sweetness are various steviol glycosides (mainly stevioside and rebaudioside), which are 200–300 times sweeter than sugar. Additionally, diterpenoids present in the leaves viz Manoyl oxide (anti-inflammatory and anti-parasitic), Labdanesclareol (anti-tumorous and cytotoxic), Phytosterols (cardiovascular advantages) have been reported.[6]

Stevia extracts are non-caloric and non-cariogenic, heat stable, pH stable, not fermentable, hence, generally used as a natural sweetening agent.[7] Stevia has low Glycemic Index & aids activation of beta cells of the pancreas which helps to evade blood sugar spikes[7,8]. Steviol Glycosides are poorly absorbed in the body and pass unaltered through the upper gastrointestinal

tract. In colon, steviol glycosides get converted to steviol, metabolized by the liver and are completely excreted in the urine. Hence there is no accumulation of Stevia in the body during and after metabolism. Stevia helps to improve the function of pancreas when taken on a regular basis.[7]

Preclinical and clinical studies have proven it to be non-genotoxic, non-mutagenic and encourage its therapeutic and pharmacological applications.[8]Genotoxicity and clinical toxicity even at dosage levels beyond 300 times the recommended dose, have been ruled out.[9] Since it has been approved and marketed as a sweetener, it is routinely and preferentially used over other artificial sweeteners currently. Other therapeutic benefits (apart from hypoglycaemic effect) reported are, antihypertensive, anti-inflammatory, antitumorogenic, antidiarrheal, diuretic, and immunomodulatory effects.[8] Topical application of stevia extract has yielded promising results in healing of abrasions, lacerations, blemishes and acne.[10]

Gamboa et al showed the antimicrobial activity of stevia extracts against oral microbial flora at MIC between 30-120mg/ml.[11] The antimicrobial spectrum of Stevia rebaudiana involves: Streptococcus mutans, Lactobacillus acidophilus, salmonella typhimurium, klebsiella pneumonia, eschericia coli, bacillus cereius, B. subtilis, S. aureus, M. letus, B. megaterium, S. marcensens, P. aeruginosa, E. coli, P. valgaris and fungi such as Candida albicans, R. oligosporus and A. niger.[11] These are the most commonly involved pathogens in infectious conditions in and around the oral cavity. Stevia extracts also exhibit antiviral activity and are potential rotavirus inhibitors.[12]

After a thorough review of literature, we did not find any clinical study using intra-alveolar stevia extracts as an antimicrobial agent. This split mouth study was thus designed to comparatively evaluate effects of Stevia extract powder vs amoxicillin trihydrate powder placed in third molar extraction sockets. This study tested the null hypothesis which states that 'Intra-alveolar Stevia extract powder is not an effective antimicrobial agent for healing of surgically extracted mandibular third molar wounds'.

MATERIALS AND METHODS

A Split mouth, randomized controlled trial was undertaken in the age group of 18-45years old healthy subjects, reporting to the dept. of Oral and Maxillofacial Surgery for surgical extraction of impacted mandibular third molars. Ethical clearance was obtained from the Institution's Ethics Committee (ref no. MUHS/PG/E-2/2254/2017). The trial was registered in CTRI-ICMR with reference number - CTRI/2020/08/027305.

Sample size was determined using the expected proportion of successful cases in each group values of which are estimated from literature & using the formula[13],

$$n = \frac{(Z_\alpha + Z_\beta)^2 [p(1-p) + q(1-q)]}{(p-q)^2}$$

where Z_α is the z variate of alpha error i.e. a constant with value 1.96, Z_β i.e. a constant with value 0.84,

A sample size of 40 subjects per group was thus computed for the present study. Since this was a split-mouth design, subjects with bilateral impacted mandibular third molars, indicated for surgical extractions were included and randomly assigned into one of the 2 groups by computer generated random numbers. A written consent was obtained from the subjects prior to commencing the study. One site was operated at once and a wash-out period of one month was diligently followed in all cases. Subjects, who were lost to follow-up or did not comply with post-operative instructions or did not report for the second site, were excluded. Patients with a

known medical history, an active infection elsewhere, and recent use of antimicrobial drugs (in the last 7 days), an immune-compromised state, pregnant or lactating mothers and uncooperative patients, mentally retarded patients were excluded from the study. Thus, a total of 42 subjects were initially recruited for this study. Two patients opted not to get operated on the second site, hence were excluded. The study thus evaluated 80 surgical sites in 40 subjects.

Pain was evaluated by 0-10 Visual Analogue Scale. Facial Swelling was assessed using two facial lines (in cm) using a flexible calibrated surgical scale.

- Line 1- The distance from tragus to the commissure of the mouth.
- Line 2- The distance from the lateral canthus of the eye to the gonion.

Mouth Opening was evaluated by measuring the distance between incisal edges of the upper and lower central incisors at maximum mouth opening in mm.

Absence of preoperative infection was ensured by normal white blood cell counts and axillary temperature. Facial measurements (mm) and inter-incisal mouth opening (mm) (to assess amount of swelling and trismus), WBC count, rise in axillary temperature (^{o}F), presence of suppuration and lymph node palpability (as indicators of surgical site infection), intra-oral erythema and wound dehiscence (to assess healing) and pain (VAS scores) were noted pre and post-operatively. Intra-operatively, length of the incision (mm) and amount of periosteal stripping (mm) (as indicators of surgical trauma) were also noted.

All subjects were operated under similar conditions by the same operating surgeon using standard operating aseptic protocol. After thorough irrigation, Group 1 subjects received intra-alveolar stevia extract powder (180mg) whereas, Group 2, received intra-alveolar amoxicillin trihydrate powder (500mg). Two 3-0 black silk interrupted sutures were placed in all 80 surgical sites. Pressure pack was applied on the surgical site, standard postoperative instructions were given, and routine analgesics (but not antibiotics) were prescribed to all subjects. All subjects were followed-up on postoperative days 1, 3 and 7 for evaluation of variables as stated above. WBC counts were evaluated only on the 3rd postoperative day.

Statistical Analysis

· Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States).

·Data was subjected to statistical analysis using the Statistical package for social sciences (SPSS v 26.0, IBM).

Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data has been depicted.

Normality of numerical data was checked using Shapiro-Wilk test & was found that the data for WBC count only followed a normal curve; hence Inter group comparison (2 groups) was done using t test (Table 1).

Normality of numerical data was checked using Shapiro-Wilk test & was found that the data for all other variables did not follow a normal curve; hence non-parametric tests have been used for comparisons. Inter group comparison (2 groups) was done using Mann Whitney U test. Intra group comparison was done using Friedman's (for >2 observations) followed by followed by pair wise comparison using Wilcoxon Signed rank test. Comparison of frequencies of categories of variables with groups was done using chi square test.

For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

RESULTS

The groups were demographically similar since a split-mouth study design was followed. A total of 80 surgical sites (in 40 subjects) were evaluated clinically preoperatively and postoperatively on 1st, 3rd and 7th days.

There was a statistically non-significant difference between the 2 groups for WBC count with t test ($p>0.05$, Table 1).

For all values in Table 2, there was a statistically non-significant difference between the 2 groups ($p>0.05$, Mann Whitney U test) except for, Line 2 on POD1 (1^{st} post-op day) ($p=0.010$), Pain on POD1 ($p=0.000$) and pain on POD 3 ($p=0.000$) with higher values in group 2 in all cases.

There was a statistically non-significant difference seen for the frequencies between the two groups ($p>0.05$) with Chi-square test (Tables 3a, 3b,3c,3d) for suppuration, wound dehiscence, erythema, lymph node palpability.

For all values, #= statistically non-significant difference, *= statistically significant difference and **= statistically highly significant difference

Table 1: Inter group comparison of values for WBC count (n=40 per group)

	Group	Mean	Std. Deviation	Std. Error Mean	T value	p value of t test
WBC count	1	7108.25	1317.536	208.321	.000	1.000#
	2	7103	1317.536	208.321		

Table 2: Inter group comparison of values (n=40 per group)

	Group	Mean	Std. Deviation	Std. Error Mean	Median	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Temp Preop (^{o}F)	1	97.775	.5839	.0923	98	800.000	0.000	1.000#
	2	97.775	.5839	.0923	98			

Temp POD1 (^{o}F)	1	97.715	.5668	.0896	97.7	800.000	0.000	1.000#
	2	97.715	.5668	.0896	97.7			
Temp POD 3 (^{o}F)	1	97.807	.5563	.0880	97.9	800.000	0.000	1.000#
	2	97.807	.5563	.0880	97.9			
Temp POD 7 (^{o}F)	1	97.825	.5852	.0925	98	800.000	0.000	1.000#
	2	97.825	.5852	.0925	98			
MO Preop (mm)	1	42.35	3.199	.506	42	800.000	0.000	1.000#
	2	42.35	3.199	.506	42			
MO POD1 (mm)	1	37.50	4.489	.710	37.5	623.000	-1.709	0.087#
	2	35.63	5.006	.792	36			
MO POD 3 (mm)	1	39.38	3.972	.628	40	655.500	-1.397	0.162#
	2	38.35	3.718	.588	38.5			
MO POD 7 (mm)	1	40.95	3.096	.490	41	776.500	-0.228	0.820#
	2	41.28	3.030	.479	40.5			
Line 1 Preop (mm)	1	115.18	9.120	1.442	118	800.000	0.000	1.000#
	2	115.18	9.120	1.442	118			

Line 1 POD1 (mm)	1	118.63	9.372	1.482	120	628.500	-1.653	0.098#
	2	122.13	9.318	1.473	125			
Line 1 POD 3 (mm)	1	117.20	8.936	1.413	118.5	657.000	-1.377	0.168#
	2	120.03	9.322	1.474	121.5			
Line 1 POD 7 (mm)	1	115.68	8.931	1.412	118	718.500	-0.785	0.432#
	2	117.03	9.133	1.444	119			
Line 2 Preop (mm)	1	107.00	9.816	1.552	108.5	800.000	0.000	1.000#
	2	107.00	9.816	1.552	108.5			
Line 2 POD1 (mm)	1	108.40	9.358	1.480	109	533.000	-2.573	0.010*
	2	112.58	10.270	1.624	114			
Line 2 POD 3 (mm)	1	107.63	9.591	1.516	109	611.500	-1.817	0.069#
	2	110.63	10.500	1.660	112			
Line 2 POD 7 (mm)	1	107.10	9.711	1.535	109	742.500	-0.555	0.579#
	2	108.08	9.900	1.565	109			
Pain Preop	1	0.33	1.859	.294	0.2	765.000	-0.341	0.733#
	2	0.18	1.738	.275	0.15			

Pain POD1	1	1.55	.932	.147	1	132.500	-6.534	0.000**
	2	3.98	1.310	.207	4			
Pain POD 3	1	.68	.656	.104	1	225.000	-5.837	0.000**
	2	1.85	.736	.116	2			
Pain POD 7	1	.23	.423	.067	0	720.000	-0.995	0.320#
	2	.33	.474	.075	0			
length of incision (mm)	1	30.48	4.904	.775	31	800.000	0.000	1.000#
	2	30.48	4.904	.775	31			
periosteal stripping (mm)	1	10.73	1.240	.196	11	800.000	0.000	1.000#
	2	10.73	1.240	.196	11			

Comparison of frequencies of categorical variables
A= Absent, P= Present
Table 3a: Suppuration

		group				
		1	2	Total	Chi square value	p value
suppuration	A	39	40	79		
	P	1	0	1	1.013	0.314#

Total	40	40	80		

Table 3b: wound dehiscence

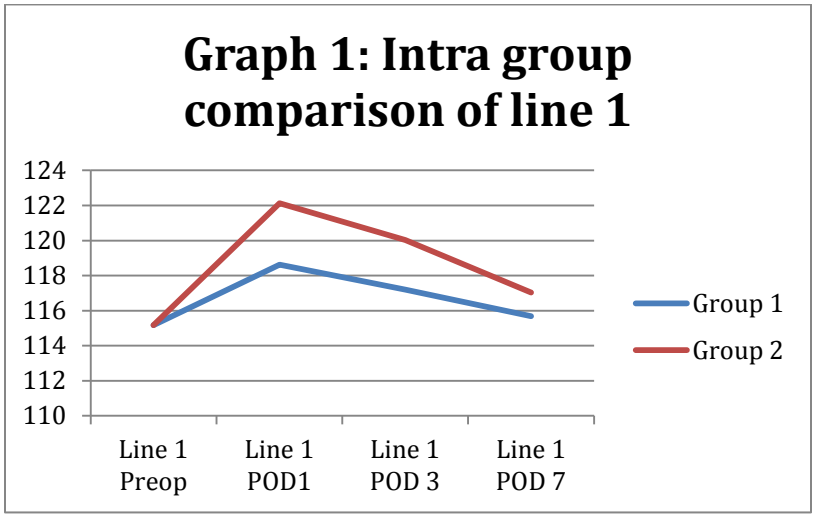
		group				
		1	2	Total	Chi square value	p value
wound dehiscence	A	39	40	79		
	P	1	0	1	1.013	0.314#
	Total	40	40	80		

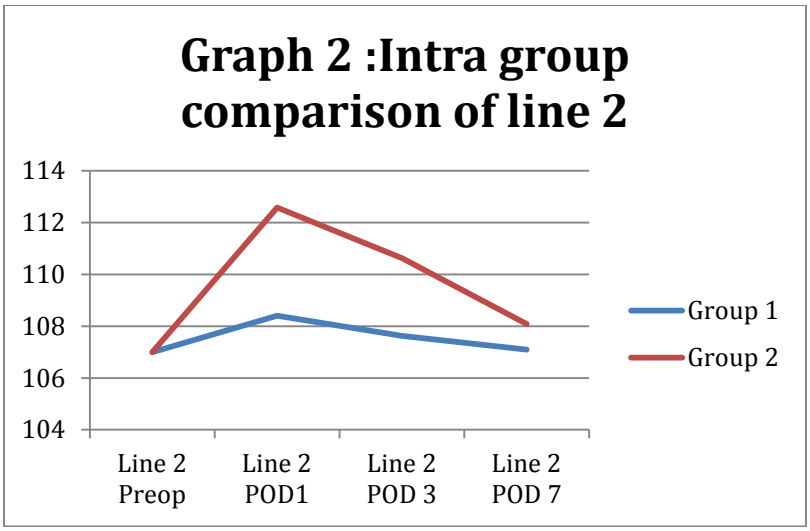
Table 3c: Erythema

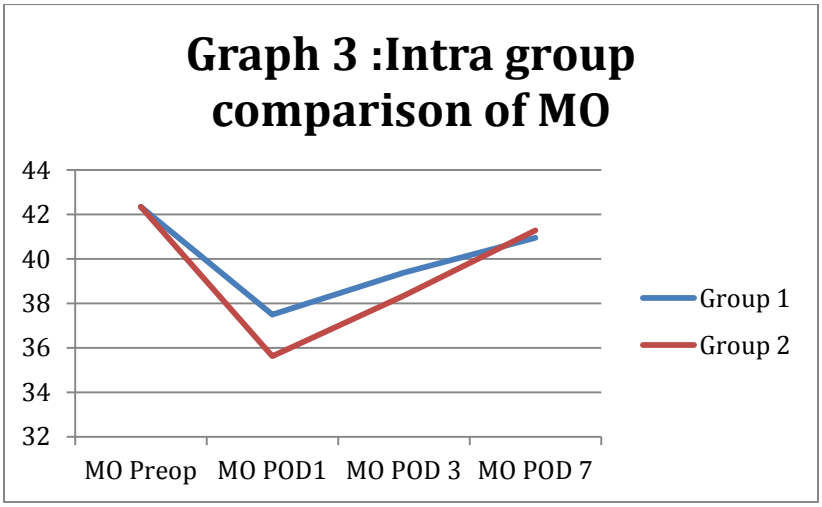
		group				
		1	2	Total	Chi square value	p value
erythema	A	39	40	79		
	P	1	0	1	1.013	0.314#
	Total	40	40	80		

Table 3d: lymph node palpability

		group				
		1	2	Total	Chi square value	p value
lymph nodes	Not palpable	40	40	80	---	---
	Total	40	40	80		







DISCUSSION

The reported frequencies of post-operative sequelae after third molar extraction surgeries are between 2.6 percent and 30.9 percent.[14]The spectrum of sequelae which range from postoperative infection, trismus, swelling and pain, depend on difficulty level of impaction, patient factors, surgical procedure, tissue handling, operating time, asepsis and medications prescribed/ administered [15,16]. The oral cavity houses maximum number of myriad of microorganisms. Hence, considering likelihood of postoperative infection and thereby, a compromised post-operative quality of life, systemic antimicrobial agents have been prescribed routinely, rather indiscriminately[17,18]. However, there is a risk of causing antimicrobial resistance and other systemic adverse effects due to these drugs.

This study was thus designed to avoid systemic antibiotic use and tested effectiveness of Stevia rebaudiana extract in comparison to Amoxicillin trihydrate powder, used in sockets of surgically extracted mandibular third molars, in subjects who did not show any preoperative signs of

locoregional infections. To the best of our knowledge, the use of Stevia extract for such a purpose has not been investigated and reported previously in the literature.

Standard operating protocols, standardized observer and similar difficulty level of impactions were ensured. Split mouth study design minimized inter-subject variability. So, the two groups were matched. It can also be noted that there is no statistically significant difference between the length of incision and periosteal stripping (p=1.000, Table 2). Thus, surgical trauma was also similar in both groups.

WBC counts, rise in axillary temperature, presence of suppuration and lymph node palpability were used as indicators of infection.
There was a statistically non-significant difference between the 2 groups for WBC counts with t test (p>0.05, Table 1).
There was a statistically non-significant difference between the 2 groups for axillary temperature with Mann-Whitney U test (p>0.05, Table 2). Even with Friedman's intra-group comparison, there was no significant difference in both groups for axillary temperature.
There was no clinically evident suppuration /lymph node palpability in both groups on all post-operative days. With chi square test, there was no statistically significant difference for suppuration and lymph node palpability (p>0.05, Table 3a, 3d) between the two groups.
The above suggest that Stevia has comparable anti-microbial effect as Amoxicillin. Gamboa et al and Tadhani et al have described antimicrobial properties of Stevia in details. [11, 19]
Evaluation of facial swelling was done using two facial lines as described. There was a statistically significant difference with Mann- Whitney U test on inter group comparison of line 2 (p=0.010, Table 2), with higher value in group 2 on POD1.
There was no statistically significant difference between 2 groups (p>0.05, Table 2) in mouth opening with Mann-Whitney U test. Intra-group comparison with Friedman's test for facial measurement lines (Graph 1,2) and mouth opening (Graph 3) did show statistically significant difference, with higher values in group 2 (ie, more facial edema) and lower values in grp2 (ie, greater reduction / more trismus). Post-surgical inflammatory response does lead to surgical edema and thereby a consequential decrease in mouth opening in all cases.
Pain as indicated by VAS Scores, shows a highly significant difference in two groups with higher values in group 2 on postoperative day 1 and 3, (p=0.000, Table 2, Mann- Whitney U test).
This denotes that Stevia not only helps in controlling the incidence of postoperative infection, but also, reduces inflammatory reaction, edema, and postoperative pain. Although, analgesic prescribed was the same in both the groups, Group 1 subjects were relatively more comfortable. The observed anti-inflammatory effect could be attributed to manoyl oxide [6], a diterpenoid content of Stevia rebaudiana. Stevia leaf extracts contain high amounts of folic acid, vitamin C and hence have radical scavenging (free radicals, hydroxyl radicals and superoxide anion) activities which promote wound healing. [11,19]
None of the surgical sites underwent wound dehiscence. There was no statistically significant difference with Chi square test (p= 0.314, Table 3b) between 2 groups.
Intra-oral erythema was comparable in the surgical sites in both groups and there was no statistically significant difference with Chi square test (p= 0.314, Table 3c) between 2 groups. Thus, wound healing was comparable and uneventful in both groups.
None of the subjects reported with any local or systemic adverse effects/drug reactions.

CONCLUSION

It can thus be concluded that the effectiveness of intra-alveolar stevia extract is comparable to that of intra-alveolar amoxicillin trihydrate powder for control of postoperative infection.

Stevia rebaudiana appears to offer a beneficial effect on the management of post-operative sequelae and improve overall patient's comfort in the postoperative period following impacted mandibular third molar surgery.

This is a simple and cost-effective method, which does not require additional skill or armamentarium and does not cause any adverse effects to the patient systemically and locally. Also, adverse systemic effects related to systemic antibiotic use can be avoided. The clinician does not have to rely on patient compliance as in with the use of oral antibiotics.

Limitations and future scope:

Present study included only healthy subjects due to ethical constraints. Future studies may be aimed at including patients with a medically compromised status.

Although this study was standardized to the maximum possible extent, similar studies with more variables and with larger sample size should be encouraged.

More complex inflammatory markers and/or culture methods could be used if adequate funding is available to execute such studies.

Conflict of interest:

No conflict of interest

Funding:

None

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