Effect of Oil Pulling on *Streptococcus Mutans* in Saliva - A Randomised, Controlled, Triple-Blind In Vivo Study

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ABSTRACT

Introduction: Ayurveda is one of the popular and accepted modality of alternative medicine. It is the system of traditional medicine native to Indian subcontinent and is practiced in other parts of the world in form of alternative medicine. This present study was designed to estimate the count of *Streptococcus Mutans* and to evaluate the effect of Oil Pulling on these counts in the saliva of adults.

Material and Methods: Each subject was assigned a specific number, and simple random sampling was done by using a table of random numbers by examiner A. Group-I (study group; sesame oil pulling) included 10 subjects and Group-II (study group; coconut oil pulling) included 10 subjects and Group III(control group; chlorhexidine) also included 10 subjects. Oral prophylaxis wasn't performed so that the subjects began the treatment regimen within normal existing level of plaque deposits. All the subjects were instructed to continue their normal home oral hygiene procedures, with Oil Pulling. All subjects had to perform Oil Pulling in morning prior to each of the clinical examination on the experimental days. Bacterial colony counts were assessed on day 0 (baseline-T0). And T1 after 30 days.

Results: The mean value for group 3 is 1.3070 that showed maximum bacterial count reduction. The mean for group 1(sesame oil) is 1.6900 that showed less count reduction as compared to group 1 but more count reduction as compared to group 2 (Coconut oil group). Whereas the group 2 showed mean of 3.4840 which showed the least count reduction as compared to the group 1 and group 3.

Conclusion: The results showed that there was bacterial count reduction in all the groups. Among these the chlorhexidine group performed best followed by sesame oil group and finally the coconut oil group.

Keywords: *S. Mutans, Species,* Mitis Salivarius Bacitracin

INTRODUCTION

Complementary and alternative medicine is gaining popularity over the conventional allopathic medicine because products and practices used are natural and safe. Ayurveda is one of the accepted modality of Complementary and alternative medicine. It is a type of traditional medicine native to Indian subcontinent and is practiced in other parts of the world as a form of alternative medicine.[1]

The concept of oil pulling is not new. It's been discussed in the Ayurvedic text *Charak Samhita* as '*kavalagraha*' or '*kavala gandoosha*.' Dr. Karach popularized the concept of oil pulling in the 1990s in Russia. Oil pulling can be done using edible oils like sunflower or sesame oil.[2,3]

The Charaka Samhita describes two types of mouthwashes, gandoosha and kavalagra, which were used for different purposes. Kavalagra was usually a type of herbal preparation in a paste or bolus form, which was then subsequently diluted to form a liquid. Oral Cavity was then filled with the kavalagra, which was then retained until nasal discharge or lacrimation occurred. Gandoosha, on the other hand, usually consisted of liquids, mostly of them were essential oils.[3]

Commonly used gandooshas consisted of herbal products like triphala, dasamoola, guggulu, pippali and sarshapashunti. These were then grounded, mixed in hot water for gargling, or otherwise mixed in honey or cow's milk before using it as a mouthwash. Mouthwashes consisted primarily of essential oils, which were sahacharadi taila and irimedadi taila, that were also used for management of periodontal disease.[3]

Mutans Streptococci possess virulent traits which supports their role in caries process. They convert dietary carbohydrates into acid, that lowers the pH, and solubilises calcium phosphate of the enamel to produce a caries lesion.[4]

Currently, five different types of media are available for the isolation of *S. mutans*. These are Mitis Salivarius with Bacitracin (MSB), Mitis Salivarius with Bacitracin and Kanamycin (MSKB), Glucose-Sucrose-Tellurite Bacitracin (GSTB), Trypticase soy with sucrose and Bacitracin (TYS20B) and Tryptone-yeast extract Cysteine with Sucrose and Bacitracin (TYCSB). The addition of 0.2U/mL bacitracin and 20% sucrose to MS led to an improved medium (MSB) that had high selection for *S. Mutans*.[5]

This present study was designed to estimate the count of *Streptococcus Mutans* and to evaluate the effect of Oil Pulling on these counts in the saliva of adults.

MATERIAL AND METHODS

The study was carried out in the Department of Conservative Dentistry and Endodontics, Darshan Dental College and Hospital, Udaipur.

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Source of Data

After screening the entire batch of veterinary students, at the Regional Disease Diagnostic Centre, Department of Animal Husbandry, Udaipur, Rajasthan and under graduate students of Darshan Dental College and Hospital, Loyara in the age range 19-21 years were recruited for a 30 days study.

Inclusion Criteria

- Subjects that were willing to participate.
- Subjects having at least 20 natural teeth in there permanent dentition.
- Subjects having mild to moderate gingivitis and plaque accumulation.
- Subjects refrained from any form of dental treatment during the study period.

Exclusion Criteria

- Subjects those were allergic to the oil used.
- Subjects having systemic diseases and under a antibiotic course.
- Subjects those were undergoing orthodontic treatment or using intraoral artificial prosthesis.
- Subjects those were using any other type of available mouth wash / rinse.

Pre-Study Procedures

The subjects were blinded about the investigation in order to avoid any bias. To participate the subjects needed to sign a witnessed consent form and committed themselves to the study. The Ethical clearance was taken from the Scientific And Ethical Review Committee, Darshan Dental College and Hospital, Udaipur.

Materials Used In The Study

- Materials Tested
- Sesame Oil (Klf Nirmal)
- Cocunut Oil (Klf Nirmal)
- Chlorhexidine, (Hexidine Icpa)

Materials For Prepration of Culture Media

- Mitis Salivarius Agar Base (Himedia)
- Sucrose (Analar)
- Potassium Tellurite 1% (Himedia)
- Bacitracin Zinc Salt (Himedia)

Accessories

- Weighing Machine
- Petridish (HIMEDIA)
- Brain Heart Infusion Broth (Himedia)
- Incubator
- Autoclave
- Laminar Flow Chamber
- Sterile Forceps
- Loops
- Sterile Swab (Himedia)
- Test Tubes (Borosil)
- Micro Pipette (tarsons)
- Microslides
- Bunsen Burner
- Sample Collecting Bottle
- Colony Counter

Methodology

Media Prepration

The culture media was prepaped by mixing 90g mitis salivarius dehydrated agar (Himedia), 20% sucrose (Analar Merck, Kilsyth, Victoria, Australia), 10g Agar (himedia) with 1 litre distilled water and sterilized then 1ml 1% potassium tellurite (Himedia), and 0.2U bacitracin (himedia) were added and stirred and were poured into agar plates. Poured agar plates were sealed and stored at 4°C.

Experimental Protocol

This study was carried out at the Department of conservative and endodontics.

Each subject that participated in study was given a specific number, and simple random sampling was done using the table of random numbers by examiner A. Group-I (study group; sesame oil pulling) included 10 subjects and Group-II (study group; coconut oil pulling) included 10 subjects and Group III(control group; chlorhexidine) also included 10 subjects.

The subjects did't underwent Oral prophylaxis so that the subjects began the treatment regimen with their normal existing level of plaque deposits. All subjects were allowed to continue their normal home oral hygiene procedures, along with Oil Pulling. All subjects were told to perform their routine morning Oil Pulling before each of the clinical examination on the experimental days. Bacterial colony counts were assessed on day 0 (baseline-T0). And T1 after 30 days.

All the subjects were told to follow the below mentioned, method of Oil Pulling.

The Oil Pulling Procedure

Take 10-15 ml of refined oil (sesame/coconut) using a tea spoon, approximately 6 gms or till the mouth is about half filled. Sip, suck and pull the oil through the teeth. Lift your chin a bit, and start swishing liquid from right to left, back to front and vice versa. Concentrate and imagine liquid moving inside the mouth. Swish for approximately 8-10 minutes or till you feel fullness in your mouth. At the end of the procedure the oil should be milky white, thin and frothy. Spit the liquid.

Sample Collection and Transportation

Approximately 2 ml of unstimulated saliva were collected from each volunteer two hours after eating, drinking or having a routine hygiene procedure as it could have affected the growth of the bacteria. A baseline saliva sample was collected by Examiner B. These samples were immediately transferred to the laboratory. The microbiologist (Examiner C) diluted the specimen serially and then spread on the culture media with sterile swab, which was incubated at 37°C for 72 hours. The number of colonies were counted using a colony counter and then the number of colonies was multiplied by the dilution factor. The colony count was then categorized into 3 different classes.

- Class 1 - $< 1.5 \times 10^8$ colony-forming units.
- Class 2 - $1.5 - 3 \times 10^8$ colony-forming units.
- Class 3 - $> 3 \times 10^8$ colony-forming units.

The study group was subjected to oil pulling with sesame Oil (Klf nirmal, Kerela) and coconut oil (Klf Nirmal, Kerela) and the control group was given 0.12% chlorhexidine mouthwash (Hexidine ICPA) for 1 minute every day in the morning before brushing for 30 days. Reassessment of the index scores (Examiner A) and collection of saliva (Examiner B) for measuring the colony count of the aerobic microorganisms was done after 30 days. Among the 30 saliva samples collected in this study, 3 samples that were contaminated with a confluent growth of Bacillus spores were repeated and reassessed.

STATISTICAL ANALYSIS

The before and after values of the total bacterial colony count between groups were compared using one way ANOVA and within group comparison was done using a paired t-test. In the present study, P<0.05 was considered as the level of significance. The statistical analysis was done using SPSS software, Version 19 (SPSS Inc, Chicago). The examiner (A), (B), and (C), and the statistician were blinded as to the division of the groups.

RESULTS

The result showed (table 2) that on comparison of before value between the groups showed no significant difference. But after group comparison showed that there was significant difference in the result between groups after application of one way ANOVA using SPSS software version 19.

On application of POST HOC test tukey B test (Table 3). The after group comparison result between group 1 and group 2 showed significant value, between group 2 and 3 showed significant value and between group 1 and group 3 showed no significant value.

On application of T test (table 4) for within group comparison.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
before	1	10	3.2890	1.78073	.56312	2.0151	4.5629
	2	10	4.0940	1.28358	.40590	3.1758	5.0122
	3	10	4.2380	1.31991	.41739	3.2938	5.1822
	Total	30	3.8737	1.48879	.27181	3.3177	4.4296
after	1	10	1.6900	.61190	.19350	1.2523	2.1277
	2	10	3.4840	.93323	.29511	2.8164	4.1516
	3	10	1.3070	.93181	.29466	.6404	1.9736
	Total	30	2.1603	1.25996	.23004	1.6899	2.6308

*The values in the table denotes the mean value of bacterial count, *P value of < 0.05 was considered to be statistically significant, *Salivary Mutans Streptococci and total viable count (CFU/ ml, mean × 108)

Table-1: Showing inter and intra group comparison of mean values.

		Sum of Squares	df	Mean Square	F	Sig.
Before	Between Groups	5.231	2	2.616	1.196	.318
	Within Groups	59.047	27	2.187		
	Total	64.278	29			
After	Between Groups	27.015	2	13.507	19.172	.000
	Within Groups	19.022	27	.705		
	Total	46.037	29			

P value of < 0.05 was considered to be statistically significant, Salivary Mutans Streptococci and total viable count (CFU/ ml, mean × 108)

Table-2: ANOVA test was applied for significance value.

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.
Before	1	2	-.80500	.66135	.454
		3	-.94900	.66135	.338
	2	1	.80500	.66135	.454
		3	-.14400	.66135	.974
	3	1	.94900	.66135	.338
		2	.14400	.66135	.974
After	1	2	-1.79400*	.37538	.000
		3	.38300	.37538	.571
	2	1	1.79400*	.37538	.000
		3	2.17700*	.37538	.000
	3	1	-.38300	.37538	.571
		2	-2.17700*	.37538	.000

*A P value of < 0.05 was considered to be statistically significant

Table-3: Showing tukey HSD test that was for mean difference, std error, and significance value.

		Paired Differences		t	df	Sig. (2-tailed)
		95% Confidence Interval of the Difference				
		Lower	Upper			
Pair 1	One before – one after	.53449	2.66751	3.396	9	.008
Pair 2	Two before – two after	.22486	.99914	3.576	9	.006
Pair 3	Three before – three after	1.92075	3.94125	6.563	9	.000
*P value of < 0.05 was considered to be statistically significant						

Table-4: Paired Samples Test of the groups for significance value.

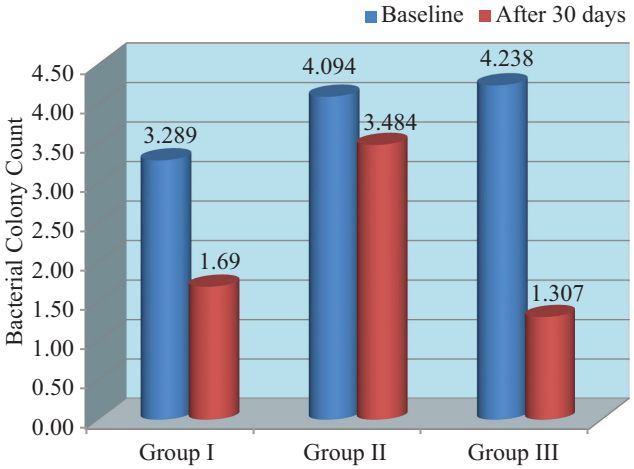

Figure-1: Showing intra and inter group comparison for group 1, group 2, group 3.

It showed that value for group 3 was highly significant. The mean value for group 3 is 1. 3070 (table 1) that showed maximum bacterial count reduction. The mean for group 1 is 1.6900 that showed less count reduction as compared to group 1 but more count reduction as compared to group 2. Whereas the group 2 showed mean of 3.4840 which showed the least count reduction as compared to the group 1 and group 3.

DISCUSSION

The mechanism of the action of oil pulling therapy is not very clear. It has been claimed that swishing of oil in the mouth activates enzymes and then draws the toxins out of the blood. The bottom line is that the oil pulling procedure actually cannot pull the toxins out of the blood because of oral mucous membrane, that does not act as a semi permeable membrane to allow the toxins to pass through. The antioxidants present in it are sesamin, sesamolin and sesaminol. These lignans have actions on the living tissues like - Detoxification of toxins, antioxidant effect, potentiating the action of vitamin E, which prevents lipid peroxidation and antibiotic effect in that it helps in the destruction of microorganisms.[1]

In the present study, group 1-sesame oil group showed no significant difference in before group comparison but after group comparison showed significant (.008) reduction in bacterial counts. (Table 4) this may be due to saponification or emulsification that occurs during oil pulling therapy.

Ashwini A. Patil et al reviewed, Oral Health and Ayurveda and found out that, Oil pulling therapy can be done using oils like sesame oil. Oil pulling therapy is very effective against plaque induced gingivitis for both in the clinical and microbiological assessment.[6]

Seema Diwan et al studied efficacy of oil pulling and found that, oil pulling therapy as an adjunct to oral hygiene protocol is efficient in treating periodontal diseases in future.[29]

Ashokan et al studied the mechanism of oil pulling therapy and checked antibacterial activity of ligans and sesame oil and whether saponification or emulsification occurs during oil pulling therapy and found that sesamin and sesamolin were not having any antibacterial effect against oral microorganisms like *S. Mutans, S.Mitis, S.Viridans.* though emulsification occurs during oil pulling therapy.[7,8]

Group 2 coconut oil pulling group showed no significant difference in before group comparison but after group comparison showed significant reduction (.006) in bacterial counts.(table 4) this may be due to monolaurin, the monoglycerides of lauric acid from coconut oil has shown antimicrobial activity against various gram positive and gram negative microrganisms.

Puneeta Duggal reviewed coconut oil and found that Oil pulling using coconut oil can help in the prevention of halitosis, decay progression and gingivitis. It exerts a saponification and emulsification on bacterias like *Streptococcus Mutans*. Coconut oil emulsifies the lipid present in the cell membranes.[9] Bruce Fife found out that the coconut oil acts like a cleanser. When you suck it in your mouth and swish it around your teeth and gums, it "pulls" out bacteria and other debris.[10]

Group 3 Chlorhexidine group showed no significant difference in before group comparison but after group comparison showed significant reduction in bacterial counts (Table 4) This is because, Chlorhexidine has a broad spectrum action and substantivity.

Shruti Balagopal reviewed Chlorhexidine: The Gold Standard Antiplaque Agent as Chlorhexidine has been used as a broad spectrum antiseptic since the 1950's. Its antibacterial action is due to its ability to disrupt the bacterial cell membrane. Increasing the permeability that results in cell lysis. It can be either bacteriostatic or bactericidal depending upon the dose.[11]

Setu Mathur reviewed Chlorhexidine, Its efficacy is attributed to its bacteriostatic and bactericidal properties and also its substantivity within the oral cavity. Chlorhexidine bi-cationic molecule attribute to its antimicrobial property.[12] Radhika Gupta et al found that Chlorhexidine is a bisbiguanide. It is active against both Gram-positive and Gram-negative strains as well as fungi.[13]

In conclusion, the present study could be a evidence to demonstrate that oil-pulling therapy with some edible oils

could be used as a preventive home therapy to maintain oral hygiene, especially in developing countries. However, further studies are needed to investigate the mechanisms of the action of the oil on these microorganisms.

CONCLUSION

An in vivo experimental study was formulated to evaluate the effect of oil pulling on *Streptococcus Mutans*.

Sesame oil, coconut oil, Chlorhexidine was used in this study for oil pulling. The results showed that there was bacterial count reduction in all the groups. Among these the chlorhexidine group performed best followed by sesame oil group and finally the coconut oil group.

Results from the present study can be a scientific evidence to demonstrate that oil-pulling with some edible oils can be used as a preventive home therapy to maintain oral hygiene, especially in developing countries. However, further studies are needed to investigate the mechanisms of the action of the oil on these microorganisms or dental plaque, as well as long-term effects in clinical trials.

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REFERENCES

1. Hebbar A, Keluskar V, Shetti A. Oil pulling – Unraveling the path to mystic cure. J. Int. Oral Health. 2010; 2:11-4.
2. Asokan S, Rathan J, Muthu M.S, Rathna P.V, Emmadi P, Raghuraman, Chamundeswari. Effect of oil pulling on streptococcus mutans count in plaque and saliva using dentocult SM strip mutans test: A randomized controlled, triple-blind study. J Indian Soc. Pedod Prevent Dent. 2008;26:12-7.
3. Surathan N, Kurumathur A.V. Traditional therapies in the management of periodontal disease in India and China. Periodontol 2000. 2011;56:14-24.
4. Jeevarathan J, Deepti A, Muthu M.S, Rathna P.V, Chamundeeswari G.S. Effect of fluoride varnish on streptococcus mutans count in plaque of caries-free children using dentocult SM strip mutans test: A randomized controlled triple blind study. J. Indian Soc. Pedod Prevent Dent. 2007;25:157-63.
5. Wan AKL, Soew H.K, Walsh L.J, Bird P.S. Comparison of five selective media for the growth and enumeration of streptococcus mutans. Aust Dent J. 2002;47:21-6.
6. Patil A.A, Deosarkar B, Niras S, Chaudhari V. Oral Health and Ayurveda. J of Interdisciplinary Dent Sciences. 2013; 2:10-14.
7. Asokan S, Rathinasamy T.K, Inbamani N, Menon T, Kumar S.S, Emmadi P, Raghuraman R. Mechanism of oil-pulling therapy- Invitro study. Ind J of Dent Res. 2011;22:34-37.
8. Sirisha K, Devi P.K. Oil pulling- a comprehensive cost-effective domiciliary remedy. Int J Of Res In Dent. 2014; 4:1-5.
9. Duggal P. Cocunut: The Life Tree. Guident. Oct 2013;60-62.
10. Fife B, Dental health with oil swishing. Well Being Journal. Dec 2008;39-42.
11. Balagopal S, Arjunkumar R. Chlorhexidine: The gold standard antiplaque agent. J. Pharm. Sci. and Res. 2013;5:270-74.
12. Mathur S, Mathur T, Srivastava R, Khatri R. Chlorhexidine: The gold standard in chemical plaque control. Natl J Physiol Pharm Pharmacol 2011; 1:45-50.
13. Gupta R, Chandravarlar V, Galgali S.R, Mishra M. Chlorhexidine, a medicine for all the oral diseases. Global J of Med and Pub Health. 2012;1:43-48.

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