Mechanism of oil-pulling therapy -*In vitro* study

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Abstract

Background: Oil pulling has been used extensively as a traditional Indian folk remedy without scientific proof for many years for strengthening teeth, gums and jaws and to prevent decay, oral malodor, bleeding gums and dryness of throat and cracked lips.

Aim: The aim of this study was to evaluate the antibacterial activity of sesame oil and lignans isolated from sesame oil on oral microorganisms and to check whether saponification or emulsification occurs during oil-pulling therapy.

Materials and Methods: The in vitro study was carried out in three different phases: (1) Antibacterial activity of the lignans and sesame oil were tested by minimum inhibitory concentration assay by agar dilution method and agar well diffusion method, respectively. (2) Increase in free fatty acid level of oil and the quantity of sodium hydroxide (NaOH) used up in the titration are good indicators of saponification process. This was assessed using analytical tests for vegetable oils. (3) Swished oil was observed under light microscope to assess the status of the oil, presence of microorganisms, oral debris and foreign bodies.

Results: Sesamin and sesamolin isolated from sesame oil did not have any antibacterial effect against oral microorganisms like Streptococcus mutans, Streptococcus mitis and Streptococcus viridans. Emulsification of sesame oil occurs during oil-pulling therapy. Increased consumption of NaOH in titration is a definite indication of a possible saponification process.

Conclusion: The myth that the effect of oil-pulling therapy on oral health was just a placebo effect has been broken and there are clear indications of possible saponification and emulsification process, which enhances its mechanical cleaning action.

Keywords: Emulsification, lingual lipase, oil-pulling therapy, sesame oil

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Oil pulling or oil swishing, in alternative medicine, is a procedure that involves swishing oil in the mouth for oral and systemic health benefits. Oil-pulling therapy can be done using edible oils like sunflower or sesame oil. [1] Oil-pulling therapy with sesame oil has been used extensively as a traditional Indian folk remedy for many years for strengthening teeth, gums and jaws and to prevent decay, oral malodour, bleeding gums and dryness of throat and cracked lips.

Online searches in PubMed and other databases showed only testimonies and literature on personal experiences, until recently. Randomized controlled trials conducted by Asokan et al. have shown that the oil-pulling therapy with sesame oil has been equally effective in reduction of Streptococcus mutans count, plaque index, modified gingival index scores and plaque-induced gingivitis as compared to chlorhexidine mouthwash. [2],[3] The myth that oil-pulling therapy was just a placebo effect has been broken but the mechanism of action remains unclear. The following in vitro research was planned in three phases with the following aims and objectives:

1. To evaluate the antibacterial activity of sesame oil and lignans (sesamin and sesamolin) isolated from sesame oil against oral microorganisms.
2. To check whether saponification or any chemical reaction occurs during oil-pulling therapy.
3. To detect whether emulsification of oil and mechanical removal of bacteria occurs during oil-pulling therapy.

Materials and Methods

Phase I

In NIIST Tiruvananthapuram, a combination of sesamin and sesamolin Compound (A), in the ratio of the 80:20 was isolated from the sesame oil (Idhayam oil, VVV Sons, Virudhunagar, India) and high-pressure liquid chromatography (HPLC) was used to check the purified sample. In MK University Madurai, the same brand of sesame oil was subjected to fractional crystallization and column chromatography to remove the saponifiable glycerides, leaving behind the unsaponifiable fraction. Two compounds were isolated - sesamin (B) from column chromatography and sesamin (C) from fractional crystallization. The three compounds were brought to Department of Microbiology, University of Madras, Taramani, Chennai to check for their antibacterial effect. The research student who did the test was blinded of the nature of the three compounds.

Antibacterial activity of the three compounds (A, B, C) were tested by minimum inhibitory concentration (MIC) assay by agar dilution method [4] using Mueller Hinton agar with 5% sheep blood (MHBA). MHBA plates of each compound with concentration ranging from 0.062 to 2 mg/mL were prepared. Ten microliters of 24-hour-old cultures (*S.mutans*, *Streptococcus mitis* and *Streptococcus viridans*) adjusted to 0.5 Mac Farland standard were spot inoculated on to the MHBA and incubated at 37°C with 5% carbon dioxide for 24 hours. MIC was determined as the lowest concentration of the compound that inhibited visible growth. None of the compounds showed antibacterial activity against any of the bacteria tested and the MIC values were more than 2 mg/mL

Antibacterial effect of sesame oil was tested by agar well diffusion method using MHBA. Using a sterile cotton swab 24-hour-old cultures (*S. mutans*, *S. mitis* and *S. viridans*) adjusted to 0.5 Mac Farland standard were swabbed on to the MHBA. Using 6-mm well cutter, four wells were made in each plate. Fifty microliters of different dilutions (1:2, 1:5, 1:10 and 1:20) diluted in Tween 20 were added into their respectively marked wells and incubated for 24 hours at 37°C in the presence of 5% carbon dioxide. After incubation the plates were observed for the zone of inhibition around the wells. None of the concentrations of the oil showed any inhibitory activity for any of the organisms tested. The cultures of micro-organisms from the agar medium were smeared on clean grease-free glass slides, Gram-stained and examined under the microscope to verify their presence.

Phase II

The sesame oil used in the study had a free fatty acid (FFA) level less than 1%. Determination of FFA is done as follows: [5] To the sample to be investigated, 70 ml of neutralized spirit is added and warmed to dissolve the oil in the spirit. A drop of phenolphthalein is added as an indicator to the warmed oil and it is titrated against

standard sodium hydroxide (NaOH) until a pink color appears. FFA is calculated based on the volume of NaOH used up in the titration. Increase in the FFA level and the titer volume of NaOH could be a good indicator of the saponification process.

The samples, which were investigated include: (1) 10 ml of oil alone, (2) 10 ml of oil and about 3 g of saliva together, (3) 10 ml of oil and about 3 g of saliva shaken (in vitro) in a conical flask for 15 minutes and (4) 10 ml of oil swished in mouth for 15 minutes (in vivo) and spit into a conical flask. Five milliliter of water is used to rinse the mouth and it is also spit into the conical flask. The saliva for the study was obtained from four healthy volunteers with good oral health and no systemic disease. Saliva from each volunteer was used independently for the tests.

For sample 1, the titer value was 3 ml of NaOH, which corresponds to a FFA value of 0.89%. With the samples 2 and 3, the titer value increased to 3.1 to 3.2 ml of NaOH. But in sample 4, the titer value increased from to 3.8 to 4.4 ml of NaOH. Chemist B repeated all the tests and the same results were obtained. There was clear evidence that after oil pulling the amount of NaOH used up increased appreciably. It was surprising to note that the titer value did not increase in the in vitro sample 3. This indicated that some component in saliva when reacted with sesame oil in the mouth increased the amount of NaOH used up. The increased titer value could be due to the formation of phenolic compounds like sesamol or FFAs during oil pulling. To 1 g of sample 4, a few drops of neutral ferric chloride were added. Blue color appears in the presence of phenol. [5] No color appeared in the test indicating that the phenol test was negative. To 2 g of sample 4 furfural was added. Pink color appears in the presence of sesamol. [5] In this study, sample 4 obtained after oil pulling had no sesamol and hence no color change was evident. It can be concluded that there was a definite increase in NaOH used up, indicating a possibility of the formation of FFAs as a result of saponification process during oil pulling.

Phase III

Oil pulling was done for 30 minutes and a drop of the swished sample was taken from the mouth every 5 minutes and observed under a light microscope using oil immersion lens (Ca 1500?). Each sample was smeared on a clean grease-free slide and stained using Gram stains. During the observation, status of the oil, presence of micro-organisms, other cells and foreign bodies were assessed. After 30 minutes the entire sample was collected in a hard glass test tube and centrifuged at 3000 rpm for 10 minutes. The oil separated on the top leaving a soapy layer in the middle and the sediments at the bottom.

Under the microscope, in the 5-minute sample the oil remained as relatively large globules. Very less number of Gram-positive bacteria and fibrous foreign particles were

seen. As time progressed from 10 to 15 minutes, the oil droplets became smaller, more colonies of Gram-positive bacteria and few squamous epithelial cells were seen. The decrease in size of the oil globules was a clear indication of the ongoing emulsification process. From 15 to 30 minutes there was progressive reduction of oil globule size but after 25 minutes only isolated bacteria were visible. The sediments in the bottom layer of the final collected sample had squamous epithelial cells and oral debris. The middle soapy layer had more of isolated bacteria and the top layer was purely oil globules. From the present study it may be inferred that the emulsification process of oil started in 5 minutes after oil-pulling therapy. The emulsification was a result of the agitation of the oil in the mouth because of the swishing process and this process may be responsible for the formation of a soapy layer. The emulsification process could alter the adhesion of the bacteria on the tooth surface, remove the superficial worn-out squamous cells and improve oral hygiene.

Discussion

Sesame oil is obtained from the seeds of the plant Sesamum indicum (Pedaliaceae family) largely by pressing methods. Sesame oil is relatively high in unsaponifiable substances, but these consist of sterols and other substances not removable by refining. The unsaponifiable fraction contains a class of substance, sesamin, sesamolin and sesamol, not found in other fats. Sesamin and sesamolin differ in their molecular formula by one oxygen atom and are mostly isolated together as a compound. Sesamol is not freely available in the oil and is formed as a result of hydrolysis of sesamolin. Sesamol has a potent antioxidant property. Sesamolin and sesamin are present in sesame oil in a very small amount (<1%) and the isolation of the compound involves a time-consuming technique sensitive procedures. The phase I study was planned with an idea that the unsaponifiable fraction of the sesame oil could have some antibacterial effect against the oral micro-organisms. But there was no antibacterial activity for the unsaponifiable fractions, sesamin and sesamolin. We cannot totally rule out the antibacterial effect of the whole oil, as complete solubility of the whole oil in an organic solvent, which had no antibacterial effect, was not possible. More research has to be carried out to prove or disprove the antibacterial effect of whole sesame oil on oral micro-organisms.

Fats of the diet and edible oils like sesame oil are mostly triglycerides, each molecule of which is composed of a glycerol nucleus and three fatty acid side chains. [6] The first step in fat digestion is to break fat globules into very small sizes physically so that the water-soluble digestive enzymes can act on the globule surfaces. [6],[7] This process is

called emulsification of fat and it begins by agitation. Each time the diameters of the fat globules are significantly reduced the total surface area of the fat increases manifold. An increase in total surface area as much as 1000-fold is caused as a result of emulsification process. [6],[8]

Lipase is any enzyme that cleaves a fatty acid anion hydrolytically from a triglyceride or phospolipid. Only the ester bonds at carbons 1 and 3 (?-positions) are attacked and the products of the reaction arte 2 mol of fatty acids and 1 mol of 2-acylglycerol (?-monoglyceride) per mole of substance. [9],[10] Free fatty acids are formed as a result of lipase action. [11] Lipases act only when the substrate is present in an emulsified form at the interface between water and the substrate. The rate of lipase action depends on the surface area of the dispersed substrate. Lipase enzymes are water-soluble compounds and can attack the fat globules only on their surfaces. Small amount of triglyceride is digested by lingual lipase that is secreted by lingual glands (von Ebners) in the mouth. [12],[13],[14] The amount of digestion could vary from 10 to 30%. [6],[13],[15] Various studies by Hamosh [16],[17],[18] have proved the localization of salivary lipase in the zymogen granules of the serous glands of the tongue and their role in digestion of fat. When acted upon by lingual lipase enzyme, the emulsified oil is broken down into two monoglycerides and FFAs. The increase in NaOH used up in the phase II study could be because of the FFA produced by the lingual lipase action on the emulsified sesame oil. Hydrolysis of fat is a highly reversible process [6] and this explains why the oil could be separated as a whole on centrifugation in the phase III study. There is a definite indication of a possible saponification and emulsification process during oil-pulling therapy, which enhances the cleansing action of the sesame oil during oil-pulling therapy.

Conclusions

- Sesamin and sesamolin isolated from sesame oil did not have any antibacterial effect against oral microorganisms like S. mutans, S. mitis and S. viridans.
- Emulsification of sesame oil occurs during oil-pulling therapy.
- Increased consumption of NaOH in titration is a definite indication of a possible saponification process.

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