Assessment of Viability and Function of Periodontal Ligament Fibroblasts Isolated from Periodontally Affected Human Teeth as Compared to Healthy Ones Upon Exposure to Sesame Oil (In *Vitro* Study)

Original Article

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ABSTRACT

Objective: The aim of this study was to investigate the effect of sesame oil on proliferation and function of periodontal ligament fibroblasts isolated from either healthy teeth or teeth with chronic periodontitis.

Design: The current study included a total of 16 teeth. Eight periodontally healthy teeth indicated for extraction for orthodontic treatment and 8 teeth with chronic periodontitis. Fibroblasts were isolated from periodontal ligaments and were characterized via flowcytometry. Cultured cells from both healthy and diseased teeth, were then divided into two groups, sesame oil group treated by sesame oil in a dose 2.5 µl of oil/ml of media at 24 hours and untreated group which served as control. MTT assay was used to assess fibroblasts' proliferation while quantitative PCR was used for quantification of basic fibroblast growth factor to assess fibroblasts' function.

Results: Within the present study, the highest mean cell proliferation value was recorded in group 2 (healthy treated with oil) while the lowest value was recorded in group 3 (periodontitis) with statistically significant difference between groups. The highest mean basic fibroblast growth factor expression was recorded in group 4 (periodontitis treated with oil) and the lowest value was recorded in group 3 (periodontitis) with statistically significant difference between groups demonstrating that sesame oil possesses a positive effect on fibroblasts proliferation and function.

Conclusion: Sesame oil is a promising biocompatible antioxidant and anti-inflammatory natural extract that can stimulate periodontal ligament fibroblasts proliferation and function through increasing basic fibroblast growth factor expression in *vitro* and therefore it may hold the potential to enhance periodontal regeneration in *vivo*.

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Key Words: Basic fibroblast growth factor, human, periodontal ligament fibroblast, sesame oil.

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INTRODUCTION

Periodontal disease is a chronic inflammatory disease of the supporting tissues surrounding the tooth[1]. It is considered as one of the most concerning global oral health burdens affecting almost 10-15% of world's population[2,3] and 89.8% of Egyptian population[4]. It is mostly prevalent in adults (35 years or older) but may also occur in younger individuals[5]. Pathogenic anaerobic microflora adhering to the tooth surfaces have been recognized as the causative agents of periodontitis[6]. Gingival bleeding, apical migration of epithelial attachment, connective tissue breakdown, alveolar bone loss and eventually tooth loss are common sequalae if left untreated[7,8].

The principal cells in the periodontal ligaments (PDL) are the fibroblasts (PDLFs), they are responsible for the synthesis of collagen bundles in the PDL in addition to renewal and replacement of old and damaged collagen fibrils[9,10]. PDLFs also secrete growth factors in the extracellular environment essential for homeostasis[11,12]. Fibroblast proliferation and basic fibroblast growth factors (bFGF) expression can be promoted by a number of influencing factors[13] which subsequently can promote the regeneration of periodontal tissues[14].

Alternative medicine is a promising branch of medicine that sought the management of different diseases. Numerous natural extracts are under continuous investigation in the research field as a replacement to conventional, chemical-based medications[15]. Yet, the application of natural extracts needs further research to test the different modes of action and side effects if present. Sesame oil is a natural extract with antioxidant and anti-inflammatory properties[16,17]. It consists of saturated and unsaturated fatty acids, phytosterols, antioxidants including tocopherols and vitamin E, in addition to oleic and linoleic acid which act as anti-inflammatory agents[18–20].

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Moreover, sesame oil is rich in are methylene dioxyphenyl compounds called lignans. Sesame seed contains two major groups of lignans glycosylated water-soluble lignans (sesaminol triglucoside, pinoresinol triglucoside, sesaminol monoglucoside, pinoresinol monoglucoside and oil-soluble lignans (sesamin, sesamolin, sesaminol, sesamolinol, and pinoresinol)[21]. Lignans and their derivatives are responsible for the antioxidative property of sesame seed, they also prevent oxidation of the oil and give it a long shelf life and stability[21,22].

Sesame oil has been used for the management of number of ailments like pain, fever, inflammation, constipation, it has been also used to accelerate healing of burns and wounds and to prevent number of disorders including hypertension, hypercholesterolemia, aging and can be used as a potential anti-cancerous agent[23,24]. Moreover, sesame oil was found effective in decreasing plasma triacylglycerol and arachidonic acid levels it also displays anti-inflammatory and estrogenic activities[25,26]. Considering the regenerative potential of sesame oil, the present study was designed to test the effect sesame oil application on cultured PDLFs cultivated from healthy and periodontally affected teeth regarding cellular proliferation and bFGF expression.

MATERIAL AND METHODS

Study population and ethical statement

According to previous study[27], a total sample size of 16 human teeth (n=16), 4 within each group, were found appropriate to detect an effect size of 1.09, power of 0.8 and 95% confidence interval.

Teeth were collected from non-smokers, healthy individuals free from any systemic conditions (diabetes mellitus, hypertension, heart disease, liver disease), including both males and females with no previous antibiotic's intake for the past 6 month. While patients with systemic or metabolic disorders, smokers, pregnant or lactating women and patients under regular treatment that may modify or alter the periodontal or oral condition were not eligible to participate in the study.

This research was conducted following approval and following guidance of research ethics committee of the faculty of dentistry, Cairo University (approval number 18-9-21). PDLFs were obtained by collaboration with out-patient clinics of the oral surgery department of faculty of dentistry -Cairo University. Written consents were obtained from all the patients prior to samples collection. Patients who were not able to give their written consent, the accompanying guardian signed a written consent in their place. The in*vitro* experiment was performed at the unit of biochemistry and molecular Biology (UBMB), medical biochemistry and molecular biology department faculty of medicine, Cairo University.

Study design

Eight human first premolars (n=8) with clinically healthy periodontium were collected from patients undergoing extraction for orthodontic treatment. PDLFs were isolated from the periodontal tissue on the roots of theses teeth and were randomly assigned to the following groups: group 1 (control): isolated PDLFs from four healthy teeth (n=4). Group 2 (healthy treated with oil): isolated PDLFs from four healthy teeth and treated with 2.5 µl of oil/ml of media (n=4).

Another eight human teeth (n=8) diagnosed with chronic periodontitis and considered hopeless and indicated for extraction were collected. Periodontally hopeless teeth were diagnosed by a trained periodontist in the Periodontology department- faculty of dentistry, Cairo University. Criteria of periodontally hopeless teeth included:

- Presence of clinical signs of periodontal disease: Edema, erythema, gingival recession, bleeding upon probing and pock depth 6 mm or more, measured by periodontal probe[28].
- Clinical attachment loss ≥5.0 mm[29].
- Radiographic evidence of bone loss showing 75% or more alveolar bone loss[30].
- Grade three tooth mobility[31].
- Grade 4 furcation involvement in multi-rooted teeth[32].
- Crown to root ratio less than 1: 1[33].

PDLFs isolated from periodontally affected teeth were randomly assigned into the following groups: group 3 (periodontitis): isolated PDLFs from four teeth with periodontitis (n=4). Group 4 (periodontitis treated with oil): isolated PDLFs from four teeth with periodontitis and treated with 2.5µl of oil/ml of media (n=4). Study design is summarized in (Table 1).

Both normal and periodontitis teeth were randomly distributed into subsequent groups by Random Sequence Generator program (random.org).

Table 1: The study design

	Group 1 (control)	Group 2 (healthy treated with oil)	Group3 (periodontitis)	Group 4 (periodontitis treated with oil)
No. of teeth	4	4	4	4
Dose	NO	Sesame oil concentrations 2.5 µl of oil/ml of media	NO	Sesame oil concentrations 2.5 µl of oil/ml of media
Incubation time	24h	24h	24h	24h

Laboratory methods and procedures

Culture of human periodontal ligament fibroblast

PDLFs were isolated from the remaining periodontal tissues attached to the roots of donor human teeth after extraction, the excised tissues were washed in phosphate buffer serum (PBS) three times, under the laminar –flow hood and under aseptic condition. The minced pieces were collected in sterile labeled 1.5 ml Eppendorf tubes to which a digesting solution consisting of 3mg/ml collagenase II enzyme was added for 60 minutes at 37°C[34]. The digestive reaction was stopped by the addition of complete culture media (DMEM (PAN Biotech, Germany) supplemented with 10% fetal bovine serum, 100U/Ml penicillin and 100mg/Ml streptomycin (PAN Biotech, Germany)). Then cultured in 75 cm^2 flasks at 37°C in humidified 5% CO2 incubator. When the cells were approximately 80% confluent, they required sub-culturing. After that, cultured cells were either treated by sesame oil in a dose 2.5 µl of oil/ml of media for 24 hours[35] in group 2 (control) and group 4 (periodontally affected cell) or left untreated (group1 & 3).

Immunophenotyping of cells using flow cytometry

Passage 2 cells were trypsinized, washed and resuspended in PBS at a concentration of 1×10^6/ml. The cells were stained for 20 minutes in the dark with monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) to CD4, CD40 and phycoerythrin (PE). 7-amino-actinomycin D (7 AAD-Sigma Aldrich) was added for 20 minutes in all tubes to ensure gating on viable cells. 10,000 cells were analyzed by CYTOMICS FC 500 flow cytometer (Beckman Coulter, FL, USA) using CXP Software version 2.2.

Sesame oil preparation

Sesame oil was obtained from Al Nasr production factory for sesame oil in Sudan. The sesame oil was sterilized and then diluted in concentration of 2.5 µl of oil/ml of complete media[35]. Then sesame oil was added to cell culture of group 2 (healthy treated with oil) and group 4 (periodontitis treated with oil). The cell culture was maintained at a temperature of 37 °C in a 5% CO2 incubator for 24 h.

Inverted Microscope examination

Following 24 hours incubation with or without sesame oil at concentrations of 2.5µl of oil/ml of media, the medium was discarded, and cells were washed once with PBS. The morphological changes of the PDLFs cells were observed using inverted light microscope (Olympus, USA) with digital camera images (Nikon, Japan) at 100x magnifications.

MTT cell proliferation assay

Cell proliferation was assessed using MTT assay. Cells ($1x10^3$) were placed in 96 well micro plates for 24 hour. The MTT 96-well cell proliferation assay is high-throughput method to measure cell proliferation and viability. The assay involves mitochondrial reaction product measurement which is directly correlated to cell number. NADH and NADPH -dependent oxidoreductase enzymes present in metabolically active cells reduce MTT, to an insoluble formazan product, which has a dark purple color. Intensity of purple color is directly proportional to the cell number and thus indicating the cell viability. The absorbance of this product can be quantified by measuring the optical density at 570 nm using a spectrophotometer[36].

RNA extraction and quantitative PCR of bFGF

Total RNA was isolated from collected cultured PDLFs and the master mix was then added. The master mix contains thermostable Taq DNA Polymerases, SYBR Green dye, ROX dye as well as MgCl2 and buffer components at optimal concentrations. The kit PCR was provided by Vivantis, ViPrimePLUS One Step Taq RT-qPCR Green Master Mix I with ROX (SYBR Green Dye) (cat no #QLMM14-R). Primer pair selection criteria included melting temperature (Tm: 60–65 °C), GC content (40%–60%) and amplicon length of about 90 - 200 bp (Table 2). The Relative Quantification of each target gene is quantified according to the calculation of delta- delta Ct.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 22. Data were statistically described in terms of mean, standard deviation. Analysis of variance (ANOVA) with multiple comparison post hoc test were used to compare quantitative variables between the studied groups. Data analysis was carried out by a blinded statistician

RESULTS

Flow cytometric analysis of CD40 and CD4 expression

Using flow cytometric analysis, the expression of CD40 and CD4 was quantified after isolation. Flow cytometry confirmed the expression of both CD4 and CD40 on PDLFs (Figure 1).

Cell in inverted microscope assessment

Upon microscopic examination of unstained PDLFs without oil in group 1 (control), the cells appeared spindle in shape while in group 3 (periodontitis) cell clusters were observed. Group 2 (healthy treated with oil) revealed the presence of stellate shaped cells, spindle shaped cells in addition to multiple cell clusters. While group 4 (periodontitis treated with oil) revealed spindle shaped cells, few stellate shaped cells and multiple cell clusters (Figure 2).

Assessment of cell proliferation and viability via MTT proliferation assay

The highest mean cell proliferation value was recorded in group 2 (healthy treated with oil), significant decrease in

cell proliferation in group 3 (periodontitis) was detected. while no significant difference was detected between either group 1 (control), group 2 (healthy treated with oil) or group 4 (periodontitis treated with oil) (Tables 3,4) (Figure 3).

Gene expression results (quantitative q-PCR)

The highest mean bFGF expression value was recorded in group 4 (periodontitis treated with oil). A statistically significant decrease in bFGF gene expression was recorded in group3 (periodontitis). While no significant difference was detected between either group 1 (control), group 2 (healthy treated with oil) or group 4 (periodontitis treated with oil) (Tables 3,4) (Figure 4).

Pearson correlation test detected statistically significant positive correlation between bFGF gene expression and cell proliferation (Table 5) (Figure 5)

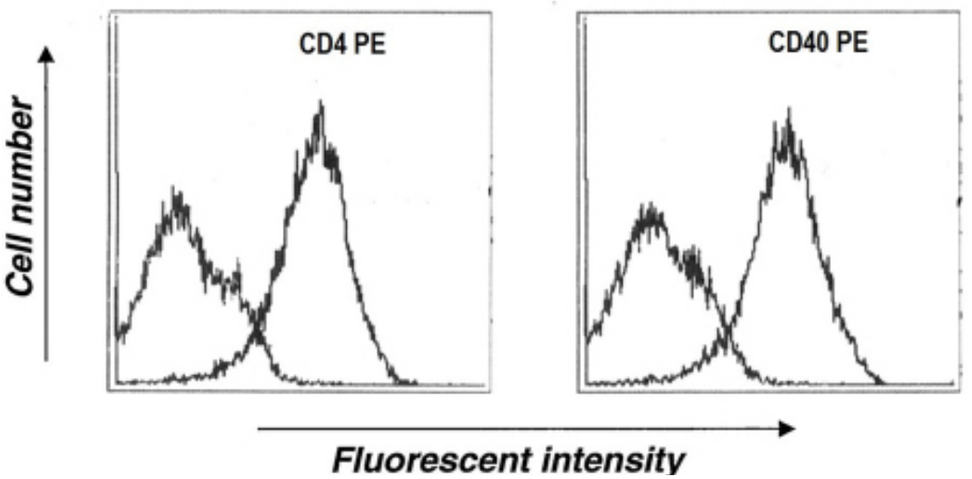

Fig. 1: CD markers on PDLFs revealed by flowcytometry. percent of positivity: CD4 with 84.5% expression in fibroblast cell in culture, CD40 with 91.3% expression in fibroblast cell in culture.

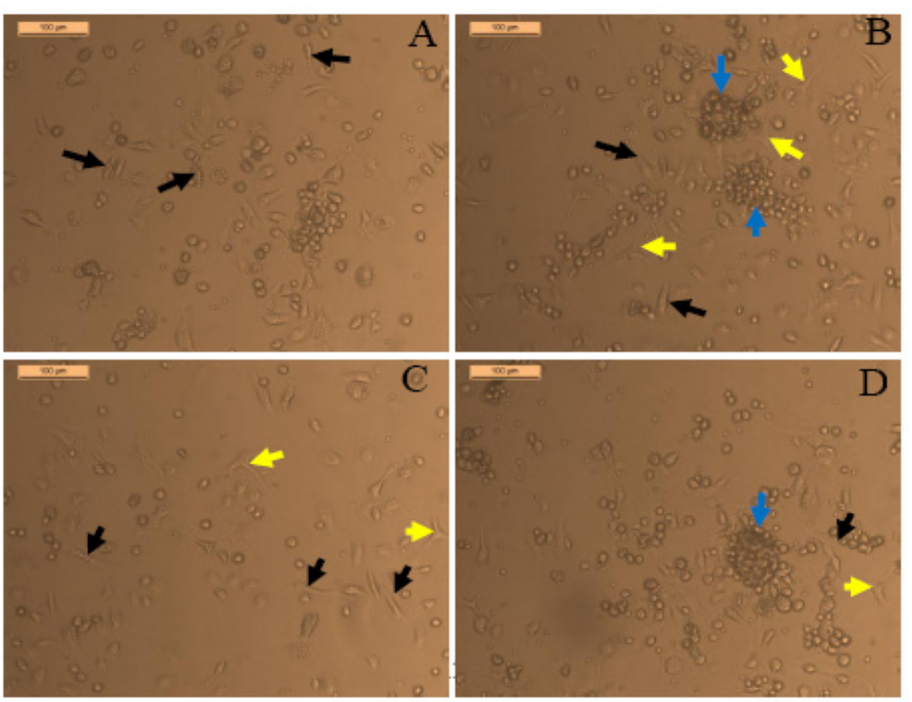

Fig. 2: Photomicrograph of unstained PDLFs (A) group1 (control) showing spindle shaped cells with central nuclei (black arrow), (B) group 2 (healthy treated with oil) Showing spindle shaped cells with central nuclei (black arrow), cell clusters (blue arrow) and stellate cells with multiple branching cell processes (yellow arrow). (C) Group 3 (periodontitis) Showing spindle shaped cells (black arrow), and central nuclei and stellate cells multiple processes (yellow arrow). (D) group 4 (periodontitis treated with oil) Showing spindle shaped cells (black arrow), cell clusters (blue arrow) and few stellate shaped cells (yellow arrow) (Original magnification x100).

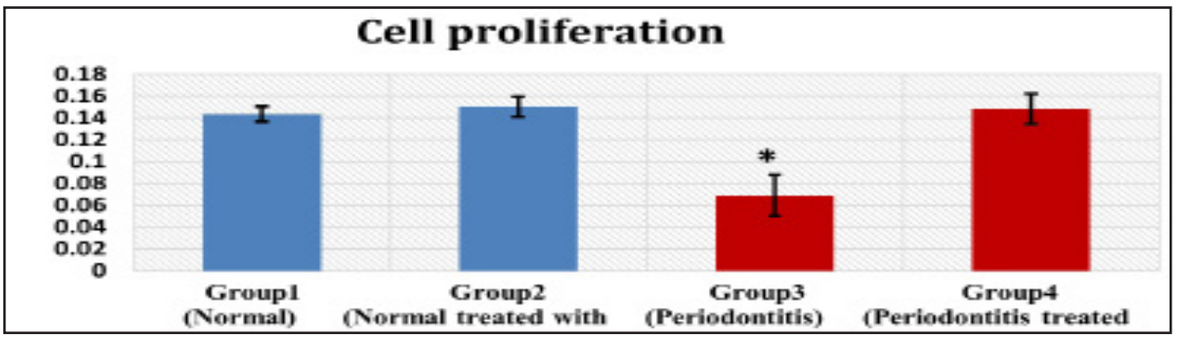

Fig. 3: Bar chart for MTT assay for PDLFs after 24h. (*) denotes significant difference versus all other groups

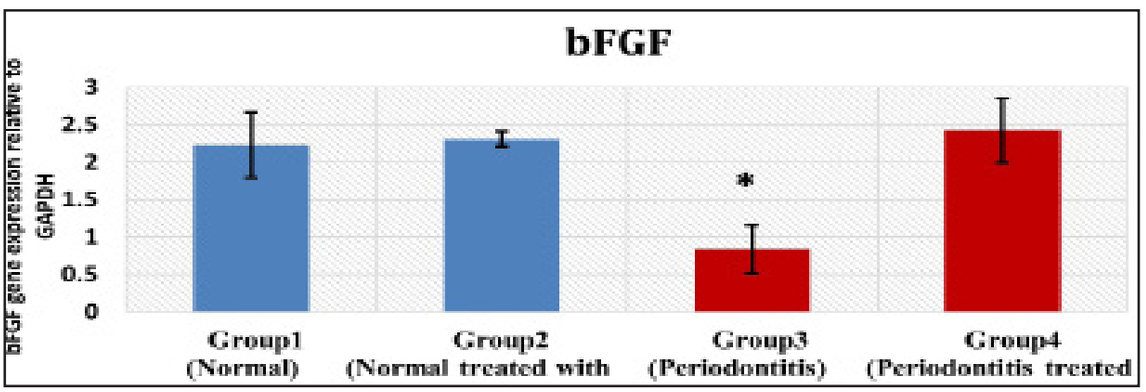

Fig. 4: Bar chart for q-PCR analysis for expression bFGF (*) denotes significant difference versus all other groups

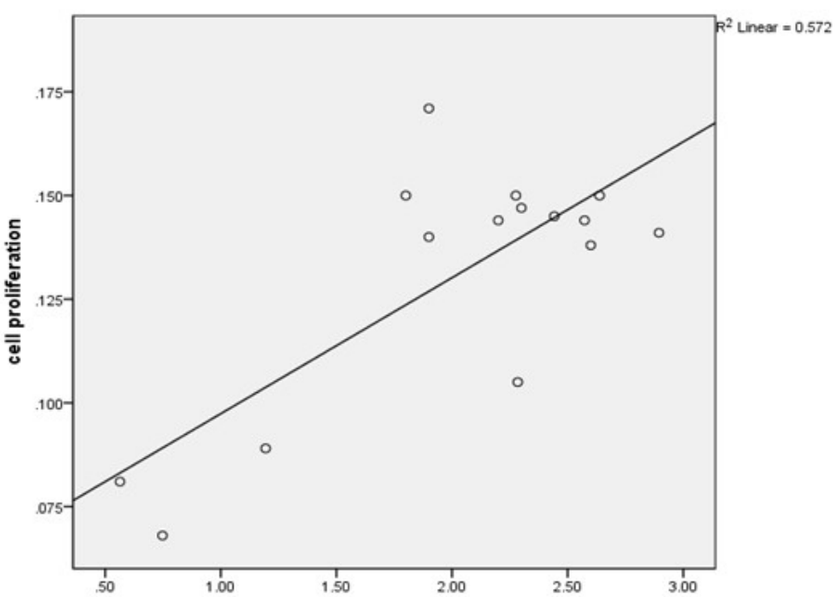

Fig. 5: scatter plot showing correlation between cell proliferation and bFGF gene expression.

Table 2: Primers' sequence of studied gene

Gene symbol	Primer sequence from 5′- 3′ F: Forward primer, R: Reverse primer
FGF	F: 5-TCAGTGGGTTGGGGGCAGAG-3 R : 5-ACGCCCCGAACCTCACCTGA-3

Table 3: Descriptive statistics for quantitative q-PCR assay and MTT proliferation assay among study groups

	Group	Mean±SD	Std. Error	Minimum	Maximum	F	*P value*
bFGF	Group 1[B]	2.227±.438	0.219	1.80	2.64	14.356	0.000*
	Group 2[B]	2.304±.101	0.050	2.20	2.44		
	Group 3[C]	0.835±.323	0.187	0.56	1.19		
	Group 4[A,B]	2.419±.426	0.213	1.90	2.90		
MTT proliferation assay	Group 1[B]	0.143±.007	0.001	0.136	0.169	215.309	0.000*
	Group 2[B]	0.150±.009	0.001	0.142	0.181		
	Group 3[C]	0.069±.018	0.003	0.031	0.104		
	Group 4[A,B]	0.148±.013	0.002	0.105	0.171		

Table 4: ANOVA and Tukey's post hoc test for quantitative q-PCR assay and MTT proliferation assay among study groups

Parameter	Difference of levels		95% Confidence Interval	Adjusted *P-Value*
bFGF	Group 1	Group2	(-.826, .673)	0.989
		Group3	(.582, 2.202)	0.001*
		Group4	(-.941, .557)	0.866
	Group 2	Group3	(.659, 2.279)	0.001*
		Group4	(-.865,.634)	0.966
	Group 3	Group4	(-2.394, -.774)	0.001*
MTT proliferation assay	Group 1	Group2	(-0.016,0.002)	0.257
		Group3	(0.064,0.084)	0.000*
		Group4	(-0.014,0.005)	0.573
	Group 2	Group3	(0.071,0.091)	0.000*
		Group4	(-0.007,0.012)	0.943
	Group 3	Group4	(-0.089,-0.069)	0.000*

Table 5: Correlation between cell proliferation and bFGF gene expression (Pearson correlation test)

	Cell proliferation	
bFGF	Pearson Correlation coefficient	0.756**
	P value	0.001*

DISCUSSION

The current work was carried out to explore the effect of sesame oil, with its potent antioxidant and anti-inflammatory effects on cellular proliferation and bFGF gene expression on PDLFs isolated from subjects with chronic periodontitis and from healthy subjects. Periodontal disease is a chronic inflammatory disease that is prevalent worldwide. Poor oral hygiene, age, smoking, low educational level, and poor economic status have been associated with increased risk of periodontal disease[37].

In *vitro* investigation is a suitable method for early testing of the direct effects of different medical agents on the cells. It is more conservative regarding saving lives of laboratory animals and paves the way to animal studies prior to the launching of clinical trials[38]. The extraction and isolation of PDLFs were performed as previously described in the literature[39,40]. In the present study, enzymatic digestion method for PDLFs isolation was carried out to release all cells from the target tissue[41,42]. Within the ongoing study, PDLFs were identified using flowcytometry via positive expression of cell marker CD4 and CD40[43,44]. Morphologic and growth characteristics were further used to confirm the identification of the isolated PDLFs[45,46]. In the present study cultured PDLFs demonstrated an elongated shape and they continued to proliferate until reaching 80% confluence confirming the isolated cells to be PDLFs.

Within the present study, the highest mean cell proliferation and viability values were recorded in group 2 (healthy treated with oil) while the lowest values were recorded in group 3 (periodontitis) with statistically significant difference between groups. While the highest mean FGF expression values were recorded in group 4 (periodontitis treated with oil) and the lowest values were recorded in group 3 (periodontitis) with statistically significant difference between groups demonstrating that sesame oil possess a positive effect on fibroblasts cell viability and function.

Sesamol, a sesame oil lignans, significantly restored cell viability and significantly reduced ultraviolet B radiation (UVB) radiation-induced cytotoxicity and cell injury on human dermal fibroblast cell culture. Sesamol pretreatment progressively decreased levels of thiobarbituric acid reactive substances and lipid hydroperoxides, which are oxidative stresses indicators, significantly inhibited intracellular reactive oxygen species (ROS) production,

increased activities of antioxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase. Further, sesamol significantly decreased the levels of DNA damage in cultured fibroblasts and decreased apoptotic morphological changes[47].

Similarly, sesamin, another sesame oil lignan significantly inhibited ROS formation, reduced upregulation of mitogen-activated protein kinase, significantly reduced the nitric oxide synthase and cyclooxygenase-2 (COX-2) protein expression levels and downregulated nuclear factor- kappa B (NF-kB) activation, a strong indicator of inflammation, following UVB irradiation in human dermal fibroblasts cell culture[48]. Sesamin and sesamolin suppress (NF-κB) activation by inhibition of I kappa B kinase (IκB) dissociation through decreasing IκB kinase activity[49–51]. Sesamin also decreased UV irradiation induced matrix metalloproteinase (MMP-1, 3, and 9) over-expressions in dermal fibroblasts and was associated with increase in tissue inhibitors of metalloproteinases expression which is a natural inhibitor of MMPs[48]. Sesamin and sesamol also demonstrated a neuroprotective effect on H_2O_2 induced human neuroblastoma apoptosis[52]. The previously mentioned data supports the antioxidant effect of sesame oil and can help explain its effect on PDLFs observed in our study.

Additionally, sesame oil and Sesamin were biocompatible[48,53], induced dental pulp cells proliferation[53] and significantly increased fibroblasts collagen synthesis by modulating Smad 3 and Smad 7 protein expression[48]. Which further augments the increase in cell proliferation rate and cell function association with sesame oil in the present work.

Sesame oil positive effect on fibroblasts can be explained by its effect on gene expression. Sesame oil application in *vivo* in rat's dermal wounds altered fibroblasts gene expression. It was associated with increased expression of antioxidant enzymes as peroxiredoxin 2, 4, superoxide dismutase C, glutathione S-transferase P 1, glutaredoxin 3 and SH3 domain-binding glutamic acid–rich protein. In addition to proteins associated with cell proliferation as pyruvate kinase isozyme type M2, spermidine synthase, probibitin, stathmin 1 and eukaryotic translation initiation factor 5A-1. It was also associated with upregulation of anti-apoptosis protein expression including heat shock protein 1 and translationally controlled tumor protein in addition to upregulation in collagen secretion, phosphoglycerate kinase 1, lactate dehydrogenase B, ATP synthase subunit delta, microtubule associated protein RP/EB family member 1, vimentin, calpain small subunit 1, actin-related protein 2/3 complex subunit 5, and cofilin-2. In addition to proliferation associated proteins including, nucleoside diphosphate kinase A, pyruvate kinase isozyme type M2, spermidine synthase, probibitin and stathmin 1. It also significantly reduced the level of ROS by up to 15% and increased production of transforming growth factor β and collagen[54]. Which further elucidate the positive role of sesame oil on fibroblasts through the current study.

Sesame oil based burn ointment local application on cutaneous excisional wounds in rats enhanced the formation of granulation tissue, neovascularization, increased fibroblasts proliferation and migration into wound bed and enhanced wound healing. It also increased the levels of VEGF and bFGF[55]. Subcutaneous daily injections of sesame oil and testosterone in rats was associated with significant increase in levels of stromal cell-derived factor-1α, platelet-derived growth factor, nitric oxide and bFGF[56]. This is in accordance with the result of the present study. However, in contrast to findings reported in the current study, sesame oil injected intrauterine to ovariectomized rats was not associated with significant increase in levels of bFGF mRNA as compared to estrogen and progesterone injection[57]. Which can be related to different experimental model used in our study.

Seaseme oil, through the current study, showed a positive effect on PDLFs proliferation and function which is probably owed to its previously documented potent anti-inflammatory and anti-oxidative properties[48,58,59]. Its noteworthy that periodontal disease is a chronic inflammatory disease associated with upregulation in inflammatory markers including interleukin-1 (IL-1), IL-6 and COX-2[60] in addition to increase in ROS production in PDL cells[61]. Therefore, the ability of sesame oil to counteract the inflammatory and oxidative damages on cellular level can account for its positive impact on PDLFs. This fact can help explain the findings of the here in study.

CONCLUSION

Sesame oil is a promising biocompatible antioxidant and anti-inflammatory herbal extract that can stimulate PDLFs proliferation and function through increasing bFGF gene expression in *vitro* and therefore it holds potential to enhance periodontal regeneration in *vivo*. Further studies are warranted to ascertain the potential effect of sesame oil on periodontal regeneration in *vivo*.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

حيوية والنشاط الوظيفي لخلايا الرباط اللثوية المعزولة من أسنان الانسان المتأثرة لثويا مقارنة بالأخري الصحيحة على أثر التعرض لزيت السمسم (دراسة في المختبر)

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مقدمه: مرض اللثة هو مرض التهابي مزمن للانسجة الداعمة المحيطة بلاسنان, وقد تم التعرف علي البكتيربا اللاهوائيه المسببة للامراض الملتصقة بأسطح الاسنان كعوامل مسببة للالتهاب مما يؤدي الي نزيف اللثة

هدف البحث: الهدف من هذه الدراسة هو التحقق من تأثير زيت السمسم على معدل تكاثر الخلايا الليفية ومستوى تعبيرعامل نمو الخلايا الليفية الأساسي.

مواد و أساليب العلاج: تم عزل الخلايا الليفية من أنسجة الرابط اللثوي المرتبطة بجذور الأسنان (عدد= ١٦) بعد خلعها، تتضمن الاسنان مجموعة الأسنان الطبيعية (عدد=٨) ،خالية من أمراض الرابط اللثوي الالتهابية والتي تم خلعها خلال تقويم الأسنان بالإضافة الي مجموعة الاسنان التي تعاني من التهاب الاربطة اللثوية المزمن الميؤوس من علاجها (عدد= ٨). تم تقسيم الاسنان عشوائيا الي المجموعات التالية: المجموعة ١: الخلايا الليفية للأربطة اللثوية مع عدم وجود علاج , المجموعة ٢: الخلايا الليفية للأربطة اللثوية وتم علاجها بزيت السمسم .

تم عزل الخلايا الليفية من الاربطة اللثوية وزراعتها عند درجة خرارة ٣٧ درجة مئوية في حضانة مخصصة. عندما كانت الخلايا متقاربة بنسبة ٨٠٪ ، تم استخلاص زراعة فرعية. بعد ذلك تمت معالجة الخلايا المستزرعة بزيت السمسم بجرعة ٥ . ٢ ميكرولتر من الزيت / مل من سائل الزراعة لمدة ٢٤ ساعة أو تركت دون علاج . تم تقييم تكاثر الخلايا باستخدام اختبار (MTT) الذي يعتمد علي قراءة امتصاص اللون باستخدام جهاز القارئ الضوئي حيث ان مقدار الامتصاص يتناسب مع عدد الخلايا. تم عزل إجمالي الحمض النووي الريبوزي من الخلايا الليفية للأربطة اللثوية عن طريق استخدام المزيج الرئيسي. يتم تحديد القياس الكمي النسبي (RQ) لكل جين مستهدف وفقًا لحساب دلتا دلتا Ct .

النتائج : تم تسجيل أعلى متوسط لتكاثر الخلايا في مجموعة الخلايا الطبيعية المعالجة بزيت السمسم مع انخفاض كبير في تكاثر الخلايا المستخلصة من الاسنان المصابة بالتهاب النسيج الرابط المزمن بدون علاج بزيت السمسم في حين لم يتم الكشف عن فرق كبير بين أي من المجموعات الأخرى. تم تسجيل أعلى قيمة لعامل نمو الخلايا الليفية الأساسي في مجموعة الخلايا المستخلصة من الاسنان المصابة بالتهاب النسيج الرابط المزمن المعالج بزيت السمسم مع تسجيل انخفاض في التعبير الجيني لعامل نمو الخلايا الليفية الاساسي في مجموعة الخلايا بدون زيت. في حين لم يتم الكشف عن فرق كبير بقية المجموعات .كشف اختبار ارتباط بيرسون عن وجود علاقة إيجابية ذات دلالة إحصائية بين التعبير الجيني لعامل نمو الخلايا الليفية الأساسي وانتشار الخلايا.

الاستنتاج : في الختام ، يعتبر زيت السمسم مستخلصًا طبيعيًا واعدًا, مضادًا للأكسدة ومضادًا للالتهابات يمكن أن يحفز انتشار الخلايا الليفية للأربطة اللثوية ويعمل من خلال زيادة مستوي تعبير عامل نمو الخلايا الليفية الاساسي في المختبر وبالتالي لديه القدرة على تعزيز تجديد الاربطة اللثوية في الجسم الحي.