Effect of subgingival application of Phytotherapeutic Agents using *Thymus Vulgaris* Essential Oil Gel along with Scaling and Root Planing on Clinical Parameters and Gingival Tissue Level of VEGF and CD34 in Patients with Chronic Periodontitis

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Abstract: Background: Non-surgical mechanical therapy is the cornerstone of periodontal treatment. However it is a technically demanding procedure and is not always efficient at eradicating all periodontal pathogens and in reducing inflammation. Therefore, local subgingival application of Phytotherapeutic (herbal) agents may be used as an adjunct to non-surgical therapy. **Objectives:** This study was conducted to evaluate the clinical, histological and immunohistochemical benefits of local subgingival application of 2%*thymus vulgaris* essential oil gel (thyme gel) along with scaling and root planing (SRP) in patients with chronic periodontitis. **Materials and Methods:** 40sites were chosen from 20 patients with chronic periodontitis. At first visit, clinical parameters were assessed and full moth supragingival scaling was done. Then SRP was done for selected sites. The sites were then randomized into control and experimental sites such that both the groups had 20 sites each. Experimental sites additionally received thyme gel subgingivally at baseline, 1st, 2nd, and 3 rd weeks. Clinical parameters were re-assessed at 4th, 6th, and 12th week. At 4th week recall, a gingival biopsy was obtained from test and control site for histological examination. Histological (H&E and Trichorome) and immunohistochemical analysis for Vascular Endothelial Growth Factor (VEGF) and CD34 (for microvessel density [MVD] count) antibodies were performed. Data obtained was subjected to appropriate statistical analysis. **Results:** The clinical parameters at all sites from baseline to 4th, 6th, and 12th weeks showed statistically significant changes. Experimental sites showed statistically significant improvement in Gingival Index (GI) and Bleeding Index (BI) at 6th and 12th weeks when compared with control sites. However, no statistically significant differences were observed in the Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) between control and experimental sites at 4th , 6th , and 12th week time interval.Histollogically the sites treated with thyme gel exhibited mild inflammatory cellular infiltrate compared to massive inflammatory infiltrate at control sites. The gingival expression of VEGF was reduced at experimental sites compared to control sites, but this reduction was not significant while that of CD34 was significantly higher in the sites treated with Thyme gel compared to the control sites. **Conclusion:** Subgingival application of 2% thyme gel along with SRP provided a significant improvement in gingival parameters. However, no additional benefit was found in periodontal parameters. Histologically, there was reduction in inflammatory infiltrate and gingival expression of VEGF, while CD34 was significantly higher in the sites treated with thyme gel compared to the control sites. This indicates that thyme possessing an anti-inflammatory role induces proliferation, viability and angiogenesis of human microvascular cells, thus promoting wound healing.

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1. Introduction

Periodontal tissues represent a unique system, where epithelial, non-mineralized, and mineralized connective tissues exist in harmony.(1) The integrity of this system is essential in providing an effective barrier against microbial invasion and preventing the destruction of the underlying periodontal tissues by bacterial toxins and enzymes. However, this integrity is lost during chronic inflammation associated with periodontal disease leading to detrimental effects upon the extracellular matrix components of underlying periodontal tissues. (2)

Non-surgical mechanical therapy is the cornerstone of periodontal treatment. However, increasing pocket depth and complicating anatomical factors limit the effectiveness of scaling and root planing (SRP), thereby compromising the results.(3)

In addition to the better-known chemotherapeutic antimicrobials and biomaterials, there are number of substances belong to the alternative complementary therapeutic agents have gain special interest, and at the same time, have the potential to augment results of periodontal therapy. These products can be categorized as standard dental product made with natural ingredients, herbal or (phytotherapeutic) products, homeopathic products, and synthetic alternative products. (4)

The key issue of phytotherapy is the use of healing plants' features in the treatment of various diseases. (5) The rational of the scientific examination of the use of phytotherapeutic agents is elaboration of a drug of a specific composition and dose, in a modern form which is easy to administer. (6)

Essential oils have recently received increased attention for their antibacterial and antifungal activities.(7) WHO in (1999) stated that, *Thymus vulgaris* is a perennial herb indigenous in central and southern Europe, Africa and Asia that are rich in essential oils and antioxidative phenolic substances.(8) It is widely used in folk medicine for the treatment of a variety of diseases including gastroenteric and bronchopulmonary disorders as antispasmodic agent. (9) It has been reported that thyme possesses numerous biological activities including antimicrobial (10), antioxidant (11), antifungal (12), and anti-inflammatory potentials (13)

Angiogenesisis an essential component of normal wound healing and repair in which endothelial cells and their precursors actively participate, and facilitate the removal of debris andassists in the development of a granulation tissue framework for wound closure. The major angiogenic factors include fibroblast growth factors, platelet derived growth factor, and vascular endothelial growth factor (VEGF). (14) The latter increases microvascular permeability, stimulates endothelial cell proliferation, and induces proteolytic enzyme expression and the migration of endothelial cells, monocytes, and osteoblasts, which are essential for angiogenesis. (15) Recently VEGF has attracted the attention as being involved in the pathogenesis and progression of several chronic inflammatory diseases.(16)

Furthermore CD34, a glycosylated transmembrane protein present on progenitor endothelial cells, has been used to highlight the microvasculature vessel density (MVD) as a direct marker of the degree of neoangiogenesis and wound healing. (14)

The present study was conducted to evaluate the effect of subgingival application of 2% thymus vulgaris essential oil gel (thyme gel) along with scaling and root planing (SRP) on the clinical parameters and gingival tissue levels of vascular endothelial growth factor (VEGF) and CD34 in patients with chronic periodontitis.

2. Material and methods

This randomized, controlled, parallel design study was conducted on patients attending the Dental clinics, college of dentistry, Qassim University KSA. Twenty chronic periodontitis patients providing a total of 40 sites were selected. A written informed consent was obtained from all patients prior to their participation in this study. The Institutional Internal Review and Ethics committee at the college of dentistry, Qassim University approved the study.

Inclusion criteria for the study were patients with severe chronic periodontitis based on the classification of periodontal diseases by the International Workshop for Classification of Periodontal Diseases and Conditions of the American Academy of Periodontology in 1999(17), adequate attached gingiva, and age range between 25 to 55 years. The exclusion criteria included patients with poor systemic health like uncontrolled diabetes, osteoporosis, collagen disorders etc. This was confirmed by taking thorough patient's medical history and by doing general as well as oral examination. The patients who were on and expected to be taking antibiotics and anti inflammatory drugs within the duration of the study were not included.

Clinical parameters including, Gingival Index (GI), Bleeding Index (BI) (18,19), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) were recorded at baseline (0 day before SRP), 4^{th} , 6^{th}, and 12^{th} week.

At first visit, clinical parameters were assessed and full mouth supragingival scaling was done. Then subgingival scaling and root planing (SRP) was done for selected sites by use of a combination of ultrasonic scalers and curettes. The sites were then randomized into control and experimental sites such that both the groups had 20 sites each.

Experimental sites additionally received 1 ml of 2% thyme gel application subgingivally, till the depth of pocket. This was done by taking the gel into a syringe and applying it with wide gauge needle assuring that the tip reaches the depth of the pocket.

The gel preparation

Thymus vulgaris L. (Lamiaceae) flowers and leaves were bought from Sekem Co for medicinal plant (Cairo, Egypt). The essential oil of thyme was isolated by the hydrodistillation method using Clevenger-type apparatus .The recovered oil was dried over anhydrous sodium sulfate and stored in darkness between 4 and 6C. Thyme gel was prepared by dispersion of 0.75%(w/w) Carbopol in preserved water (methyl paraben 0.18% and propyl paraben

0.02%) and 5% glycerin over night. 2% of the extract was dissolved in 20% propylene glycol and was added to the polymer dispersion and stirred for 10 min, and neutralized by triethanolamine to pH 6.4.(20)

Thyme gel application subgingivally was repeated at experimental sites at the end of 1 st , 2 nd , and 3 rd week. At the 4 th week recall, clinical parameters were assessed and recorded at all sites. A marginal gingival biopsy of the size ranging from 1mm to 1.5mm maintaining the scalloped contour was obtained from experimental and control sites, providing tissue for histological examinations. Marginal gingiva of the tooth with residual deep pocket was the preferred site for biopsy.

The samples were fixed in 10% nature buffered formalin for 6-12h, embedded in paraffin and sectioned in 6um thickness. Part of the specimens was stained with hematoxylin and eosin (H&E) and Masson's trichrome stain (MTC). Deparffinized sections from the paraffin blocks tissue specimen were prepared for immunohistochemical staining using monoclonal antibodies for VEGF and CD34. The sections were additionally treated with 0.5% casein in Tris-buffered saline (TBS) for 10 min to block non-specific binding sites and counterstained with Mayer's hematoxylin. All of the stained tissue sections were examined under light microscope, photographed and subjected to software program to count & analyze histological &immunohistochemical results.

All patients providing the test and control sites were recalled at 6th and 12th week for the assessment of clinical parameters at the remaining sites.

Method of morphometry

Images were viewed and recorded using Olympus microscope – equipped with Spot digital camera, using computer program MATLAB software. The image of each slides of groups were captured using a 20 X objective (Bar = 100) with numerical aperture of a high resolution of (16-bit digital camera (1280X1024 pixel). The MVD was determined by counting all the vessels stained by CD34 positive cell even a single CD34 positive cell visible without lumen(capillaries) within an examination area of 1mm2). The measure of the image optical density (IOD) changes of VGEF in the animal groups was analyzed. The maximum, minimum and integrity of color intensity based on Gray-level acquisition. Analysis of the data was carried out by (reading 10 fixed areas in one image, five images for each case). The mean values of each reaction were based on the mean of pixel number. All data were collected and analyzed with SPSS statistical software (SPSS for Windows, release 15.0, version 20; SPSS, Inc., Chicago, IL).

3. Results

Data obtained was subjected to appropriate statistical analysis. At the time of baseline examination, no significant differences were observed in GI, BI, PPD and CAL in the test and control groups allowing the assumption of homogeneity of the patient population

The mean GI at 4th week at control sites was 1.28±0.28, while at experimental sites, it was 1.07±0.23. The differences in GI scores between control and experimental sites were not statistically significant (P>0.05) at this point of time. At 6th week, the mean (GI) at control sites was 1.22±0.27, while at experimental sites, it was 0.93±0.24. Higher mean GI was noticed at control sites as compared to the experimental sites at 6th week time interval. The differences between experimental and control sites were found to be statistically significant (P<0.05).At 12th week, the mean GI at control sites was 1.19±0.24, while at the experimental sites, it was 0.89±0.21. The differences in the GI scores between control and experimental sites were statistically significant (P=0.01) table 1.

Table 1: Mean gingival index

GI	Base line	4th week	6th week	12thweek
Control	2.03±0.08	1.28±0.28	1.22±0.27	1.19±0.24
Exp.	2.04±0.09	1.07±0.23	0.93±0.24	0.89±0.21
P ≤ 0.05	Not Sig	Not sig	Sig	Sig

p: *p* value for Post Hoc test (Scheffe) for comparing between control and experimental sites, Sig:Statistically significant at $p \leq 0.05$

The mean bleeding index (BI) at 4th week, at control sites was 0.28±0.27, while at the experimental sites, it was 0.14±0.15. This differences in BI scores were not statistically significant (P>0.05). At 6th week, the mean BI at control sites was 0.23±0.25, while at experimental sites, it was 0.05±0.11. Higher mean BI was noticed at control sites compared with experimental sites at 6th week time interval, and the difference between the two scores were statistically significant (P<0.05). At 12th week, the mean BI at control sites was 0.21±0.22; while at the experimental sites, it was 0.02±0.05. The differences in the BI scores between control and experimental sites were statistically significant (P=0.01). (Table2).

Table 2: Mean bleeding index

BI	Base line	4th week	6th week	12thweek
Control	1.00	0.28±0.27	0.23±0.25	0.21±0.22
Exp	1.00	0.14±0.15	0.05±0.11	0.02±0.05
$P \leq 0.05$	Not sig	Not sig	Sig	sig

p: p value for Post Hoc test for comparing between control and experimental site, Sig: Statistically significant at $p \leq 0.05$

At 4thweek, the PPD values reduced to 5.56±0.40 at control sites and 5.41±0.45 at the experimental

sites. At 6th week, the PPD values were 5.12±0.25 at control sites and 5.02±0.41 at experimental sites. At 12th week, the PPD further reduced to 4.92±0.22 at control sites and 4.82±0.32 at experimental sites. When comparisons were made between control and experimental sites, higher mean PPD was observed at control sites at 4th, 6th, and 12th week interval. However, no statistically significant differences were observed in PPD values between experimental and control sites at 4, 6, and at 12 week time interval (P>0.05).table 3

Table 3: Mean probing pocket depth

PD	Base line	4th week	6th week	12th week
Control	6.45±0.43	5.56±0.40	5.12±0.25	4.92±0.22
Exp	6.57±0.45	5.41±0.45	5.02±0.41	4.82±0.32
$P \leq 0.05$	Notsig	NotSig	Notsig	NotSig

p: p value for Post Hoc test (Scheffe) for comparing between control and experimental sites, Sig: Statistically significant at $p \leq 0.05$

At 4th week, the mean attachment level at the control sites reduced to 8.06±0.56 and 7.92±0.45 at the experimental sites. At 6th week, the mean attachment level was 7.75±0.54 at control sites and 7.64±0.58 at experimental sites. At 12th week, mean CAL further reduced to 7.64±0.53 at control sites and 7.60±0.59 at experimental sites. When comparisons were made between control and experimental sites, higher mean CAL was observed at control sites; however, no statistically significant differences were observed in the CAL values of experimental and control sites at 4th , 6th , and 12th week interval (P>0.05). table4

Table 4: Mean Clinical attachment level

CAL	Base line	4th	6th	12th
Control	8.45±0.41	8.06±0.56	7.75±0.54	7.64±0.53
Exp	8.57±0.44	7.92±0.45	7.64±0.58	7.60±0.59
$P \leq 0.05$	Not sig	Not sig	Not sig	Not sig

p: p value for Post Hoc test for comparing between control and experimental sites, Sig: Statistically significant at $p \leq 0.05$

To summarize, when all the four clinical parameters at control and experimental sites were subjected to intra group analysis from baseline to 4th week, baseline to 6th week and baseline to 12th week, results showed statistically significant changes in all clinical parameters (P=0.05) at all-time intervals. Analysis also showed that changes in the clinical parameters from baseline to 4th, 6th, and 12th week were higher at the experimental sites than at the control sites. On comparing the clinical parameters between experimental and control sites at 4th , 6th , and 12th week interval, statistically significant differences were observed in GI and BI scores at 6th and 12th week, whereas, differences between PPD and CAL values were not statistically significant.

Histological results:

H & E results:

Histologically, gingival sections of the control sites exhibited an epithelium lining that varied in thickness with areas of discontinuity and elongated rete processes. At the connective tissue (CT) level massive inflammatory cellular infiltrate is present. (Figs.1 and 2). On the other hand, reduced inflammatory cellular infiltrate was observed in the treated sites with evident fibroplasia and prominent blood vessels. (Fig. 3)

Masson trichrome results:

Masson trichrome stain showed mild intensity of collagen fibers, few fibroblasts and decreased CT thickness in the control sites.(Fig.4)While, the treated specimens showed moderate staining reaction of well-developed collagen bundles with organized proliferating fibroblasts and endothelial cells. Also there were numerous capillaries & few inflammatory cells (Fig. 5)

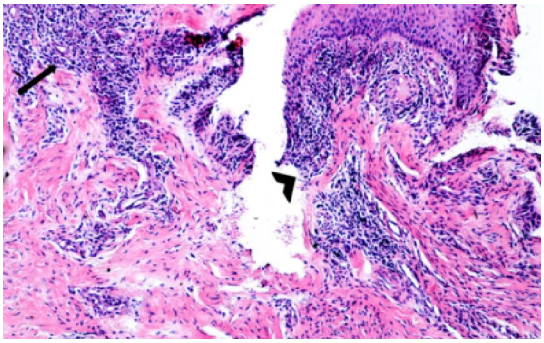

Fig 1: Photomicrograph of control gingival tissue showing a discontinuity of the lining epithelium (arrow head) with varying thickness, inflammatory cellular infiltrate is massive at the underling C.T (arrow), normal blood vessels. (H&E stain, Mic. Mag. × 100)

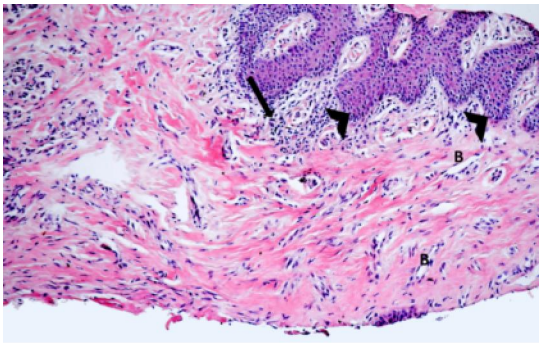

Fig 2: Photomicrograph of control gingival tissue showing thickened epithelial lining and elongated rete processes (arrow head), blood vessels with regular shape and normal dimensions, also note subepithelial inflammatory infiltrate (arrow).(H&E stain, Mic. Mag. × 200)

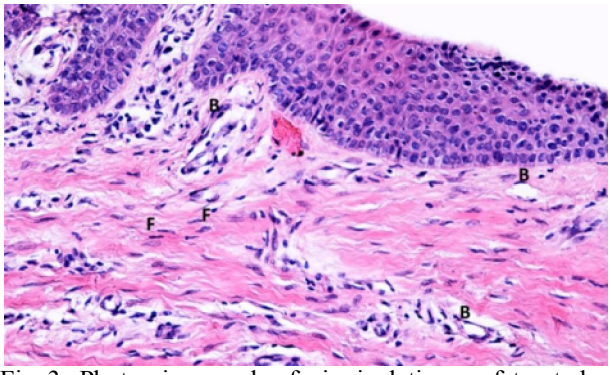

Fig 3: Photomicrograph of gingival tissue of treated site showing nearly normal epithelial lining, mild inflammatory cell infiltration, many fibroblasts/ fibroplasia (F) & increased vascularity (B). (H&E stain, Mic. Mag. × 400)

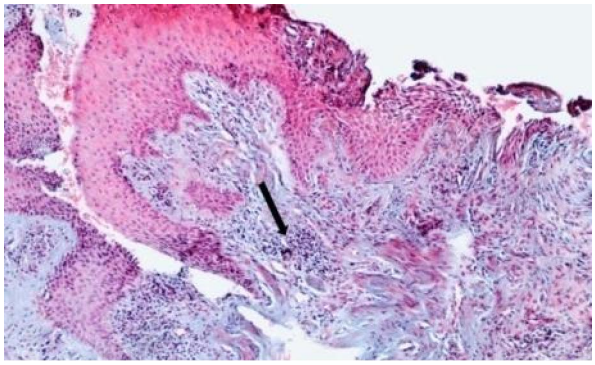
Fig 4: Photomicrograph of gingival tissue of control site showing increased thickness of the lining epithelium, inflammatory cellular infiltrate is massive at the underlying C.T (arrow), few fibroblasts and few collagen fibers. (T.C stain, Mic. Mag. × 100)

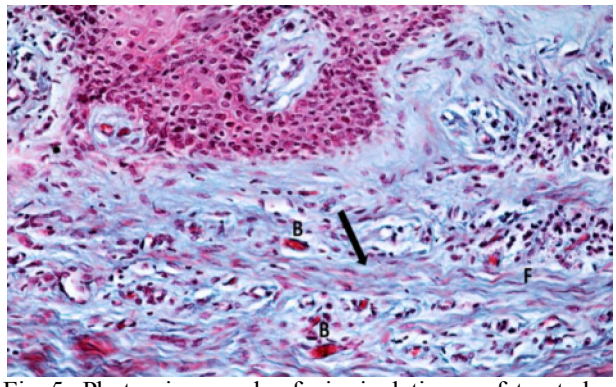

Fig 5: Photomicrograph of gingival tissue of treated site showing abundant thick collagen fibers (arrow), many fibroblasts (F), mild inflammatory cell infiltration and increased vascularity (T.C stain, Mic. Mag. × 200)

Immunohistochemical results:

CD34 immunohistochemical results:

Mild positive staining of blood capillaries was observed in the control sites.(Fig 6) The treated sites showed, intense brown staining of endothelial cells of blood capillaries.(Fig7&8)

VEGF immunohistochemical results:

Control sites show, blood vessels with regular shape and normal dimensions.(Fig 9) On the other hand, the treated sites showed brown stain indicating the distribution of VEGF. Increase in the number of blood vessels indicating increased vascularization. (Fig 10&11)

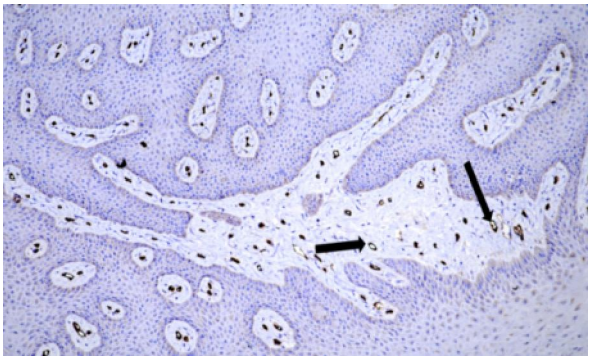
Fig 6: Photomicrograph of gingival tissue of control site showing, mild positive staining of blood capillaries (arrow). CD34 immunohistochemical staining. Mic. Mag. X100)

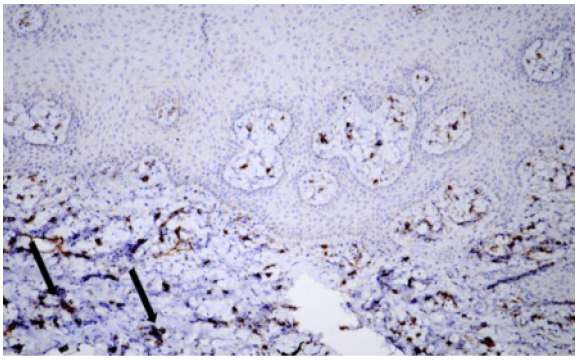
Fig 7: Photomicrograph of gingival tissue of treated site showing, intense brown staining of endothelial cells of blood capillaries (arrow). (CD34 immunohistochemical staining. Mic. Mag. X100)

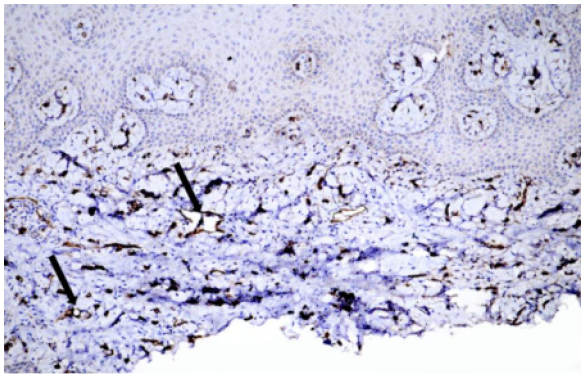

Fig 8: Photomicrograph of gingival tissue of treated site showing, numerous blood capillaries with intense positive reaction (arrow).(CD34 immunohistochemicalstaining. Mic. Mag. X100)

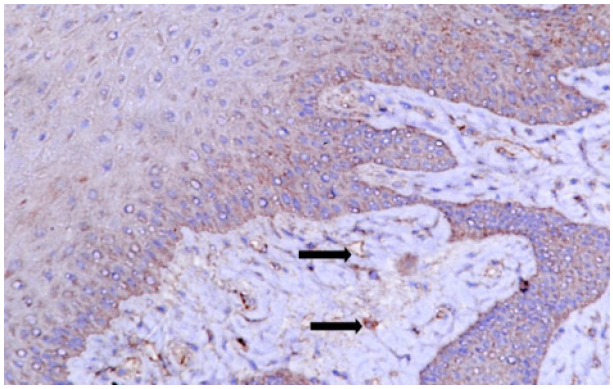

Fig 9: Photomicrograph of gingival tissue of control site showing, mild reaction of the blood vessels with regular shape and normal dimensions (arrow). (VEGF immunohistochemical staining .Mic. Mag. X400)

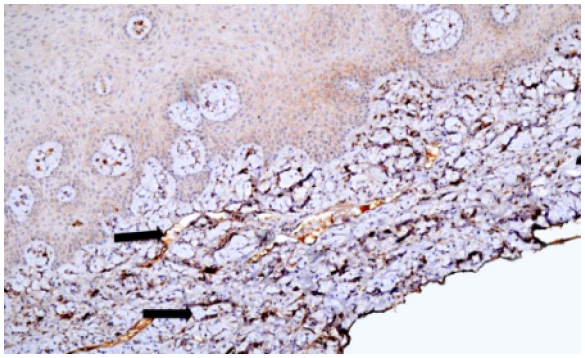

Fig 10: Photomicrograph of gingival tissue of treated site showing brown stain indicating the distribution of VEGF. Increase in the number of blood vessels indicating increased vascularization (arrow). (VEGF immunohistochemical staining. Mic. Mag. X100)

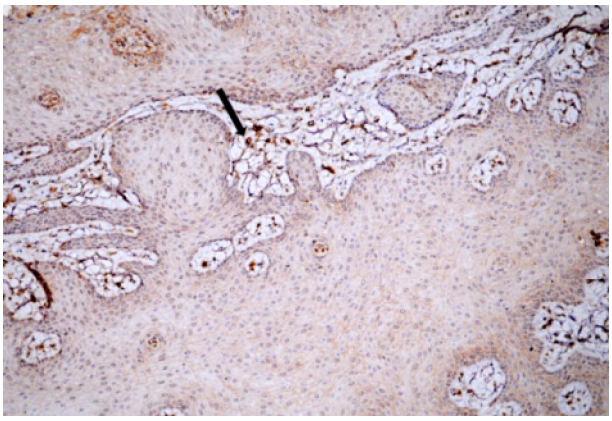

Fig 11: Photomicrograph of gingival tissue of treated site showing, numerous blood capillaries lined by positive stained endothelial cells (arrow).(VEGF immunohistochemicalstaining. Mic. Mag. X200)

Statistical results:

CD34 immunostaining was observed in all gingival samples. The mean percentage of MVD was (52.0 ± 9.97) in the test site and was significantly increased compared to the control site.(31.92 ± 5.40; $P<0.05$).(Table5)

In the control site, immunohistochemical analysis showed diffuse VEGF-positive cells (130.19 ± 24.64). In the experimental site, VEGF-positive cells were not significantly decreased in endothelium and C.T cells (118.71 ± 15.0; $P>0.05$) compared to the control site. (Table7)

Morphometric results:

For confirming immunohistochemical staining, image analysis studies were used for counting the number of microvesicles and image optical density of the VEGF brown color stain (IOD).

A- Micorvesicles count (MVC):

The number of the microvesicles in all studied groups was counted under the microscopy used the immunohistochemical stains of CD34 / constant area ($1mm^2$). The statistical analysis evaluation showed increased significant difference of microvesicles number (MVD mm^2) stained byCD34 reaction in the experimentalsite (p=0.015) table 5.

Table (5): Comparison between studied groups according to MVD

MVD	Control	Experimental
Min. – Max.	26.25 – 37.0	42.0 – 62.0
Mean ± SD.	31.92 ± 5.40	52.0 ± 9.97
p		0.015*

p: p value for Post Hoc test (Scheffe) for comparing between control and experimental sites

*: Statistically significant at $p \leq 0.05$

B- Image Optical Density (IOD)

The image analysis of control and experimental sites revealed the image optical density (IOD) of immunohistochemical stains and calculated the relative density of VEGF by means of integrate density and area calculated (from intense to weak). The intense positive expression of the VEGF was reduced in the treated sites (Table 6), but there was no statistical significance finding. (Table7.)

Table (6): mean of IOD/pixel

Cases	Mean of IOD/pixel
Control site	130.1918
Treated site	118.7123

Table (7): Comparison between studied groups according to IOD of VEGF expression

VEGF	Control	Experimental
Min. – Max.	105.26 –180.16	95.18 – 137.40
Mean ± SD.	130.19 ± 24.64	118.71 ± 15.0
p		0.743

p: p value for Post Hoc test (Scheffe) for comparing between control and experimental sites.

4. Discussion

Local subgingival applications of phytotherapeutic agents have emerged and gain special interest as adjunctive modalities to enhance the clinical and microbial benefits of mechanical debridement. (21)

Thyme is an aromatic herb extensively used to add a distinctive aroma and flavor to food. It is rich in volatile oil. Several biological properties are attributed to this oil; it presents fungicidal, antiseptic, and antioxidant activities, and is an excellent tonic, besides carminative, antispasmodic and expectorant properties.(22) In folk medicine the main applications of thyme have been in the treatment of digestive complaints and respiratory problems, and in the prevention and treatment of infection.(23) The major phenolic components in thyme extracts, especially thymol and carvacrol, present higher antioxidant activity than the well-known α-tocopherol antioxidants. (24,25)

In this study, to evaluate the clinical benefits of thyme gel in periodontitis, various indices were used. GI provides an assessment of gingival inflammatory status that can be used to compare gingival status at recall visits. Comparison between experimental and control sites showed no statistically significant difference in GI at 4th week time interval, but a statistically significant difference in GI was shown at 6th week and 12th week time interval. A statistically significant reduction in GI was seen in both experimental and control sites at all intervals of time (4th, 6th, and 12th week), when compared to baseline, indicating that debridement itself results in significant reduction in clinical signs of periodontal disease. This may be because supra and sub-gingival calculus removal results in reduction of microbial load and thereby the endotoxins. This resulted in reduced severity of inflammatory infiltrate in the periodontal soft tissues, thereby providing favorable environment for diseased gingival tissues to heal. The change being higher in the experimental group signifies additional beneficial effect of thyme gel in reducing inflammation and promoting healing.

Bleeding on probing provides an objective, easily reproducible assessment of gingival status. It is extremely useful for detecting early inflammatory changes and the presence of inflammatory lesions located at the base of the pocket, an area which is usually inaccessible to visual examination.(1) When compared with baseline, a statistically significant reduction in BI scores were observed in both experimental and control sites at all points of time during the study. Comparison between experimental and control sites showed a statistically significant difference in BI scores at 6th week and 12th week time interval, but not at the 4th week interval. The improvement is possibly due to the beneficial effects of SRP, which helps sulcular epithelium regain its structural integrity and optimum thickness. The blood vessels attain normal tonus as inflammatory mediators diminish. Thyme gel is additionally known to possess antimicrobial (11) and anti-inflammatory properties (26, 27) leading to non-inflamed and healthy periodontal tissue that is less susceptible to bleeding on probing.

Periodontal pockets are pathognomonic signs of periodontal disease and therefore attain critical status in diagnosis of periodontitis. When compared from baseline to 4th, 6th, and 12th week, a statistically significant reduction in probing pocket depth was observed at both experimental and control sites. No statistically significant differences were observed in PPD values between control and experimental sites at 4, 6, and at 12 weeks time interval (P>0.05). Higher PPD was observed at control sites compared with experimental sites at 4, 6, and 12 weeks time interval. Changes in PPD and CAL may be attributed to the reduction in gingival inflammation and resolution in inflammatory exudates along with the formation of new collagen fibers. Favorable changes in PPD and CAL values at the experimental sites may be attributed to the beneficial effect of thyme gel.

Thus, in terms of clinical parameters, when comparisons were made between experimental and control sites, a significant improvement was found in GI and BI scores at experimental sites, while the improvement in PPD and CAL were not statistically

significant, indicating the ability of thyme gel in preventing progression of lesion and maintaining the pocket in an inactive state.

Wound healing is a complex process involving a number of interdependent stages including hemostasis, inflammation and angiogenesis, proliferation, and remodeling (28). Different cell types, complex signaling events and numerous growth factors are involved in each phase of wound healing. In this study, to evaluate the histological benefits of thyme gel in reducing inflammation and stimulating wound healing in periodontitis, gingival sections were evaluated histologically with H & E and Masson trichrome stain and immunohistochemically with vascular endothelial growth factor VEGF and CD34.

Histologically, gingival sections stained with H&E of the control sites exhibited massive inflammatory cellular infiltrate in the connective tissue. (Figs.1 and 2). on the other hand, mild inflammatory cellular infiltrate was observed in the thyme treated sites with evident fibroplasia and prominent blood vessels. (Fig3) Masson trichrome stain showed mild intensity of collagen fibers, few fibroblasts and numerous inflammatory cells and decreased CT thickness in the control sites.(Fig.4) While, the thyme treated specimens showed moderate staining reaction of well-developed collagen bundles with organized proliferating fibroblasts and endothelial cells. Also there were numerous capillaries & few inflammatory cells (Fig. 5)

These results confirmed the anti-inflammatory role of thyme as recently reported by Braga *et al.*(26) and Juhas *et al.*(27) who pointed out the pharmacological properties and anti-inflammatory effects of thyme essential oil. Moreover our findings are also in accordance with the previous studies conducted by Skold *et al.* and Yucel *et al.* who proven that thyme was shown to have strong anti-inflammatory action by decreasing the release of inflammatory metabolites like prostanoids, interleukins, and leukotrienes. (13,29)

VEGF is one of the major endothelial-cell specific stimulatory factors; it was detected within vascular endothelial cells, neutrophils, plasma cells and junctional, pocket and gingival epithelium. It is a multifunctional angiogenic cytokine of importance in inflammation and wound healing. (30)

Recently VEGF has attracted attention as a potential inducer of periodontal disease progression (31) and correlates with inflammatory resolution and periodontal tissue healing. (32)

In this study, the gingival expression of VEGF in endothelium and CT was reduced in the sites treated with thyme compared to the control sites. However this reduction was not statistically significant. This finding was in agreement with the study of Prapulla and coworkers (2007) (33) who reported that, VEGF levels increased from health to periodontitis, and periodontal treatment resulted in a reduction in their concentrations. Our findings are also in accordance with more recent studies by Thais *et al.*(2008) and Pradeep *et al.*(2011) who indicated that VEGF plays a key role in periodontal disease progression and can be considered a biomarker of periodontal disease progression and effectiveness of therapy. (34,35)

One method for detection of the degree of neovascularization is the microvascular density (MVD). The assessment of microvessel density was developed by Weidner *et al.*, initially using antibodies against factor VIII-related antigen that stain mainly matures vessels. (37)The more recent is the use of antibodies against CD34 to evaluate the degree of neovascularization and subsequently degree of wound healing. (38)

The current work showed significant increase in endothelial and CT expression of CD34 in gingival tissue samples at sites treated with thyme compared to control sites.

The present study was aimed to evaluate the clinical and histological benefits of thyme gel in conjunction with SRP in patients with chronic periodontitis. The results at both control and experimental sites were significant when compared to baseline; this reinforces the already established importance of SRP in non-surgical periodontal therapy (39,40). When comparisons were made between the control and experimental sites, clinical results were significant only in terms of gingival parameters, but not that of periodontal parameters. Moreover histological and immunohistochemical findings revealed that subgingival application of thyme gel seems to promote in vivo angiogenesis, thus enhancing the healing process of the periodontal soft tissue wounds as evidenced by significant increase in the expression of CD34 in sites treated with thyme. Furthermore an anti inflammatory effect has been exhibited by thyme as evidenced by the reduction in inflammatory cellular infiltrate and VEGF expression in thyme treated sites compared to control sites.

So, it can be concluded that thyme gel enhanced the results of SRP by improving the gingival parameters. Additionally, it also helped in preventing progression of periodontal lesion owing to its anti-inflammatory properties. In patients with periodontitis, more favorable results may be expected if thyme gel is used in conjunction with surgical periodontal therapy.

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