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Lepidium Sativum Extract versus Simvastatin as an Adjunctive Local Delivery Agents to Non-Surgical Periodontal Therapy: A Randomized Controlled Clinical Trial with Biochemical Analysis

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Abstract

Background: *Adjunctive local periodontal therapy is one of the best options to improve the health of the pocket tissue. The comparison of local effects of simvastatin and Lepidium sativum as an aid in reinforcing the healing of periodontal tissue is a point of importance.The objective of this work was to evaluate theeffectiveness of the local application of Lepidium sativum versus simvastatin gel on clinical parameters and nuclear factor kappa B biomarker.*

Subjects and methods: *The current study was conducted on thirty patients selected in this study with localized periodontitis stages (II, and III) and grade (A). Patients havehaphazardlydivided into two groups; with fifteen subjects in each. Group (I): (Control group) candidates treated with scaling and root surface debridement with local delivery of 1.2% of simvastatin gel. Group (II): (Test group) they were treated with scaling and root surface debridement with local delivery of Lepidium sativum gel.Clinical and biochemical indices were gathered at time intervals; baseline (after 1 week from the last debridement), 1 month, and 3 months postoperatively.*

Results: *Statistical results of our study showed advancement in MSBI, PD, and CAL parameters in both groups (I) and (II) after 1 month and 3 months follow-ups. There was a higher significant minimization in NF-κB level in the SMV group in comparison with the LS group.*

Conclusion: *Hence, we concluded from this present study that the test group has almost similar significant impact to the control one on the periodontal disease indicators as an adjunctive agent to non-surgical therapy, which requires more investigations and different concentrations used for LS efficacy.*

Keywords: *Periodontitis; Non-surgical therapy; Lepidium sativum; Simvastatin, Nuclear factor kappaB.*

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I. Introduction

Periodontal disease is a common oral disease that is characterized by severe inflammation and destruction of the periodontal attachment apparatus.The disease involves a wide range of endemic inflammatory conditions that affect the supporting structures of teeth (clinical attachment and bone loss) leading to tooth loss and inflammation of the system.The pathogenesis of periodontitisis characterized by microbial-associated, host-mediated inflammation that leads to the destruction of periodontal tissue***(Kinane et al., 2017).***

In an effort to align it with arising scientific evidence; the classification of periodontitis had been frequently updated.The 2017 world workshop accepted that three types of periodontitis can be identified, consistent with current knowledge of pathophysiology: necrotizing periodontitis, periodontitis as a manifestation of systemic diseases, and types of the disease previously known as ‘chronic’ or ‘aggressive’ and now grouped into a single category, ‘periodontitis’.This modern classification is also based on multi-dimensional staging and grading systems according to the severity, complexity, distribution, rate of progression, and modifiable risk factors ***(Caton et al., 2018).***

The pathogenesis of periodontitis includes antagonistic actions between subgingival biofilm and host immune responsecausing a loss of balance between the virulence of bacteria and the host defense and leading to changes in the function and structure of the periodontium. The bacterial plaque has a major role in exacerbating these responses and causing the disease ***(Cekici et al., 2014).***

NF- κB plays a major role in soft tissue inflammation and collagen degradation through MMP activation.Periodontal tissue remodeling is also mediated by the activation of NF- κB- dependent genes encoding inducible forms of COX- 2 and iNOS enzymes participating in the production of prostaglandins, and NO and its metabolites. This factor also has another important function in bone destruction by stimulating osteoclast production. NF- κB- dependent cytokines such as IL- 1α and IL- 1 β, TNF- α, IL- 6, and IL- 17 induce osteoclast differentiation and activation mediated by RANK***(Gölz et al., 2015)***.

Although Non-surgical periodontal therapy (NSPT) is considered the gold standard and the first route to periodontal disease management, its inability to completely eliminate periodontal pathogens from the soft and hard tissue surfaces and may cause re-colonization leads to reinfection. To overcome these deficiencies, adjunctive use of systemic or local chemotherapeutic agents becomes an indispensable treatment modality ***(Ehizele et al., 2013).***

The rationale for using locally delivered drugs (LDD) in periodontal disease is to chemically kill or reduce the plaques within the biofilm in the pocket. This is achieved by applying high concentrations of an antibiotic or antiseptic in direct contact with the root surface without remarkable systemic effect***(Arunachalam et al., 2017).***

Statins such as simvastatin (SMV) have been considered as an ancillary remedy to non-surgicalperiodontal therapy. Statins were initially imported as cholesterol-reducing drugs, which were performed by hindering the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Statins also have additional properties including; anti-inflammatory, antioxidant, antibacterial, and other pleiotropic effects such as blocking the release of pro-inflammatory mediators and matrix metalloproteinases (MMPs). Therefore,statins have been shown to promote bone formation and have proven to be effective in periodontal therapy***(Zeiser, 2018)***.

There are many researcherswho use plants without any regard for their benefits, efficacy, safety, and therapeutic prominence. Lepidium Sativum (LS) is an annual, edible herb belonging to the family of Brassicaceae. It was found in many countries such as Saudi Arabia, India, and Egypt. It had other names such as Garden cress andHab Al-Rashad ***(Prajapati et al., 2014; Moro et al, 2018).***

LSis composed of seeds, leaves, and roots. LS seeds contain many several phenols, minerals, proteins, and fatty acids. LS oil (LSO) which is extracted from seeds, consisting primarily of polyunsaturated fatty acids (linolenic acid (LA) andα-linolenic acid (ALA)). Due to its high antioxidant and phytosterol content, this oil is relatively stable leading to a decrease in reactive oxygen species (ROS) generation and their nitric oxide metabolites ***(Al-Sheddi et al., 2016).***

LS extract can be used in many therapeutic domains asan anti-hyperglycemia, anti-hyperlipidemia, anti-diarrhea, anti-rheumatic, hepatoprotective, anti-oxidant, anti-inflammatory, and anti-microbial and in gastrointestinal, skin and airway diseases and some bone fractures healing. Moreover,LS is used in common medically to treat inflammatory diseases such as hepatitis, diabetes mellitus and joint inflammation.***(Shabbir et al., 2018).***

II. Subjects And Methods

Study Design: This study was designed as a randomized, controlled, and phase (IV) clinical trial using computer-generated random tables. The patients, researcher, and outcome assessor were blinded from the type of intervention.

Patient selection and grouping: Thirtyperiodontitis patients were chosen from the out- patient clinic of Oral Medicine, Periodontology, Oral Diagnosis and Radiology Department, Faculty of Dentistry, Ain Shams University. Total number of patients was randomly divided into two groups:
Group I (Control group): included (15) patients with stage II and III, grade A periodontitis; were treated with scaling and root surface debridement followed by application of 1.2% simvastatin gel.
Group II (Test group): included (15) patients with stage II and III, grade A periodontitis;were treated with scaling and root surface debridement followed by application of lepidium sativum gel.

Patients were selected considering the following criteria :(they were free from any systemic disease according to the modified Burkett's health history questionnaire, both genders with age ranged from 25-50 years, pocket depth ≥5mm with horizontal bone loss and less than 30% of teeth were affected, and must be able to return to recall visits). Smokers, pregnant and lactating women, prisoners or abused individuals, uncooperative patients, patients with history of allergy, and previous periodontal treatment or any use of antibiotic/anti-inflammatory drugs within the last 6 monthswere excluded from this study.

All patients in this study were received conventional scaling and root debridement using hand and ultrasonic instruments followed by oral hygiene instructions.The study was approved by the Research Ethical Committee,

Faculty of Dentistry, Ain Shams University. All eligible patients were thoroughly learned of the nature, possible risks and their auxiliary aids in the research and signed written informed consent documents.The whole study was carried out from September 2021 to July 2022.

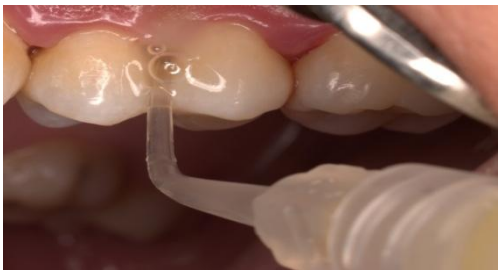
Methods:

Preparation of 1.2% simvastatin gel: A 4% methyl cellulose 4000 C*ps*gel was prepared by dispersing 2 gm of methyl cellulose powder in 50 ml of hot distilled water 50 - 60 o C (as the methyl cellulose starts to melt at 65.7oC). 1.2mg of SMV was added to distilled water to produce 1.2% concentration of the drug in the gel. Continuous stirring is performed until cooling to obtain the gel form of homogeneous mixture of polymer and ensure complete dissolution of methyl cellulose, water and drug at room temperature. The produced gel is white in color with viscous nature***(Pradeep and Thorat 2010).***

Preparation of lepidium sativum in situ gel: Carbopol-poloxamer gel of LS was prepared by the cold method (Schmolk, 1972). Carbopol P940 (1% w/v) was firstly solved in non-ionized water using a magnetic stirrer. About (30% w/w) of LSO was added and stirred for 5 min at 200 rpm. After total dissolution, the solution was cooled in an ice bath, pluronic F127 (30% w/v) was then added slowly with continuous stirring. The mixture was transferred to fridge at 4 degree Celsius for 24 hours to ensure complete drying and removal of entrapped air bubbles***(Nasra et al., 2017).***

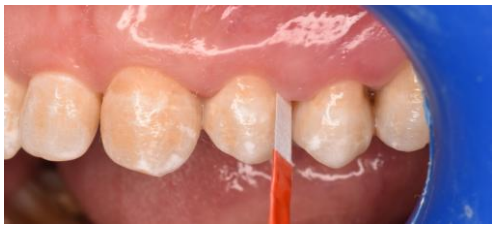
Local gel application into the periodontal pocket:LS and SMV gels were locally delivered in the deepest periodontal pockets. Local application of the gel was done at baseline. The area around the injected materials was isolated by cotton rolls. The two gels were injected into the pockets using a syringe with blunted needle. The pockets were filled until the materials were detected at the gingival margin ***(Agarwal et al., 2016).***

Figure (1):application of LS gel using a plastic blunted tip



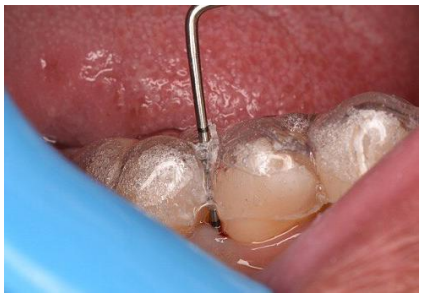
Gingival crevicular fluid sample collection:GCF samples were taken at baseline, 1 month, and 3 months after therapy. The GCF sample was collected by inserting periopaper (Oraflow Inc., Smithtown, NY, USA) parallel to the long axis of the tooth, into the sulcus/pocket for 30s until a minimal resistance was felt. Periopaper strips visually contaminated with blood and saliva were discarded. Level of nuclear factor kappa b in GCF samples will be determined by using a commercially available means of Enzyme Linked Immune sorbent Assay (ELISA) ***(Escobar et al., 2018).***

Figure (2): Insertion of a periopaper strip parallel to the long axis of the tooth, just 0.5 mm into the sulcus, and kept for 30 seconds for GCF sample collection



Clinical assessment: The periodontal conditions of each patient were evaluated at baselineand after 3 months follow-up. Plaque Index (PI) used to assess plaque accumulation around gingival margin. Mean Sulcus Bleeding Index (SBI) used to assess gingival inflammation. Probing Depth (PD) and Clinical Attachment Level (CAL) were measured by the University of Mish O'probe with Williams' graduations.TheOcclusal stent was used as an auxiliary tool for the proper placement of the probe.

Figure (3): Measurement of probing pocket depth equal to 5mm using the University of Mish O'probe with stent placement



III. Results:

This clinical trial was done in 30 patients with a mean age of 35.5 years whom suffered from mild to moderate periodontitis. All of them continued the study with no allergic reactions or any adverse effects.

A. Clinical evaluation:

Regarding oral hygiene status, plaque index score (PI) in both groups of the study was showed a significant advancement of plaque amount condition at baseline. However, PI showed no significant improvement in each group after 3 months of the treatment. By comparing the two studied groups, there was no statistically significant difference between them at baseline and after 3 months of therapy. (Table 1, figure 4)

Additionally, the test and control groups showed no significant decrease in the mean sulcus bleeding index (MSBI) at baseline. While these two studied groups showed significant advancement in SBI and improvement in the bleeding status after 3 months of treatment. But in spite of all that, when comparing both groups there was no noticeable difference at baseline and after 3 months follow up. (Table 1, figure 4)

Furthermore, the reduction of probing depth (PD) and clinical attachment level (CAL) were statistically significant within test and control groups from baseline until 3 months. Thus, the periodontal condition was improved after intervention in each group. When comparing SMV versus LS intended, the reduction in PD and CAL at each investigation period was not statistically significant. (Table 1, figure 4)

Table (1): Mean and standard deviation (SD) for PI, MSBI, PD, and CAL percentage change (%) for two studied group at baseline and after 3 months follow up:

Measurement	Percentage change (%) (Mean±SD)		p-value
	Simvastatin group	Lepidium Sativum group	
PI	0.25±0.50	0.50±0.71	**0.780ns**
MSBI	0.86±0.21	0.81±0.21	**0.552ns**
PD	0.42±0.08	0.35±0.09	**0.063ns**
CAL	0.35±0.09	0.30±0.14	**0.583ns**

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

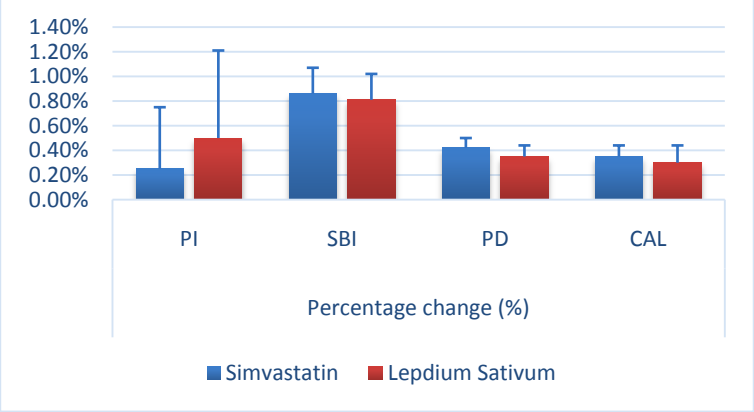


Figure (4): Bar chart showing average PI, MSBI, PD, and CAL percentage change (%) for two studied group at baseline and after 3 months follow up

B. Biochemical evaluation:

Regarding the change in the level of NF-κB between two studied groups, SMV group showed higher values at two time interval (baseline-1 month) and (baseline- 3month) than LS group. But the statistical difference was not significant. Also, there is no statistically substantial difference between two groups at time interval (1 month – 3 month).(Table 2, figure 5)

Table (2): Mean and standard deviation (SD) for NF-κB percentage change (%) for two studied groups

Interval	NF-κB percentage change(%) (Mean±SD)		p-value
	Simvastatin group	Lepidium Sativum group	
Baseline- 1 month	24.63±6.50	19.69±7.36	**0.071ns**
1-3 months	37.74±13.82	36.62±7.00	**0.789ns**
Baseline- 3 months	53.59±8.48	49.10±7.48	**0.149ns**

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

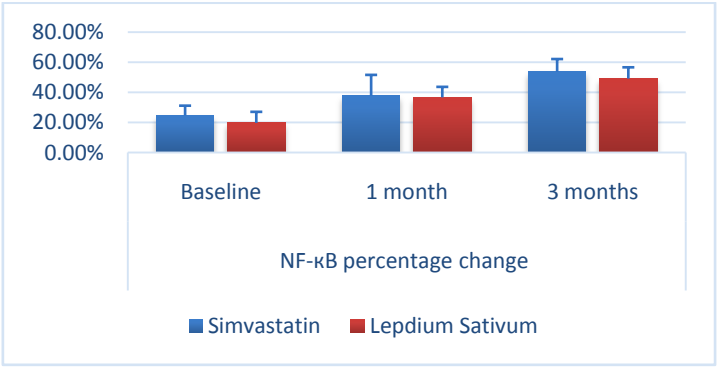


Figure (5): Bar chart showing average NF-κB percentage change (%) for the two studied groups

IV. Discussion

The primary aim of periodontal therapy is to inhibit fragmentation and replaces the periodontal apparatus with its initial form and structure. Non-surgical periodontal therapy (NSPT), including scaling and root surface debridement (SRD), has been suggested as the ideal initial treatment for patients suffering from periodontitis. This phase I therapy alone cannot guarantee remission of the disease and achieve healing of tissue completely. Hence, SRD should be augmented with adjunctive methods systemically or locally ***(Laleman et al., 2017; Graziani et al., 2018).***

Local delivery of antibacterial therapeutic agents in periodontitis can be effective when they are placed directly in periodontal pockets. LDD has numerous properties including minimally invasive, direct application at the site of infection, avoidance of gastrointestinal issues, and devaluation in the dose. The essential advantage is that the drug has a higher concentration than the minimum inhibitory concentration (MIC) after being delivered into the pocket and persists for up to several weeks **(*Szulc et al., 2018; Rajeshwari et al., 2019).***

The host modulation treatment (HMT) aims to reduce periodontal tissue damage and stabilize or even regenerate periodontium. This can be achieved by improvements in themodulatory behavior of immune responseagainst perio-pathogens. HMT can improve therapeutic outcomes, slow the progression of the disease, allow for more predictable management of patients, and possibly even work as a preventive agent against the development of periodontitis ***(Deore et al., 2014; Sulijaya et al., 2019)***.

LS accelerates the speed of bone fracture healing. It possesses strong pharmacological properties which resemble those of non-steroidal anti-inflammatory drugs (NSAIDs). It was suggested that the extract produced anti-inflammatory and anti-ulcerative effects by inhibiting *P.g* synthesis like other NSAIDs ***(ElSayed et al., 2017).***

According to a new study by ***Bathish et al., 2021,***Lepidium sativum seeds possess strong pharmacological properties which are anti-inflammatory immune-modulatory effects. In addition, due to the antioxidant properties of seeds, the beneficial effect of LS on bone-forming cells relies on the phenolic content in the seeds of garden cress (GCS) essentially Tocopherols. They contribute to disease prevention, besides having an essential nutritional role as a source of vitamin E for humans.In addition,L.S has immunomodulatory properties and wound healing activities. It decreases nuclear factor (NF-κB), nitric oxide (NO), COX-2 and leukotriene B4 (LB4) which are thought to play roles in the pathogenesis of inflammatory diseases. L. sativum prevented the rise of RANKL/OPG ratio, which is an essential component in the regulation of bone metabolism ***(Abdallah et al., 2020).***

The anti-inflammatory effect of SMV comes from the down-regulating expression of the pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α and decrease levels of tissue destruction mediators like as MMP-1, 3, 8, 9, and 13 through inhibiting activation of NF-κB that regulates the expression of diverse inflammatory cytokines and destructive enzymes.The anti-inflammatory effect of simvastatin as a locally delivered chemotherapeutic agent showed a substantial improvement in the clinical indicators of periodontal disease. Also, SMV has an antimicrobial effect which is considered to be the best statin against periodontal pathogens such as *P.gingivalis* and *A.a*. In addition,some studies have proved that statins have the capability to elevate levels of both OPG and BMP-2; and inhibit RANK/RANKL factors. Thus SMV can hinder bone resorption, and encourage osteoblast differentiation and neovascularization***(Martande et al., 2017; Roca-Millan et al., 2019; Gupta et al., 2019; Rajagopalet al., 2021).***

A recent study confirmed the impact of SMV and LS with NSPT on the RANKL/OPG ratio in the crevicular fluid. The effects of RANKL are blocked by its soluble decoy receptor osteoprotegerin (OPG), thus inhibiting osteoclast differentiation, activation, and survival ***(Rajagopal et al., and Hegazy et al., 2021).***

Regarding PI score, it was recorded that the LS group (which is the test one) showed higher PI than the SMV group (which is the control one) after 3 months of treatment in comparison with baseline, which is after one week of the last SRD, but this difference was not statistically significant between the two studied groups. In addition, the mean difference and percentage change in SMV are not significantly differentfrom that of LS. Therefore, it was noticed that patients strove to be compliant and follow proper oral hygiene instructions.

These results are in accordance with ***Vijayapremakumar et al., 2021*** who noted a slight increase in plaque scores after 90 days. This might be due to the reduction in the concentration of the drug. None of the patients reported any long-term side effects and no adverse reactions were observed during the study.

Despite the mean percentage change of MSBIshowing no statistically substantial difference between these groups after 3 months of follow-up, there was a statistically significant decrease in each of the two studied groups from baseline to after 3 months with a little advantage for the LS group. This was due to the resolution of the inflammation and reduction of pro-inflammatory markers. This result is similarly coinciding with a study conducted by ***Gupta et al., 2021***who demonstrated that a significant reduction in clinical signs of inflammation can be achieved by NSPT in combination with SMV in patients with chronic periodontitis.

Furthermore, studies conducted by ***El Kilani, et al., 2019*** and ***Bathish et al., 2021*** showed clinically the important role of LS seeds powder extract with local debridement. The bleeding index was significantly reduced after 3 and 6 months follow-up periods.

With reference to probing depth (PD), the results of the present study showed a significant decrease in pocket depth in both groups from baseline to three months. However, by comparing the study group with the control one, there was no statistically significant difference between them at baseline. After 3months, SMV showed a more significant decrease in PD than LS. Although the mean percentage degree of PD showed a significant decrease in the SMV group, there was no statistically substantial difference between the two studied groups. These results are compatible with the studies demonstrated by ***Donos et al. 2020***and ***Cecoro et al., 2021*** who showed a significant reduction in PPD after local application of 1.2% SMV combined with NSPT at time intervals from baseline up to 3 months.

Moreover, in agreement with a study demonstrated by ***Bathish et al., 2021,*** the local delivery of LS gel showed a significant decrease in PD after 3 months of treatment. This study indicated that LS gel was applied twice to ensure its efficacy.

In our study, the clinical attachment loss was decreased significantly in each group with a slight preference for the SMV group after 3 months of treatment. Thus clinical attachment level was gained after 3 months postoperatively. Despite that, there was no statistically significant difference in the mean difference and percentage degree of CAL between the test and control groups at two-time intervals. This improvement of CAL in each group was noticed in a study by ***Vemanaradhya et al., 2017***who confirmed that SMV is ranked the best in CAL gain than among statins family. Another recent study was done by ***Bathish et al., 2021*** who showed that Lepidium sativum extract gel can result in a significant reduction in clinical attachment loss and CAL gain.

By comparing NF-κB (ng/ml) levelsbetween the study and control group, each one of these two groups showed a significant decrease in inflammatory marker value from baseline to after 3 months follow up. The mean difference revealed that the SMV group showed significant differences at time intervals (baseline-1 month) and (baseline-3 months). But in the time interval from one month to three months, there was no statistically substantial difference between L.S and SMV groups. In spite, SMV had higher change than LS at three-time intervals (baseline, 1 month, and 3 months); the mean percentage change of NF-κB demonstrated that there was no statistically significant difference between the two groups of the study.

These results are in accordance with a study done by ***Hegazy et al., 2021***who proved the efficacy of L.S in decreasing the expression of NF-κB through their effects on markers of inflammation such as IL-6 and TNF-α which played a fundamental role in bone degradation and inflammation.

A systematic review conducted by ***Petit et al., 2019*** showed the importance of statins such as SMV in the suppression of severe pathways in inflammation including NF-κB activation. In addition, this study confirmed that osteoclastogenesis was decreased by down-regulation of RANKL/RANK signaling and NF-κB.

V. Conclusion

Withinthe limitations of the current study, we concluded thatlocally delivered LS and SMV can be used as effective local adjunctive agents to NSPT because of their important direct impact on periodontitis clinical parameters and NF-κB level reduction. In addition, we also recommended more studies and investigations to evaluatemultiple applications and different concentrations of LS in situ gel in the management of periodontitis.

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