



Effect of *Lepidium Sativum* Gel as an Adjunct to Non- Surgical Treatment in Management of Periodontitis Patients Stage (II, III) and Grade (A, B)

Yusra M. Bathish^{1*}, Naglaa S. Elkilani², Aya M. Dawaba³, Zienab S. Farid⁴

Codex : 14/21.04

azhardentj@azhar.edu.eg

http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2021.48100.1315

Oral Medicine & Surgical Sciences
(Oral Medicine, Oral & Maxillofacial
Surgery, Oral Pathology, Oral Biology)

ABSTRACT

Purpose: The current study was conducted to elaborate the clinical and transforming growth factor beta biomarker effect of local application of lepidium sativum gel as an adjunct to scaling and root planing in the therapeutic management of periodontitis patients stage(II, III) and grade(A, B). **Subjects and Methods:** The current study was conducted on twenty patients were selected in this study with stage (II, III) and grade (A, B) periodontitis. Patients were randomly divided into two group's ten subjects on each. Group A: (control group) were treated with scaling and root planing alone, group B: (study group) were treated with scaling and root planing with local delivery of lepidium sativum gel. **Results:** study group showed a significant increase in the clinical parameter like clinical attachment level (CAL), probing depth (PD), bleeding on probing (BOP) and plaque index (PI). Clinical parameters were recorded after one month and three months, transforming growth factor beta biomarker level after one week and four weeks in comparison with control group follow up. **Conclusion:** Adjunctive use of lepidium sativum with non-surgical therapy showed an improvement in the treatment outcome of periodontitis patient's stage (II, III) and grade (A, B).

KEYWORDS

Periodontitis,
Lepidium Sativum Gel,
Transforming Growth Factor Beta,
Non-Surgical Treatment.

INTRODUCTION

Periodontal infection is an immuno-provocative ruinous disease triggered by reduction of attachment of soft tissues in periodontal

• Paper extracted from Master Thesis titled "Effect of *Lepidium Sativum* Gel as an Adjunct to Non- Surgical Treatment in Management of Periodontitis Patients Stage (II, III) and Grade (A, B)"

1. Dentist at Faculty of Oral and Dental Medicine 6 October University, Cairo, Egypt
2. Professor of Oral Medicine, Periodontology, Oral Diagnosis and Radiology Faculty of Dental Medicine for Girls' - Al-Azhar University, Cairo, Egypt
3. Lecturer of Pharmaceutic, Faculty of Pharmacy' - Al-Azhar University, Cairo, Egypt
4. Lecturer of Oral Medicine, Periodontology, Oral Diagnosis and Radiology Faculty of Dental Medicine for Girls-Al-Azhar University, Cairo, Egypt.

* Corresponding author email: dryusramahmoud103@gmail.com

tissues and resorption of alveolar bone triggered by pathogenic micro-organisms leading to gingival recession and pocket formation⁽¹⁾. It is the product of host-microbial interaction where significant destruction is caused by the inflammatory component⁽²⁾.

In an effort to reconcile it with new scientific evidence, the classification of periodontitis had been repeatedly updated. The 2017 world workshop accepted that three types of periodontitis can be identified, consistent with current knowledge of pathophysiology: necrotizing periodontitis⁽³⁾, periodontitis as a manifestation of systemic diseases⁽⁴⁾ and types of the disease previously known as 'chronic' or 'aggressive' and now grouped into a single category, 'periodontitis'⁽⁵⁾.

Non-surgical periodontal therapy is known to be the gold standard and is the first path to periodontal disease management that is recommended. However, not all periodontopathic bacteria in such inaccessible areas are eliminated by traditional mechanical debridement. However, mechanical debridement has demonstrated clinical advancement in the periodontal disorder therapy, along with adjunctive use of anti-infective agents and other host modulatory agents⁽⁶⁾.

Several different pathways can begin an inflammatory response, including B cells, T-cells, and macrophages. In order to organize the immune response, they generate pro-inflammatory cytokines or signaling proteins, resulting in either pro- or anti-inflammatory results⁽⁷⁾. The host modulation therapies resolve the host immune response to antigens. They seek to interrupt the immune inflammatory pathways within the response of the host to certain pathogens that perpetuate the inflammatory response⁽⁸⁾.

In periodontal disease pathogenesis, cytokines show a significant act. These are found in GCF and help to diagnose the activity of periodontal disease and to resolve the impact of periodontal therapy⁽⁹⁾.

More recently, research on a new lineage of T cells, regulatory T cells (Tregs) has gained prominence. Cytokines such as Transforming Growth Factor β (TGF- β) are produced by these cells that,

by regulating exacerbated immune responses, regulate the induction and activity of effector T cells⁽¹⁰⁾.

TGF- β is a bio functional growth regulator; this plays an important role in controlling inflammation, embryonic growth, tissue repair, immune system, cell growth, and differentiation⁽¹¹⁾.

Lepidium sativum is an annual herb that grows quickly originating in Egypt and West Asia, commonly known as garden cress. Its seeds are high in protein, omega-3 fatty acids, dietary fiber, phytochemicals, iron and other essential nutrients. L.S has been given different Arabic names in Arabic countries, such as Rashad / Thuffa. In European cultures, the plant was well known as *Herba Lepidii Sativii*⁽¹²⁾.

Oil is contained in *Lepidium sativum* seeds, consisting primarily of linolenic acid (LA) (12percent) and α -linolenic acid (ALA) (32 percent)⁽¹⁶⁾. Due to its high antioxidant and phytosterol content, this oil is relatively stable. L. Synergistic impacts of restraint of thromboxane B2 and aggregation of platelets focuses in the spleen and lung tissues of wistar rodents have been recorded in *sativum* oil (LSO)⁽¹³⁾. In a further study in rats, LSO was developed to decrease lymphocyte proliferation and inflammatory production⁽¹⁴⁾.

L. Sativum is used in common medically to treat inflammatory diseases such as hepatitis, diabetes mellitus and joint inflammation⁽¹⁵⁾. Some studies have shown that *L. extract* has been used. *Sativum* has antidiarrheal, antioxidant, antispasmodic, hepatoprotective, anti-inflammatory and antimicrobial impacts against oxidative damage^(16,17).

SUBJECTS AND METHODS

Study design

This current study was a randomized clinical trial on 20 patients with stage (II, III) and grade (A,B) periodontitis according to new classification and age scope of 20-45 years chosen from the out-patient clinic of Periodontology, Oral Medicine and Radiology Department at Faculty of Dental Medicine for Girls, Al-Azhar University.

Sample Size:

According to sample size calculation ⁽¹⁸⁾, the sample size of 20 patients (10 patients in each group) were sufficient to detect the difference. Total numbers of patients were alienated randomly in to two groups.

Group A (Control group): Ten (10) patients with stage (II, III) and grade (A, B) periodontitis .These patients were subjected to scaling and root planning only.

Group B (Study group): Ten (10) patients with stage (II, III) and grade (A,B) periodontitis. These patients were subjected to scaling and root planing with local delivery of *lepidium sativum* gel.

The patients were selected according to following criteria; (The patients were free from any systemic diseases as evaluated by modified Cornell medical index ⁽¹⁹⁾, no history of allergy or hypersensitivity reaction, non-smoker and non-pregnant women). The selected patients signed an informed consent.

Study procedures:

Prior to any procedure, all subjects were informed about the nature of their participation in the study. A satisfactory written consent was taken from all the patients denoting them about the scheduled research program and experimental design. Research ethics committee (REC) approval of the faculty Dental Medicine for Girls were obtained

All patients in this study were received conventional scaling and root planning using hand instruments and ultrasonic scalar followed by oral hygiene instructions.

Preparation of *lepidium sativum* gel ⁽²⁰⁾:

By watering 1% carbopol in 25 ml of water, the gels were primed for 24 hours and subsequently neutralized with an appropriate amount of triethanolamine. The medication dissolved in sufficient methanol. The neutralized carbopol solution with continuous stirring was precisely weighed in sum to

obtain a sparkling clear gel for about 30 minutes. Finally, the volume was 50ml with continuous stirring distilled water. The stirring was constantly stopped to expel the air caught during the stirring process.

Application of *lepidium sativum* gel in the periodontitis patient ⁽²¹⁾:

- Local delivery of *lepidium sativum* gel was injected in the deepest periodontal pockets.
- *Lepidium sativum* gel was injected into the pockets with a syringe with blunt needle around the selected teeth in the treatment test sites. The pockets were filled until the materials were detected at the gingival margin.
- After the first week, *Lepidium sativum* gel was re-applied only at the access point of the periodontal pocket at the selected location.

Clinical evaluation:

- Bleeding index (BI) ⁽²²⁾ and Plaque index (PI) were recorded at baseline, 1 and 3 months for each patient around the healing site.
- Probing depth (PD): The distance from the base of the pocket to the gingival margin was measured using periodontal probe a UNC-15 probe that has markings from 1-15 at 1 mm interval. It has colour coding at markings 5, 10 and 15.
- Clinical attachment level (CAL): The distance from the cement-enamel junction (CEJ) to the base of the periodontal pocket. The readings were recorded at the same location of PD.
- Collection of GCF samples and transforming growth factor beta anti-inflammatory markers⁽²³⁾:

At each three appointment (baseline, one week and four weeks), GCF samples were collected from one pocket site. Level of transforming growth factor beta in GCF samples will be determined by using a commercially available means of Enzyme Linked Immune sorbent Assay (ELISA).

RESULTS

Clinical evaluation results:

Plaque Index: by comparing the plaque index at baseline between study and control group revealed no statistically significant difference ($P=1$). Comparing the plaque index at one month between study and control group revealed a statistically significant difference ($P=0.0077$). Similarly, by comparing the plaque index at 3 months between study and control group there was a significant difference ($p=0.00$).

Bleeding Index: by comparing the bleeding index at baseline between study and control group revealed no statistically significant difference ($P=1$). Comparing the bleeding index at one month between study and control group revealed no statistically significant difference ($P=0.136$). Moreover, by comparing the bleeding index at 3 months between study and control group there was a significant difference ($p=0.00$).

Probing depth: by comparing the probing depth at baseline between study and control group revealed no difference statistically relevant ($P=0.11$). Comparing the probing depth at one month between study and control group revealed a significant difference ($P=0.042$). Moreover, by comparing the probing depth at 3 months between study and control group there was a significant difference ($p=0.011$).

Clinical attachment loss: by comparing the Clinical attachment level at baseline between study and control group revealed no difference statistically relevant ($P=0.6$). Comparing the Clinical attachment level at one month between study and control group revealed no difference statistically relevant ($P=0.179$). Moreover, by comparing the CAL at 3 months between study and control group there was a significant difference ($p=0.011$).

Table (1) Descriptive analysis of Absolute change in clinical parameters within the study groups through the whole study period.

		Baseline	After 1month	After 3 months	F value of ANOVA test	P value
Plaque index (mean \pm SD)	Study group	3 ^a \pm 0	1.11 ^b \pm 0.31	0.22 ^c \pm 0.12	547.18	0.00*
	Control group	3 ^a \pm 0	1.78 ^b \pm 0.63	1.22 ^c \pm 0.43	42.72	0.00*
Bleeding index (mean \pm SD)	Study group	3 ^a \pm 0	1.22 ^b \pm 0.44	0.33 ^c \pm 0.10	282.67	0.00*
	Control group	3 ^a \pm 0	1.56 ^b \pm 0.53	1.22 ^b \pm 0.47	53.38	0.00*
Probing depth (mean \pm SD)	Study group	5.11 ^a \pm 0.33	3.89 ^b \pm 0.49	3.00 ^c \pm 0.43	63.11	0.00*
	Control group	5.67 ^a \pm 1.0	4.89 ^{a,b} \pm 1.36	4.22 ^b \pm 1.30	3.48	0.045*
Clinical attachment loss (mean \pm SD)	Study group	3.78 ^a \pm 0.67	2.56 ^b \pm 0.73	1.22 ^c \pm 0.67	34.38	0.00*
	Control group	4.00 \pm 1.12	3.22 \pm 1.30	2.56 \pm 1.33	3.29	0.056ns

Significance level $p \leq 0.05$, *significant, ns=non-significant

Transforming growth factor beta: by comparing the anti-inflammatory marker at baseline between study and control group revealed no significant difference ($P=0.31$). Comparing the value at one week between study and control group revealed no significant difference ($P=0.111$). However, by comparing the value at 4 weeks between study and control group, there was a significant difference ($p=0.0004$).

Table (2) Descriptive analysis of absolute change in ELISA from zero point to 1&4 weeks between groups

	Study group	Control group	t value of independent t test	P value
Transforming growth factor beta at zero point (mean±SD)	379.14±54.03	412.20±79.87	1.05	0.31ns
Transforming growth factor beta after one week (mean±SD)	456.76±38.90	493.34±56.42	1.68	0.11ns
Transforming growth factor beta after four weeks	533.81±36.08	467.62±31.93	4.339	0.0004*

Significance level $p \leq 0.05$, *significant, ns=non-significant

DISCUSSION

Periodontitis is the sixth chronic disorder that affects more than 743 million persons worldwide and has an adverse effect on an individual's oral capacity, self- assurance, fundamental wellbeing and general prosperity. Recently, some systemic disorders and syndromes have been shown to be associated with a rise in immune system cell function and progression of clinical periodontal conditions⁽²⁴⁾.

The aim of periodontal disease care is to decrease probing pocket depth (PPD), enhanced levels of the clinical attachment (CAL) and reduce bleeding on probing (BOP) incidents⁽²⁵⁾.

The host modulation treatment (HMT) aims to reducing periodontal tissue damage and stabilizing or even regenerating periodontium by improvements in immune response to preventative and corrective behavior. The ability to alter the host response to periopathogens in an immunomodulation manner to avoid damage to periodontal tissue allows for rapid recovery from periodontal homeostasis⁽²⁶⁾.

From ancient times to today, complementary treatments, such as traditional folk medicine, have used natural elements. This has been achieved in various cultures for the treatment of several ailments. In particular, *Lepidium sativum* and its seeds were widely used in Saudi Arabia as herbal medicine mainly to treat recent traumatic fractures and less commonly for delayed or unitary fractures⁽²⁷⁾.

TGF- β represents an important growth factor used for the development of a variety of tissues, including the process of cure for the bone, via extracellular protein formation, the matrix control of metalloproteinase and the spread of fibroblast during the wound treatment process⁽²⁸⁾.

In the current study used *Lepidium sativum* seeds possesses strong pharmacological properties which anti-inflammatory effect⁽²⁹⁾. Due to the antioxidant properties of garden cress, the beneficial effect of L.S on bone forming cells relies on the phenolic content in the seeds of garden cress. Tocopherols are the major phenolic compounds that found in GCS extracts. Tocopherols also contribute to disease prevention, besides having an essential nutritional role as a source of vitamin E for humans⁽³⁰⁾.

TGF- β is a biochemical marker selected in the current study to detect the response of periodontitis to various treatments, as the mediator can contribute to tissue repair by stimulating production of collagen, neovascularization and proliferation of fibroblasts. TGF- β can prevent inflammatory cell synthesis of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6⁽³¹⁾.

The present study L.S was used because several studies showed that the use of *lepidium sativum* in periodontal therapy provide improvement of the clinical parameters of L.S treated groups⁽³²⁾.

The result of current study showed there were no significant difference in the study group and control group regarding the bleeding index from baseline

to one month. The decrease in bleeding is due to the resolving of gingival inflammation following the elimination of bacterial deposits by scaling and root management. However the between both groups in the interval from baseline to 3 months was significant difference in study group. These results are expected as lepidium sativum exhibits an anti-inflammatory effect, immunomodulatory properties and good wound healing activities⁽³³⁾.

As regard to plaque index score recorded in the present investigation the results showed that there were a significant differences in the study group and control group at 1 and 3 months observation period when compared to baseline. This was clarified in a study conducted in Egypt that revealed that *L. Sativum* extract shows antimicrobial activity against various gram-negative and gram-positive bacteria⁽³⁴⁾.

With reference periodontal pocket depth (PD) and clinical attachment level (CAL), the results of the present study showed significant reduction in pocket depth and gain in clinical attachment level in both groups from baseline to three months. However by comparing the study group with the control group, study group showed more significant improvement at different observation period. This is in accordance to previous studies indicating that *Lepidium sativum* can be used in the treatment of osteoporotic postmenopausal women with chronic periodontitis who refuse treatment with ALN. The combined use of LS and ALN may have a synergistic effect resulting in a more favorable clinical response, increased bone mass than using ALN alone when combined with conventional therapy in treatment of chronic periodontitis in postmenopausal osteoporotic women⁽³⁵⁾.

Transforming growth factor beta level in the current study is the most significant biomarker that has a modulator role, increasing healing and remodeling of connective tissue, and inducing angiogenesis. The lack of TGF- β may lead to periodontal damage, as the GCF of patients with chronic periodontitis has been documented to have decreased levels of TGF- β ⁽³⁶⁾.

By comparing TGF- β between study and control group regarding the mean change of biochemical value in the interval (baseline-1 week) revealed no statistically significant difference. Thus the fact that the levels of TGF- β depend on local inflammatory conditions and the host response characteristics are dynamic⁽³⁷⁾.

However in the present study, by comparing transforming growth factor beta level from baseline to 4 weeks this result is parallel to the previous study observed that TGF- β levels increased significantly in the treated group reported that an increase in TGF- β levels was associated with a decrease in periodontal inflammation, leading to improved clinical outcomes⁽³⁸⁾. With other study findings suggesting that high TGF- β production may be a protective factor for periodontitis, accelerating connective tissue remodeling and angiogenesis, which could contribute to inflammatory regulation and/or to bone remodeling events⁽³⁹⁾.

CONCLUSION

The adjunct use of *lepidium sativum* gel as host modulation improved the treatment outcomes when used with mechanical debridement in periodontitis patients.

RECOMMENDATIONS

- Further studies should be conducted, to determine time of release of local delivery *lepidium sativum* gel in vitro study.
- Further studies to determine optimal concentration of local delivery *lepidium sativum* gel in vitro study.
- Further studies to determine optimal concentration of polymeric carrier in vitro study.

ACKNOWLEDGMENT

I would like to express my deep gratitude to Dr. Naglaa S. El Kilani, Dr. Aya M. Dawaba, Dr. Zienab S. Shalaby.

DECLARATION STATEMENT

Authors declare no conflict of interest.

REFERENCES

1. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J. Periodontol.* 2018; 89: 159–72.
2. Figueredo CM, Lira-Junior R, Love RM. T and B Cells in periodontal disease: new functions in complex scenario. *Int. J. Mol. Sci.* 2019; 20:1-13.
3. Herrera D, Retamal VB, Alonso B, Feres M. Acute periodontal lesions (periodontal abscesses and necrotizing periodontal diseases) and endo□periodontal lesions. *J clin periodontol.* 2018; 45:78-94.
4. Albandar JM, Susin C, Hughes FJ. Manifestations of systemic diseases and conditions that affect the periodontal attachment apparatus: case definitions and diagnostic considerations. *J Clin Periodontol.* 2018; 45:171–89.
5. Needleman I, Garcia R, Gkraniias N. Mean annual attachment, bone level and tooth loss: A systematic review. *J Clin Periodontol.* 2018; 45:112–29.
6. Tanwar J, Hungund SA, Dodani K. Nonsurgical periodontal therapy: A review. *J Oral Res Rev* 2016; 8:39-44.
7. Eke PI, Thornton-Evans GO, Wei L, Borgnakke WS, Dye BA, Genco RJ. Periodontitis in US adults: national health and nutrition examination survey 2009-2014. *J Am Dent Assoc.* 2018; 149: 576–88.
8. Preshaw PM. Host modulation therapy with anti-inflammatory agents. *Periodontol* 2000. 2017; 76:131–49.
9. Escalona LA, Mastromatteo-Alberga P, Correnti M. Cytokine and metalloproteinases in gingival fluid from patients with chronic periodontitis. *Investigacion clinica.* 2016; 57:131-42.
10. Poniatowski ŁA, Wojdasiewicz P, Gasik R, Szukiewicz D. Transforming growth factor Beta family: insight into the role of growth factors in regulation of fracture healing biology and potential clinical applications. *Mediators of inflammation.* 2015; 1:1-17.
11. Tang YC, Shi YJ, Huang SG. Expression of transforming growth factor-β in mast cells in human chronic periapical diseases. *International Journal of Clinical and Experimental Pathology.* 2017; 10:9243.
12. Sharma S., Singh A. K., Singh R. P., Singh M. K., Singh P., Mohapatra C. Effects of plant growth regulator on in vitro callogenesis of garden cress (*Lepidium sativum* L.). *The Bioscan.* 2015; 10:167–71.
13. Kumar V, Tomar V, Ranade SA, Yadav HK, Srivastava M. Phytochemical, antioxidant investigations and fatty acid composition of *lepidium sativum* seeds. *J Environ Biol.* 2020; 41:59-65.
14. Abdel-Baky ES. Efficiency of *lepidium sativum* seeds in modulation the alterations in hematological parameters induced by sodium nitrite in rats. *Egypt J Hospit Med.* 2019; 74:396-402.
15. Bigoniya, P., Shukla, A. Phytopharmacological screening of *lepidium sativum* seeds total alkaloid: hepatoprotective, antidiabetic and in vitro antioxidant activity along with identification by LC/MS/MS. *Pharm. Nutrit.* 2014; 2:90-7.
16. Al-Sheddi ES, Farshori NN, Al-Oqail MM, Musarrat J, Al-Khedhairi AA, Siddiqui MA. Protective effect of *lepidium sativum* seed extract against hydrogen peroxide-induced cytotoxicity and oxidative stress in human liver cells (HepG2). *Pharm. Biol.* 2016; 54:314–21.
17. Raish M., Ahmad A., Alkharfy K.M., Ahamad S.R., Mohsin K., Al-Jenoobi F. et al. Hepatoprotective activity of *lepidium sativum* seeds against D-galactosamine/lipopolysaccharide induced hepatotoxicity in animal model. *Complem. Altern. Med.* 2016; 16:501-7.
18. Phatale SK, Chavda M. Adjunctive local delivery of aloe vera gel in the treatment of chronic periodontitis: a clinical study with 3 month follow up. *Int J Sci Res.* 2019; 9:1-9.
19. Abramson JH. The Cornell Medical Index as an epidemiological tool. *Am J Public Health Nations Health.* 1966;56:287-98.
20. Sharma D, Agarwal S. Thermoreversible insitu gel for nasal drug delivery. *Int J Cur Res Life Sci.* 2018;7:1629-31.
21. Warad SB, Kolar SS, Kalburgi V, Kalburgi NB. Lemongrass essential oil gel as a local drug delivery agent for the treatment of periodontitis. *Ancient science of life.* 2013;32:205-11.
22. Mombelli A, Van Osten MA, Schruoch EJ, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol.* 1987; 2:145-51.
23. Escobar GF, Abdalla DR, Beghini M, Gotti VB, Junior VR, Napimoga MH, et al. Levels of pro and anti-inflammatory cytokines and C-reactive protein in patients with

- chronic periodontitis submitted to nonsurgical periodontal treatment. *Asian Pac J Cancer Prev*.2018; 19:1927-33.
24. Patini R, Cantiani M, Spagnuolo G, Cordaro M, Call à CAM, Amalfitano A, et al. Metabolic syndrome and periodontitis: association with reactive oxygen species production. A pilot study. *Open Dent J* 2017; 11: 621-7.
 25. Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease? *Periodontology* 2000. 2017; 75:152-88.
 26. Deore GD, Gurav AN, Patil R, Shete AR, Naiktari RS, Inamdar SP. Herbal anti-inflammatory immunomodulators as host modulators in chronic periodontitis patients: a randomised, double-blind, placebo-controlled, Clinical trial. *J Periodontal Implant Sci*. 2014; 44:25-30.
 27. JR III VD, Kumar I, Palandurkar K, Giri R, Giri K. *Lepidium sativum*: Bone healer in traditional medicine, an experimental validation study in rats. *J Fam Med Prim Car*. 2020; 9:812-8.
 28. Wang R, Xu B, Xu HG. Up-regulation of TGF- β promotes tendon-to-bone healing after anterior cruciate ligament reconstruction using bone marrow-derived mesenchymal stem cells through the TGF- β /MAPK signaling pathway in a new zealand white rabbit model. *Cell Physiol Biochem*. 2017; 41:213-26.
 29. Chatoui K, Talbaoui A, Aneb M, Bakri Y, Harhar H, Tabyaoui M. Phytochemical screening, the antioxidant and antibacterial activity of *lepidium sativum* seeds from morocco. *J Mater Environ Sci* 2016; 7:2938-46.
 30. Bukhari HM, Wahb HM, Header EA, Sharkar AA, Al-saegh NA, Bukhari SM, et al. Effects of *lepidium sativum* (Tuffa) on bone mineral density and symptoms in female university students in makkah, saudi arabia: single-blind randomized trial. *Am J Food Nutr Res*. 2018; 5:48-54.
 31. Araujo-Pires AC, Francisconi CF, Biguetti CC, Cavalla F, Aranha AM, Letra A, et al. Simultaneous analysis of T helper subsets (Th1, Th2, Th9, Th17, Th22, Tfh, Tr1 and Tregs) markers expression in periapical lesions reveals multiple cytokine clusters accountable for lesions activity and inactivity status. *JAppl Oral Sci*. 2014; 22:336-46.
 32. ElSayed H, Elkilani N, Farid M. The effects of different extract of *lepidium sativum* on induced periodontitis in dexamethasone immunosuppressed rats. *Al-Azhar Dental Journal for Girls*. 2017; 4:249-53.
 33. Alqahtani FY, Aleanizy FS, Mahmoud AZ, Farshori NN, Alfaraj R, Al-sheddi ES, et al. Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of *Lepidium sativum* seed oil. *Saudi journal of biological sciences*. 2019; 26:1089-92.
 34. Abo El-Maati M.F., Labib S, Al-Gaby A.M., Ramadan M.F. Antioxidant and antibacterial properties of different extracts of garden cress (*Lepidium sativum* L.). *Zagazig J. Agric. Biochem. Appl*.2016; 43:1685–97.
 35. Elkilani N, Hazzaa HH, Hemoud HE, Imam O. Clinical and radiographic assessment of single or combined treatment with *lepidium sativum* and alendronate of non-surgically treated chronic periodontitis in postmenopausal osteoporotic women. *J Int Periodontol*. 2019; 21:20-8.
 36. Sabarish R, Rao SR, Lavu V. Natural T regulatory cells (n Treg) in the peripheral blood of healthy subjects and subjects with chronic periodontitis – A pilot study. *J Clin Diagn Res*. 2016; 10: 36-9.
 37. Naufel AO, Aguiar MC, Madeira FM, Abreu LG. Treg and Th17 cells in inflammatory periapical disease: a systematic review. *Braz Oral Res*. 2017; 31:103-9.
 38. Agrali ÖB, Kuru BE, Yarat A, Kuru L. Evaluation of gingival crevicular fluid transforming growth factor- β 1 level after treatment of intrabony periodontal defects with enamel matrix derivatives and autogenous bone graft: a randomized controlled clinical trial. *Niger J Clin Pract*.2016; 19:535-43.
 39. Cirano FR, Pimentel SP, Ribeiro FV, Casati MZ, Casarin RC, Gallafassi DF, et al. Impact of history of periodontitis on gene expression of bone-related factors in young patients. *Brazilian Oral Research*. 2020; 34:1-9.