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## Periodontal Regeneration Related to Periodontitis Treated by Garden Cress and Ozone Therapy. (Randomized Clinical & Experimental Study)

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## ORIGINAL STUDY

# Periodontal regeneration related to periodontitis treated by garden cress and ozone therapy: Randomized clinical and experimental study

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## Abstract

**Objective:** In this article, garden cress and ozone were evaluated for their potential therapeutic properties of periodontal ligament and resorption of alveolar bone in the periodontal tissues based on rats' experimental periodontitis and to assess the clinical effectiveness of local application of garden cress and ozone for treatment and management of periodontitis patients using scaling and root planing.

**Patients and methods:** Thirty mature Wistar male albino rats were used. Three groups of rats were formed: periodontitis without treatment, periodontitis with garden cress, and periodontitis plus ozone therapy. The present study also included 30 periodontitis patients who were randomly allocated to one of three groups of 10 people each. Group A just had scaling and root planing done; group B and C received scaling and root planing plus garden cress therapy and ozone therapy, respectively. After 1 month, clinical parameters were recorded.

**Results:** Clinical parameters included clinical attachment level, probing depth, bleeding on probing, and plaque index increased significantly across the research groups (groups B and C) after 1 month of treatment. It is also shown that garden cress applied topically quickens alveolar bone and periodontal ligament repair in albino rats with periodontitis that has been produced, while ozone gel improves osteoplastic activity and new bone production.

**Conclusion:** Using garden cress and ozone in conjunction with alternative treatments improved the treatment success of periodontitis patients.

**Keywords:** Garden cress, Histopathological study, Ozone therapy, Periodontal repair, Periodontitis

## 1. Introduction

The most prevalent chronic infectious inflammatory disease is periodontitis, which cause pathological changes in the structures that support teeth and, if ignored, can result in tooth loss. The main objective of periodontal therapy is to restore the functional attached tissues [periodontal ligament (PDL), alveolar bone, and cementum], which are destroyed by periodontal defects [1]. Clinical therapies for periodontitis, including scaling, root planing and surgical treatments, focus on local inflammation management, and plaque removal.

These treatments aim to prevent disease development and reduce symptoms, but they cannot restore the attachment of periodontal tissue to teeth or the original periodontal tissues. As a result, the normal functioning of the teeth and dentition are still impacted after the treatments [2].

Garden cress, sometimes known as *Lepidium sativum*, is a plant seed native to West Asia and Egypt. Phytochemicals, protein, iron, omega-3 fatty acids, dietary fiber, and other vital elements are abundant in the seeds of this plant. In medicine, garden cress is frequently used to treat inflammatory conditions like hepatitis, diabetes mellitus, and joint

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inflammation. According to certain investigations, L. extract has been applied. Sativum possesses effects against oxidative damage that include antidiarrheal, antioxidant, antispasmodic, hepatoprotective, anti-inflammatory, and antibacterial properties [3].

Ozone is an unsteady gas that degrades rapidly and produces new compounds of oxygen; hence, it has long been utilized in treatment. It is a potent germicidal agent against bacteria, viruses, spores, and fungi. Additionally, it can eradicate all recognized Gram positive and Gram negative bacteria, such as antibiotic-resistant *Pseudomonas aeruginosa* and *Escherichia coli*. Ozone's wide spectrum germicidal actions are due to its high oxidizing capacity. Ozone gel as extra management is a novel method in periodontitis patients' care and might be regarded as a complementary therapeutic choice. It is classified as an adjuvant therapy (a therapy provided besides the primary treatment to enhance its efficacy) [4]. Due to its powerful germicidal properties in addition to its capability to reactivate immunity through macrophage activation and the release of cytokines, which in turn boosts the immune system and stimulates regenerative processes, ozone therapy has a clinically effective role in treating diseases caused by bacteria, fungi, and viruses [5]. Ozone potential application uses in clinical dentistry depend on its immune-modulating, antimicrobial, biosynthetic, analgesic, anti-inflammatory, anti-hypoxic, and its effect in hemostasis [6].

Ozonized olive oil creates more stable and long-lasting ozone than other forms. Additionally, ozonized compounds found in ozonized olive oil can promote tissue regeneration. Ozone applied topically promotes the formation of fibroblasts and collagen at the wound of skin while also improving vascular endothelial growth factor (VEGF), transforming growth factor (TGF), and platelet derived growth factor (PDGF) growth factor expressions [7].

The present research purposed to elaborate on the role of garden cress (GC) and ozone therapy on periodontal disease (clinical and histological study).

## 2. Patients and methods

### 2.1. Study design and ethical aspects

In this study, patients were recruited from the outpatient clinic of the Oral Medicine and Periodontology Department, Faculty of Oral and Dental Medicine, Al-Azhar University, Girls branch in Nasr City, Egypt. The study was conducted according to the Declaration of Helsinki (1964, revision 2008). All participants participated voluntarily and received detailed information about the study. The execution

of the research was approved by the Committee of Scientific Ethics at the Faculty of Oral and Dental Medicine, Al-Azhar University, Girls branch in Nasr City, Egypt. The Research Ethics Committee of the Faculty of Dental Medicine for Girls, Al-Azhar University is constituted and operates according to ICH GCP guidelines and applicable local and institutional regulations and guidelines which govern IRB operation. The committee met on May 25, 2023. Approval number was: REC-PD-23-10.

### 2.2. Sample size

Sample size calculation was done using G\*Power, version 3.1.9.2, Faul *et al.* [8,9] University Kiel, Germany. Copyright (c) 1992–2014.

$$f = \frac{\sigma_{\mu}}{\sigma_k}$$

$$\sigma_{\mu}^2 = \frac{\sum_{i=1}^k n_j (\mu_i - \mu)^2}{N}$$

Whereas;

$F$ : is the effect size;  $\alpha = 0.05$ ;  $\beta = 0.05$ ; Power =  $1 - \beta = 0.95$ .

The effect size  $f$  was 0.70 using an alpha ( $\alpha$ ) level of 0.05 and beta ( $\beta$ ) level of 0.05, i.e., power = 95%; the estimated sample size ( $n$ ) should be 30 samples and be divided equally into 10 samples each.

### 2.3. For clinical study

Thirty patients with periodontitis who were sent to the Faculty of Dental Medicine for Girls, Al-Azhar University, Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology for periodontal treatment had undergone a randomized trial.

After being informed of the study's goal, respondents provided written informed permission. Dental Medicine for Girls received permission from the faculty's research ethics committee (REC).

### 2.4. Randomization

The patients were asked to choose an opaque sealed envelope from a collection before being enrolled in the trial. The patient's assigned group was listed on the envelope. The patient is not exposed to an radiograph.

### 2.5. Study groups

The 30 patients were separated equally into:

Group A: 10 periodontitis patients who had scaling and planing of root.

Group B: 10 patients with periodontitis who had scaling and planing of root as well as topical garden cress gel.

Group C: 10 periodontitis patients who used ozone gel, underwent scaling, and root planing.

## 2.6. Clinical examination

The following parameters were evaluated at baseline and after 1 month.

- (1) Plaque index (PI).
- (2) Bleeding index (BI).
- (3) Probing pocket depth (PPD).
- (4) Clinical attachment level (CAL).

A periodontal probe was used to take all clinical measures.

### 2.6.1. For experimental animals

In this investigation, 30 adult Wistar male albino rats (8 weeks old), weighing between 200 and 250 g, were employed. The animals were housed in controlled-temperature laboratory settings (22–24 °C), humidity (45–55%), and 12-h light and dark cycles. They were housed in normal rat cages with four to five rats per cage. All animals have unlimited access to water and a typical rat chow diet.

## 2.7. Induction of periodontitis

The production of experimental periodontitis resulted in developing a periodontitis model induced by ligature. Under general anaesthesia, rats were given intraperitoneal injections of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight), and experimental periodontitis was induced by placing sterile 4/0 silk ligatures in '8' around the mandibular first molars (left and right) in each group for 14 days, causing gingival inflammation and plaque accumulation, and thus the development of periodontal disease [1,10].

## 2.8. Animal grouping

Following periodontitis induction, the animals were categorized into:

### 2.8.1. Group I: negative G without treatment

Group II: treated G with the gel of garden cress's aqueous extract.

Garden cress gel preparation [3]: with 1% carbopol diluted in 25 ml of water, the gels were primed for 24 h, then neutralized with the necessary proportion of triethanolamine. Methanol was present in sufficient quantities to dissolve the medicine. For around 30 min, the neutralized carbopol solution was carefully weighed to make a dazzling clear gel while stirring continually. The capacity was ultimately attained with persistent whirling and 50 ml of distilled water. The stirring was regularly paused to liberate the trapped air during the stirring operation [11]. The garden cress gel was made by the Ministry of Agriculture in Dokki.

Group III: treated G with ozone gel (OXaktiv, Pharmoxid Arznei, GmbH & Co KG, Germany): its ingredients include ozonized olive oil, polyethylene and paraffin liquidum. Both garden cress and ozone gel were applied topically daily by syringe for 4 weeks.

## 2.9. Euthanasia

The animals were put to sleep using 20 mg/kg of thiopental (0.5 g Thiopentax, Crista'Lia, Sao Paulo) [12]. The animals were euthanized 6 weeks after the ligature was taken off. The mandible of each rat's was dissected, with the associated gingiva remaining intact with the bone while the muscle and soft tissue were removed. Only some of the lower molar teeth and the alveolar bone around them were prepared for histological analysis. The way to discard the euthanized rats at Medical crematorium.

## 3. Results

### 3.1. Clinical results

#### 3.1.1. Plaque index

According to Table 1, there is no statistically significant difference between the groups at the baseline, although there was a highly statistically significant difference between groups after 1 month. The mean values of PI of all the study groups at baseline was  $3.0 \pm 0.0$ , while after 1 month, were  $2.40 \pm 0.70$ ,  $0.40 \pm 0.52$ , and  $1.40 \pm 0.52$  for groups A, B, and C, respectively. Regards to changing by time, the PI values decreased with the increasing periods for all groups, with 20.0, 86.7, and 53.3% for groups A, B, and C, respectively, and there was a statistically significant change in the mean of PI within the same group after 1 month compared with the baseline for all groups ( $P = 0.00$ ,  $P < 0.001$ , and  $P < 0.001$ ) (Fig. 1).

#### 3.1.2. Probing depth

There is no statistical difference between the groups, according to the data in Table 1 at the

Table 1. Clinical parameters within research groups during the trial.

	Group A	Group B	Group C	P value
<b>Plaque index</b>				
Baseline				
Mean	3.00	3.00	3.00	NS
SD	0.00	0.00	0.00	
1 month				
Mean	2.40	0.40	1.40	<0.001**
SD	0.70	0.52	0.52	
RD%	-20.0	-86.7	-53.3	
P value	0.012**	<0.001*	<0.001*	
<b>PD</b>				
Baseline				
Mean	5.70	5.40	5.90	0.250
SD	0.95	0.97	0.99	
1 month				
Mean	5.00	2.50	3.40	<0.001**
SD	1.33	0.71	0.70	
RD%	-12.3	-53.7	-42.4	
P value	0.08	<0.001*	<0.001*	
<b>CAL</b>				
Baseline				
Mean	3.60	4.80	4.10	0.011**
SD	0.84	0.63	0.88	
1 month				
Mean	2.80	1.90	2.30	0.088
SD	1.03	0.74	0.48	
RD%	-22.2	-60.4	-43.9	
P value	0.04*	<0.001*	<0.001*	
<b>BOP</b>				
Baseline				
Mean	3.00	3.00	3.00	Ns
SD	0.00	0.00	0.00	
1 month				
Mean	1.80	0.80	1.40	0.008**
SD	0.63	0.63	0.52	
RD%	-40.0	-73.3	-53.3	
P value	<0.001*	<0.001*	<0.001*	

BOP, bleeding on probing; CAL, clinical attachment level; PD, probing depth.

baseline, despite the fact that there was highly significant difference between groups after 1 month. The mean values of probing depth (PD) of all the study groups at baseline were  $5.70 \pm 0.95$ ,  $5.40 \pm 0.97$ , and  $5.90 \pm 0.99$ , while after 1 month, were  $5.0 \pm 1.33$ ,  $2.50 \pm 0.71$ , and  $3.40 \pm 0.70$  for groups A, B, and C, respectively. Regarding changing time, the PD values decreased with the increasing periods for all groups, with 12.3, 53.7, and 42.4% for previous groups, respectively, and there was a statistically significant change in the mean of PD within the same group after 1 month compared with the baseline for all groups except group A ( $P = 0.08$ ,  $P < 0.001$ , and  $P < 0.001$ ) (Fig. 1).

### 3.1.3. Clinical attachment level

The results in Table 1 revealed no significant difference between groups at the baseline, while there was highly significant difference between groups after 1 month. The mean values of CAL of the all the study groups at baseline were  $3.60 \pm 0.84$ ,  $4.80 \pm 0.63$ , and  $4.10 \pm 0.88$ , while after 1 month were  $2.80 \pm 1.03$ ,  $1.90 \pm 0.74$ , and  $2.30 \pm 0.48$  for groups A, B, and C, respectively. Regarding changing time, CAL values decreased with the increasing period for all groups, with 22.2, 60.4, and 43.9% for previous groups, respectively, and there was statistically significant change in the mean of CAL within the same group after 1 month compared with the baseline for all groups ( $P = 0.04$ ,  $P < 0.001$ , and  $P < 0.001$ ) (Fig. 1).

### 3.1.4. Bleeding on probing

Table 1 findings showed no noticeable difference between the groups. At the baseline, while there

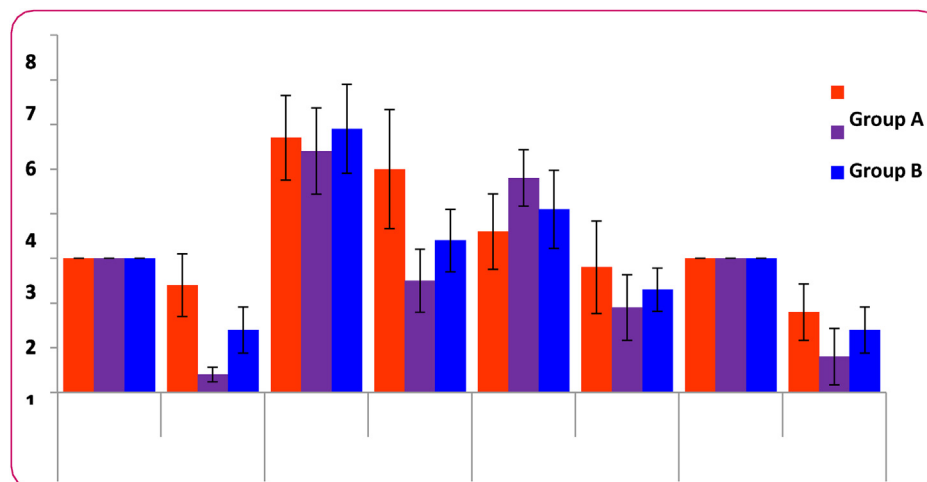


Fig. 1. Bar chart illustrating mean value of clinical parameters within research groups during the course of the trial.

was a highly significant difference between groups after 1 month. The mean values of bleeding on probing (BOP) of all the study groups at baseline were  $3.0 \pm 0.0$ , while after 1 month, were  $1.80 \pm 0.63$ ,  $0.80 \pm 0.63$ , and  $1.40 \pm 0.52$  for groups A, B, and C, respectively. Regards to changing by time, the BOP values decreased with the increasing periods for all groups, with 40.0, 73.3, and 53.3% for previous groups, respectively, and there was a statistically significant change in the mean of BOP within the same group after 1 month compared with the baseline for all groups ( $P < 0.00$ ,  $0.001$ , and  $0.001$ ) (Fig. 1).

### 3.2. Histological results

#### 3.2.1. Group I (periodontal defect G)

The histologic examination of the specimens of this group showed thin, wiry bone trabeculae dispersed in large, wide cellular bone marrow spaces. The trabeculae appeared with areas of clefts. The osteocytes showed a decrease in average number and lacked normal arrangement and architecture. Most of the osteocytes showed degeneration with empty osteocytic lacunae. The PDL showed a change in organization just. There was discontinuation between the PDL/bone interface by

the presence of many clefts and empty spaces (Fig. 2a–c).

#### 3.2.2. Group II (periodontitis + garden cress G)

The histologic examination of the specimens of this group showed obvious signs of new bone formation where multiple resting lines were seen beside the presence of osteoid tissue, denoting active bone formation. Relatively normal PDL architecture was also clearly observed in the PDL where the collagen fibers were properly arranged and organized. The PDL–bone interface showed normal attachment. The alveolar bone showed normal structure and architecture with slight thickening, normal average size of osteocytes, shape, and arrangement, and the appearance of Zuker candle canals (Fig. 3d, e).

#### 3.2.3. Group III (periodontitis + ozone gel)

The histologic examination of the specimens of this group showed that the bone trabeculae of the surrounding alveolar bone showed variable findings where some specimens showed thinning of bone trabeculae that appeared dispersed in wide areas of bone marrow spaces. The bone trabeculae of those specimens showed multiple reversal lines (Fig. 4f). Regarding the PDL examination, there were

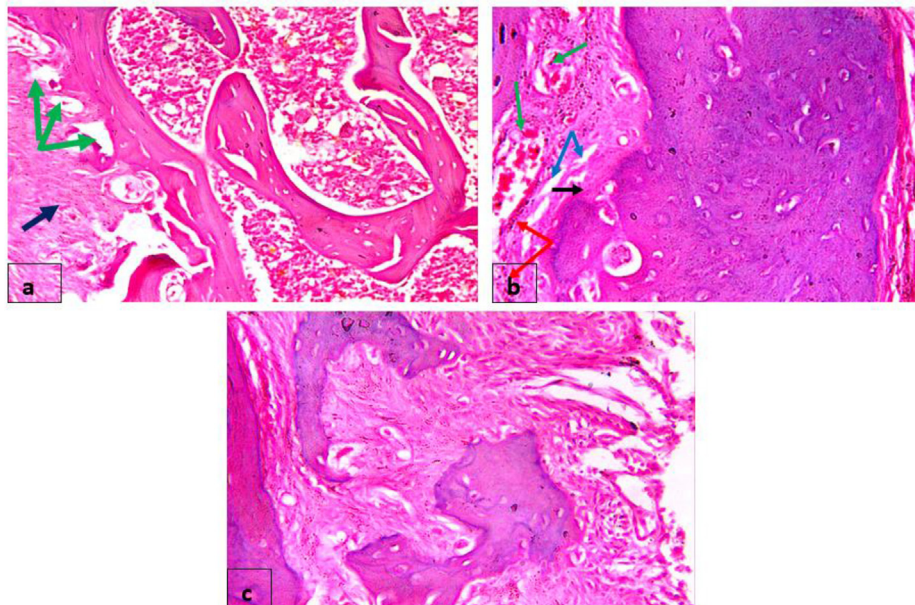


Fig. 2. (a) Periodontal defect G showing detachment of periodontal ligament fibers from bone surface (green arrows), periodontal ligament with apparent normal arrangement of fibers (blue arrow), (b) disorganized periodontal ligament fibers, large dilated blood vessels encouraged with RBCs (green arrow), hyalinized periodontal ligament fibers (black arrow), tissue clefts (blue arrow), and chronic inflammatory cells (red arrow) (original magnification,  $\times 200$ ), and (c) areas of tissue degeneration (blue arrow), thin scattered trabeculae. The bone trabeculae showed loss of normal structure where the osteocytes number appeared sparsely distributed, beside the presence of areas that lack the osteocytes as they were completely degenerated (black arrow). The bone trabeculae showed also signs of bone resorption represented in the existence of multiple reversal lines (red arrow) (original magnification,  $\times 200$ ).

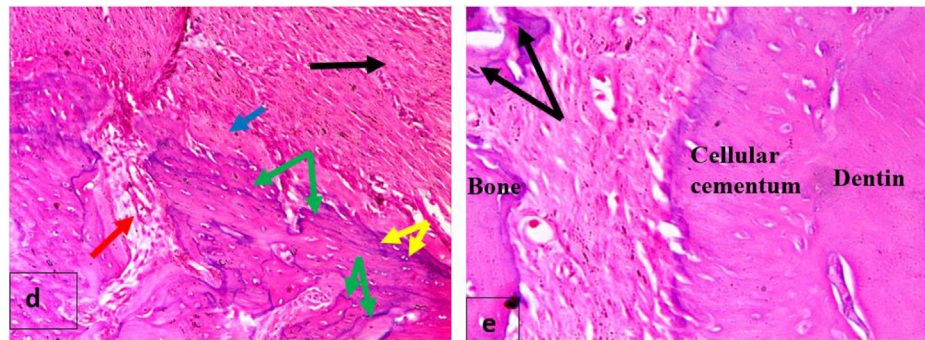


Fig. 3. (d) Periodontitis treated with garden cress gel showing multiple resting lines (green arrows), normal structure and architecture of alveolar bone with the appearance of Zuker candle canals (red arrow), properly organized periodontal ligament fibers (black arrow), normal osteocytes (yellow arrow) and periodontal ligament bone interface showed formation of new tissue (blue arrow) (original magnification,  $\times 100$ ) and (e) a normal arrangement of periodontal ligament fibers, continuous normal tooth periodontal ligament–bone interface and bone with multiple resting lines (black arrow) (original magnification,  $\times 200$ ).

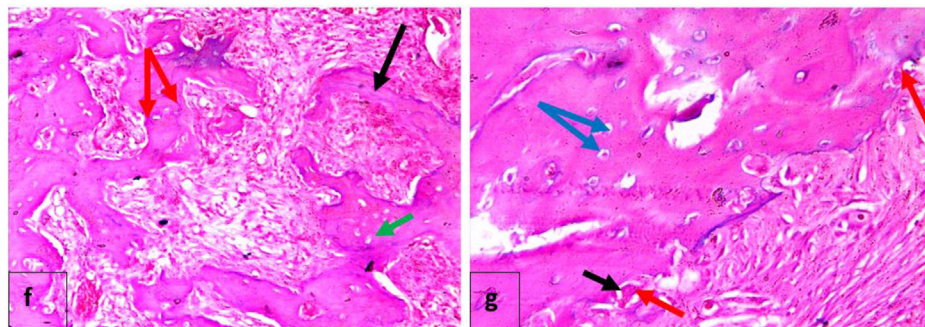


Fig. 4. (f) A periodontitis treated with ozone gel showing thin bone trabeculae dispersed (black arrow) in wide areas of bone marrow spaces. Sparse osteocytes appeared to be normal size and shape (green arrow). Multiple reversal lines (red arrow) (original magnification,  $\times 100$ ) and (g) Howship's lacuna (black arrow), osteoclasts (red arrow), and sparse normal appearing osteocytes (blue arrow) (original magnification,  $\times 200$ ).

multiple large blood vessels encouraged with red blood cells. The collagen fibers were not properly aligned but did not show any hyalinization. The alveolar bone margins that face the PDL showed multiple Howship's lacunae, some of which were occupied by osteoclasts (Fig. 4g).

#### 4. Discussion

Periodontitis is a general term for any condition harming the periodontium, the tissues around the teeth, including disease of the PDL, bone, and cementum. A set of oral inflammatory disorders known as periodontal diseases are impacted by host response reaction variables. Gingival inflammation that affects the gums and inflammation of periodontal tissues (characterized by apical migration of the attachment of PDL and connective tissue and alveolar bone deterioration) are the two basic diseases that affect periodontal tissues [12,13].

The healing of periodontal pocket was a significant task that was clinically, experimentally, and

traditionally investigated with the goal of supporting and documenting this phenomenon using different techniques. The effects of several variables and drugs on periodontal pocket healing were also documented, along with biomechanical assessments, surgical procedures, and other impacts [14,15].

Periodontitis was selected as the acute model to construct an exact defect model to test and evaluate alternative treatment approaches without significant bias utilizing a ligature. Actually, the diseased root surface was created [16].

The goal of periodontal disease treatment is to minimize PPD, increase CAL, and reduce BOP occurrences. The aim of this research is to evaluate the clinical and histological effects of garden cress and ozone gel treatment on healing periodontitis caused by ligation in rats.

Rats, dogs, and primates have all been given ligature-induced periodontitis to investigate the variables influencing the severity of the condition. Albino rats were used as experimental animals in

our study to help garden cress and ozone gel in the healing of periodontitis because rats were adequate to assess the impact of bacteria, diet, or other factors in periodontal inflammation at the histological level, with statistical significance and preclinical relevance [17].

Natural ingredients have been employed in alternative therapies, including traditional folk medicine, since history. Garden cress and its seeds are one of these materials [18]. Seeds of garden cress have strong anti-inflammatory properties [19] because of the antioxidant qualities and the phenolic content of garden cress seeds, it enhances beneficial effect on bone-forming cells [20].

The clinical parameters improvement in the garden cress treated groups are consistent with earlier research that found the content of cells has a significant improved and enhancement of fiber orientation of the PDL in rats with diabetes given garden cress seeds [21].

Ozone treatment is not an original concept in dentistry; previous studies and medical evaluations have examined the value of using ozone instead of antiseptics to treat dental infections [22]. The gel usage was chosen for local administration in this investigation because it is simple to apply, requires no complicated equipment for production or storage, and includes an accurate dosage of ozone molecules with an extended lifespan that can be measured in years [23].

In the existing study, results displayed no statistically significant difference between groups at the baseline, while after a month, there was a significant difference between the groups. Regarding changing time, clinical parameters like PI, BOP, PD, and CAL were improved.

In agreement with our results Ramzy *et al.* [24], as well as Kshitish and Laxman [25], presented positive finding for CAL and GI following ozonized water irrigation. Issac *et al.* [26] investigated 4 weeks of ozonized water subgingival irrigation in patients with PDs higher than 6 mm. The PD was greatly lowered after therapy. Dengizek and colleagues compared nonmedical gaseous ozone with scaling and planning of root with a placebo. Increases in PD and GI were equivalent in both groups after treatment, while the ozone group exhibited a much greater elevation in TGF-levels than the control group [27].

Histologically, in group I, the pathophysiology of the periodontal inflammatory process was thought to be responsible for both the disorientation of PDL fibers and the loss of alveolar bone. When inflammatory mediators such as prostaglandins, TNF, IL-1, and cytokines are released, inflammatory cells are

drawn in, lytic enzymes are produced, and osteoclastic differentiation occurs. As a result, connective tissue is destroyed, and alveolar bone gradually resorbs [28].

After the experimental investigation was completed, there was a favorable role of LS on osteoblasts in group II. This may be because garden cress has antioxidant features that depend on phenolic chemicals found in garden cress seeds. The primary phenolic substances found in garden cress seed extracts are tocopherols. The potential of garden cress is modulated upon by tocopherols. In addition to providing essential vitamin E for human nutrition, tocopherols also aid in preventing diseases [20].

In group III, the role of ozone to stimulate osteoplastic activity, which was discovered to be much higher in groups that treated by ozone than the untreated group, may be responsible for improving alveolar bone and PDL regeneration. Another factor is that ozone increases osteoblasts' alkaline phosphatase enzyme activity and expression of bone morphogenic protein-2, which benefits bone production and mineralization, respectively [29].

Additionally, ozone has angiogenic and biosynthetic properties that stimulate cellular proliferation and protein synthesis, increasing cell activity and the ability of organs and tissues to regenerate. Earlier Wang *et al.* [30] study had estimated the influence of ozone gel on human gingival fibroblasts' capability to produce type-I collagen, which is strongly related to the ability of periodontal tissues to regenerate. It demonstrated that ozone increases type-I collagen production by about 1.4 times, which is supposed to positively impact the repair of periodontal tissues [31,32].

#### 4.1. Conclusions

The results of this research show that GC applied topically quickens alveolar bone and PDL repair in albino rats with periodontitis that has been produced. Compared to ozone gel, it improves osteoplastic activity and new bone production. Therefore, in addition to the traditional treatment for periodontitis, it may be used.

#### Institutional review board (IRB) approval number

REC-PD-23-10

#### Conflict of interest

There are no conflicts of interest.



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