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RESEARCH ARTICLE

The Effect of Egg Yolk Oil in Repairing Tight Junction Claudin-1 in Periodontitis in a *Wistar* Rat

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A B S T R A C T

The leading factor in tooth loss, periodontitis is a global public health concern. Common periodontal treatments include medication and mechanical therapy. Regarding the use of natural medicines to treat periodontitis, there has been much advancement. Cell proliferation, migration, differentiation, and homeostasis are modulated by tight junction (TJs). The present study reports the effectiveness of egg yolk oil (EYO) as a treatment for ligature-induced periodontitis by analyzing its microstructure and its effect on claudin-1. Eighteen mature male and female rats were used in the investigation, and they were categorized: Group 1 (Control Negative, n=9), which received no treatment; Group 2 (Control Positive, n=9), which received a silk ligature to cause periodontitis. The EYO treatment+ periodontitis for rats in Group 3 (EYO, n=9) was applied by ligature for 30 days. The incisor teeth with periodontal tissues were collected on days 7, 14, and 30 of the experiment and sections were prepared for both H&E staining and IHC for claudin-1 gene utilization. There was a significant improvement in alveolar bone loss, reduction in the inflammatory reaction, regeneration of PDL thickness, reduction of osteoclast numbers, and activation of claudin-1 tight junction expression compared to the control group in the entire experiment period. Our research concluded that EYO may have a critical role in alveolar bone formation with the regeneration of periodontal tissue damage that is associated with ligature-induced periodontitis via up-regulation of the expression of claudin-1 tight junction proteins that provide regeneration and repair processes, hence reducing pathological damages.

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INTRODUCTION

Periodontitis is a chronic inflammatory condition that is characterized by the development of periodontal pockets caused by the destruction of periodontal connective tissue, cementum, and alveolar bone following the presence of polymicrobial subgingival oral biofilms (Di Benedetto *et al*., 2013; Aral *et al*., 2015). As a result of inflammation, there is vasodilation, edema, infiltration of inflammatory cells, and release of pro-inflammatory cytokines. Resorption of the alveolar bone could result in tooth loss from this process (Kesavalu *et al*., 2002). It is still a huge global public health burden and one of the most common inflammatory disorders affecting adults (Jin *et al*., 2016).

Recent studies have revealed that a number of substances produced from natural products have anti-inflammatory, antioxidant, and antibacterial properties. By modifying host inflammatory cascades and bone resorption, it may be possible to utilize these properties as pharmacological drugs to treat periodontitis (Prasad and Kunnaiah, 2014).

The hen's egg is a productive foodstuff because it contains high-quality proteins, minerals, vitamins, and lipids like polyunsaturated fatty acids (PUFA) and phospholipids (Zdrojewicz *et al*., 2016). Egg oil generated

from the yolk is mostly composed of high fat, protein, glucose, ash, and water that appears to be helpful in the healing process (Rastegar *et al*., 2011). Eggs are rich in ovalbumin, ovotransferrin, and phosvitin, which are examples of egg proteins that exhibit antioxidant and nutraceutical qualities, as do phospholipids, vitamin A, vitamin E, selenium, and carotenes (Abeyrathne *et al*., 2013; Lee *et al*., 2017).

Tight junctions, desmosomes, and adherens junctions are a few examples of the several types of cell-cell junctions that can exist. Three significant transmembrane protein families that together account for more than 40 molecules that make up TJs include occludin, claudin, and junctional adhesion molecules (Groeger and Meyle, 2015; Hajishengallis and Lamont, 2014). Tight junctions, which are located apically to adherens junctions and are used to restrict the paracellular pathway, are in charge of sustaining cell-cell adhesion. The epithelial barrier, a fundamental element of innate immunity, is controlled by the plasma membrane and intercellular tight junctions (Schroeder and Listgarten, 2003). A barrier and the first line of defense against the bacteria connected to dental biofilms and their virulence factors within the periodontium is provided by the gingival junctional epithelium (Shimono *et al*., 2003).

The largest and best-understood family of tight-junction proteins is the claudins, which have at least 27 members (Markov *et al*., 2015). Claudin-1 overexpression results in increased transepithelial electrical resistance in cells. Thus, claudins are essential for regulating the epithelial barrier at tight junctions and septate junctions (Schneeberger and Lynch, 2004).

TJ proteins are recognized to be involved in proliferation and differentiation, according to earlier investigations. This work emphasized the regenerative effects of egg yolk oil against induced periodontitis by silk ligature using histomorphometric and IHC staining. The role of claudin-1 in the perspective of alveolar bone and periodontal connective tissue regeneration and repair was also evalauted.

MATERIALS AND METHODS

Experimental animals and study design: Thirty-six male white *Sprague Dawley* rats weighing between 300 and 350 gm at 9 weeks old were adopted. The animals were purchased from the veterinary teaching hospital's laboratory animal house at the University of Sulaimani's College of Veterinary Medicine. The College of Veterinary Medicine/Ethics Committee approved all operations involving the care, handling, and sampling of animals (permission 030521, dated July, 01, 2022). Before the trial, the animals had one week of acclimatization. The room was maintained tidy and had good ventilation. All rats are given normal laboratory food pellets and have access to filtered water in bottles at all times. Based on a physical assessment, all rats are found to be healthy.

Rats were randomly allotted into three main groups as follows: Negative control (n=9): rats with normal periodontium and not exposed to 4/0 silk suture ligature induced periodontitis or treatment; Positive control (n=9): rats exposed to silk-induced periodontitis; Treatment group with EYO (n=9): rats were subjected to periodontitis with silk ligature+EYO treatment. Each group was subdivided into 3 replications (n=3) (small groups) for 7, 14 and 30 days of experiments.

Experimental periodontitis (EPD) induction in a rat model: After giving the rats general anesthesia with a combination of intraperitoneal (IP) ketamine (50 mg/kg) and xylazine (5 mg/kg) administration, experimental periodontitis was produced via a ligature. Using an eye speculum to keep the mouth open, ligatures in the shape of 8 were put in the cervical region of the lower incisor teeth, resulting in a supragingival position labially and a subgingival position lingually. As stated before, this ligature triggered gingival irritants, aided in the buildup of plaque, and ultimately facilitated the onset of periodontal disease (Ionel *et al*., 2017). Throughout the 30-day study period, the ligatures were checked every two to three days.

Preparation of egg yolk oil (EYO): The approach of Herring (1980) was employed to obtain EYO (Herring, 1980). Only solid egg yolks were added to the clean, sanitized egg white after the shells of thirteen organic chicken eggs had been removed and separated. The egg yolks were then cooked to 190-200°C for 20 minutes to release the EYO. The egg yolk was agitated for an additional five minutes after the EYO emerged. The leftover egg yolks were then thrown away, and pure EYO was obtained by filtering it using sterile gauze. EYO was stored until further usage in this investigation in an amber flask at 4°C.

Intra-gingival injection of EYO: Each rat was placed under general anesthesia before having EYO treatment using a microsyringe. The maximum volume that the tissue could receive without being repulsed was 10µL every day for 30 days, which was the volumetric equivalent of all injections.

Collection of teeth samples for histological evaluation: Rats were sacrificed on days 7, 14, and 30. Periodontal connective tissue samples were collected from the teeth after the mandibles were removed and bisected from both sides posterior to the incisor teeth and were preserved with a 10% of neutral buffer formalin for 24 hr and decalcified in a chelating agent 10% EDTA solution for 8 weeks at 4°C, then managed by routine paraffin embedding technique in the Histopathology Lab of Anwar Shexa Medical City/Sulaimani Governorate, two thin sections 4 µm were mounted on glass slides and stained with hematoxylin and eosin (H&E), also for an IHC claudin-1 marker.

Histopathologic examination: All slides were inspected histologically using an eyepiece grid under a microscope at 20-100x magnification by a pathologist who was blind to the previous treatment. Re-epithelialization, dermal matrix deposition, regeneration, granulation tissue development, remodeling, and angiogenesis were among the criteria that were assessed and graded. According to information from the literature about experimental model wound healing, the histological score used in this study was carried out and evaluated as seen in Table 1 (Altavilla *et al*., 2005).

Histometric analyzer software was used to measure the thickness of the alveolar bone and periodontal ligament in three locations (the furcation area or crest, middle and cervical section) perpendicular to the alveolar bone surface and the cementum surface (Amscope™, Japan).

Osteoclasts were counted in tissue slices in each rat group at 130 µm from the alveolar bone surface using histometric analyzer software (Amscope™, Japan) and were recognized as (multiple nuclei, ruffled border, and granular cytoplasm).

Immunohistochemical analyses: For immunohistochemistry evaluation, serial sections of 4µm thickness were taken from paraffinized blocks for days 7, 14, and 30. Paraffin-embedded slides were dewaxed in xylene and hydrated. Sections were heated for 25 minutes in a microwave oven in a sodium citrate buffer (pH 6.5) and then cooled in de-ionized water. Slices were exposed to 5 percent hydrogen peroxide for 12 minutes in order to lower endogenous peroxidase activity. Claudin-1 rabbit polyclonal antibody was incubated for two hours with primary antibodies (1:100; DAKO, Denmark). As instructed by the manufacturer, reaction was improved by using biotin-labeled anti-rabbit secondary antibodies and streptavidin coupled to horseradish peroxidase (DAKO Cytomation, Denmark). To observe the results of the process, diaminobenzidine was utilized (DAKO, Denmark). Hematoxylin was counterstained, dried using the standard procedure, and covered with coverslips.

Immunostainings were evaluated manually with a conventional light microscope (Leica, Germany) via computer-assisted image analysis software to examine slices (Am Scope™, Japan). The nuclei were not stained remaining blush color, while the cytoplasm and membrane were stained with brownish granules of Claudin-1. No staining or 0 for 5% positive staining, (1) for 6-25 percent positive staining, (2) for 26-50 percent positive staining, (3) for 51-75 percent positive staining, and (4) for >75 percent positive staining were used to quantify the degree of positively stained epithelial cells and endothelial cells in IHC staining of Claudin 1. The intensity of Claudin 1 staining was rated on a scale of weak (+1), moderate (+2), moderate-strong (+3) and strong (+4). The positive reactivity extent and level of staining intensity were multiplied to get a total staining score, which ranged from 0 to 16.

RESULTS

The periodontium in the negative control group underwent histologic evaluation and the results showed normal architecture and organization without any signs of inflammation or bone loss (Fig. 1a, b) vs. to the control positive group on day 7 which showed marked damage of periodontal tissue. It shown distinctive junctional epithelial disruption in which detached periodontal ligament (PDL) from cementum, also marked edema, vascular congestion, and infiltration of neutrophil inflammatory cells in the gingival connective tissue above the crestal bone, thin granulation tissue with excessive fibroblast proliferation was seen in PDL (Fig. 1c-f). The incisor teeth section in control positive showed marked bone loss with irregular bone surface, absence of the Sharpey's fibers and migration of mesenchymal cells toward the alveolar bone to compensate for the damaged tissue with mild re-epithelialization (4 layers were formed), with intense inflammatory cells in the sub-epithelial region, few numbers of angiogenesis with granulation tissue (score 0; immature and inflammatory tissue), in comparison to the rat that treated with egg oil revealed intact junctional epithelium with irregular newly formed bone trabeculae noted as randomly oriented collagen fibers and plates of bones to their actual length, fibroblast proliferation was prominent in PDL with scant collagen fibers but Sharpey's fibers were not produced yet (Fig. 1g-i). The healing score for the egg oil-treated group was recorded as (1); Moderate re-epithelialization, thin granulation tissue with angiogenesis (average 1-2 per field) with mild-moderate edema. Inflammatory cells (average 12 per site). On day 14, the tooth's section in EYO greatly enhanced healing (Fig. 2e-h) and showed advanced re-epithelization and early remodeling phase characterized by well-organized AB with evidence resorption area that contains osteoclasts which surrounded by large numbers of mesenchymal stem cells, organized and intact PDL with cementum, thick granulation tissue with angiogenesis (5 per field), inflammatory cells (4 per field), the healing score was (score 3) in comparison to the control positive group revealed moderate re-epithelialization characterized by attaching disorganized periodontal ligament with cementum, disorganized with less mineralization of AB, and little collagen fibers were formed, absence of Sharpey's fibers (Fig. 2a-d), the healing score regarded as (Score 2), vascular granulation tissue, angiogenesis (3 per field), inflammatory cells (9 per field).

After 30 days, EYO improved bone healing and restored the periodontal tissue to a high degree (Fig. 3d,e) that showed alveolar bone more organized with an osteoid tissue layer, marked remodeling, periodontal ligament more organized and attached to a regular cementum surface (score 4) vs. control positive group exhibited irregular bone surface with bone resorption in the 80% of cases, with narrow widths of the periodontal ligament, well-formed proliferating periodontal tissue that attached to the regular cementum surface with a patch of Sharpey's fiber, and thick fibrovascular granulation tissue (Fig. 3a-c). Additionally, stem cells were also found in their borders of AB with newly formed osteoblasts (score 3).

Histometric assessment of PDL thickness, alveolar bone loss, and osteoclast activity: Throughout the investigation, the periodontal ligament thickness considerably increased in the control positive group and EYO vs. control negative. EYO reduced PDL thickness vs. the control positive group non-significantly on days 7 and 30 ($P=0.7$ and $P=0.06$) respectively, while significantly ($P=0.04$) decreased on day 30 vs. to the control positive group (Table 2 and Fig. 4a-c).

The EYO-treated group significantly reduced the alveolar bone loss vs. the control positive group on days 7 and 14 respectively, while both groups particularly the control positive group showed significantly marked bone loss ($P=0.05$, 0.001, and 0.05) respectively on days 7, 14, and 30 vs. control negative group, whereas subsequently at day 30 the bone loss in EYO reduced non-significantly vs. control positive ($P=0.2$) (Table 2 and Fig. 4d-f).

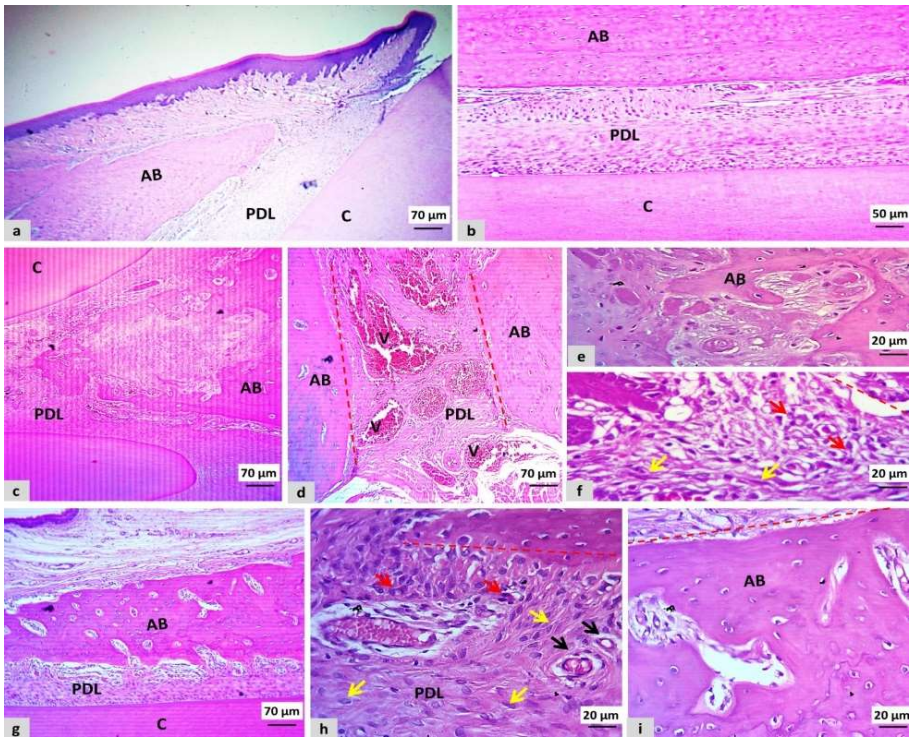

Fig. 1: The microscopic section of an incisor tooth and periodontal tissue of rat at day 7: a and b: Normal histological arrangement of the intact gingival lining epithelium, alveolar bone, periodontal ligament attached to a regular cementum layer, c-f: Marked disruption of the junctional epithelium with granulation tissue in the insertion point also beyond the bone crest, severe edema and vascular congestion (V) in the periodontal ligament that detached from cementum, disorganized bone trabeculae and irregular bone surface, mild-repithelization (red line) with inflammatory reaction in the periodontal ligament, and proliferation of fibroblast (yellow arrows) g-i: Intact periodontal ligament and cementum, moderate re-epithelialization (red dash line), granulation tissue with few angiogenesis (black arrows), mild-moderate edema with inflammatory reaction (red arrows) and proliferation of fibroblast (yellow arrows), randomly oriented collagen fibers and plates of alveolar bones in section (i) in EYO treated group, (AB; alveolar bone, PDL; periodontal ligament an C; cementum), (H&E stain).

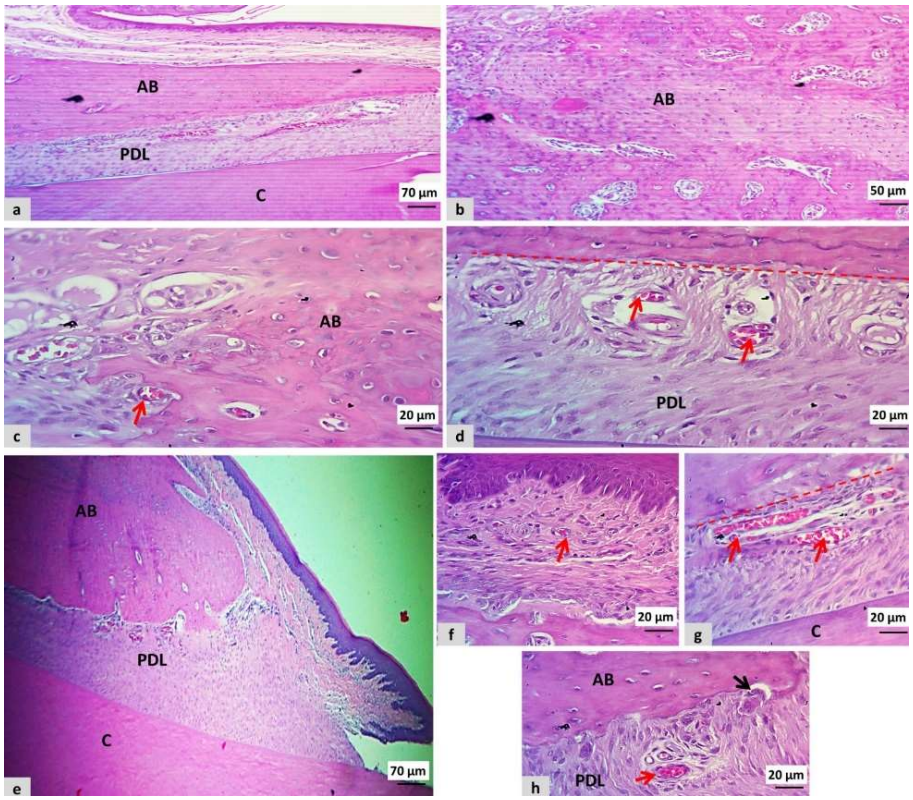

Fig. 2: The microscopic section of an incisor tooth and periodontal tissue of rat at day 14: a-d: Moderate re-epithelization (red line) and vascular granulation tissue with evident angiogenesis (red arrows), the periodontal ligament has contact with the cementum, disorganized bone trabeculae and irregular bone surface, proliferation of fibroblast (yellow arrows) in disorganized PDL in control positive group e-h: Complete re-epithelization (red dash line) and intact periodontal ligament with cementum, vascular granulation tissue with the high number of neovascularization (red arrows), inflammatory reaction (yellow arrows), well-oriented collagen fibers and plates of alveolar bones in section (h) with osteoclasts in large bone matrix resorption as indicated by the black arrow in EYO-treated group, (AB; alveolar bone, PDL; periodontal ligament and C; cementum), (H&E stain).

Periodontitis increased the activity of osteoclast throughout the experimental period and showed a maximum number in the control positive group vs. normal and EYO-treated periodontal tissues as seen in Table 3. On day 7 osteoclast number increased significantly in control positive vs. control negative (P=*0.000*) and EYO-treated group (P=*0.001*). Additionally, on day 14 significantly raising in osteoclast activity was seen in the control positive vs. control negative and treated group (P=*0.000* and *0.01*) respectively. The maximum number was detected on day 30 in the control positive group vs. both groups (P=*0.000* and *0.002*) respectively in the control negative and EYO-treated groups (Table 3 and Fig. 4g-i).

Table 1: The parameters that were utilized to determine the histology scores of wound healing (Altavilla *et al*., 2005).

Score	Re-epithelialization	Granulation Tissue	Inflammatory Cells per histological field	Angiogenesis
0	Absence of epithelial proliferation in ≥70% of the tissue	Immature in ≥70% of the tissue	13-15 inflammatory cells	Absence of angiogenesis,
1	Poor epidermal organization in ≥60% of the tissue	Thin immature in ≥60% of the tissue	10-13 inflammatory cells	1-2 vessels per site,
2	Incomplete epidermal organization in ≥40% of the tissue	Moderate remodeling in ≥40% of the tissue	7-10 inflammatory cells	3-4 vessels per site,
3	Moderate epithelial proliferation in ≥60% of the tissue	Thick granulation layer and well-formed collagen matrix in ≥60% of the tissue	4-7 inflammatory cells	5-6 vessels per site
4	Complete epidermal remodeling in ≥80% of the tissue	Complete tissue organization in ≥80% of the tissue	1-4 inflammatory cells	More than 7 vessels per site vertically disposed toward the epithelial surface

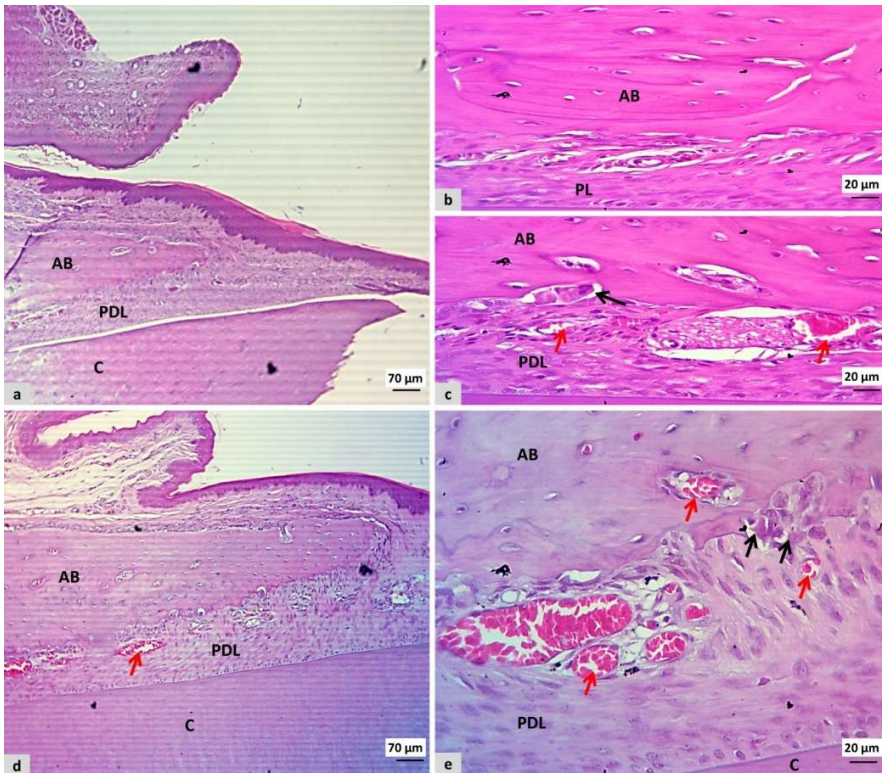

Fig. 3: The microscopic section of an incisor tooth and periodontal tissue of rat at day 30: a-c: Well-formed thin bone that had an irregular bone surface with resorption area that contain osteoclast (black arrows), a wide space of less organized proliferating periodontal ligament tissue attached to a regular cementum surface and angiogenesis (red arrows) in control positive group, d and e: Well-organized dense bone with resorption area containing osteoclast (black arrows), uniform thickness of PDL filled with organized proliferating PD tissue attached to a regular cementum surface with angiogenesis in EYO-treated group, (AB; alveolar bone, PDL; periodontal ligament and C; cementum), (H&E stain).

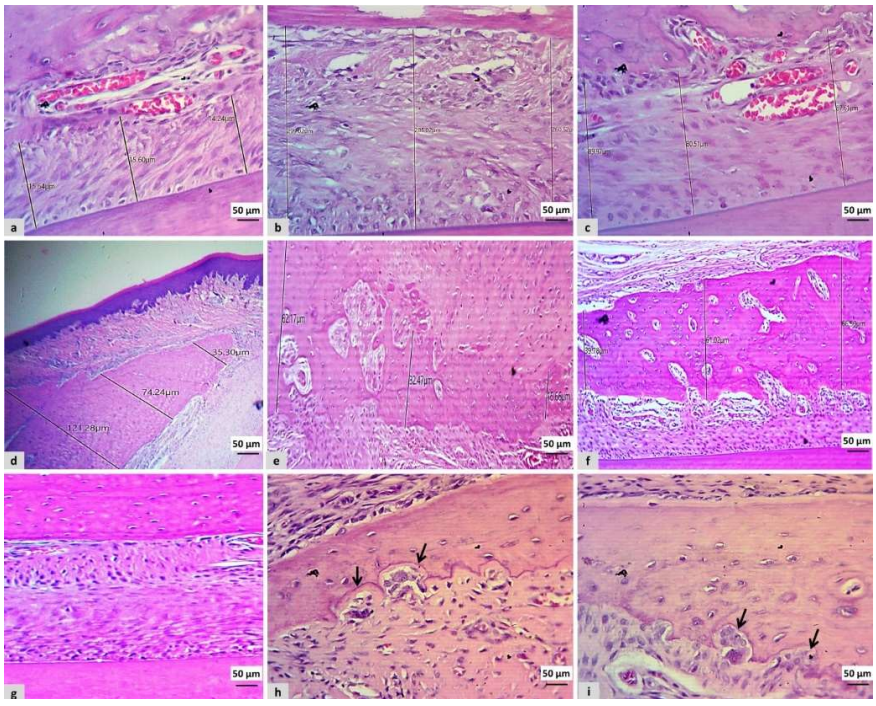

Fig. 4: The microscopic section of an incisor tooth and periodontal tissue of rat at day 14: a-c: Alveolar bone thickness in control negative, control positive, and EYO groups respectively. d-f: PDL thickness in control negative, control positive, and EYO groups correspondingly. g: No osteoclast cells in the control negative group. h and i: Large multinucleated osteoclast cells within Howship's lacuna in control positive and EYO groups individually (H&E stain).

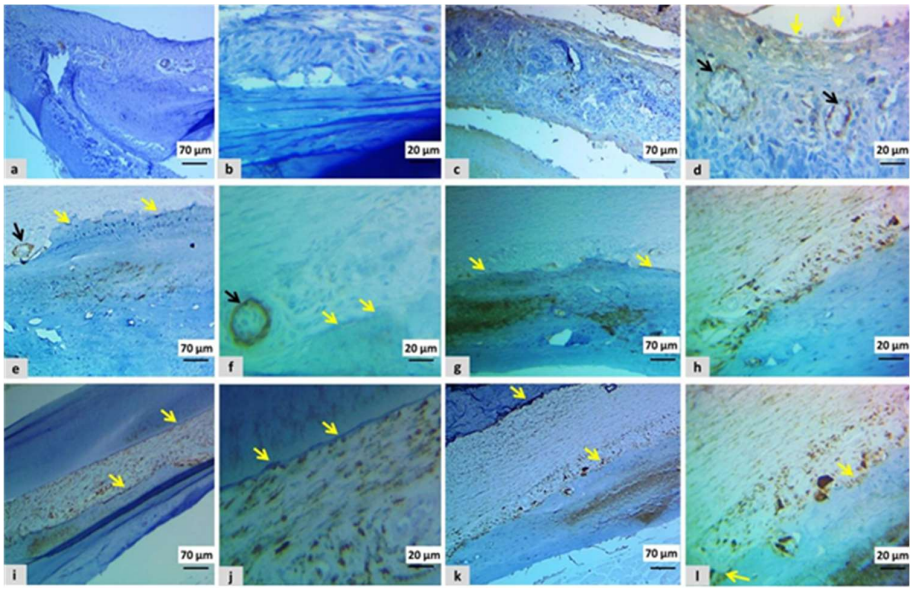

Fig. 5: Immunohistochemical staining of Claudin-1 cytoplasmic-membranous immunostaining of an incisor tooth and periodontal tissue of rat. a and b: Negative staining (score 0) in control negative group at day 7. c and d: Weak staining (score 1) in the EYO-treated group on day 7. e and f: Focal-weak staining (score 2) in the control negative group on day 14. g and h: Diffuse moderate staining (score 6) in the EYO-treated group on day 14. i and j: Diffuse moderate staining (score 6) in the control negative group at day 30. K and l: Diffuse moderate-strong staining (score 9) in EYO-treated group on day 30, with black arrows for blood vessels, and yellow arrows for the tight junction.

Table 2: The thickness of the periodontal ligament and alveolar bone in various groups.

Groups	PDL	Alveolar bone
Control Negative	50.64±1.44^{a}	74.11±0.3^{a}
	53.04±1.21^{a}	75.44±2.74^{a}
	53.73±1.33^{a}	75.94±2.58^{a}
Control Positive	63.9±4.06^{b}	50.75±8.02^{b}
	61.24±2.52^{b}	57.16±3.37^{b}
	58.65±2.31^{a}	65.91±4.52^{a}
EYO	62.21±1.75^{b}	65.09±11.55^{b,c}
	54.73±1.98^{b}	68.13±3.32^{b,c}
	52.59±1.56^{a}	71.65±2.89^{a}

Within each row, values expressed by Mean ±SE, each different alphabetical letter by P≤0.05 considered significant. In each group, the first row for day 7, the second row for day 14, and the third row for day 30.

Table 3: The osteoclast number between various studied groups.

Time	Control Negative	Control Positive	EYO
7	0.00±0.00^{a}	2.83±0.30^{b}	1.66±0.21^{b,c}
14	0.00±0.00^{a}	3.33±0.21^{b}	2.00±0.25^{b,c}
30	0.00±0.00^{a}	4.83±0.30^{b}	2.25±0.22^{b}

Within each row, values expressed by Mean ±SE, each different alphabetical letter by P ≤0.05 considered significant.

Effect of EYO in the expression of Claudin-1 tight junction (TJ): At cell-cell contact locations (periodontal ligament to alveolar bone and cementum with gingival epithelium), there was intense staining of claudin-1 in the junctional epithelium in the EYO-treated group vs. control positive group (Fig. 5), for example, at day 7 in EYO group (Fig. 5c, d), claudin-1 expressed in all locations by weak score (1) vs. control positive group that showed no positive reaction (Fig. 5a, d). Also on day 14, claudin-1 was detected on immunohistochemical analysis highly expressed in the EYO group in comparison to the control positive group periodontal tissue more specifically in the junctional epithelium at cell-cell contact (Fig. 5e-h). Additionally, at day 30 the high score (score 9) in the EYO-treated group, immunostaining claudin-1 was substantially expressed in the junctional epithelium, sulcus epithelium, and oral epithelium at the cell-cell junctions in the basal and suprabasal layers. (Fig. 5k, l) vs. the control positive group (Figure 5i, j) that showed low score (score 6).

DISCUSSION

Chronic inflammatory lesions, which are the hallmark of periodontal diseases, are caused by the buildup of sub-gingival biofilm as a result of the host's inflammatory response to periodontal pathogens. Periodontitis is characterized by the resorption of alveolar bone, the development of pockets, and inflammation (Ionel *et al*., 2017). The creation of a novel therapy that may inhibit or limit bone loss opens the possibility of discovering a drug that will not only treat the inflammatory response but also have an impact on the destruction of bone loss that occurs in periodontitis (Bartold *et al*., 2010).

In the present work, we discovered that these clinical features are well mimicked by silk ligature-induced periodontitis; bone loss happened quickly, in line with prior studies (Campi *et al*., 2016; Matsuda *et al*., 2016), who validated the experimental periodontitis model in which a silk ligature is compressed locally, causing plaque to build up over time, the periodontal connective tissues to deteriorate more quickly, and bone resorption. Following other reports, ligature-induced periodontitis in animals led to changes in the tissue that are similar to those in human periodontal tissues, as a result of causing considerable plaque accumulation, epithelial ulceration, and junctional epithelium disintegration (de Molon *et al*., 2013).

We also observed a significant increase in the inflammatory reaction, alveolar bone loss, and disruption of junction epithelium with attachment loss in the control positive group with a low score of healing at day 7 vs. those of the EYO group confirmed advanced healing with a high score, our explanation for this result agree with the previous report (Shinn *et al*., 2018), who discovered that the benefits of the main source of omega-3 found in EYO, eggs, dramatically reduced allergic and inflammatory reactions. Due to the presence of phosphatidylcholine and gamma-linolenic acid and choline, another study also supported the anti-inflammatory effect of EYO (Mahmoudi *et al*., 2013; Gao *et al*., 2014).

On days 14 and 30 the EYO-treated groups improved the healing (score 4) characterized by alveolar bone

remodeling, epithelial reattachment, and regeneration of periodontal tissue vs. control positive group that exhibited irregular bone surface with bone resorption in the 80% of cases (score 3). Our findings are similar to those of (Rastegar *et al*., 2011), who established that EYO biological components are in charge of the decrease in catabolism, as well as the rise in matrix synthesis and encouragement of re-epithelialization with the proliferation of cellular components, would shed light on the process of rapid wound healing.

The present study utilized morphometric analyses to prove the impact of EYO in repairing the periodontal ligament thickness significantly throughout the experiment vs. the control positive group, our hypothesis is associated with the antioxidant component of egg proved by many studies (Abeyrathne *et al*., 2013; Surai and Kochish, 2019), whereby these components can enhance vascularity, collagen synthesis (which is the primary component of the PDL), and collagen fiber cross-linking. They can also help lower lipid peroxidation.

In the current study, the EYO-treated group significantly reduced the alveolar bone loss vs. the control positive group more specifically on days 7 and 14, it was associated with elevation of osteoclast number in line with the earlier research, which showed that osteoclast activity was growing and driving up bone resorption (Li *et al*., 2021), while the egg's antioxidant properties, which include β-carotene, β-carotene, β-cryptoxanthin, lycopene, zeaxanthin, total phenols, and flavonoids, may have a role in the reduction in the number of osteoclasts (Nimalaratne and Wu, 2015; Omri *et al*., 2019), and an oil's ability to inhibit osteoclast genesis is due to its antioxidant content (Verma *et al*., 2012).

Claudins are proteins linked to tight junctions that play a role in maintaining the equilibrium of the epithelial barrier. A frequent component of healthy junctional epithelium, claudin-1 plays a crucial role in the function of the epithelial barrier (Fujita *et al*., 2010). An interesting point in this study that EYO increased regeneration of alveolar bone damage and periodontal tissues by expressing claudin-1 throughout the experiment is intriguing because claudin-1 is crucial for wound healing and the role that TJs protein plays in the function of the oral mucosa may provide insight into these proteins' involvement in the wound healing process (Chen and DiPietro, 2017), in comparison to the control positive group that showed low expression of claudin-1 that lead to less improvement regarding alveolar bone and periodontal tissues repair, our findings, which were in line with earlier research, showed that the loss of periodontal tissue may be related to the inability to recruit and breakdown of tight junction and cell-cell contact, as well as a decrease in claudin expression that raises oral epithelial permeability (Fujita *et al*., 2018). Reduced claudin-1 expression leads to decreased TEER and suggests enhanced permeability as a result of increased paracellular tracer flux for sodium fluorescein, which indicates disruption of the barrier function (De Benedetto *et al*., 2011).

This is in line with research from other teams, which revealed that oral tissue with damage or disease expresses less claudin-1 (Rybakovsky *et al*., 2017). Additionally, claudin-1 is required for wound healing, suggesting that both as a standalone protein and as part of the TJ complex, it is essential for cell proliferation and differentiation (Shi *et al*., 2018).

Conclusions: Our research is the first to demonstrate that EYO may have a critical role in alveolar bone formation and reverses alveolar bone with the regeneration of periodontal tissue damage. Our experimental findings suggest that EYO may be used as a therapeutic option for the treatment of bone loss associated with ligature-induced periodontitis via up-regulation of the expression of claudin-1 tight junction, proteins that provide regeneration and repair processes, hence reducing pathological damages.

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Authors contribution: The experiment was carried out by Hana HM, Sozan AM, and Mardin OM. SMA Hassan supervised, came up with the initial concept, examined the data, produced the article, and double-checked the Histopathological usage and paper. The paper is also written by Nahla MS and Shilan FMS. The work has been read and approved by all authors.

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