

EFFECT OF GARDEN CRESS ON ALVEOLAR BONE HEALING IN RATS WITH LIGATURE INDUCED PERIODONTITIS (Histological & Ultrastructural study)

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ABSTRACT

INTRODUCTION: *Lepidium sativum*, commonly known as garden cress is a herb that is native to Egypt and West Asia. Its seeds are rich source of proteins, dietary fiber, omega-3 fatty acids, iron, other essential nutrients and phytochemicals. Garden cress is widely used in folk medicine for the treatment of many diseases. Periodontitis is defined as an inflammatory bacterial-induced disease that damages the soft tissue and gradually destroys the periodontal ligaments and supporting alveolar bone. Periodontitis can cause teeth to loosen or lead to tooth loss. Different methods, such as biomechanical measurements, surgical approaches are used to treat periodontitis.

OBJECTIVE: to evaluate histologically and ultra-structurally the possible effect of garden cress therapy on alveolar bone healing in rats with ligature induced periodontitis.

MATERIALS AND METHODS: Forty-five adult male Sprague–Dawley rats of 6 months old (200-250 grams in weight) was used in this study. They were divided into 3 equal groups: group A; (control group), group B; (ligature induced periodontitis) and group C :(ligature induced periodontitis with garden cress administration). Ligature was removed after 11 days. Garden cress was applied after ligature removal systemically by using oral gavage. Rats were sacrificed after six weeks from the ligature removal. Mandibles were dissected out and were prepared for histological and scanning electron microscopic evaluation.

RESULTS: After 6 weeks the specimens of group B (periodontitis group) showed alveolar bone loss, while in group C (garden cress group) showed relative restoration of alveolar bone. Results of scanning electron microscope revealed an irregular & porous bone surface of group B, while Group C showed relative smooth and regular alveolar bone.

CONCLUSION: Garden Cress systemic administration accelerates alveolar bone healing and enhances bone formation in periodontal diseases.

KEYWORDS: Garden Cress, periodontitis, alveolar bone, rats.

RUNNING TITLE: Garden Cress in rats with induced periodontitis.

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INTRODUCTION

Lepidium sativum, commonly known as garden cress is a fast-growing annual herb that is native to Egypt and West Asia. Its seeds are rich source of proteins, dietary fiber, omega-3 fatty acids, iron, other essential nutrients, and phytochemicals. Different Arabic names, such as Rashad/Hurf/Thuffa, were given to *L. sativum* in Arabic countries, including Saudi Arabia. The plant was well recognized in European communities as *Herba Lepidii Sativi*. Garden cress is widely used in folk medicine for the treatment of many diseases. (1)

However, with the inception and upcoming of the science of Foods and Nutrition, a number of non-conventional food stuffs have been explored, analyzed, processed and used up in the development of food products.

Garden cress is one such food stuff that abounds not only in nutrients but also in health enhancing phytochemicals. (2)

Periodontium is the tissues that support the teeth. The periodontium consists of four tissues: gingiva, or gum tissue, cementum, or outer layer of the roots of teeth, alveolar bone, and periodontal ligaments (PDLs), which are the connective tissue fibers that run between the cementum and the alveolar bone. (3)

The alveolar process is that bone of the jaws containing the sockets (alveoli) for the teeth. The alveolar process consists of an outer (buccal and lingual) cortical plate, a central spongiosa, and bone lining the alveolus (alveolar bone proper). The cortical plate and alveolar bone meet at the alveolar crest (usually 1.5 to 2 mm below the level of the cemento-enamel junction on the tooth it surrounds). (4,5)

Periodontitis is an inflammatory diseases affecting the tissues surrounding the teeth. Periodontitis involves progressive loss of the alveolar bone around the teeth, and if left untreated, can lead to the loosening and subsequent loss of teeth. (6)

The application of Garden cress has long been used in healing of bone and wound healing but the literature regarding the direct effect of garden cress on the oral tissues is still obscure. This stimulated the idea of the present work in studying the effect of using garden cress therapy on the healing of the alveolar bone of rats after induction of periodontitis.

MATERIALS AND METHODS

This study was conducted following the ethical guidelines for conduct of research on experimental animals, by the Faculty of Dentistry, Alexandria University (IRB NO:00010556-IORG 0008839).

Forty-five adult male Sprague–Dawley rats of 6 months old (200-250 grams) were used in this study. Animals were obtained from the animal house of Faculty of Dentistry, Pharos University. They were kept under the same nutritional and environmental conditions in the experimental animal house.

Rats were divided randomly into 3 equal groups 15 rat each:

Group A: control group

Group B: ligature induced periodontitis

Group C: ligature induced periodontitis with garden cress administration

Induced periodontitis preparation (7)

Animals of groups B & C were subjected to ligature placement under general anesthesia using a mixture of ketamine and xylazine anesthesia (Nikon Instruments Inc., NY, USA), a 4-0 silk ligature (Roboz Surgical Instrument Co., MD, USA) was secured at the gingival sulcus level of the mandibular right first molar (M1) of all animals. After 11 days the silk ligature was removed, the signs of periodontitis were evaluated clinically through bleeding and inflammation of the gingiva. The gingival tissues become swollen, with pocket formation, accumulation of debris, and ulceration appeared.

Preparation of aqueous extract of garden cress

The aqueous extract of Garden cress (Garden Cress seeds by Fareast flora garden center, website, USA) was prepared by boiling 10g of dried seeds of *L. sativum* in 100 ml of distilled water for 10 min and left for 15 min to infuse. Thereafter, the extract was cooled and filtered to remove particulate matter, and then the solution was returned back to 100 ml volume by the adding of distilled water. For group C the oral administration of the aqueous extract of garden cress by using oral gavage. 1.6 gm/kg was taken in the same day after ligature removal and continuous daily until the day of scarification.

For group A&B the rats had received distilled water orally by using oral gavage to control the influence of any stress or buffer-induced effects on the animals through the experimental period.

Scarification

- The animals were euthanized with 20 mg/kg thiopental (0.5 g Thiopentax, Crista 'Lia, Sa'õ Paulo. (8)
- Animals were sacrificed after 6 weeks from removal of ligature. The mandible of each rat was dissected, and separated from muscle and soft tissue, keeping the attached gingiva intact with the bone. Only the segment of the mandibular molar teeth with the surrounding

alveolar bone was prepared for Histological and SEM evaluation.

Histological evaluation (9)

Specimens were prepared to be examined by the light microscope. Segments of molar area of the biopsies were fixed in 10% neutral buffered formalin. The fixation of the specimens was followed by washing, decalcification, washing, dehydration, clearance, infiltration, embedding in paraffine wax, cutting in 5 µm thick sections, mounting and stains preparation. The stains used in this study were Hematoxylin and Eosin stain.

Scanning Electron Microscopic (SEM) examination: (10)

Specimens were fixed in 2.5% glutaraldehyde in phosphate buffer (PH 7.3) for 48 hours and washed twice in the same buffer. The specimens were then dehydrated in the graded series of aqueous ethanol solution 50%, 70%, 90%, and 100% for one hour for each specimen. Then they were airdried, mounted on aluminum SEM stubs with silver paint and sputter coated with gold using an ion coater (sputter coater). The specimens were examined by SEM using a 20-kV accelerating voltage; to observe the alveolar bone surface integrity buccally using magnifications X75. The alveolar bone was judged based on the smoothness of its surface; normally bone shows a smooth surface penetrated by nutritive canals, resorption is indicated by irregularities, pits and depressions as osteoclasts form How ship's lacunae, and level of alveolar bone crest between 1st and 2nd molars.

RESULTS

Light microscopic results:

Control group

Examination of alveolar bone revealed normal architecture of alveolar bone. Regular bone surface facing the periodontal ligament interrupted by Volkmann canal, healthy and continuous PDL fibers inserted in the alveolar bone. Normal size, number and distribution of osteocytes was seen, well defined resting lines with osteoblasts lining narrow bone marrow cavity were also seen. Figure (1).

ligature induced periodontitis group:

Microscopic examination of this group revealed alveolar bone destruction and more aggressive loss in bone height with detachment of PDL fibers, irregular resorbed alveolar bone surface, wide bone marrow spaces with fatty tissues infiltration. Discontinuity of osteoblast on bone surface was seen. Empty osteocytes and osteocytes with pyknotic nucleus were also observed. Multinucleated osteoclasts in Howship's lacunae and resting lines were evident. Figure (2). **ligature induced periodontitis with garden cress administration group:**

The result was relatively similar to group A (control group). It showed the normal orientation of PDL fibers. The group showed dense bone with relatively regular and smooth boundary, normal size and distribution of osteocytes. Well defined resting line were seen. Figure (3).

Results of the scanning electron microscope:

Control group:

An intact alveolar crest in the interproximal area between 1st & 2nd molars was seen, without any destruction. The surface topography of the buccal cortical plate of alveolar bone was seen uniform, smooth and regular, Figure (4).

Ligature induced periodontitis group:

Alveolar bone destruction was noted, and the surface topography exhibited a generalized pattern of surface porosity, roughening and irregularities. Figure (5).

Ligature induced periodontitis with garden cress administration group:

Better improvement in alveolar bone surface in the form of Relative restoration of alveolar crest level between 1st and 2nd molars into a level comparable to the control group. The surface topography of the buccal cortical plate of alveolar bone was seen uniform, smooth and regular Figure (6).



Figure (1): A. Light photomicrograph of interstitial bone (control group six weeks) showing normal architecture of the alveolar bone, regular bone surface facing the periodontal ligament interrupted by Volkman canal (Arrow), healthy and continuous PDL fibers inserted in the alveolar bone. AB: alveolar bone. D: dentin. PDL: Periodontal ligament. (H&E) x100.

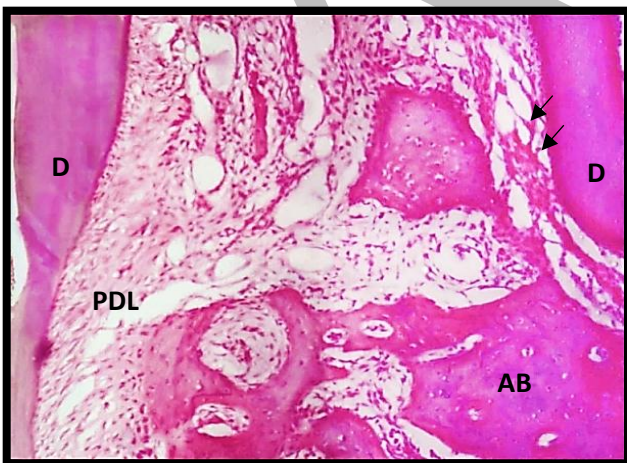


Figure (2): Light photomicrograph of interstitial done in ligature induced periodontitis (group B six weeks) showing irregular resorbed alveolar bone, wide bone marrow spaces with and areas of detachment of PDL fiber (small arrows). AB: alveolar bone. D: dentin. PDL: Periodontal ligament. (H&E) x100.

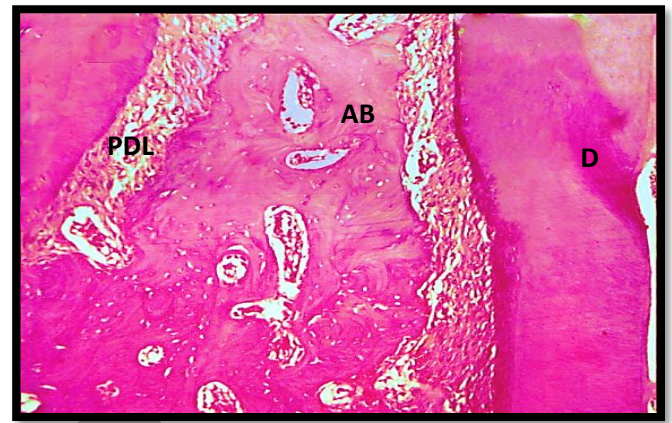


Figure (3): Light photomicrograph of interstitial bone in ligature induced periodontitis with garden cress administration (group c) at six weeks showing dense bone with relatively regular boundary, relatively healthy and continuous PDL. AB: alveolar bone. D: dentin. PDL: Periodontal ligament. (H&E) x 100.

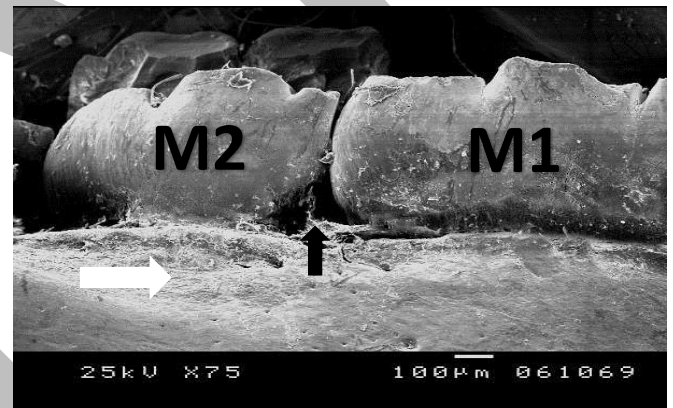


Figure (4): Scanning electron micrograph (SEM), group (A), showing: Intact buccal cortical plate and alveolar crest between 1st & 2nd molars (black arrow). The surface topography of the buccal cortical plate of alveolar bone is uniform, smooth, and regular (white arrow). (X75)

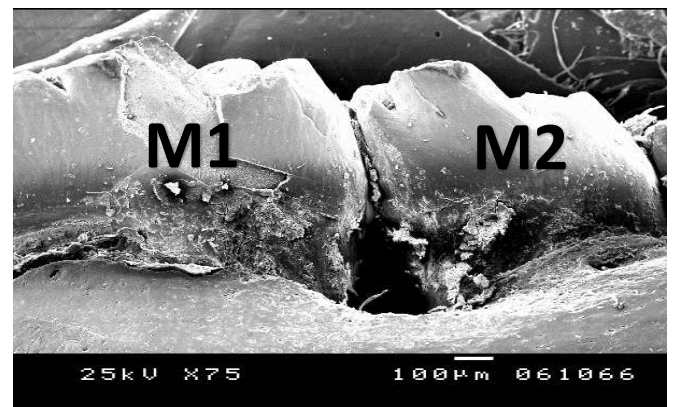


Figure (5): SEM, group (B), showing: reduction in the level of alveolar bone crest between mandibular 1st and 2nd molars Alveolar bone destruction was noted and the surface topography exhibited a generalized pattern of surface porosity, roughening and irregularities are seen. (X75)

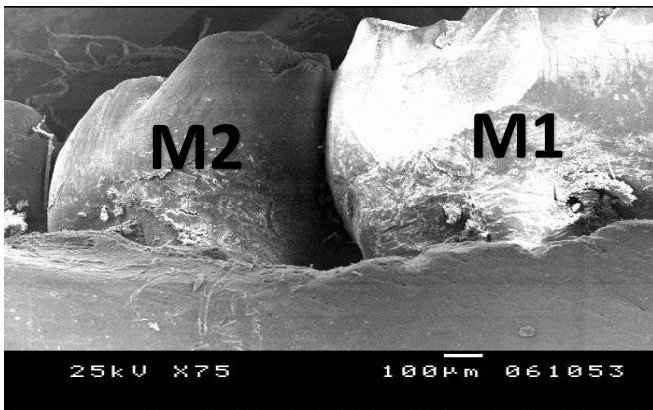


Figure (6): SEM, group (C), showing: Alveolar crest between 1st and 2nd molars, and buccal cortical plate restored to normal architecture. The surface topography of the buccal cortical plate of alveolar bone was seen uniform, smooth and regular. (X75)

DISCUSSION

Periodontal disease is an inclusive term describing any disease of the tissues surrounding the teeth, including gingival diseases and diseases of the supporting structures. Periodontal diseases are a group of oral inflammatory diseases that are influenced by host–response factors. The two main types of periodontal disease are gingivitis, which affects only the gums, and periodontitis, which is characterized by apical migration of the periodontal ligament attachment and destruction of the connective tissue and alveolar bone that support the teeth. (11,12)

Healing of periodontitis was a major task that was studied clinically, experimentally, and traditionally aiming at facilitating this phenomenon positively and documenting it by different methods, such as biomechanical measurements, surgical and the influences of many factors and medications on the healing of periodontal pockets were noted as well. (13, 14)

However, alternative medicines, such as traditional folk medicine, have used natural elements from ancient times to now. This was practiced for the treatment of many ailments in different societies. *L sativum* and its seeds, in particular, were publicly used in Saudi Arabia as a traditional medicine, mostly for the treatment of recent traumatic fractures and less commonly in delayed or non-united fractures. (15)

This study aimed to evaluate histologically and ultrastructurally the possible effect of garden cress therapy on alveolar bone healing in rats with ligature induced periodontitis.

Animal models have been used to investigate the pathologic processes of infectious diseases such as periodontitis. Ligature-induced periodontitis has been used in primates, dogs, and rats to study factors affecting the severity of periodontitis (8) in most cases small animal models such as rats or hamsters will be sufficient to assess the role of bacteria, diet or other factors in periodontal inflammation at the histological level, providing sufficient statistical significance and preclinical relevance. In this study rats were used as experimental animal model to assist role of garden cress in the healing of periodontitis. (7)

In this study locally induced periodontal defects were chosen over systemically induced periodontitis because systemic

bone resorption was found in previous studies with using systemic induced periodontitis. It has been reported that bone loss affects the jaw bones, cranial bones, ribs, and long bones in descending order. The alveolar bone is affected first due to the highest rate of remodeling. (8)

Garden Cress was giving systemically because its challenging to remain in its position in local administration. It was given at day 11 from ligature placement, in the same day after ligature removal.

The results of the control group showed normal structure and architecture of alveolar bone, regular bone surface facing the periodontal ligament interrupted by Volkmann canal, healthy and continuous PDL fibers inserted in the alveolar bone, this result is in agreement with the results of (Heinz et al .2015).

After 6 weeks from ligature removal, the results of this study showed alveolar bone destruction and loss of height in ligature induced periodontitis group, and impaired new bone formation compared to the control group .Early pathological findings in previous study about ligature induced periodontitis include an acute inflammatory response, with polymorphonuclear cells infiltrating beneath the junctional epithelium, followed by macrophages infiltrating into the affected epithelium. They added that connective tissues are destroyed, and the epithelial attachment migrates apically along the root surface, deepening the pocket. The advanced lesions present as a destruction of the alveolar bone with fibrosis and granulation of the gingival connective tissues and periodontal ligament space. (8)

On the other hand, the administration of garden cress in the present study, inhibited alveolar bone loss & restoring its normal original architecture compared with the normal control group.

One of the traditional uses of *Lepidium sativum* is for increasing the speed of bone fracture healing. According to the authors of several articles on this aspect, clinical observations were supportive for this use. Among the studies, which aimed to investigate the validity of the plant for this use, (Juma et al.2007) carried a study on rabbits. *Lepidium sativum* seeds were incorporated in the diet of the test group (undergone induced fracture of mid shaft of left femur), observations showed that *Lepidium sativum* has a significant role in accelerating bone fracture healing which supports the rationality of its traditional use for this purpose. (16) Several other studies showed similar results which supports the need of more researches on this aspect. (16, 17)

The positive effect of Garden cress on bone forming cells is due to the antioxidant properties of garden cress depend on the phenolic compounds present in garden cress seeds. The main phenolic compounds present in GCS extracts are tocopherols. Tocopherols act as The Potential of Garden Cress. Tocopherols also help in preventing diseases, besides possessing an important nutritional function for human beings as a source of vitamin E. (17).

Consequently, the tensile strength, stiffness of fractures, and other inhibitory factors – which are also important elements influencing fracture healing together with all previously mentioned factors – will open a major field of further studies to be carried out under the influence of *L sativum* seeds. Note, *L sativum* seeds had no significant effects on the weight of rabbits, which was similarly noted by others. (17, 18)

CONCLUSIONS

The findings of this study provide evidence that Garden Cress administered systemically, accelerates alveolar bone healing in rats with induced periodontitis. It enhances osteoblastic activity and new bone formation. So, it may be used as adjunct treatment besides conventional treatment of periodontitis.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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