Original Article

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Experimental investigation of the effect of ellagic acid on bone healing

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ABSTRACT

Aims: Some of the most effective medicines discovered to date have been derived from natural products derived from microorganisms, plants and animals. Phenolic compounds have been recognized as the most important bioactive compounds responsible for bone health effects. Ellagic acid (EA), a member of the flavonoids, is a natural phenol antioxidant found in many fruits and vegetables. The anti-proliferative and antioxidant properties of EA have prompted research into its potential health benefits.[1] The aim of our study is to investigate the effect of EA on hard tissue healing after tooth extraction in rats.

Methods: For this purpose, the lower left mandibular teeth of 16 Wistar Albino male rats were extracted under general anesthesia. Among the rats randomly divided into four groups, the 1st study group was given EA by gavage once a day for 14 days (14EA), and the 2nd study group was given EA by gavage once a day for 21 days (21EA). The 1st control group was given 0.9% Isotonic NaCl by gavage once a day for 14 days (14K), the 2nd control group was given 0.9% Isotonic NaCl by gavage once a day for 21 days (21K). Afterwards, the rats were sacrificed and prepared for histopathological examination.

Results: As a result of histopathological examination, the inflammation level of the 21EA group was found to be significantly lower than the 14K (p:0.042) and 21K (p:0.004) groups ($p<0.05$). The epithelial proliferation level of the 21EA group was found to be significantly higher than the 21K, 14K (p:0.001) and 14EA (p:0.009) groups ($p<0.05$). The new bone formation level of the 21EA group was higher than the 14K (p:0.001), 21K (p:0.008) and 14EA (p:0.018) groups; The new bone formation level of the 14EA group was statistically significantly higher than the 14K group (p:0.014; $p<0.05$). The healing score of the 21EA group was found to be significantly higher than the 14EA (p:0.013) (14K (p:0.017) and 21K (p:0.009) groups ($p<0.05$)

Conclusion: As a result of the study, it was found that EA had a statistically significant positive effect on bone healing, especially on the 21st day.

Keywords: Elagic acid, tooth extraction, bone healiing, rat, polyphenols

INTRODUCTION

In dentistry, it is important to preserve sufficient alveolar bone volume in order to make a prosthesis that can provide the increasing demand for aesthetics and function. For this reason, research to accelerate the healing process of the alveolar bone has become an important topic of discussion. Therefore, it is increasingly important that the healing process promotes the formation of alveolar bone with sufficient amounts of hard and soft tissue to allow for an ideal implant-supported restoration.

Tooth extraction is a surgical procedure that is frequently performed in clinics and involves hard tissue and soft tissue. However, tooth extraction can cause complications such as bleeding, infection, fracture and alveolitis. The healing process of the alveolar bone is affected by osteoblasts, osteoclasts and osteocytes, as well as hormones, nutrients, growth factors and inflammatory cytokines.[2] Impaired wound healing after tooth extraction may hinder subsequent treatments and the patient's ability to function. For this reason, it is important that the wound healing process after tooth extraction occurs smoothly.

Some of the most effective drugs discovered to date have been obtained from microorganisms, plants and animals.[3] Polyphenols constitute one of the most common and widely distributed groups of substances

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found in plants and are considered the most important bioactive compounds responsible for bone health effects.[4] They are secondary metabolites involved in the chemical defense of plants against predators and in plant-plant interventions. Several thousand plant-derived polyphenols are known, covering a wide variety of molecules containing at least one aromatic ring with one or more hydroxyl groups. The biological properties of polyphenols include anti-oxidant, anti-cancer and anti-inflammatory effects.[5,6]

EA was first discovered by French chemist and pharmacist Henri Braconnot in 1831.[7] EA is a dimeric derivative of gallic acid and is rarely found free in dietary products and usually forms part of ellagitanins.[8,9] Considering the level of conversion of ellagitannins to EA, the highest concentrations of EA are found in berries, pomegranates, muscadines, and tropical fruits in plants of the raspberry rubus genus.[7] In addition, dates, goji berries and green tea also contain high amounts of EA.[10,11]

EA is a highly thermostable molecule with a melting point of 350°C, a molecular weight of 302.197 g/mol, slightly soluble in water, alcohol and ether, and soluble in potassium hydroxide. EA is a weak acid that ionizes at physiological pH. Structurally, it has four rings representing the lipophilic domain, four phenolic groups, and two lactones representing the hydrophilic domain, which function as electron acceptors, forming the hydrogen bond sides, respectively.[12] The hydrophilic part of the EA molecule plays an important role in biological activity due to the presence of both hydrogen-bonding acceptor (lactone) and donor (–OH) sites (phenolic hydroxyl groups that can dissociate into negatively charged phenolate ions under physiological conditions).[7] EA has effective anti-oxidant activity, radical scavenging capacity, chemopreventive and anti-apoptotic, anti-mutagenetic, anti-viral, anti-fibrosis and anti-inflammatory properties.[4]

EA scavenges free electrons present in the last orbital shell and therefore acts as a potent antioxidant and therefore reduces oxidative stress of the cellular component.[13]

Ionic metals such as copper, iron, nickel and cadmium are a potential source of oxidative stress, and EA provides an additional protection mechanism against this situation by chelating these metals.[14]

EA can not only clear pro-oxidant agents, but also increases the expression/activity of enzymes such as antioxidant superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase.[15]

Bone damage triggers the bone healing process, which begins with inflammation. Inflammation is a normal part of the bone healing process. Long-term inflammation causes bone resorption due to osteoclast activity modulated by pro-inflammatory cytokines such as TNF-α.[15] This inflammation increases the activity of osteoclasts, which play a role in bone resorption. Suppression of inflammation is expected to reduce osteoclast activity and therefore increase the rate of new bone formation.[16]

METHODS

The study was carried out with the permission of İstanbul University Animal Experiments Local Ethics Committee (Date: 29.01.2021, Decision No: 2021/01). All procedures followed are based on animal rights (Guide for the Care and Use of Laboratory Animals).

Our study was carried out in the Laboratory of Experimental Medicine Research Institute and the experimental animals were housed in the Experimental Animal Care and Housing Unit of Experimental Medicine Research Institute, Department of Laboratory Animal Science. In our study, Ketanest vial (Ketamine hydrochloride, Parke Davis, Berlin, Germany) (50 mg/kg) and Rhompun vial (Xylazine hydrochloride, Bayer, İstanbul, Turkey) (5 mg/kg) were administered intramuscularly (i.m.) to rats under the supervision of a veterinarian for general anesthesia. 2% lidocaine (Lidocaine 2% E80, Vem, Ankara, Turkey) containing 1:100,000 epinephrine was applied to the lingual fold to create local anesthesia and hemostasis. After the left mandibular first molar tooth of each rat was extracted with the help of a hemostat, a defect was created in the extraction socket with a 2 mm steel round bur under cooling. The extraction wound was sutured with 4.0 silk suture (Doğsan, İstanbul, Türkiye). Compression was applied with a sterile tampon for 2 minutes. Xylazine HCl at a dosage of 5 mg/kg was administered intramuscularly for pain control in the pre-operative period. In the post-operative period, Cefazolin at a dosage of 50 mg/kg and Tramadol HCl at a dosage of 5 mg/kg were administered intraperitoneally twice a day for pain and infection control for 2 days.

EA solution was prepared by dissolving 12.5 mg of EA powder (E2250, Sigma Aldrich, USA) in 1 ml of distilled water, following the methodology described in the studies of Al-Obaidi et al.[4] The solution was shaken before each application to ensure homogeneous distribution and was administered to rats at a dosage of 10 ml/kg.

1. The control group received 0.9% Isotonic NaCl by oral gavage once a day for 14 days.

2. The study group was administered EA powder (E2250, Sigma Aldrich, USA) dissolved in distilled water (10 ml/kg) via oral gavage once a day for 14 days.

3. The control group was given 0.9% Isotonic NaCl by oral gavage once a day for 21 days.

4. EA was administered to the study group via oral gavage once a day for 21 days.

Sacrifice was performed by administering 150 mg/kg of sodium pentothal (Pental Sodium, Ulagay, Istanbul, Turkey) to all groups on the 14th or 21st day. The alveolar bone in the extraction area was then removed and sent to the I.U. Oncology Institute Pathology Laboratory in formaldehyde solution for histopathological examination.

Histopathological Evaluation

The mandibles of the animals were extracted, and the soft tissues and skin were meticulously cleaned before being transported to the pathology laboratory in 10% formaldehyde solution. The bone tissue surrounding the extraction sites of the mandibular left first molar teeth was fixed in formalin and subsequently immersed in solutions containing 32 sodium citrate (20%) and formic acid (50%) for the decalcification process. Following decalcification, the rat mandibles underwent standard tissue processing procedures and were then embedded in paraffin blocks. Sections measuring 5 µm in thickness were obtained from the paraffin blocks, followed by deparaffinization and transparency procedures prior to mounting on slides. These sections were then stained with hematoxylin eosin dye and examined under a light microscope. Digital photographs were taken and analyzed using the Olympus ANAlysis 5 (Tokyo, Japan) program. Inflammation, new bone formation, fibrosis, epithelial proliferation, necrosis, and healing scores were assessed by a single researcher across predetermined areas within the sections using the software.

Inflammation: Scored from 0 to 4, with increasing scores indicating higher levels of inflammation.

Epithelial proliferation: Scored from 0 to 3, with increasing scores indicating greater epithelial proliferation.

Necrosis: Scored as 0 for absent and 1 for present.

Fibrosis: Scored from 0 to 3, with increasing scores reflecting greater amounts of fibrosis.

Bone healing score: Graded from 0 to 6, with the following interpretations:

0: No defect healing

1: Incomplete cartilage healing

2: Complete cartilage healing

3: Incomplete bone healing in its initial stage

4: Moderate incomplete bone healing

5: Late incomplete bone healing

6: Complete bone healing

Statistical Analysis

The findings obtained in this study were evaluated using IBM SPSS Statistics 22 (IBM SPSS, Tukey) program for statistical analysis. The suitability of the data parameters in the study to normal distribution was evaluated with the Shapiro Wilkstest. While evaluating the study data, descriptive statistical methods (Mean, Standard deviation, frequency) were used, as well as when comparing quantitative data, one-way ANOVA test was used to compare normally distributed parameters between groups, and TukeyHDS test was used to determine the group causing the difference. Kruskal Wallis test was used for intergroup comparisons of parameters that did not show normal distribution, and posthoc Dunn's test was used to determine the group causing the difference. While evaluating the study data, Mann Whitney U test was used to compare the parameters between two groups. Kruskal Wallis test (post hoc Dunn's test) was used to compare the parameters between the four groups. FFisher's exact test, chi-square test and Fisher Freeman Halton exact test and chi-square test were used to compare qualitative data. Significance was evaluated at $p<0.05$ level.

RESULTS

This study was conducted on a total of 16 male Wistar Albino rats weighing between 250-300 g. The control group, named "14K," received 0.9% isotonic NaCl for 14 days after tooth extraction and was sacrificed on the 14th day post-extraction. The study group, named "14EA," received EA solution for 14 days and was also sacrificed on the 14th day. Similarly, the control group named "21K" received 0.9% Isotonic NaCl for 21 days and was sacrificed on the 21st day, while the study group named "21EA" received EA solution for 21 days and was also sacrificed on the 21st day.

Inflammation

Inflammation plays a crucial role in biological processes aimed at eliminating pathogens and maintaining tissue homeostasis. In the context of bone defect regeneration through osteogenesis, the process begins with inflammation. During the inflammatory phase, phagocytic cells such as monocytes, macrophages, and neutrophils situated on the bone surface generate oxygen-based free radicals. These free radicals contribute to the formation of osteoclasts and subsequent bone resorption. This process hinders the remineralization of the bone defect or tooth extraction socket.

In our study, significant differences in inflammation levels between groups were observed ($p:0.026$; $p<0.05$) (Table). Post hoc analyses conducted to determine significance revealed that the inflammation level of the 21EA group was notably lower compared to both the 14K ($p:0.042$) and 21K ($p:0.004$) groups ($p<0.05$) (Figure 1, Figure 2).

Table. Comparison of four groups in terms of study parameters

	Study 14 (n=4)		Control 14 (n=4)		Study 21 (n=4)		Control 21 (n=4)		
	Min-Max	Mean±SD (median)	Min-Max	Mean±SD (median)	Min-Max	Mean±SD (median)	Min-Max	Mean±SD (median)	p
Inflammation	2-4	2.8±0.84 (3)	3-4	3.25±0.5 (3)	1-3	2±0.71 (2)	3-4	3.75±0.5 (4)	0.026*
Fibrosis	2-3	2.2±0.45 (2)	1-2	1.75±0.5 (2)	2-2	2±0 (2)	2-3	2.5±0.58 (2.5)	0.138
Epitel proliferation	0-2	1.4±0.89 (2)	1-1	1±0 (1)	3-3	3±0 (3)	1-3	2±0.82 (2)	0.007*
New bone formation (%)	0.5-0.75	0.66±0.09 (0.68)	0.02-0.08	0.05±0.03 (0.05)	0.8-1.7	1.14±0.35 (1.1)	0.11-0.3	0.21±0.08 (0.21)	0.001*
Healing score	3-4	3.4±0.55 (3)	1-3	2.25±0.96 (2.5)	4-6	5±0.71 (5)	2-3	2.25±0.5 (2)	0.004*
Necrosis n (%)	4	%80	4	%100	2	%40	4	%100	+0.137

Kruskal Wallis Test, +Fisher Freeman Halton Exact Ki-kare test, *p<0.05

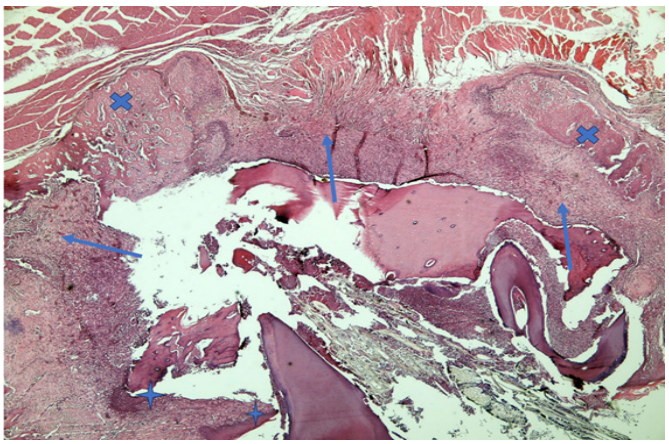

Figure 1. Study group sacrificed on the 14th day (14EA): food residues in the cavity, new bone formation (x's) in the fibrous connective tissue (arrows) at the cavity floor and in the surrounding area. Surface epithelium (stars) are observed at the edges of the cavity surface (H&E X40).

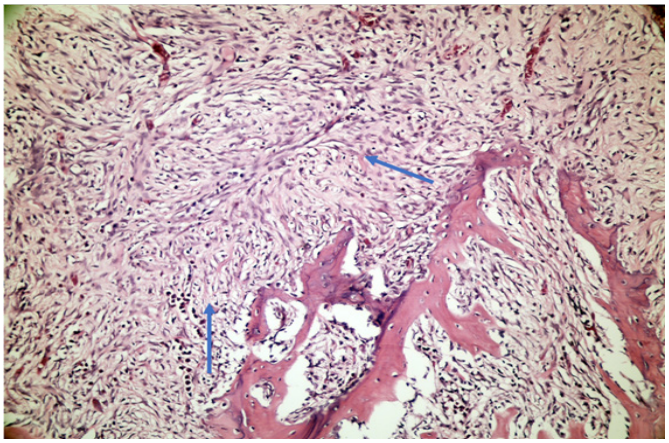

Figure 2. Control group sacrificed on the 21st day (21K): New bone formation within the active fibrous tissue (arrows) within the extraction socket (H&EX200).

Additionally, the inflammation level of the 21EA group was statistically significantly lower than that of the 21K group (p:0.016; p<0.05). However, there were no statistically significant differences in inflammation levels among the other groups (p>0.05) (Figure 3).

21EA group was statistically significantly lower than that of the 21K group (p:0.016; p<0.05). However, there were no statistically significant differences in inflammation levels among the other groups (p>0.05) (Figure 3).

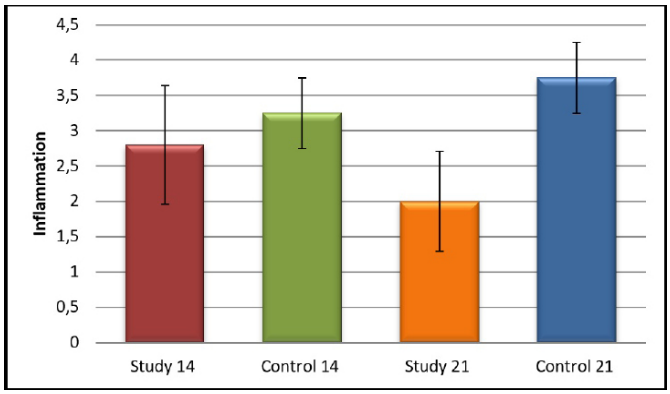

Figure 3. Graph showing inflammation levels across all groups

Epitel Proliferation

A statistically significant difference was observed in epithelial proliferation levels between groups (p:0.007; p<0.05). Post hoc analyses were conducted to determine significance, revealing that the epithelial proliferation level of the 21EA group was significantly higher than that of the 21K group (p:0.007) (Figure 4), as well as the 14K group (p: 0.001) and the 14EA group (p:0.009) (p<0.05). However, there were no statistically significant differences observed between the other groups in terms of epithelial proliferation levels (p>0.05) (Figure 5).

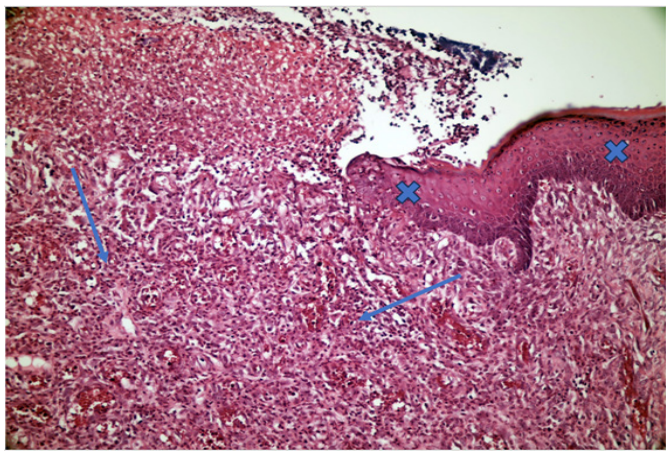

Figure 4. Control group sacrificed on the 21st day (21K): Connective tissue (arrows) containing dense lymphocyte, plasma cell, neutrophil polymorphous infiltration in the cavity and stratified squamous epithelium (x's) proliferating in the direction of closing the extraction socket on the surface (H&EX200)

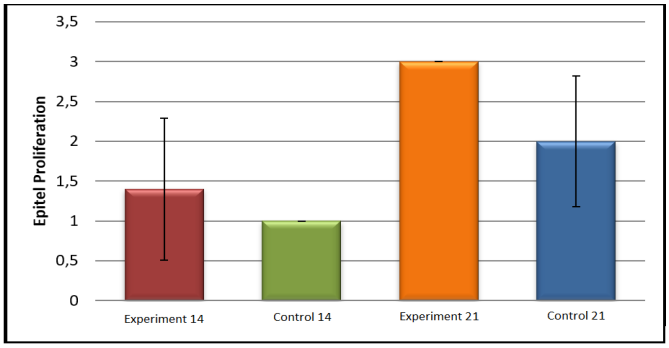

Figure 5. Graph showing epithelial proliferation levels between all groups

There is no statistically significant difference between the 14EA and 14K groups in terms of epithelial proliferation levels (p>0.05). The epithelial proliferation level of the 21EA group is statistically significantly higher than the 21K group (p:0.028; p<0.05).

New Bone Formation

A statistically significant difference was observed between the groups in terms of new bone formation levels (p:0.001; p<0.05). Post hoc analyses were conducted to determine significance, revealing that the new bone formation level of the 21EA group (Figure 6, Figure 7, Figure 8) was significantly higher (p<0.05) than that of the 14K group (p:0.001), the 21K group (p:0.008), and the 14EA group (p:0.018) (Figure 1, Figure 9, Figure 10). However, there was no statistically significant difference in new bone formation levels between the other groups (p>0.05).

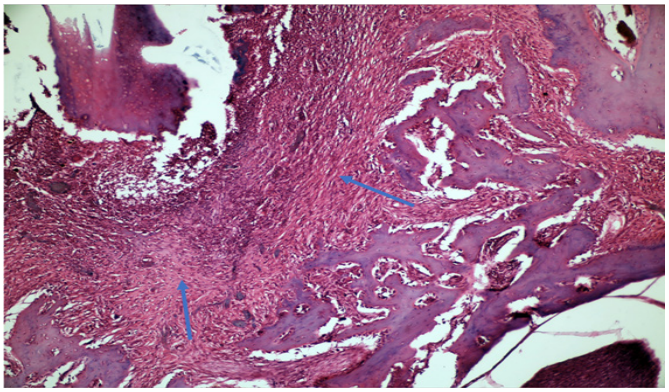

Figure 6. 21. Control group sacrificed on the first day (21EA): Fibrous connective tissue (arrows) and new bone trabeculae (H&EX100) in the extraction cavity

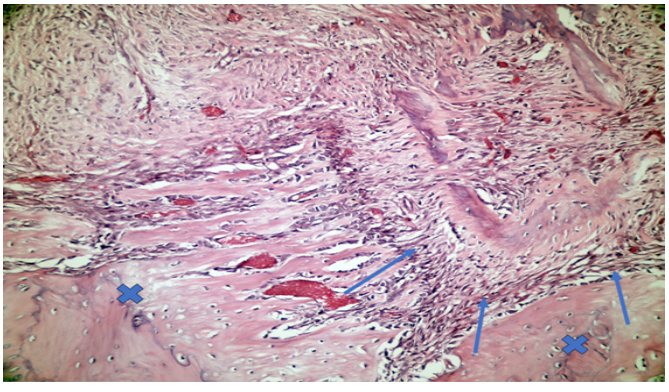

Figure 7. EA group sacrificed on the 21st day (21EA): Fibrous connective tissue (arrows) within the extraction cavity and new bone trabeculae (x's) filling most of the cavity (H&EX200)

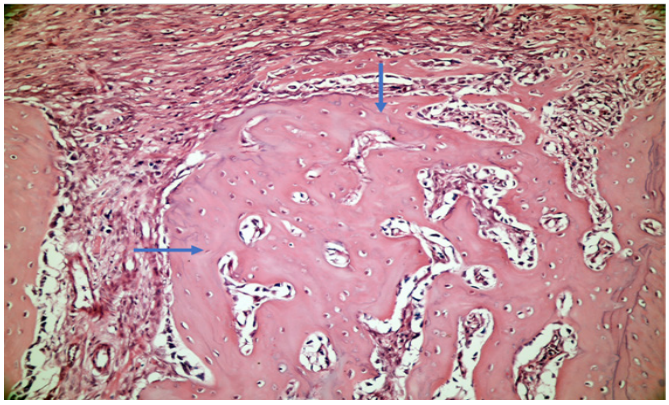

Figure 8. Study group sacrificed on the 21st day (21EA): New bone formation filling the cavity in the extraction socket (arrow marks) (H&EX200)

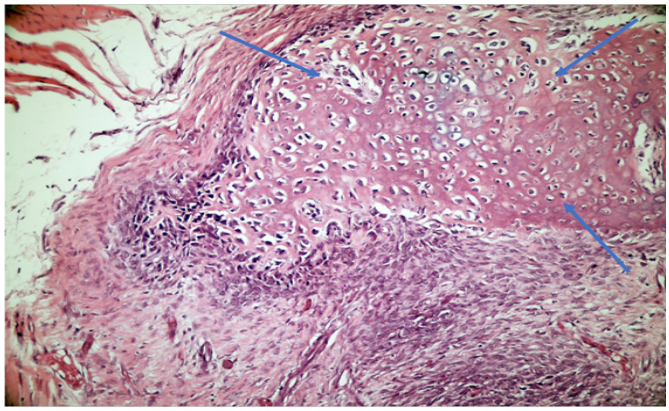

Figure 9. Study group sacrificed on day 14 (14EA), new bone formation (arrow marks) (H&EX200)

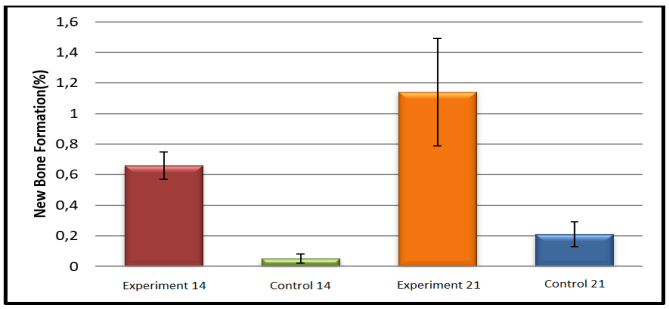

Figure 10. Graph showing new bone formation levels among all groups

The new bone formation level of the 14EA group was statistically significantly higher than the 14K group (p:0.014; p<0.05). The new bone formation level of the 21EA group is statistically significantly higher than the 21K group (p:0.014; p<0.05) (Figure 11).

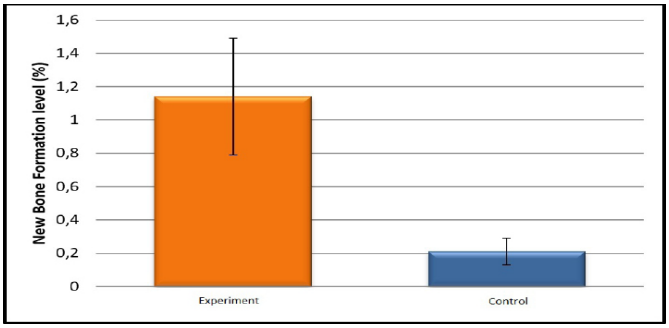

Figure 11. Graph showing new bone formation levels between 21EA and 21K groups

Bone Healing Score

In this study, a statistically significant difference was observed between the groups in terms of healing (p:0.004; p<0.05). Post hoc analyses were conducted to determine significance, revealing that the healing score of the 21EA group was significantly higher than that of the 14K group (p:0.017) and the 21K group (p:0.009) (p<0.05) (Figure 12).

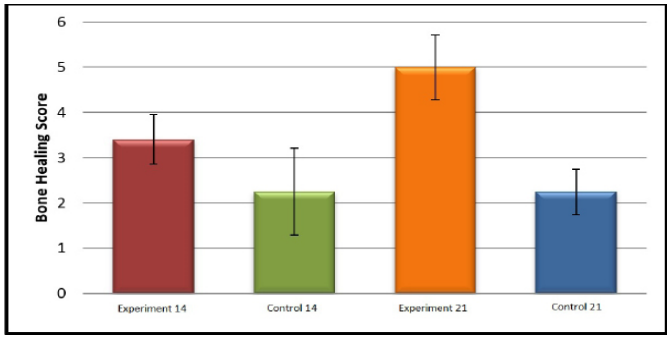

Figure 12. Graph showing healing score levels among all groups

Although the healing score of the 14EA group was higher than that of the 14K group, this difference was not statistically significant, although it was close to significance (p>0.05).

The healing score of the 21EA group is statistically significantly higher than the 21K group (p:0.011; p<0.05).

Fibrosis

There are studies showing that EA has a stimulating effect on the proliferation of osteoblasts and fibroblasts.[4,10] However, in this study, no statistically significant difference was seen between the groups in terms of fibrosis level. (p>0.05) (Table).

Between 14EA and 14K groups; Between 21EA and 21K groups; Between 14EA and 21EA groups; There is no statistically significant difference in fibrosis levels between the 14K and 21K groups (p>0.05).

Necrosis

Although the rate of necrosis did not show a statistically significant difference between the groups in this study (p>0.05), the rate of necrosis was observed as 80% in the 14EA group, 100% in the 14K group, 40% in the 21EA group, and 100% in the 21K group.

DISCUSSION

Since bone healing can be influenced by sex hormones, our study experiments were conducted on male rats. This choice was made because sex hormones are generally less impactful in males compared to females, and male rats do not experience monthly periods.[17] However, it's important to note that bone healing was evaluated in adult, mature rats.

In their study involving mice, Ito et al.[8] observed significantly different pathological features during the healing processes of semi-stabilized femur fractures, attributed to the absence of cartilage appearance in tooth extraction socket healing. They highlighted the distinct cartilage plasticity between periodontium and periosteum, noting the absence of cartilage formation in socket healing post tooth extraction.[18] This absence of cartilage appearance and the evident pathological features observed during extraction socket healing served as the rationale for utilizing a tooth extraction model in our study.

In their study involving 24 male rats, Al-Obaidi et al.[4] divided the rats that underwent left upper incisor tooth extraction into two groups, each consisting of twelve rats. The first group served as the control group and received only normal saline, while the second group received EA intragastrically once a day for 28 days. Their histopathological examination concluded that EA administration on days 14, 21, and 28 accelerated the healing process of tooth extraction sockets in rats. Similarly, in our study, the healing score of the 21EA group was significantly higher compared to the 14K group (p:0.017) and the 21K group (p:0.009) (p<0.05). Although the healing score of the 14EA group was higher than that of the 14K group, this difference was close to significance but not statistically significant. In our study, molar tooth extraction was performed in rats due to the difficulty in extracting anterior teeth caused by their root length, which significantly affected post-operative nutrition. However, as the molar teeth in rats have a root form that prevents complete removal, the extraction socket was milled after extraction. To eliminate the possibility of residual roots affecting the study results, the socket was meticulously cleaned from any remaining roots using a round bur.Plant-derived drugs are seen as a promising source with fewer side effects and their compounds are non-toxic.[2] In our study, no rats were lost during the experimental procedures, and it was observed that EA did not cause any side effects, toxicity, or allergic reactions on rats.

In their study, Dede et al.[18] aimed to evaluate the effect of EA on the periodontal repair process associated with experimental periodontitis in rats. They measured the levels of alveolar bone resorption, inflammatory markers, and oxidative stress markers in periodontal tissue and serum. The results of their study revealed that EA led to significant positive improvements in gingival oxidative stress, inflammatory markers, and alveolar bone resorption during the repair process associated with experimental periodontitis. Therefore, they concluded that EA may hold therapeutic potential for periodontitis. In our study, consistent with the findings of Dede et al.[18], the inflammation level of the 21EA group was significantly lower compared to the 14K group (p:0.042) and the 21K group (p:0.004). Additionally, the epithelial proliferation level of the 21EA group was significantly higher than that of the 21K group, the 14K group (p:0.001), and the 14EA group (p:0.009). These results suggest that EA may have a positive effect on periodontitis and bone healing by reducing inflammation levels and increasing epithelial proliferation.

Nirwana et al.[19] aimed to investigate the effect of pomegranate extracts on the wound healing process in tooth extraction wounds in their experimental study on Cavia Cobayas. Their statistical analysis revealed significant differences between the control and treatment groups, leading to the conclusion that the application of pomegranate fruit extract increased the expression of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) in the wound after tooth extraction. In our study, similar to the findings of Nirwana et al.[19], the recovery score of the 21EA group was significantly higher compared to the 14K group (p:0.017) and the 21K group (p:0.009). Additionally, the epithelial proliferation level of the 21EA group was found to be significantly higher compared to the 21K group, the 14K group (p:0.001), and the 14EA group (p:0.009). Therefore, it can be concluded that both the healing score and epithelial proliferation increase with the duration of EA use in our study.

In their study, Nirwana et al.[20] investigated the effect of the combination of hydroxyapatite (HA) grafts and EA in preventing graft-induced inflammation. They demonstrated that the HA+EA combination could effectively prevent bone damage and promote bone tissue renewal after tooth extraction. Similarly, Primasari et al.[21] explored the effectiveness of EA and hydroxyapatite in bone remodeling. Their findings revealed that the combination of HA+EA reduced the levels of TNF-α, the primary pro-inflammatory cytokine involved in bone inflammation, leading to increased bone growth factors.[21] In our study, consistent with the findings of Primasari et al.[21] and Nirwana et al.[20], the inflammation level of the 21EA group was significantly lower compared to both the 14K group (p:0.042) and the 21K group (p:0.004). Additionally, the new bone formation level of the 21EA group was significantly higher ($p<0.05$) than that of the 14K group (p:0.001), the 21K group (p:0.008), and the 14EA group (p:0.018). Moreover, the new bone formation level of the 14EA group was statistically significantly higher than that of the 14K group (p:0.014; $p<0.05$). Based on these findings, it is suggested that the use of EA in combination with grafts may accelerate bone healing and mitigate graft-induced inflammatory reactions.

In the study by Gül et al.[22], they conducted an experiment involving 24 male Wistar rats, wherein a 7 mm critical size calvarial bone defect was surgically created. The rats were divided into three groups: the first group had the defect left empty as a control, the second group received only a bone graft placed in the defect, and the third group had 0.325 mg/kg EA applied topically to the defect in addition to the bone graft. Following histological and biochemical examinations, the researchers observed that the topical application of 0.325 mg/kg EA graft material did not yield a positive effect on the applied area. However, significant positive differences were noted in the analyses conducted on serum samples obtained from rats in both the graft group and the EA+graft group. As a limitation of their study, Gül et al.[22] highlighted the need for further investigations at various doses to elucidate the relationship between topical application of EA and bone healing.

In another study conducted by Al-Obaidi et al.[23], involving 24 rats as a diabetes model, maxillary incisor teeth were extracted from the rats. Subsequently, the sockets were filled with Rosuvastatin. The animals were then divided into three groups: the first group served as the control and received normal saline, the second group, where a diabetes model was induced using streptozotocin, also received normal saline, and the third group, with a streptozotocin-induced diabetes model, received EA via gastric feeding. The rats were sacrificed on the 14th and 28th days. The study concluded that EA is an effective natural compound in preventing bone loss caused by tooth extraction in diabetic rats. The treatment with EA resulted in reduced levels of pro-inflammatory cytokines in the serum of diabetic rats after tooth extraction, and a decrease in oxidative stress levels in the gingival tissue of diabetic rats was also observed. In our study, similar to the findings of Al-Obaidi et al.[23], the new bone formation level of the 21EA group was significantly higher compared to the 14K group (p:0.001), the 21K group (p:0.008), and the 14EA group (p:0.018) ($p<0.05$). In a study by Wardhana et al.[24], a bone defect was created in the left femur of 30 Wistar rats, where the defect in the study group was filled with EA-HA in powder form mixed at a ratio of 3:97, while the control groups were filled with polyethylene glycol (PEG) or HA in gel form. It was observed that defects treated with EA-HA exhibited fewer osteoclasts and decreased RANKL staining on days 7 and 14. The findings of our study support the positive effect of EA on bone healing, consistent with the studies by Wardhana et al.[24] and Al-Obaidi et al.[23] The new bone formation level of the 21EA group was significantly higher than the 14K group (p:0.001), the 21K group (p:0.008), and the 14EA group (p:0.018). Furthermore, the epithelial proliferation level of the 21EA group was significantly higher compared to the 21K group, the 14K group (p:0.001), and the 14EA group (p:0.009) ($p<0.05$).

Limitations

The limitations of this study include the fact that tooth extraction in experimental animals is more difficult and not standardized compared to humans and factors such as wound healing disorders and infection that may occur in experimental animals cannot be fully prevented. In addition, since the genetic and physiological structure of experimental animals is not exactly similar to humans, the clinical generalization of the results of the study to humans is limited. In addition, the complexity of the models used

in experimental animals may not fully reflect real clinical situations and therefore the clinical applicability of the findings may be limited.

CONCLUSION

According to the histopathological findings of this study, EA was observed to decrease the level of inflammation on days 14 and 21.

In this study, larger areas of thin bone trabeculae and areas covered by connective tissue and osteoblasts were observed around the newly formed bone trabeculae in the EA groups compared to the control groups. Based on histological observations, this study suggests that EA could expedite the healing process by reducing inflammation and promoting epithelial proliferation on days 14 and 21.

Although there are currently food supplements and cosmetic products containing EA, further studies and clinical trials are necessary to fully comprehend their effects.

Future studies could include longer observation periods, exploration of conditions that hinder wound healing such as diabetes, investigating the combination of EA with other biomaterials, prolonged use of EA, and examining both topical and systemic applications.

Consequently, it has been observed that the administration of EA for 21 days post tooth extraction accelerates wound healing.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of İstanbul University Animal Experiments Local Ethics Committee (Date: 29.01.2021, Decision No: 2021/01).

Informed Consent

Since this is an animal study, there is no need for an informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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