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ORIGINAL ARTICLE

Histopathologic Study of the Repair of a Minor Defect in Femur Using *Elaeagnus Angustifolia* Extract in Rabbit

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Abstract

Objective- Veterinary and human orthopedics is widely applied to stimulate fracture healing, accelerate junction connection, and repair bone defects. In this regard, several studies have been conducted on selecting the best material for bone repair. This study aimed to evaluate the effect of aqueous extract of *Elaeagnus angustifolia* fruit on the process of bone repair in a rabbit animal model.

Design- Experimental Study

Animals- For this purpose, 12 rabbits were obtained.

Procedures- Rabbits randomly divided into 3 groups. The control group, in which a two-sided hole was created in the femur and did not receive the treatment. The second group underwent surgery and received an aqueous extract of *Elaeagnus angustifolia* fruit at a dose of 250 mg/kg, and the third group received 500 mg/kg intraperitoneal. On weeks 2 and 4 following the bone defect, a histopathologic sample was taken from the site. The bone tissue samples were stained with hematoxylin and eosin.

Results-According to histopathologic results, the best and weakest performances occurred in the group treated with 500 mg/kg of extract and in the control group, respectively. This group had a significant difference with other groups in terms of osteoplasia ($p < 0.05$).

Conclusion and Clinical Relevance- In general, the results showed that the aqueous extract of *Elaeagnus angustifolia* can play an important role in the healing of bone fractures.

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1. Introduction

Bone fracture is a chronic disorder in the quality of the animal's life. It causes pain, a reduction in physical activity, and permanent or temporary functional defects. Fracture recovery includes a set of systemic and focal factors.[1,2] The biological process of fracture recovery includes some reconstruction factors such as cellular arrangement, cytokines, periosteum molecular events, external soft tissue, and growth factors. The fracture of a diaphysis area often is recovered through a combination of intramembranous and endochondral ossifications. This process begins with the periosteum and involves the formation of an external callus.[3]

Bone fracture consists of two parts of pathological and traumatic fracture. Traumatic fractures occur directly through some events like accidents and crashes and indirectly through the power of bone pressure, muscles, tendons, and ligaments.[4] An inefficient organ does not affect the life of the animal but results in some complications, such as appetite reduction, exhaustion, and reduction in the productive efficiency of economical and industrial animals. Moreover, it may lead to the killing of an animal by its owner.[5] According to the state of the animal, the recovery speed plays a key role in bone healing. The material used for bone fracture recovery must be able to prevent infection in open fractures, decrease the recovery time, increase the restoration of bone tissue, and be affordable.[6,7]

Russian olive (*Elaeagnus angustifolia*) provides many unique properties in pharmaceutics and industry.[8] The root, wood, fruit, and skin of this plant are used because of its sedative, anti-inflammatory, and anti-dermatitis effects. Fruit and the leaf of this plant contain a considerable amount of flavonoids, terpenoids, cardiac glycosides, carvacrol, and sitosterol compound.[9] Phenol compounds including isorhamnetin and dihydroxy, phenol beta-galactosidase pyranoside, and tetra caffeic acid are some other materials in the *Elaeagnus angustifolia*.[10] *Elaeagnus angustifolia* fruit has astringent, diuretic, stomach-enhancing, anti-cough, and anti-diarrhea properties. Infusion or decoction of its flower is useful for the treatment of illnesses such as paralysis, tetanus, shortness of breath, and jaundice.[11] Pharmacological studies of the aqueous extract of its core have shown an analgesic and muscle relaxant effect.[4] Studies have shown analgesic and anti-inflammatory effects of some flavonoids and sitosterols.[12] These compounds may accelerate the healing process of the bones.[13]

This study aimed to evaluate the effect of aqueous extract of *Elaeagnus angustifolia* fruit on the bone repair process of a rabbit animal model.

2. Materials and Methods

In this study, 12 male rabbits with a weight of 2.5 kg, regardless of their sex, were used. The rabbits were kept at a temperature of 22 ± 2° C, at a 12 h light-darkness cycle under optimal and standard conditions with free access to water and food. They were kept in the animal houses under the condition of the Institutional Animal Care and National Advisory Committee for Laboratory Animal Research, Iran, after the approval of the ethics committee. The rabbits were randomly assigned to one of three groups. The first group was the control group. A two-sided hole was formed in the diaphysis area of the femur of these rabbits. The rabbits in the control group did not receive any treatment. The second and third groups were the experimental groups, were undergone surgery and the holes were created on their femur, as well. They received an aqueous extract of the *Elaeagnus angustifolia* fruit intraperitoneally at doses of 250 and 500 mg/kg, daily.

Extract Preparation

To prepare an aqueous extract, the *Elaeagnus angustifolia* fruit was purchased from the store and its extract was removed after its confirmation by the botanical expert of the Medicinal Plants Research Center of Shahrekord Azad

University. To prepare the aqueous extract, 100 grams of *Elaeagnus angustifolia* fruit was collected, poured into 1 liter of boiled water, and placed in the laboratory for 2 hours. Finally, the filter paper was placed in a colander and the extract was filtered.

During the study, the animals were sedated by intramuscular injection of acepromazine (KELA Co., Belgium) at a dose of 0.2 mg/kg. General anesthesia was induced intramuscularly by ketamine (Trittau Co., Germany) at a dose of 30 mg/kg of body weight before the surgery. To create bone defect, the outer surface of their left femur area was shaved and disinfected by povidone-iodine and ethanol. Then, a 3 cm incision was created in the lateral surface area of the rabbits' leg and after putting bone muscles aside, the femur was exposed. Next, a two-sided 1.5 mm diameter hole was created in the diaphysis area of the femur using a 1.5 mm orthopedic drill. Finally, the muscles were sutured by an absorbable suture material, the skin was sutured by nylon suture material, and post-operative care was provided for animals. One-half of the animals were euthanized in week 2 and the others were euthanized without pain in week 4 by injecting high doses of chloroform. After an autopsy, the femur was removed and sent to the pathology laboratory after fixation.

Twenty slides of each bone at a thickness of 0.5 µm were stained with hematoxylin and eosin (H&E). The prepared slides were examined quantitatively and qualitatively and were rated using a scoring system.[14] Then, performing a histopathology study on the slides, we determined the inflammatory cells, cartilage tissue, fibrous tissue, collagen sedimentation, and the rate of bone formation of callus (Figure 1).

Statistical Analysis

The results were statistically analyzed by the Kruskal-Wallis non-parametric test. When the *p*-value was less than 0.01, they were re-analyzed statistically by the Mann-Whitney U test. In this test, if the values were less than 0.01, they were considered statistically significant. SPSS version 16 software was applied to perform statistical tests.

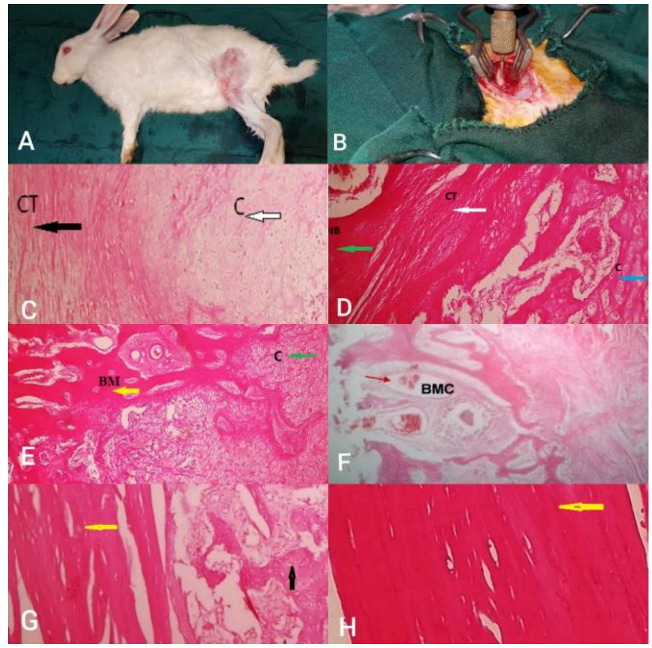

Figure 1. Histological evaluation of bone healing after 2 and 4 weeks. **B:** Skin suturing after surgery. **A:** Creating bone defect. **C:** Control group, in week 2, the formation of connective tissue (CT) (black arrow) and the formation of cartilage tissue are visible. **D:** The group treated with a dose of 250 mg/kg in week 2, cartilage tissue (C) (blue arrow) and connective tissue (CT) (white arrow) formed. New bone tissue (NB) (green arrow). **E:** The group treated with a dose of 500 mg/kg in week 2 Cartilage tissue (C) (yellow arrow) and new bone tissue (NB) formation is visible. **F:** Control group in week 4, Bone formation is observed, and bone marrow cavities (BMC) (red arrow) are also visible. **G**: the group treated with a dose of 250 mg/kg in week 4 Bone tissue is formed (yellow arrow) and lamellae are apparent in the tissue (black arrow). **H:** The group treated with a dose of 500 mg/kg in week 4 dense bone tissue is well-formed (yellow arrow). H&E staining, 100×.

3. Results

The results of week 2 and week 4 after the surgery were studied in terms of the bone tissue pathology using an optical microscope.

In week 2, the rate of connective tissue formation in the group treated with 500 mg/kg was lower compared to the control group and showed better performance ($p < 0.01$). Both of the groups treated with 500 and 250 mg/kg dose showed a reduction in chondrodysplasia, but this difference was not significant between the two groups ($p > 0.01$).

The osteoplasia rate was zero in the control group in week 2 and it was lower compared to that in the groups treated with 250 and 500 mg/kg. However, a significant difference was observed between the control group and the other two groups in terms of the rate of osteoplasia variations ($p < 0.01$) (Table 1, Figure 1C-E). The results of osteoplasia variations in week 4 are presented in Table 2 and Figure 1F-H. As can be seen, in both the control group and the group treated with 250 mg/kg of *Elaeagnus angustifolia* extract, a nonsignificant increase occurred in the bone formation process ($p > 0.01$). In the group treated with a dose of 500 mg/kg, the rate of chondrodysplasia was less than that of the group treated with a dose of 250 mg/kg, and the difference was statistically significant ($p < 0.01$). As it was observed, the rate of connective tissue formation in the group treated with a dose of 250 mg/kg was lower than that of the control group, indicating a significant difference ($p < 0.01$).

Table 1. Comparison of the mean of different factors in different treatments in week 2 (Mean ± SEM)

Group	fibroplasia	chondrodysplasia	osteoplasia
control	49.29 ± 0.2^{c}	00.00 ± 0.1^{a}	00.00 ± 0.0^{a}
250	38.14 ± 0.1^{b}	38.14 ± 0.1^{b}	00.00 ± 0.1^{b}
500	00.00 ± 0.0^{a}	38.14 ± 0.14^{b}	38.14 ± 0.0^{c}
P1	0.001**	0.001**	0.001**
P2	0.001**	0.004**	0.001**
P3	0.001**	0.71	0.00**

P1: significance level between control and 250 mg/kg group
P2: significance level between the control group and 500 mg/kg group
P3: significance level between the 500 and 250 mg/kg groups
**: The difference between the two groups is significant ($p < 0.01$)

Table 2. Comparison of the mean of different factors in different treatments in week 4 (Mean ± SEM)

Group	fibroplasia	chondrodysplasia	osteoplasia
control	1.71 ± 0.49^{b}	1.29 ± 0.49^{b}	$0/86 \pm 0.38^{a}$
250	0.14 ± 0.38^{a}	1.00 ± 0.00^{b}	1.43 ± 0.53^{a}
500	0.00 ± 0.00^{a}	0.14 ± 0.38^{a}	3.00 ± 0.00^{b}
P1	0.001**	0.38	0.13
P2	0.001**	0.002**	0.001**
P3	0.71	0.004**	0.001**

P1: significance level between control and 250 mg/kg group
P2: significance level between the control group and 500 mg/kg group
P3: significance level between the 500 and 250 mg/kg groups
**: The difference between the two groups is significant ($p < 0.01$)

4. Discussion

Nowadays, several studies are conducted on the effects of physiologic and pharmacologic effects of plant extracts because of their lower side effects, lower economic costs, diversity of ineffective compounds, development of industries related to medicinal plants, and especially the recommendations of the World Health Organization to use herbs. Therefore, this study was conducted to investigate the effect of *Elaeagnus angustifolia* fruit on the healing process of the bone partial defect in the animal model of the rabbit. Our results were evaluated morphologically and histopathologically. Several studies have shown that *Elaeagnus angustifolia* fruit can be effective in reducing inflammatory cells and increasing bone density because of its existing compounds.[13] Moreover, these studies have reported that *Elaeagnus angustifolia* fruit is useful and effective in the treatment of osteoporosis and stimulation of bone formation. These results are consistent with those of the present study. The results of this study indicated an increase in bone density in the groups treated with a dose of 250 mg/kg of *Elaeagnus angustifolia* extract and the group treated with a dose of 500 mg/kg of *Elaeagnus angustifolia* extract after the surgery. Bone density was examined after 2 and 4 weeks by optical microscope and the difference in bone density at the cortex level after injury was compared in the control and treated groups with different doses. The results showed a significant change in the groups treated with *Elaeagnus angustifolia* extract compared to the control group. Notebaert *et al*. studied the healing effects of aqueous extract of *Elaeagnus angustifolia* leave on experimental skin wounds in rats. This study showed that the aqueous extract of *Elaeagnus angustifolia* leaves has healing effects on experimental skin wounds in rats. Therefore, the leaves of this plant can be used as a low-cost method in wound treatment.[11] Rabiei *et al*. investigated the effect of *Elaeagnus angustifolia* and ginger extract on knee osteoarthritis. They showed that taking 200 mg of this extract reduces the symptoms of osteoarthritis in the patients.[10]

Recent studies cast doubts on the effects of some common drugs in the bone healing process.[2] Therefore, it is necessary to compare these compounds with hormones, pure plants, and animal compounds. The studies on bone healing indicate the importance of these compounds, as they involve no secondary complications and accelerate the healing process. Almost all the studies conducted on *Elaeagnus angustifolia* fruit and other parts of this plant have emphasized its antimicrobial, anti-inflammatory, and healing effects.[13] These effects have been further proven by identifying the compounds of plants and their secondary metabolites. Also, *Elaeagnus angustifolia* fruit has phenolic and flavonoids compounds that can accelerate the process of wound healing.[7] In general, the results of this study showed that the aqueous extract of *Elaeagnus angustifolia* can play an important role in the healing of bone fractures.

Conflict of Interests

There is no conflict of interest to declare regarding this study.

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