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Original Article

Hazelnut Has Potency on Wound Healing Associated with Immunohistochemical Biomarkers

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ABSTRACT

Background: In this study, it was aimed to evaluate the impact of hazelnut on wound healing by using its immunohistochemical impacts on Claudin-5, Tumor Necrosis Factor-alpha (TNF-α), tumor Protein-63 (p63), and Insulin-like Growth Factor-1 (IGF-1) expression. **Materials and Methods:** Seventy-two male Wistar-Albino rats were used. Nine groups with 8 rats each group, and 3, 7, and 14 days groups were formed as oral, local, and control groups. Samples obtained from rats were evaluated with these immunohistochemical biomarkers. **Results:** Claudin-5 scores were found to be statistically significant in the oral group on all days (p=0.007, p=0.002, p=0,014). TNF-α scores were found to be statistically significant on the 3rd and 7th days in the local and oral groups (p=0.040, p=0.001). p63 scores were found to be statistically significant in the oral group when looking at the 14th day values (p=0.007). IGF-1 scores were found to be statistically significant on the 7th and 14th days in the local group (p=0.040). **Conclusion:** It was determined that hazelnut has positive impact on wound healing by stimulating the claudin-5, TNF-a, p63, and IGF-1expression levels.

Keywords: Wound, Healing, Hazelnut, Immunohistochemical, Biomarkers.

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INTRODUCTION

Plant or plant-derived products have been tried and used in medicine for centuries.[1] The health benefits of hazelnuts are also known. However, it is seen that the number of scientific studies conducted with hazelnuts is quite low. In these studies, positive impacts of hazelnut on wound healing have been reported histochemically.[2,3] It is thought that hazelnut has this impact because of its antioxidant contents, especially selenium and Vitamin-E. The demonstration of its beneficial impacts with new scientific studies, may enable it to be used as a food supplement for wound healing.

The development of immunohistochemical methods has opened a new field for wound healing studies. Because of that many studies have been reported. Ortiz-Ray *et al.*[4] stated that fibronectin and tenascin were detected in most of the vital injuries in rat skin and muscle. In another study, platelet-derived growth factor can stimulate the repair defects of articular cartilage in an animal model was expressed.[5] But there is no study conducted with hazelnut on wound healing associated with immunohistochemical biomarkers in the literature. We aim to determine the impact of hazelnut on wound healing with claudin-5, TNF-a, p63, and IGF-1.

MATERIALS AND METHODS

Study design

The study was performed with the approval of the Ordu University Local Committee (dated 16.02.2021, number 1/ decision 2 and 3), and animal rights were protected. Seventy-two male Wistar-Albino rats (200-250 g) were used. The rats were randomly divided into 9 groups. So, it was prepared as 8 rats in each group. Hazelnut was applied to these groups on different days (3, 7, and 14).

In the oral group, hazelnuts were mixed with the feed of the animals and their consumption was controlled. In the local group, hazelnut was mixed with a sponge and placed on the wounds. The impact of hazelnut on wound healing was evaluated by claudin-5, TNF-α, p63, and IGF-1 immunohistochemically.

Surgical procedure

Rats were shaved and disinfected under anesthesia. Approximately 1 cm skin incision was generated. Saline impressed sponge (Clinisponge®, Turkiye) was applied in groups control and oral. and hazelnut was fed to oral group at 500 mg/kg per day according to the quantity from Shahidi and Alasalvar.[6] For the local group, 1 cm^3 hazelnut impressed sponge was applied.

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Wounds were primarily repaired with 3/0 polypropylene sutures (Doğsan, Turkey). The rats were kept at normal room temperature and humidity by being fed standard pellet chow and tap water on a 10/14 hr light/dark light cycle, 4 in each cage. Samples obtained from rats were evaluated.

Immunohistochemical evaluation

The samples were previously removed with tissue adjacent to the suture site. They were fixed with 4% buffered formalin, and properly blocked with paraffin. Four sections, five micrometer thick, were taken from these blocks on different slides. These slides were stained with claudin-5, TNF-α, p63, and IGF-1. The slides were examined with light microscopy (Nikon, Japan) at 400× magnification. The sections for immunohistochemical study were marked with Leica Bond-Max IHK staining device (Vision Biosystems, Australia).

Claudin-5 (ab15106) (diluted at a ratio of 1:100), TNF-α (1 mL, lyophilized, Santa Cruz-sc130349, USA) (diluted at a ratio of 1:250), p63 (leica) (diluted at a ratio of 1:100), and IGF-1 (EMD Millipore CBL52 M23) (diluted at a ratio of 1:100) were used.

Claudin-5, TNF-α, p63, and IGF-1 were analyzed. Cells were enumerated with light microscope (magnification ×400) and the percentage of stained cells were counted in all visual fields. The values were calculated and grouped according to the scoring levels from Aslan *et al.*[7] (0-3): without marked cells, 0; <25% marked cells, 1; 25-50% marked cells, 2; >50% marked cells, 3.

Statistical evaluation

The data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., USA) 22.0. The conformity of the variables to the normal distribution was examined with Histogram Graphs and Kolmogorov-Smirnov Test. Mean, standard deviation, median values, number and percentage were used when presenting descriptive analyzes. The Mann Whitney U Test was used when evaluating non-normally distributed variables between two groups, and the Kruskal Wallis Test was used when evaluating between more than two groups. *p*-value under 0.05 was accepted statistically significant.

RESULTS

When the results were evaluated by days, Claudin-5 and TNF-α scores were found to be statistically significant in the oral group when looking at the 3rd day values (p=0.027, p=0.033) (Figures 1 and 2). Claudin-5 and TNF-a scores were also found to be statistically significant in the oral group when the 7th day values were examined (p=0.013, p=0.002). Claudin-5 ve p63 scores were found to be statistically significant in the oral group when looking at the 14th day values (p=0.047, p=0.007) see Figure 3 and Table 1).

When the results were evaluated by groups, TNF-α scores were found to be statistically significant on the 3rd and 7th days in the local and oral groups (p=0.040, p=0.001) (Table 2).

When the results were evaluated based on whether there is staining or not; TNF-α scores were found to be statistically significant on the 7th day in the local group (p=0.030). IGF-1 scores were found to be statistically significant on the 7th and 14th days in the local group (p=0.040) (Figure 4). TNF-α scores were found to be statistically significant on the 3rd and 7th days in the oral group (p=0.002) (Table 3).

When the results are evaluated on average values; Claudin-5 was statistically significant in the oral group on all days (p=0.007, p=0.002, p=0,014). TNF-α was statistically significant in the oral group on 3rd day (p=0.015), in the oral and local groups on the 7th day (p=0.002). p63 was statistically significant in the oral group on 14th day (p=0.002) (Table 4).

DISCUSSION

Wound healing includes different stages of inflammatory response, which are intertwined with each other and affected by many factors.[8] Since antioxidant substances prevent free radical formation, they have positive impacts on wound healing.[9] Hazelnut has antioxidant contents, especially selenium and Vitamin-E. For this reason, it could make positive action on wound healing.

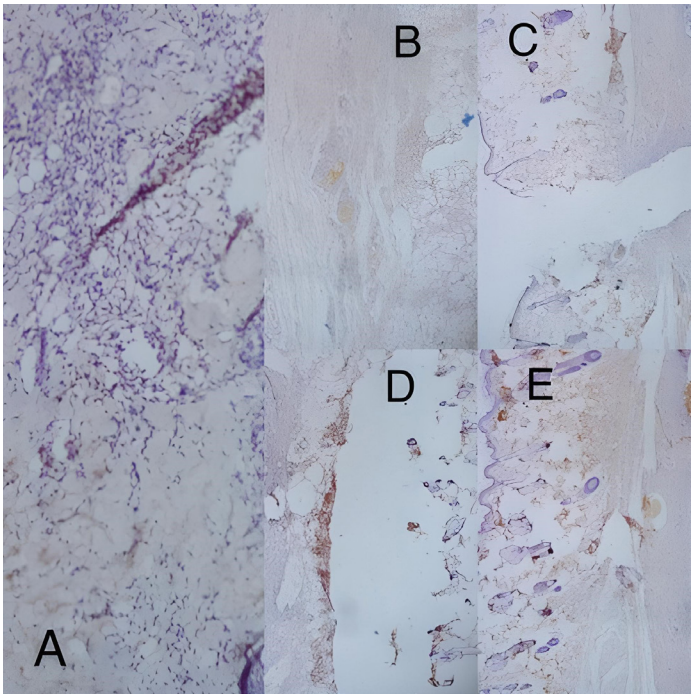

Figure 1: Grade-2 staining of Claudin-5 is clearly observed in the oral group on the 7th day in E part, (claudin-5 x40).
A: 3rd day control group (no staining), B: 3rd day oral group (grade 1 staining), C: 3rd day local group (grade 1 staining), D: 7th day control group (grade 1 staining), E: 7th day oral group (grade 2 staining).

Immunohistochemical examinations are currently the most preferred method in the evaluation of wound healing.[10] Lee *et al.*[11] determined that it did not contribute to the recovery when the use of silver sulfadiazine alone, but improved wound healing when applied with epidermal growth factor.[11] Saarista *et al.*[12] found that topically applied vascular endothelial growth factor increased angiogenesis and lymph angiogenesis, so it significantly accelerated wound healing. Mohammed *et al.*[13] noted that transforming growth factor-β has regenerative impacts on wound healing in sheep. The impact of hazelnut on wound healing was evaluated in our study with biomarkers consisting of Claudin-5, TNF-a, p63, and IGF-1. We found that Claudin-5, TNF-a, p63, and IGF-1 expression levels were significantly higher in the oral and local hazelnut groups compared to the control groups.

Claudins are important for normal epithelial function, studies have shown that variations in claudin expression can change the function of tight junctions in the epithelium.[14] Anh *et al.*[15] determined that expression levels of Claudin-5 are increased when compared to other claudin family members in mouse plecenta. Claudin-5 is present in the vascular endothelia of the skin, brain, and lung in mice.[16] Morita *et al.*[17] stated that the impacts of claudins on epithelial and endothelial function may influence the wound healing process because the epithelium and endothelium are directly involved in dermal injury and repair. In our study, it was observed that Claudin-5 scores were found to be statistically significant in the oral group on all days.

TNF-a is a cytokine formed by the use of 212 amino acids mainly synthesized by monocytes and macrophages.[18] It has important properties in the initiation and maintenance of inflammation in autoimmune diseases such as rheumatoid arthritis, that is, different impacts are known at different stages during inflammatory diseases.[19] It proceeds as a proinflammatory cytokine leading to the damage of the joint.[20] Zahid and Ghafoor[21] demonstrated that increased TNF-a levels in alveolar and osteotois cause a delay in the bone healing process. Ren *et al.*[22] also stated that TNF-α was found to inhibit vascular endothelial growth factor, which is an important factor in the progression of wound healing. Although its negative impacts on the literature, TNF-α scores were found to be statistically significant on the 3rd and 7th days in the local and oral groups in our study.

p63 is a tumor protein that is expressed from the basal layer of the epidermis, which controls the proliferation of cells, prevents the elimination of autonomous cells and thus prevents tumor formation.[23] Su *et al.*[24] stated that p63 expressed in response to stresses such as wound healing. Romano *et al.*,[25] and Botchkarev and Flores[26] stated that p63 has very important role in maintaining the quiescence of stem cells, and in mediating the impacts on epidermal development. In our study, p63 scores were found to be statistically significant in the oral group when looking at the 14th day values in line with the literature.

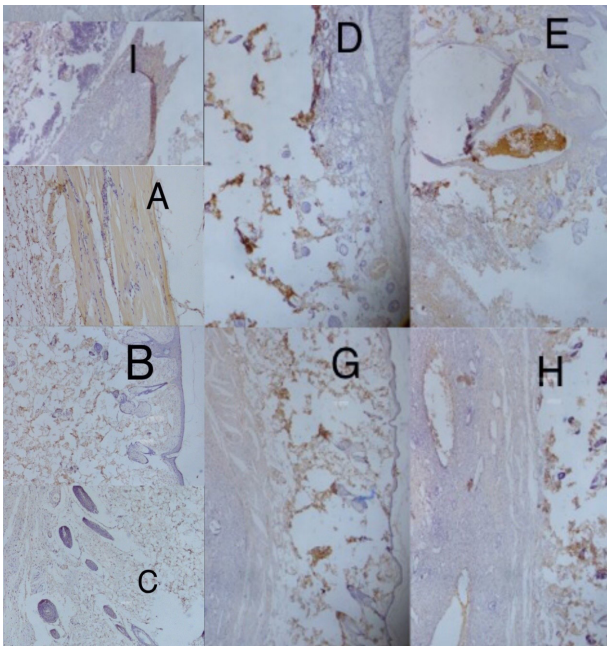

Figure 2: Grade-1 staining of tumor necrosis factor-alpha is observed in the oral and local groups on the different days in B, C, E, H, and I parts, (tumor necrosis factor-alpha x40).
A: 3rd day control group (grade 2 staining), B: 3rd day oral group (grade 1 staining), C: 3rd day local group (grade 1 staining), D: 7th day control group (grade 1 staining) E: 7th day oral group (grade 1 staining), G: 14th day control group (grade 1 staining), H: 14th oral group (grade 1 staining), I: 14th local group (grade 1 staining).

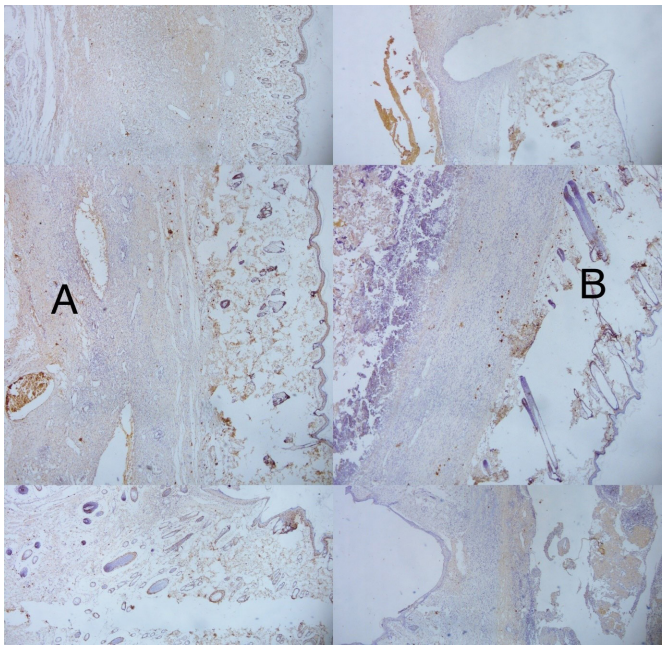

Figure 3: Grade-2 staining of tumor protein-63 is observed in the oral group on the 14th day in B part, (tumor protein-63 x40).
A: control group (grade 1 staining), B: oral group (grade 2 staining).

Table 1: **Accordingly, Claudin-5, TNF-α and p63 scores give significant results in oral groups on different days.**

		3rd day						7th day						14th day					
		Control		Local		Oral		Control		Local		Oral		Control		Local		Oral	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Claudin-5	0	6	(75.00)	2	(25.00)	0	(0.00)	1	(12.50)	0	(0.00)	0	(0.00)	1	(12.50)	1	(12.50)	0	(0.00)
	1	2	(25.00)	5	(62.50)	6	(75.00)	7	(87.50)	3	(37.50)	1	(12.50)	7	(87.50)	6	(75.00)	3	(37.50)
	2	0	(0.00)	1	(12.50)	2	(25.00)	0	(0.00)	5	(62.50)	5	(62.50)	0	(0.00)	1	(12.50)	5	(62.50)
	3	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	2	(25.00)	0	(0.00)	0	(0.00)	0	(0.00)
p value		0.027						0.013						0.047					
TNF-α	0	6	(75.00)	5	(62.50)	0	(0.00)	6	(75.00)	1	(12.50)	0	(0.00)	3	(37.50)	6	(75.00)	5	(62.50)
	1	1	(12.50)	2	(25.00)	5	(62.50)	2	(25.00)	7	(87.50)	8	(100.0)	5	(62.50)	2	(25.00)	3	(37.50)
	2	1	(12.50)	1	(12.50)	3	(37.50)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)
p value		0.033						0.002						0.301					
p63	1	6	(75.00)	6	(75.00)	5	(62.50)	5	(62.50)	6	(75.00)	2	(25.00)	8	(100.0)	6	(75.00)	1	(12.50)
	2	2	(25.00)	1	(12.50)	3	(37.50)	2	(25.00)	2	(25.00)	5	(62.50)	0	(0.00)	1	(12.50)	5	(62.50)
	3	0	(0.00)	1	(12.50)	0	(0.00)	1	(12.50)	0	(0.00)	1	(12.50)	0	(0.00)	1	(12.50)	2	(25.00)
p value		0.538						0.287						0.007					
IGF-1	0	5	(62.50)	5	(62.50)	3	(37.50)	4	(50.00)	1	(12.50)	0	(.00)	2	(25.00)	1	(12.50)	4	(50.00)
	1	3	(37.50)	2	(25.00)	3	(37.50)	2	(25.00)	6	(75.00)	6	(75.00)	5	(62.50)	6	(75.00)	3	(37.50)
	2	0	(0.00)	1	(12.50)	1	(12.50)	2	(25.00)	1	(12.50)	2	(25.00)	1	(12.50)	1	(12.50)	1	(12.50)
	3	0	(0.00)	0	(0.00)	1	(12.50)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)
p value		0.695						0.096						0.558					

TNF-a: tumor necrosis factor-alpha, p63: tumor protein-63, IGF-1: insulin-like growth factor-1.

Table 2: Accordingly, TNF-α scores give significant results in oral and local groups.

		Control						Local						Oral					
		3rd day		7th day		14th day		3rd day		7th day		14th day		3rd day		7th day		14th day	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Claudin-5	0	6	(75.00)	1	(12.50)	1	(12.50)	2	(25.00)	0	(0.00)	1	(12.50)	0	(0.00)	0	(0.00)	0	(0.00)
	1	2	(25.00)	7	(87.50)	7	(87.50)	5	(62.50)	3	(37.50)	6	(75.00)	6	(75.00)	1	(12.50)	3	(37.50)
	2	0	(0.00)	0	(0.00)	0	(0.00)	1	(12.50)	5	(62.50)	1	(12.50)	2	(25.00)	5	(62.50)	5	(62.50)
	3	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	2	(25.00)	0	(0.00)
p value		0.009						0.109						0.054					
TNF-α	0	6	(75.00)	6	(75.00)	3	(37.50)	5	(62.50)	1	(12.50)	6	(75.00)	0	(0.00)	0	(0.00)	5	(62.50)
	1	1	(12.50)	2	(25.00)	5	(62.50)	2	(25.00)	7	(87.50)	2	(25.00)	5	(62.50)	8	(100.0)	3	(37.50)
	2	1	(12.50)	0	(0.00)	0	(0.00)	1	(12.50)	0	(0.00)	0	(0.00)	3	(37.50)	0	(0.00)	0	(0.00)
p value		0.168						0.040						0.001					
p63	1	6	(75.00)	5	(62.50)	8	(100.0)	6	(75.00)	6	(75.00)	6	(75.00)	5	(62.50)	2	(25.00)	1	(12.50)
	2	2	(25.00)	2	(25.00)	0	(0.00)	1	(12.50)	2	(25.00)	1	(12.50)	3	(37.50)	5	(62.50)	5	(62.50)
	3	0	(0.00)	1	(12.50)	0	(0.00)	1	(12.50)	0	(0.00)	1	(12.50)	0	(0.00)	1	(12.50)	2	(25.00)
p value		0.315						0.827						0.209					
IGF-1	0	5	(62.50)	4	(50.00)	2	(25.00)	5	(62.50)	1	(12.50)	1	(12.50)	3	(37.50)	0	(0.00)	4	(50.00)
	1	3	(37.50)	2	(25.00)	5	(62.50)	2	(25.00)	6	(75.00)	6	(75.00)	3	(37.50)	6	(75.00)	3	(37.50)
	2	0	(0.00)	2	(25.00)	1	(12.50)	1	(12.50)	1	(12.50)	1	(12.50)	1	(12.50)	2	(25.00)	1	(12.50)
	3	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(12.50)	0	(0.00)	0	(0.00)
p value		0.323						0.144						0.26					

TNF-a: tumor necrosis factor-alpha, p63: tumor protein-63, IGF-1: insulin-like growth factor-1.

Table 3: Groups according to whether there is staining or not. TNF-α and IGF-1 scores give significant results.

		Control						Local						Oral					
		3rd day		7th day		14th day		3rd day		7th day		14th day		3rd day		7th day		14th day	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Claudin-5	0	6	(75.00)	1	(12.50)	1	(12.50)	2	(25.00)	0	(0.00)	1	(12.50)	0	(0.00)	0	(0.00)	0	(0.00)
	1-2-3	2	(25.00)	7	(87.50)	7	(87.50)	6	(75.00)	8	(100.0)	7	(87.50)	8	(100.0)	8	(100.0)	8	(100.0)
p value		0.009						0.319											
TNF-a	0	6	(75.00)	6	(75.00)	3	(37.50)	5	(62.50)	1	(12.50)	6	(75.00)	0	(0.00)	0	(0.00)	5	(62.50)
	1-2-3	2	(25.00)	2	(25.00)	5	(62.50)	3	(37.50)	7	(87.50)	2	(25.00)	8	(100.0)	8	(100.0)	3	(37.50)
p value		0.202						0.030						0.002					
p63	0	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)
	1-2-3	8	(100.0)	8	(100.0)	8	(100.0)	8	(100.0)	8	(100.0)	8	(100.0)	8	(100.0)	8	(100.0)	8	(100.0)
p value																			
IGF-1	0	5	(62.50)	4	(50.00)	2	(25.00)	5	(62.50)	1	(12.50)	1	(12.50)	3	(37.50)	0	(0.00)	4	(50.00)
	1-2-3	3	(37.50)	4	(50.00)	6	(75.00)	3	(37.50)	7	(87.50)	7	(87.50)	5	(62.50)	8	(100.0)	4	(50.00)
p value		0.309						0.040						0.073					

TNF-a: tumor necrosis factor-alpha, p63: tumor protein-63, IGF-1: insulin-like growth factor-1.

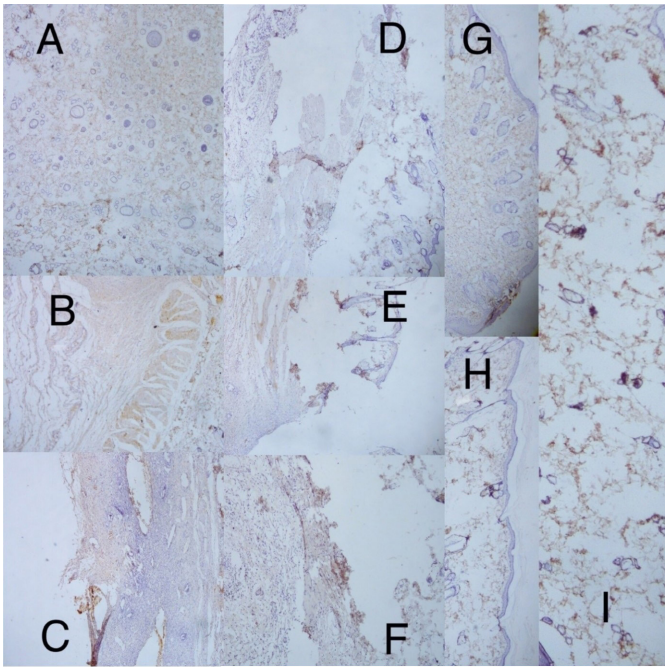

Figure 4: Grade-1 staining of insulin-like growth factor-1 is observed in the oral and local groups on the different days in B, C, E, F, H, and I parts, (tumor necrosis factor-alpha x40), (insulin-like growth factor-1 x40).
A: 3rd day control group (no staining), B: 3rd day oral group (grade 1 staining), C: 3rd day local group (grade 1 staining), D: 7th day control group (no staining) E: 7th day oral group (grade 1 staining), F: 7th day local group (grade 1 staining), G: 14th day control group (no staining), H: 14th oral group (grade 1 staining), I: 14th local group (grade 1 staining).

IGF family is important in growth and development.[27] IGF-1 modulate cellular proliferation and differentiation throughout the body.[28] According to the other 3 markers, more articles about wound healing are in the literature. Because of its major role in cellular migration and proliferation. Stuard *et al.*[29] show that IGF-1 promotes wound repair. Pierre *et al.*[30] also shown that IGF-1 stimulates local collagen formation, thus creating its wound healing impacts. In our study, IGF-1 scores were found to be statistically significant on the 7th and 14th days in the local group in line with the literature.

There are some limitations of this study. Although biomechanical evaluation is important in wound healing, unfortunately, it was not available in our study. Another limitation of the study is that vitamin-e and selenium, which impact wound healing we think, could not be applied alone. With new studies, hazelnut can be used as a food supplement in wound healing.

CONCLUSION

Effective healing process mechanisms and factors that promote healing understanding enable the approaches will provide better treatment in wound healing. Hazelnut ensure wound healing with immunohistochemical impacts on Claudin-5, TNF-α, p63, and IGF-1. Thus, the positive impact of hazelnut on wound healing was demonstrated immunohistochemically. Evaluation analysis

Table 4: Claudin-5, TNF-a and p63 scores give significant results according to average values.

		Control			Local			Oral			*p* value
		Average	Standard Deviation	Median	Average	Standard Deviation	Median	Average	Standard Deviation	Median	
Claudin-5	3rd day	0.25	±0.46	0.00	0.88	±0.64	1.00	1.25	±0.46	1.00	0.007
	7th day	0.88	±0.35	1.00	1.63	±0.52	2.00	2.13	±0.64	2.00	0.002
	14th day	0.88	±0.35	1.00	1.00	±0.53	1.00	1.63	±0.52	2.00	0.014
TNF-a	3rd day	0.38	±0.74	0.00	0.50	±0.76	0.00	1.38	±0.52	1.00	0.015
	7th day	0.25	±0.46	0.00	0.88	±0.35	1.00	1.00	±0.00	1.00	0.002
	14th day	0.63	±0.52	1.00	0.25	±0.46	0.00	0.38	±0.52	0.00	0.317
p63	3rd day	1.25	±0.46	1.00	1.38	±0.74	1.00	1.38	±0.52	1.00	0.871
	7th day	1.50	±0.76	1.00	1.25	±0.46	1.00	1.88	±0.64	2.00	0.134
	14th day	1.00	±0.00	1.00	1.38	±0.74	1.00	2.13	±0.64	2.00	0.002
IGF-1	3rd day	0.38	±0.52	0.00	0.50	±0.76	.00	1.00	±1.07	1.00	0.386
	7th day	0.75	±.89	0.50	1.00	±.53	1.00	1.25	±0.46	1.00	0.317
	14th day	0.88	±.64	1.00	1.00	±.53	1.00	0.63	±0.74	0.50	0.437

TNF-α: tumor necrosis factor-alpha, p63: tumor protein-63, IGF-1: insulin-like growth factor-1.

of such markers can lead to a better understanding of the events that occur in maintaining more precise results. Documentation of the expressions of these markers will provide new ideas.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TNF-α: Tumor necrosis factor-alpha; **P63:** Tumor protein-63; **IGF-1:** Insulin-like growth factor-1.

AUTHOR CONTRIBUTIONS

All steps: Alper Çirakli and Havva Erdem.

SUMMARY

- Antioxidant substances prevent free radical formation, so they have positive effects on wound healing.
- The aim of this study is to determine the effect of hazelnut extract in wound healing on different periods with claudin-5, TNF-α, p63, and IGF-1.
- Seventy two male Wistar-Albino rats were used. 1 cm length wound was generated in the back regions of the rats and surgically repaired. Rats were sacrificed at 3rd, 7th, and 14th days to obtain samples. Immunohistochemical findings of claudin-5, TNF-α, p63, and IGF-1 were evaluated.
- It was determined that hazelnut has positive effect on wound healing by stimulating the claudin-5, TNF-α, p63, and IGF-1expression levels.

REFERENCES

1. Gürbüz K, Yerer MB, Gürbüz P, Halici M. Icariin promotes early and late stages of fracture healing in rats. Eklem Hastalik Cerrahisi. 2019;30(3):282-8. doi: 10.5606/ehc.2019.66796, PMID 31650926.

2. Rojo LE, Villano CM, Joseph G, Schmidt B, Shulaev V, Shuman JL, *et al*. Wound-healing properties of nut oil from *Pouteria lucuma*. J Cosmet Dermatol. 2010;9(3):185-95. doi: 10.1111/j.1473-2165.2010.00509.x, PMID 20883291.
3. Çirakli A, Gürgör PN, Uzun E, Erdoğan E, Baş O, Yarilgaç T. Orally-applied hazelnut extract accelerates wound healing in the early period. An experimental study in rats. Ann Ital Chir. 2019;90(6):590-6. pii: S0003469X19029257.
4. Ortiz-Rey JA, Suárez-Peñaranda JM, Muñoz-Barús JI, Alvarez C, San Miguel P, Rodríguez-Calvo MS, *et al*. Expression of fibronectin and tenascin as a demonstration of vital reaction in rat skin and muscle. Int J Legal Med. 2003;117(6):356-60. doi: 10.1007/s00414-003-0403-6, PMID 14586623.
5. Sarban S, Tabur H, Baba ZF, Işikan UE. The positive impact of platelet-derived growth factor on the repair of full-thickness defects of articular cartilage. Eklem Hastalik Cerrahisi. 2019;30(2):91-6. doi: 10.5606/ehc.2019.64018, PMID 31291855.
6. Shahidi F, Alasalvar C. Findik ve Findik Yan Ürünlerinde Fitokimyasal Maddeler ve Biyoaktif Bileşikler. Findik Tanitim Grubu Araştirma Sonuç Raporu; 2004. Ankara. Available from: https://www.ftg.org.tr. /files/downloads/findikta-fitokimyasalmaddeller-ve-bioaktifle.doc.
7. Aslan A, Erdem H, Çelik MA, Şahin A, Çankaya S. Investigation of insulin-like growth Factor-1 (IGF-1), P53, and Wilms' tumor 1 (WT1) expression levels in the colon polyp subtypes in colon cancer. Med Sci Monit. 2019;25(7):5510-7. doi: 10.12659/MSM.915335, PMID 31341157.
8. Arik M, Ekinci Y, Gürbüz K, Batin S. The effects of focal brain damage on fracture healing: an experimental rat study. Eklem Hastalik Cerrahisi. 2019;30(3):267-74. doi: 10.5606/ehc.2019.66041, PMID 31650924.
9. Tek M, Toptas O, Akkas I, Kazancioglu HO, Firat T, Ezirganli S, *et al*. Effects of energy drinks on soft tissue healing. J Craniofac Surg. 2014;25(6):2084-8. doi: 10.1097/SCS.0000000000001013, PMID 25304141.
10. Grellner W, Madea B. Demands on scientific studies: vitality of wounds and wound age estimation. Forensic Sci Int. 2007;165(2-3):150-4. doi: 10.1016/j.forsciint.2006.05.029, PMID 16806766.
11. Lee AC, Leem H, Lee J, Park KC. Reversal of silver sulfadiazine-impaired wound healing by epidermal growth factor. Biomaterials. 2005;26(22):4670-6. doi: 10.1016/j.biomaterials.2004.11.041, PMID 15722137.
12. Saaristo A, Tammela T, Farkkilā A, Kärkkäinen M, Suominen E, Yla-Herttuala S, *et al*. Vascular endothelial growth factor-C accelerates diabetic wound healing. Am J Pathol. 2006;169(3):1080-7. doi: 10.2353/ajpath.2006.051251, PMID 16936280.
13. Mohammed S, Pack AR, Kardos TB. The effect of transforming growth factor beta one (TGF-beta 1) on wound healing, with or without barrier membranes, in a Class II furcation defect in sheep. J Periodont Res. 1998;33(6):335-44. doi: 10.1111/j.1600-0765.1998.tb02208.x, PMID 9777584.
14. Günzel D. Claudins: vital partners in transcellular and paracellular transport coupling. Pflugers Arch. 2017;469(1):35-44. doi: 10.1007/s00424-016-1909-3, PMID 27888337.
15. Ahn C, Yang H, Lee D, An BS, Jeung EB. Placental claudin expression and its regulation by endogenous sex steroid hormones. Steroids. 2015;100(8):44-51. doi: 10.1016/j.steroids.2015.05.001, PMID 25982333.
16. Morita K, Sasaki H, Furuse K, Furuse M, Tsukita S, Miyachi Y. Expression of claudin-5 in dermal vascular endothelia. Exp Dermatol. 2003;12(3):289-95. doi: 10.1034/j.1600-0625.2003.120309.x, PMID 12823443.
17. Shi J, Barakat M, Chen D, Chen L. Bicellular tight junctions and wound healing. Int J Mol Sci. 2018;19(12):1-20. doi: 10.3390/ijms19123862, PMID 30518037.
18. Rozas P, Lazcano P, Piña R, Cho A, Terse A, Pertusa M, *et al*. Targeted overexpression of tumor necrosis factor-α increases cyclin-dependent kinase 5 activity and TRPV1-dependent Ca2+ influx in trigeminal neurons. Pain. 2016;157(6):1346-62. doi: 10.1097/j.pain.0000000000000527, PMID 26894912.
19. Ursini F, Leporini C, Bene F, D'Angelo S, Mauro D, Russo E, *et al*. Anti-TNF-alpha agents and endothelial function in rheumatoid arthritis: a systematic review and meta-analysis. Sci Rep. 2017;7(1):5346. doi: 10.1038/s41598-017-05759-2, PMID 28706194.
20. Williams A, Wang ECY, Thurner L, Liu CJ. Review: novel insights into tumor necrosis factor receptor, death Receptor 3, and progranulin pathways in arthritis and bone remodeling. Arthritis Rheumatol. 2016;68(12):2845-56. doi: 10.1002/art.39816, PMID 27428882.
21. Zahid T, Ghafoor S. Molecular events in the clinicopathological diagnosis of alveolar osteitis. J Pak Med Assoc. 2021;71(2(A)):508-13. doi: 10.47391/JPMA.491, PMID 33819239.
22. Ren S, Chen J, Duscher D, Liu Y, Guo G, Kang Y, *et al*. Microvesicles from human adipose stem cells promote wound healing by optimizing cellular functions via AKT and ERK signaling pathways. Stem Cell Res Ther. 2019;10(1):47. doi: 10.1186/s13287-019-1152-x, PMID 30704535.
23. Qian Y, Chen X. Senescence regulation by the p53 protein family. Methods Mol Biol. 2013;965:37-61. doi: 10.1007/978-1-62703-239-1_3, PMID 23296650.
24. Su X, Chakravarti D. Flores ER. p63 steps into the limelight: crucial roles in the suppression of tumorigenesis and metastasis. Nat Rev Cancer. 2013;13(2):136-43. doi: 10.1038/nrc3446, PMID 23344544.
25. Romano RA, Smalley K, Magraw C, Serna VA, Kurita T, Raghavan S, *et al*. ΔNp63 knockout mice reveal its indispensable role as a master regulator of epithelial development and differentiation. Development. 2012;139(4):772-82. doi: 10.1242/dev.071191, PMID 22274697.
26. Botchkarev VA, Flores ER. p53/p63/p73 in the epidermis in health and disease. Cold Spring Harb Perspect Med. 2014;4(8):a015248. doi: 10.1101/cshperspect.a015248, PMID 25085956.
27. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and plateled derived growth factor on *in vivo* cartilage healing and repair. Osteoarthritis Cartilage. 2006;14(5):403-12. doi: 10.1016/j.joca.2005.10.011, PMID 16413799.
28. Todorović V, Pesko P, Micev M, Bjelović M, Budec M, Mićić M, *et al*. Insulin-like growth factor-I in wound healing of rat skin. Regul Pept. 2008;150(1-3):7-13. doi: 10.1016/j.regpep.2008.05.006, PMID 18597865.
29. Stuard WL, Titone R, Robertson DM. The IGF/insulin-IGFBP axis in corneal development, wound healing, and disease. Front Endocrinol (Lausanne). 2020;11(24):24. doi: 10.3389/fendo.2020.00024, PMID 32194500.
30. Pierre EJ, Perez-Polo JR, Mitchell AT, Matin S, Foyt HL, Herndon DN. Insulin-like growth factor-I liposomal gene transfer and systemic growth hormone stimulate wound healing. J Burn Care Rehabil. 1997;18(4):287-91. doi: 10.1097/00004630-199707000-00002, PMID 9261692.

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