

# Orally-applied hazelnut extract accelerates wound healing in the early period.

## An experimental study in rats



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## Orally-applied hazelnut extract accelerates wound healing in the early period. An experimental study in rats

**AIM:** The aim of this study was to evaluate the effect of oral and local use of hazelnut extract on wound healing on different periods.

**MATERIAL OF STUDY:** 72 male Wistar-Albino rats were used in this study. The rats were divided into 9 groups with similar weights. According to the days (3; 7; 14) and administration of hazelnut groups were formed as oral (3O, 7O, 14O), local (3L, 7L, 14L) and control (3C, 7C, 14C) groups. Under anesthesia 1 cm length wound was formed in the back regions of the rats and surgically repaired with sutures. Rats were sacrificed at 3th, 7th and 14th days and samples were obtained from the surgical regions. Histopathological analyzes were performed according to the wound healing scoring system.

**RESULTS:** According to the histopathological analysis, re-epithelization and collagen organization wound healing scores were significantly superior in the group 3O when compared to other groups (re-epithelization  $p=0.002$ , collagen organization  $p=0.003$ ).

**DISCUSSION:** The most important finding explored in this study is that orally administered hazelnut accelerated wound healing in the early period. It is thought that this effect is achieved due to antioxidant substances contained in hazelnut by increasing the re-epithelization and collagen organization and preventing free radicals formed during wound healing.

**CONCLUSION:** In this study it was determined that oral nutrition accelerated wound healing in the early period. However, further immunohistochemical and biomechanical studies are needed to support these results.

**KEY WORDS:** Hazelnut, Recovery, Wound

## Introduction

The aim of this study is to investigate the effect of hazelnut on wound healing at different periods. It was hypothesized that the hazelnut has a positive effect on wound healing thanks to the various antioxidants it contains.

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Medicinal plants or phytochemicals possess many wound healing properties, anticancer potential and more than a thousand are known<sup>1-3</sup>. After the announcement that the nuts approved by the United States Food and Drug Administration (FDA) reduce cardiovascular disease risk, interest in nuts including hazelnuts, has increased<sup>4</sup>. Turkey is one of the largest hazelnut producer and exporter in the world. In this respect, hazelnut directly affects the world economy for Turkey. Despite this, the num-

ber of scientific studies on hazelnut is very small in number. In these studies, it has been shown that hazelnut and by-products contain effective ingredients in the prevention of many diseases. In subsequent scientific studies, investigating the clinical effects of these substances on animals and humans, it may be great importance for Turkey to investigate the real effects and clinical use on the prevention and protection from diseases and improvement and acceleration of diseases.

Scientific demonstration of the effect of hazelnut on wound healing will contribute to the literature on the use of hazelnut as national product of Turkey. However, there is no study on the effect of wound healing of oral or local application of hazelnut in the literature. This study was performed using the well-known rat wound model. The aim of this study is to determine the effect of oral and local use of hazelnuts on wound healing in different periods.

## Materials and Methods

The study started with the approval of the Local Ethics Committee of Experimental Animals (dated 12.05.2016, number 4/decision 7). This study was conducted in accordance with the principles of Care and Use of the Laboratory Animals and the animal rights were protected. Wistar-Albino type 300-350 g. 72 rats were weighed before starting the experiment and divided into nine groups so that the weights of the animals were close to each other. According to the application of hazelnut extract (*Corylus avellana* L and *Corylus maxima* mill) for days of 3, 7 and 14, groups were formed as oral (3O, 7O, 14O), local (3L, 7L, 14L) and control (3C, 7C, 14C) groups. The reason of administration of the samples on days 3, 7 and 14 in the study is to show the effect of hazelnut on the different phases of healing.

### SURGICAL PROCEDURE

Operations were performed under general anesthesia with intraperitoneal injection of ketamine 90 mg/kg (Ketalar;

Eczacıba 1, Istanbul, Turkey) and xylazine hydrochloride 3 mg/kg (Rompun; Bayer, Turkey). After appropriate anesthetic conditions, animals were shaved with care to avoid damaging the skin with a razor blade and disinfected with polyvinyl pyrrolidone-iodine (Batticon®, Adeka, Samsun, Turkey). The surgical field was covered with sterile compresses (Fig. 1a). Under anesthesia 1 cm skin incision was formed in the back regions of the rats (Fig. 1b). A saline (SF) impregnated sponge (Clinisponge®, Yücel Medical, Turkey) was placed in groups C and O and oral hazelnut was given to O group as 500 mg / kg per day. For the L group, 1 cm<sup>3</sup> hazelnut extract impregnated sponge was placed (Fig. 1c). The surgical wounds were closed primarily with 2/0 polypropylene skin sutures (Propilen, Do san, Trabzon, Turkey) (Fig. 1d). Wound dressing was done. During the experiment, rats were kept at normal room temperature and humidity by feeding with standard pellet feed and tap water for 10/14 hours in a light/dark cycle of light, 4 subjects per cage. After the surgical procedure, all animals were regularly treated with wound dressing. At days 3, 7 and 14, the rats were sacrificed.

### HISTOPATHOLOGICAL EXAMINATION

All wound specimens were taken exposing suture area and fixed with 10% buffered formaldehyde solution. Then the samples were decalcified in 10% formic acid solution, histological tissue monitoring was performed and paraffin tissue blocks were prepared. Sections with a thickness of 5 µm were taken longitudinally from the blotted tissues. Gamori's trichrome staining was performed and histopathological evaluation was performed. The specimens separated into nine groups were examined objectively by two specialist histologists working in two different clinics. No information was given about which group the preparations belong to. Preparations were examined under a BX-53 model Olympus microscope (Olympus BX-53, Tokyo, Japan) and the necessary images were recorded (Fig. 2). The samples were evaluated according to the wound healing scoring system described by Li et al.<sup>5</sup> This classification consists of re-

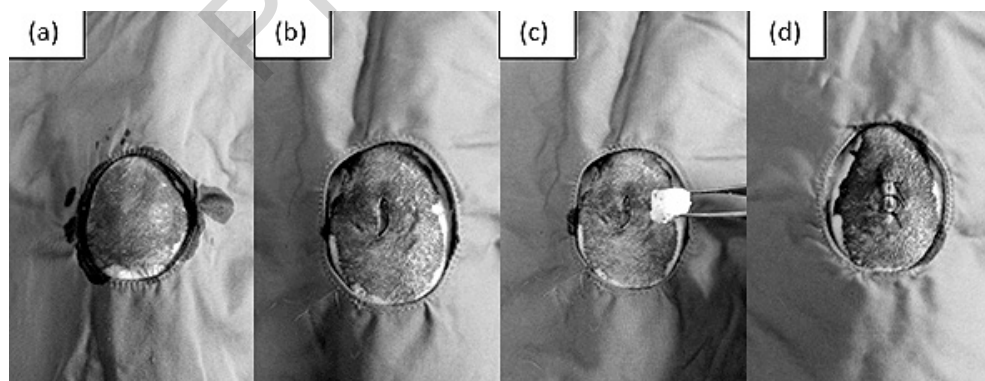


Fig. 1: A) Surgical areas were covered with sterile compresses; B) 1 cm length wound was formed in the back regions of the rats; C) Sponges were impregnated with saline or nuts extract according to the groups; D) Wounds were surgically repaired with sutures.

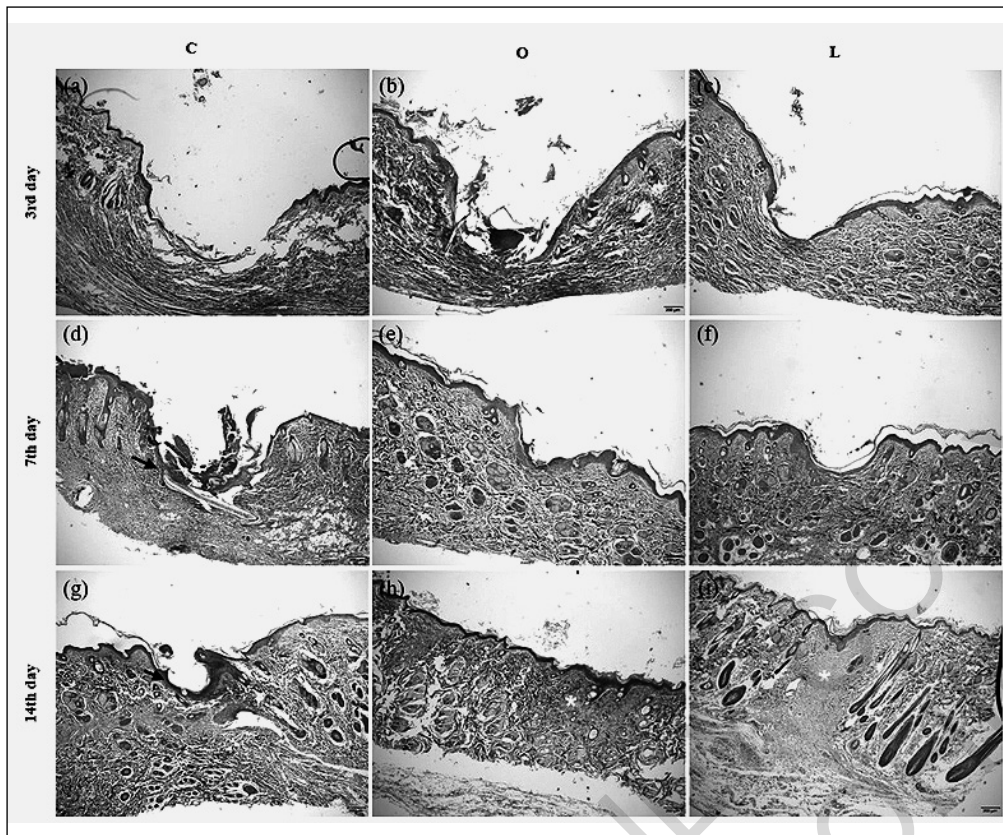


Fig. 2: Wound healing according to weeks and groups (Gomori's trichrome stain, x40 magnification). Re-epithelization, granulation tissue formation and collagen organization were observed to be better in the following weeks in each group. C: Control, O: Oral, L: Local application of hazelnut. Black arrow: areas of re-epithelization. Yellow stars: areas of granulation tissue formation.

epithelialization, granulation tissue formation, and collagen organization parameters. Each parameter is evaluated between 0 and 4 points. In this classification, low scores indicate bad wound healing, whereas high scores indicate good wound healing.

#### STATISTICAL ANALYSIS

The data were firstly analyzed using Levene's test and the Shapiro-Wilk test for equality of variance and for normality assumption, respectively ( $p > 0.05$ ). Secondly the differences between the study and control groups were analyzed by using Mann Whitney U test, and Kruskal-Wallis test. According to this test results, the data were expressed as a sample size, mean with standard deviation, median, IQR (interquartile range) and minimum and maximum values. The results were accepted as significant at the level of  $p < 0.05$ . All the statistical calculations were made with the SPSS (SPSS Inc., Chicago, IL, USA) 22.0 V. statistical program.

#### Results

When the parameters of the wound healing scoring system were examined, it was observed that the wound healing scores were better for each group on the follo-

wing days. It was observed that both oral and local hazelnut use were better at all stages than the control groups. With a slight difference it was determined that the oral application provided a better improvement than the local application.

When the data were evaluated statistically, there was a statistically significant superiority in re-epithelization and collagen organization wound healing scores in the group treated with oral hazelnut on the 3rd day (re-epithelization  $p = 0.002$ , collagen organization  $p = 0.003$ ). Other results according to wound healing scoring system are summarized in Table I.

#### Discussion

The most important finding explored in this study is that orally administered hazelnut accelerated wound healing in the early period. It is thought that this effect is achieved due to antioxidant substances contained in hazelnut by increasing the re-epithelization and collagen organization and preventing free radicals formed during wound healing.

The importance of hazelnut is becoming increasingly known in the world because of the benefits of hazelnut to human health. This special feature of hazelnut is important because of its special fat, protein, carbohydrate, fiber, vitamins (especially vitamin E), minerals (especially sele-

TABLE: I - Histological results of the groups

| Groups                       | Means<br>Re-epithelization | SD    | Min  | Max  | p values |
|------------------------------|----------------------------|-------|------|------|----------|
| 3C                           | 0.375                      | 0.517 | 0.00 | 1.00 | 0.002*   |
| 3O                           | 1.500                      | 0.755 | 1.00 | 3.00 |          |
| 3L                           | 1.375                      | 0.517 | 1.00 | 2.00 |          |
| 7C                           | 1.750                      | 0.707 | 1.00 | 3.00 | 0.158    |
| 7O                           | 2.375                      | 0.744 | 1.00 | 3.00 |          |
| 7L                           | 2.125                      | 0.353 | 2.00 | 3.00 |          |
| 14C                          | 3.000                      | 0.534 | 2.00 | 4.00 | 0.133    |
| 14O                          | 3.750                      | 0.462 | 3.00 | 4.00 |          |
| 14L                          | 3.250                      | 1.035 | 1.00 | 4.00 |          |
| Granulation tissue formation |                            |       |      |      |          |
| 3C                           | 0.250                      | 0.462 | 0.00 | 1.00 | 0.240    |
| 3O                           | 1.125                      | 0.640 | 0.00 | 2.00 |          |
| 3L                           | 1.000                      | 0.755 | 0.00 | 2.00 |          |
| 7C                           | 1.500                      | 0.755 | 0.00 | 2.00 | 0.059    |
| 7O                           | 2.250                      | 0.707 | 1.00 | 3.00 |          |
| 7L                           | 2.125                      | 0.353 | 2.00 | 3.00 |          |
| 14C                          | 2.750                      | 0.462 | 2.00 | 3.00 | 0.194    |
| 14O                          | 3.375                      | 0.744 | 2.00 | 4.00 |          |
| 14L                          | 3.000                      | 0.755 | 2.00 | 4.00 |          |
| Collagen organization        |                            |       |      |      |          |
| 3C                           | 0.125                      | 0.353 | 0.00 | 1.00 | 0.003*   |
| 3O                           | 1.250                      | 0.707 | 0.00 | 2.00 |          |
| 3L                           | 0.875                      | 0.640 | 0.00 | 2.00 |          |
| 7C                           | 1.250                      | 0.707 | 0.00 | 2.00 | 0.024    |
| 7O                           | 2.125                      | 0.640 | 1.00 | 3.00 |          |
| 7L                           | 2.000                      | 0.534 | 1.00 | 3.00 |          |
| 14C                          | 2.625                      | 0.517 | 2.00 | 3.00 | 0.194    |
| 14O                          | 3.250                      | 0.707 | 2.00 | 4.00 |          |
| 14L                          | 3.000                      | 0.755 | 2.00 | 4.00 |          |

C: Control; O: Oral; L: Local; SD: Standard deviation

nium) and other antioxidant components<sup>4</sup>. Hazelnut also contains most essential amino acids. The effects of these components on bone development and health are well known. From another perspective, the formation of free radicals, which can lead to the formation of many diseases such as cancer, vascular occlusion and diabetes, is hampered by the presence of antioxidant-specific components<sup>6</sup>. Studies have shown that hazelnuts decrease blood pressure, lower the level of low density lipoprotein (LDL) cholesterol, increase the level of high density lipoprotein (HDL) cholesterol, and decrease the risk of developing cardiovascular diseases. In this study the effect of hazelnuts on wound healing on the animal model was investigated and found that oral application improved the wound healing statistically in the early period.

Wound healing process is an active dynamic process that starts from the moment of injury and consists of three phases as inflammation, proliferation and maturation-remodeling. These phases are intertwined with each other

and are not able to draw successive boundaries with complex effects<sup>7-9</sup>. Delays or negativity in one of the phases involved in this process results in openness of the wound or prolongation of the healing, which is still a problem. For this reason, using these agents topically and systemically the target is that to shorten wound healing time and achieve ideal scar formation affecting the factors involved in wound healing process<sup>10</sup>. Antioxidant substances that prevent the formation of free radicals are important in this process. Tek et al.<sup>11</sup> reported the positive effect of energy drink containing several components on wound healing by increasing re-epithelization, collagen organization and new vascular formation. Leonel et al.<sup>12</sup> in their study reached a decision that not-oil also known as "lucuma" in Chile and Peru which contains many of the ingredients increased the wound regeneration and they argued that it was the reason why lucuma could be used for wound care. In this study it is also aimed to demonstrate the different effects of the hazelnut on the wound healing process in the course of wound healing and by ascertaining the various constituents (especially the antioxidant vitamin-e and selenium) which are present in the hazelnut. In the early period oral administration of hazelnut had positive effects on wound healing.

There are some limitations of this study. First, there is the lack of biomechanical analyses. Although histological evaluation is important for wound healing process, biomechanical test results are also important for the evaluation of wound repairs. A second limitation of this study is lack of markers about immunohistochemical evaluation. Another limitation is that application of the active ingredient as not a single component but co-administration of other ingredients may result as study-clinical incompatibility in terms of effect and dose.

The results of this study showed that wound healing was achieved in the following days for each group, but the orally administered hazelnut accelerated wound healing in the early period. This effect was attributed to early re-epithelization and increased collagen organization.

## Acknowledgments

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## Riassunto

Scopo di questo studio è la valutazione dell'effetto per via orale o per uso locale di estratti di nocciole sulla guarigione delle ferite a diversi intervalli, sperimentando

su 72 ratti Wistar-Albino di sesso maschile divisi in 9 gruppi di peso simile.

I gruppi sono stati formati suddividendo gli animali in relazione ai giorni 3, 7, 14, postoperatori e secondo il trattamento per via orale (3O, 7O, 14 O), per via locale (3L, 7L, 14L), ed il gruppo di controllo (3C, 7C, 14C).

Si è proceduto praticando in anestesia una incisione della lunghezza di 1 cm sul dorso dei animali, quindi suturata chirurgicamente. I ratti sono stati sacrificati in 3°, 7° e 14° giornata postoperatoria prelevando campioni dalla zona operatoria, e con analisi istopatologica secondo il sistema di punteggio del processo di guarigione delle ferite.

Da queste analisi istopatologiche è risultato che il punteggio della guarigione della ferita per quanto riguarda la riepitelizzazione e l'organizzazione del collagene è significativamente superiore nel gruppo 3O se paragonato agli altri gruppi (riepitelizzazione  $p=0.002$ , organizzazione del collagene  $p=0.003$ ).

Il reperto più importante risultato da questo studio è che la somministrazione orale con nocciole ha accelerato la guarigione della ferita nel periodo precoce. Si ritiene che questo effetto sia dovuto alle sostanze antiossidanti presenti nelle nocciole nell'accrescere la riepitelizzazione e l'organizzazione del collagene, e nel contrastare i radicali liberi che si formano durante il processo di guarigione.

Con questo studio si conclude che la nutrizione orale con nocciole accelera nelle fasi precoci la guarigione delle ferite. Ciononostante sono necessari ulteriori studi immunoistochimici e biomeccanici per confermare questi risultati.

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## Commento e Commentary

PROF. NICOLA PICARDI  
Professore Ordinario di Chirurgia Generale

*Pur riconoscendo la corretta impostazione dello studio sperimentale, i risultati ottenuti sono meno significativi di quanto appare perchè gli effetti positivi sulla guarigione delle ferite riscontrati negli animali alimentati con estratti di nocciole sono soprattutto evidenti nella fase precoce della guarigione. Ad una e due settimane dall'inizio della sperimentazione tale effetto appare rallentato anche se le immagini riportate a 14 giorni per quanto riguarda gli animali alimentati oralmente descrivono risultati migliori. È possibile che intervengano altri fattori che necessitano un approfondimento.*

\* \* \*

*While recognizing the correct setting of the experimental study, the results obtained are less significant than it appears because the positive effects on wound healing found in animals fed with hazelnut extracts are especially evident in the early phase of healing. At one and two weeks from the beginning of the experimentation, this effect appears to be slowed down even if the images reported at 14 days for the orally fed animals describe better results. It is possible that other factors may occur that require further study.*

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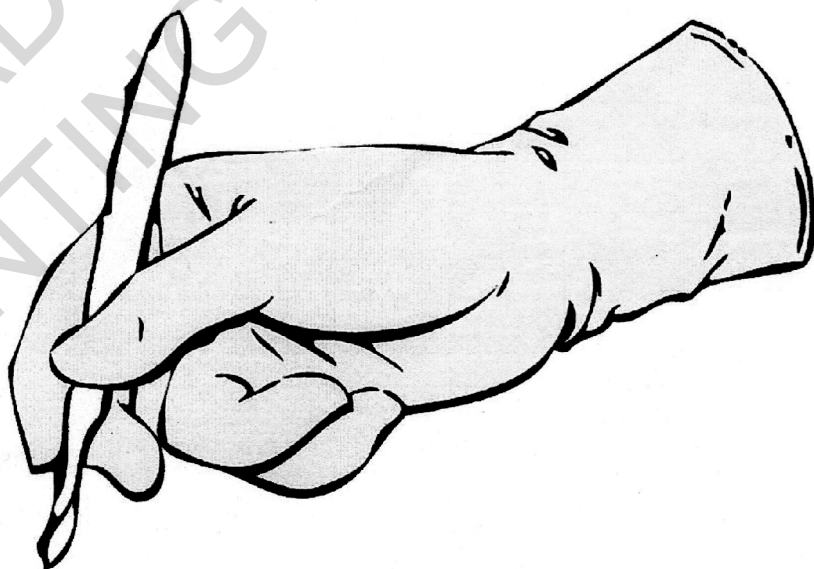
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