

Wound healing effects of Persian walnut (*Juglans regia* L.) green husk on the incision wound model in rats

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Abstract

Walnut green husk (WGH) has been mentioned as a wound-healing agent in traditional Iranian medicine. Although previous studies indicated that WGH is a good source of pharmaceutical ingredients, they did not assess its wound healing activity; so the present study set out the scientific validation of the wound healing potential of the Persian walnut. Total phenolic content, reducing power, DPPH, and nitric oxide scavenging activity of aqueous ethanol extract of WGH was evaluated. Forty-eight male Wistar albino rats were divided into four groups of 12 each. An incision wound was created on the dorsal region of each rat. WGH extract (20% w/w), WGH burnt residues (20% w/w), Eucerin, and Phenytoin ointments were used in each group. Wound length, contraction percentage, and histopathological evaluations were recorded on days 3, 7, 10, and 14. Total phenolic content and EC₅₀ values of reducing power, DPPH and nitric oxide scavenging activity of the WGH extract were 61.34 ± 0.64 mg/g dry extract, 0.95 ± 0.02 mg/mL, 0.35 ± 0.01 mg/mL, and 0.28 ± 0.01 mg/mL, respectively. Treated animals with WGH extract showed significantly (p ≤ 0.05) better results for physical and pathological parameters compared to the control group; overall, WGH extract showed better results than WGH burnt residues. The present study indicated that the WGH aqueous ethanol extract has a promising potential for wound healing in the animal model and could be a valuable resource for developing new wound-healing medicines for humans.

Key Words: antioxidant, *Juglans regia*, Persian walnut, traditional Iranian (Persian) medicine, wound healing.

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Wound refers to a laceration or breakdown of the skin tissues or membranes as a result of damage caused by physico-chemical and biological factors. The wound healing process includes coagulation, inflammation, proliferation, and remodeling phases.¹ Chronic wounds and delayed wound healing are among the most challenging problems in today's medicine, and impose many socio-economic issues on patients.² Recent developments in medical sciences have heightened the need for developing the healing agents to speed up the wound healing process. Therefore, the use of herbal remedies, along with conventional methods, has attracted attention in recent years. The use of complementary and alternative medicine (CAM) and medicinal plants in the treatment of various types of wounds, such as incisions, burns, etc., has a long history in many countries. In traditional Iranian (Persian) medicine (TIM/PM), there are many different ways of using medicinal plants for the

treatment of diseases, which are linked to the vast geographical area and high plant diversity of Iran; as many as 1200 species of medicinal plants have been reported for the flora of Iran.³ Plants have a wide variety of pharmaceutical ingredients with different biological activities, including anti-inflammatory, antimicrobial, and antioxidant; such properties have a crucial role in the wound healing process.⁴ The Persian walnut (*Juglans regia* L.) is a monoecious tree with imperfect flowers and belongs to the Juglandaceae family. Different parts of the Persian walnut have been used to treat a range of diseases, including skin disorders, cancer, infectious disease, diarrhea, hyperglycemia, anorexia, arthritis helminthiasis, sinusitis, eczema, asthma.⁵ Although different drug forms of the walnut green husk (WGH), the extract and burnt residues, were referred to as the wound-healing agents in the TIM/PM resources and previous reports indicated that the plant is a good source of phenolic compounds with high antioxidant potential.^{5,6}

Scientific research on effects on wound healing is lacking. WGH extract and burnt residues are evaluated on incision wound healing in rats.

Materials and Methods

Collection and identification of plant materials

Ripe walnut fruits were collected from the Saman region (32°28'59.4"N 50°54'52.4"E) in Chaharmahal and Bakhtiari province, Iran. Identification of samples was performed by academic staff in the department of horticultural science and landscape engineering, university of Tehran, Karaj, Iran, and the voucher specimen (No. 2019-1245) was preserved in the herbarium of the University of Tehran.

Preparation of extract and burnt residue

Previous studies reported that binary-solvent systems are more desirable for the extraction of phenolic compounds from plant samples than mono-solvent systems,⁷ so in the present study, we used 75% aqueous ethanol (ethanol: water, 75:25 v/v). At first, WGHs were washed under running tap water and were air-dried under shade. Then the samples were finely powdered using a mixer grinder before further studies. The powdered samples (200 g) were mixed with 2.0 L of aqueous ethanol and incubated for 72 hours in a shaker incubator at 37 °C and 80 rpm. Whatman No. 1 filter paper was used for the filtration procedure. Afterward, solvents were evaporated at 40–45 °C using a vacuum evaporator (Heidolph-Laborota 4001), and then plant extracts were stored in the dark at 4 °C until being used in the experiment. The yield of the extract was about 20% of the crude powder. For the preparation of burnt residues, the powdered samples were placed in a laboratory electric furnace (WiseTherm) at 650 °C for 5 hours. Burnt residues collected after cooling have been preserved in the appropriate containers before use.

Quantification assays

Measurement of total phenolic contents

The amount of total phenolic content in the extract was determined using the Folin-Ciocalteu method.⁸ Briefly, 40 µL of sample extract dissolved in EtOH (2 mg/mL) was mixed with 200 µL of Folin-Ciocalteu reagent (2.0 N) and 1160 µL of distilled water. After 5 min in dark condition, 600 µL of sodium carbonate (20% v/w) was added, and the mixture was incubated at 40 °C for 30 min. The mixture was subsequently centrifuged at 2000 × g for 10 min, and the absorbance of the supernatant was measured at 765 nm using a spectrophotometer. The Gallic acid (GA) calibration curve was generated using the standard GA solutions (50-500 µg/mL) and the amount of total phenolic contents was calculated based on the equation $y = 0.0078x + 0.2487$, $R^2 = 0.9885$ where y was the absorbance and x was the Gallic acid equivalent. The results were expressed as mg Gallic acid equivalents per gram of extract (GAE/g extract).

In vitro antioxidant assays: Determination of reducing power

Determination of the reducing power of the extracts was done according to the previously described procedure.⁹ Different concentrations (0.01–5 mg/mL) of the extract (1 mL) was mixed with 2.5 mL of sodium phosphate buffer (200 mmol / L) (pH = 6.6) and 2.5 mL of potassium ferricyanide solution (1% w/v). The mixture was incubated at 50 °C for 20 minutes. After that, 2.5 mL of trichloroacetic acid (10% w/v) was added, and then the resulting mixture was centrifuged at 1000 rpm for 8 minutes. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1%). The absorbance was measured at 700 nm. The extract concentration providing 0.5 of absorbance (EC₅₀) was calculated from the graph of absorbance at 700 nm against the correspondent extract concentration.

In vitro antioxidant assays: DPPH radical scavenging activity

The DPPH scavenging ability of the extracts was evaluated using the method of Hatano et al. (1988).¹⁰ Extract solutions (0.3 mL) with different concentrations (0.01–2 mg/mL) were mixed with 2.7 mL of fresh DPPH methanolic solution (6×10^{-5} M). The resulting mixture was shaken vigorously and placed at room temperature and dark for 60 min. The reduction of DPPH radicals was measured by monitoring the decrease in absorbance at 517 nm. The DPPH scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = \left[\frac{A_x - A_o}{A_x} \right] \times 100$$

where; A_x was the absorbance of the control reaction without the test material, and A_o was the absorbance of the test material. The EC₅₀ was calculated from the graph of the scavenging effect percentage against the concentration of the extract in the solution

In vitro antioxidant assays: Nitric oxide scavenging activity

The nitric oxide (NO) scavenging activity of the samples was determined using the method of Sousa et al. (2008).¹¹ Different concentrations (100 µL) of the extracts were mixed with 100 µL of sodium nitroprusside solution (SNP, 10 mM), and the mixture was kept for 60 min at room temperature and light condition. Each sample was reacted with 100 µL of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2% phosphoric acid). The resulting mixture was incubated at room temperature for 10 min, and the absorbance was measured at 562 nm. NO scavenging activity and EC₅₀ were determined according to the process described for the DPPH method.

In vivo study of wound healing: Experimental animals

In this experimental study, 48 male Wistar albino rats weighing 180 to 200 g were used. Animals were kept in

Table 1. Experimental design for *in vivo* wound healing study

Group name	Ingredients
Control	Eucerin
Standard	Phenytoin cream (1%)
Extract	20% (w/w) WGH extract ointment
Burnt residues	20% (w/w) WGH burnt residues ointment

separate cages under standard laboratory conditions (ambient temperature 25 ± 3 °C and 12 h light/dark cycle). Animals were fed standard pellets and water *ad libitum* throughout the test period (14 days). Rats were randomly divided into four groups of 12 each. The details of the statistical design are shown in Table 1. The concentration of the drug (20% w/w) was selected based on a pilot study with doses like 5%, 10%, 15%, and 20% on the incision wound model in male Wistar albino rats and the concentration of 20% (w/w) showed better results in case of wound healing. The present study followed the guidelines of the World Medical Association for the care and use of laboratory animals

In vivo study of wound healing: Incision wound model
Animals in each group were anesthetized by a mixture of ketamine 10% (80 mg kg⁻¹; Alfasan, Netherlands) and xylazine 2% (10 mg kg⁻¹; Alfasan, Netherlands), injected intraperitoneally. Then the dorsal region of rats was shaved, and a 2-cm full-thickness incision wound was made through the skin. The wounds were photographed and left open, and the day of the surgery was considered as day 0. The drugs were topically applied once daily until the 14th day

In vivo study of wound healing: Physical parameters
The physical wound parameters were measured on days 3, 7, 10, and 14 to evaluate the wound healing process. Wound length was measured using a digital caliper. For evaluating the rest of the parameters, the cross-sections of the wounds were photographed on days 3, 7, 10, and 14, and images were analyzed using ImageJ software. The formulas are as follow: ¹²

$$\text{wound length percentage} = \frac{\text{wound length on day } x \text{ (mm)}}{\text{wound length on day 0 (mm)}} \times 100$$

$$\text{wound healing ratio (\% contraction)} = \left[1 - \frac{\text{wound area on day } x \text{ (mm}^2\text{)}}{\text{wound area on day 0 (mm}^2\text{)}} \right] \times 100$$

In vivo study of wound healing: Histopathological study
Three animals of each group were sacrificed on days 3, 7, 10, and 14 and wound tissues were collected and maintained in a 10% formalin solution and then embedded in paraffin wax. A 3 µm thick cut was performed, and the samples were stained with hematoxylin and eosin (H&E).¹³ The samples were observed under a light microscope, and the parameters, including re-epithelialization, polymorphonuclear cells (PMN), macrophage, neovascularization, and fibroblast, were blindly scored by a pathologist. Scores were as follow: 0 = no evidence, 1 = minimal, 2 = slight, 3 = moderate and, 4 = severe.

Statistical analysis

Statistical analysis was performed using SPSS, version 25 (IBM, New York, USA). EC₅₀ values of the samples in the antioxidant assays were determined using GraphPad Prism 8.2.1. The data are represented as the mean ± SEM, and the values were subjected to analysis of variance (ANOVA) with Tukey post-hoc tests. A probability level of p ≤ 0.05 was considered significant.

Results

Total phenolic content and antioxidant activity

Table 2 presents results of the total phenolic content, reducing power, DPPH, and nitric oxide scavenging activity.

In vivo study of wound healing: Physical parameters
In order to investigate the effects of different treatments on wound healing, the wounds were photographed during the healing period (days 0, 3, 7, 10, 14) (Figure 1) and the percentage of wound length and wound healing ratio (contraction percentage) of different animals of four treatment groups were measured (Table 3).

Table 2. Total phenolic content, reducing power, DPPH radical, and nitric oxide scavenging activities of 75% aqueous ethanol extract of WGH

Medical part	Total phenolic content mg/g extract equivalent)	phenolic dry (GAE)	Reducing power (EC ₅₀) mg/mL	DPPH (EC ₅₀) mg/mL	Nitric oxide scavenging activity (EC ₅₀) mg/mL
Walnut green husk (WGH)	61.34 ± 0.64		0.95 ± 0.02	0.35 ± 0.01	0.28 ± 0.01

Each value was obtained by calculating average of three experiments. Data are presented as Mean ± SEM.

Table 3. Physical evaluations of 20% (w/w) WGH ointment compared to the control and standard in the incision wound model in rats

Groups	Wound Physical Parameters	
	Wound length (%)	Wound healing ratio (% contraction)
3-Day application		
Eucerin	93.26 ± 1.19 ^a	4.30 ± 1.80 ^b
Phenytoin	90.84 ± 0.67 ^a	7.51 ± 0.69 ^{ab}
Extract	81.41 ± 2.65 ^b	17.01 ± 2.70 ^a
Burnt residues	85.58 ± 1.12 ^a	12.81 ± 1.15 ^{ab}
7-Day application		
Eucerin	72.00 ± 0.61 ^a	26.88 ± 0.62 ^c
Phenytoin	63.69 ± 0.73 ^{bc}	35.22 ± 0.74 ^{ab}
Extract	58.35 ± 1.76 ^c	40.82 ± 1.79 ^a
Burnt residues	68.02 ± 1.00 ^{ab}	31.13 ± 1.02 ^{bc}
10-Day application		
Eucerin	49.41 ± 0.93 ^a	46.08 ± 1.02 ^b
Phenytoin	0.00 ^b	100.00 ^a
Extract	0.00 ^b	100.00 ^a
Burnt residues	44.49 ± 1.60 ^a	51.58 ± 1.74 ^b
14-Day application		
Eucerin	15.44 ± 1.32 ^a	83.65 ± 1.40 ^b
Phenytoin	0.00 ^b	100.00 ^a
Extract	0.00 ^b	100.00 ^a
Burnt residues	0.00 ^b	100.00 ^a

Data are means ± SEM. Means with different letters are significantly different ($p < 0.05$).

As depicted in Table 3, until day 7, the highest wound healing was observed in the extract group (40.82%); however, on day 10, both extract and standard groups showed complete wound healing. In the case of burnt residues, complete wound healing was observed on day 14th, but there was no proof of complete wound healing in the control group until the end of the experiment.

In vivo study of wound healing: Pathological parameters Figure 2 displays the results obtained from the histopathological evaluation of studied tissues. On the 3rd day, there was no significant difference in re-epithelialization between groups; however, on day 7th, re-epithelialization in the WGH treated samples either

ethanol extract and WGH burnt residues were significantly higher than the control group. On the 10th day, although WGH extract and burnt residue ointments had significant differences in re-epithelialization compared to the control group, burnt residue ointment showed significantly lower values than the standard. Both WGH extract and burnt residues ointments exhibited significant differences with the control group on the 14th day. There is a significant difference in the number of PMNs between WGH extract and the control on the 10th day. Despite that the PMNs were less when compared with the burnt residues treated samples, they were significantly higher compared to the controls.



Fig 1. Macroscopic view of the wounds in the four treatment groups at different time-points. a: Eucerin (control); b: Phenytoin (standard); c: WGH extract (20% w/w); d: WGH burnt residues (20% w/w).

Although macrophage levels have increased in all treatments during the test period, their difference was never significant. On days 3rd and 7th, neovascularization was increased in both WGH extract and burnt residue ointments samples, but the differences were not significant when compared to the control and standard groups. Although not significant, decreasing in neovascularization was observed, in WGH extract and burnt residues treatments on the 10th and 14th days. Also in fibroblasts, there were no significant differences between groups throughout the test period.

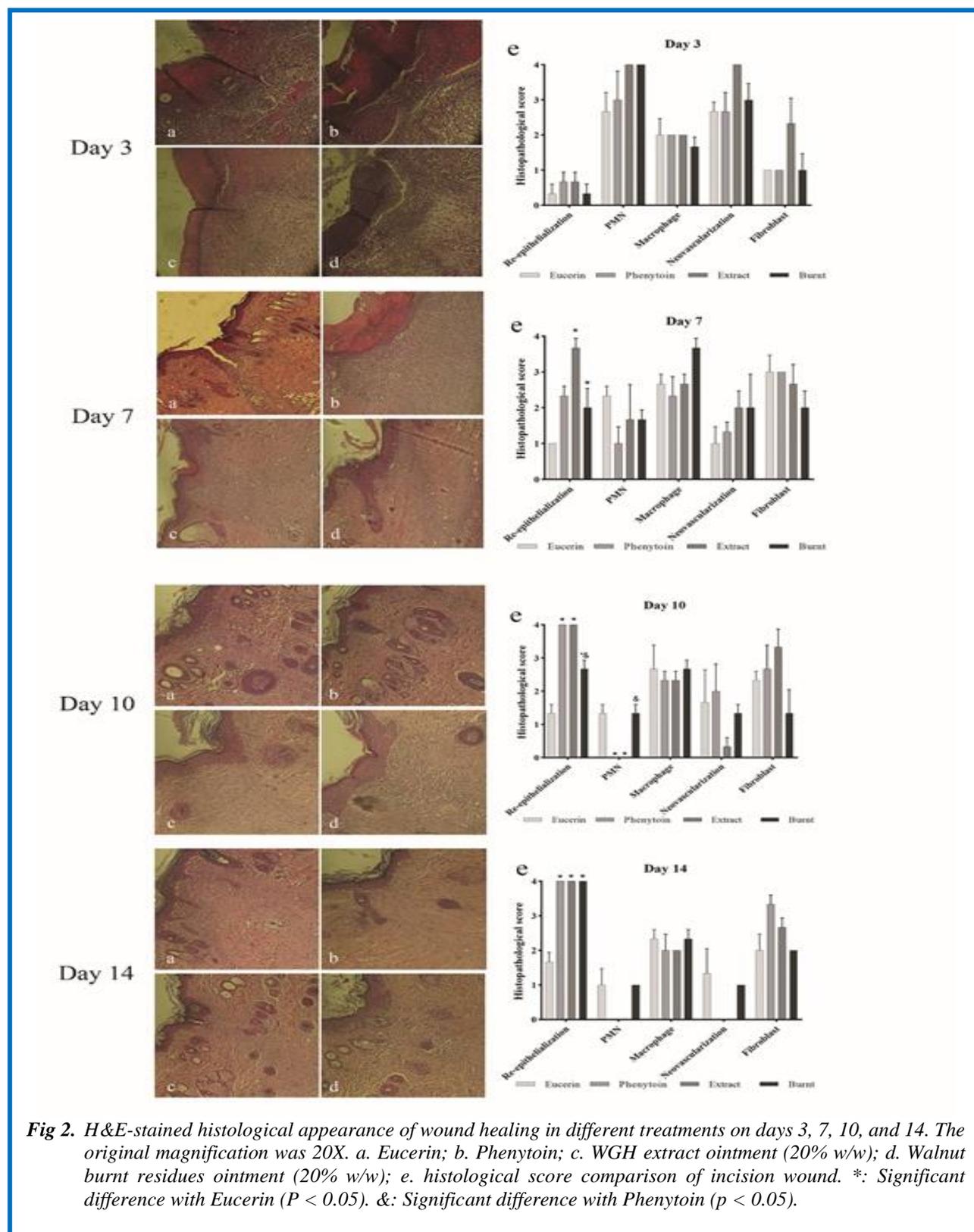
Discussion

The wound healing is a complicated process that involves the cooperation of different cell types toward restoration and re-epithelization of any discontinuity in the damaged skin. The wound healing has four stages: the first stage is the coagulation and homeostasis phase that occurs immediately after the creation of a wound in damaged skin. The second stage is the inflammatory phase, which protects the wound against the microorganisms. The third stage is the proliferation phase that involves proliferation and migration of keratinocytes, proliferation of

fibroblasts, matrix deposition, and angiogenesis. Finally, the fourth stage is the remodeling phase, which involves scar tissue replacement, starting approximately on day 21st and may take up to a year or more.¹⁴ Antioxidant compounds which are known for their free radical scavenging activity have a significant effect on the wound healing process. During the inflammation phase of wound healing, phagocytosis caused by monocytes, neutrophils, and, to a much less extent, by eosinophils leads to an oxidative burst in which there is a dramatic increase in oxygen consumption. The result of this increase is the accumulation of reactive oxygen and nitrogen derivatives. These derivatives have the ability to kill microorganisms directly, but they will also act as messengers and they are able to trigger signaling molecules; however, the damage in the affected area is inevitable by their large amount of production.¹⁵ It is, therefore, essential to determine the antioxidant effect of drugs and plants in order to provide information on their wound healing activities.¹⁶ The results of the present study indicated that the aqueous ethanol extract of WGH contains a high level of phenolic compounds (61.34 mg/g dry extract (GAE equivalent)). The WGH contains

Effects of Persian walnut green husk on rat wound healing

Eur J Transl Myol 30 (1): 210-218, 2020



various phenolic compounds; among them, juglone (5-hydroxy-1,4-naphthoquinone) is the characteristic of *Juglans spp.*¹⁷ Previous studies showed that total phenolic content could be different based on the variety,

harvesting time, plant part, and the type of solvent used for the extraction process.^{6,18,19} The redox potential of medicinal plants is mainly due to their phenolic content, which qualifies them to serve as reducing compounds,

donors of hydrogen, and scavengers of oxygen free-radicals.²⁰ Previous studies have shown that there is a relationship between the phenolic content and the antioxidant ability of plant-origin compounds.^{21,22} In this study, three different assays were used to assess the antioxidant capacity of WGH samples against reactive oxygen species (ROS) species: reducing power, DPPH, and NO scavenging activity. The findings indicated that the extract of WGH is a rich source of antioxidants which have a vital role at the forefront of the battle against free radicals. In the present study, the extract of WGH showed a great reducing power ($EC_{50} = 0.95$ mg/mL). The reducing power of a compound is an essential sign of its potential antioxidant activity.²³ It can be inferred from higher values of reducing power, that some components serve as electron donors and interact with free radicals. DPPH assay is one of the most commonly used methods for evaluation of the free radical scavenging activity. The studied extract showed a high DPPH radical scavenging activity ($EC_{50} = 0.35$ mg/mL). According to previous studies, DPPH activity in the WGH is concentration-dependent, and specimens with higher total phenols show higher DPPH activity.^{18,24} The WGH showed an EC_{50} of 0.28 mg/mL for the NO scavenging that was slightly lower compared to DPPH radical scavenging activity ($EC_{50} = 0.35$ mg/mL). The NO has a significant role as a signaling molecule in various biological processes, including blood pressure regulation, defense mechanisms, immune regulation, and smooth muscle relaxation. When NO is overproduced, the system is unable to neutralize or eliminate the molecules of free radicals in excess and therefore, NO alters the protein structures and interferes with their normal function.²⁵ From the above findings, we concluded that the extract of WGH has a high antioxidant power and could be used as a pharmacotherapeutic agent. The results from wound healing and wound repair length clearly showed that the groups treated with WGH ointment had similar results when compared with control groups. Nevertheless WGH burnt residues ointment had significantly better results than the control group, the standard did much better. Overall, the WGH extract ointment had better results than the WGH burnt residues ointment, probably due to the lack of organics like phenolic compounds in burnt residues. The presence of organics like phenolic compounds contribute to the higher antioxidant level.²⁶ The presence of antioxidants in the extract will fasten the healing process.²⁷ Probably, the improved wound healing results of the WGH extract are due to its anti-inflammatory effects, along with its effect on the maturation and organization of the granulation tissue.²⁸ For the histopathological evaluations, re-epithelialization, PMN, macrophage, neovascularization, and fibroblast were studied. Re-epithelialization is known as an essential factor in the assessment of successful wound healing; this process is characterized by the migration of keratinocytes and increased proliferation across the wound area. In the case of chronic

wounds, the process of epithelialization is disrupted, and the impaired wounds are the result of the lack of epithelialization.²⁹ In this study, re-epithelialization in standard, WGH extract, and burnt residues ointment treatments were increased on days 7, 10, and 14. Keratinocytes have several vital roles in the wound healing process including epidermis maintenance and restoration after wounding.²⁹ PMNs are also crucial for the wound healing; the fundamental role of PMNs is to release soluble mediators, including neutrophil elastase, matrix metalloproteinases (MMPs), and myeloperoxidase (MPO).³⁰ MPO acts as a mediator for the killing of bacteria by the production of reactive oxygen species (ROS). In this study, from the 3rd day to the end of the experiment, the PMNs decreased, and on the 10th day, there were significant differences between WGH extract and control group. By its dynamic plasticity, macrophage plays a critical role in the inflammation phase of tissue repair, which allows macrophages to function both as a tissue-destructive and –reparative agents.³¹ In our study, the differences between treatments were not significant throughout the experiment. The formation of new blood vessels is essential for healthy wound healing.

The reduction of neovascularization on the 14th day may suggest that WGH extract accelerates the process of wound healing. Fibroblasts are essential in all stages of wound healing; they play a vital role in the deposition of extracellular matrix (ECM) components, wound contraction and ECM remodeling.³² In the present study, fibroblasts have increased from day 3rd to 14th; although the differences were not statistically significant, standard and WGH extract groups exhibited higher values which may indicate the potential wound healing properties of WGH extract compared to the standard. In conclusion, the present study, for the first time, investigates the wound healing activity of the WGH.

In conclusion, the results of the wound healing ratio, along with histopathological evaluations, indicate that the ethanol extract of WGH has a promising wound healing capacity. These findings highlight the potential usefulness of plant-derived compounds for the treatment of wounds in both animals and humans.

List of acronyms

CAM - Complementary and alternative medicine
DPPH - 2, 2-diphenyl-1-picrylhydrazyl
ECM - Extracellular matrix
GA - Gallic acid
GAE - Gallic acid equivalent
MMP - Matrix metalloproteinase
MPO - Myeloperoxidase
NO - Nitric oxide
PMN - Polymorphonuclear cells
ROS - Reactive oxygen species
SNP - Sodium nitroprusside
TIM/PM - Traditional Iranian (Persian) medicine
WGH - Walnut green husk

Authors contributions

All authors equally contributed to manuscript.

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Conflict of Interest none

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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References

- Gonzalez AC de O, Costa TF, Andrade Z de A, Medrado ARAP. Wound healing - A literature review. *An Bras Dermatol* 2016;91:614-20.
- Nicolaus C, Junghanns S, Hartmann A, et al. In vitro studies to evaluate the wound healing properties of *Calendula officinalis* extracts. *J Ethnopharmacol* 2017;196:94-103.
- Nordensram B, Rechinger, K. H. (ed.), *Flora Iranica*, Fasc. 111-162 (1975-1987). *Nord J Bot* 1989;8:625-6.
- Barreto R, Albuquerque-Júnior R, Araújo A, et al. A Systematic Review of the Wound-Healing Effects of Monoterpenes and Iridoid Derivatives. *Molecules* 2014;19:846-62.
- Panth N, Paudel KR, Karki R. Phytochemical profile and biological activity of *Juglans regia*. *J Integr Med* 2016;14:359-73.
- Ghasemi K, Ghasemi Y, Ehteshamnia A, et al. Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (*Juglans regia* L.) green husks. *J Med Plants Res* 2011;5:1128-33.
- Chew KK, Khoo MZ, Ng SY, et al. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Orthosiphon stamineus* extracts. *Int Food Res J* 2011;18:1427.
- AĞ ŞELECI D, GÜMÜŞ ZP, YAVUZ M, et al. A case study on in vitro investigations of the potent biological activities of wheat germ and black cumin seed oil. *Turkish J Chem* 2015;39:801-12.
- Berker KI, Güçlü K, Tor İ, Apak R. Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyl triazine (FRAP), and ferricyanide reagents. *Talanta* 2007;72:1157-65.
- Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chem Pharm Bull* 1988;36:2090-7.
- Sousa A, Ferreira I, Barros L, et al. Antioxidant potential of traditional stoned table olives "Alcaparras": influence of the solvent and temperature extraction conditions. *LWT-Food Sci Technol* 2008;41:739-45.
- Ozay Y, Kasim MC, Guzel-Ozay S, et al. Effects of *Equisetum arvense* Ointment on Diabetic Wound Healing in Rats. *Wounds a Compend Clin Res Pract* 2013;25:234-41.
- Sirak A, Abie G. Manual of standard operating procedure (SOP) for tissue processing. *Natl Anim Heal Investig Cent (NAHDIC), Ethiop* 2009:8-32.
- Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* 2009;37:1528-42.
- Tümen İ, Akkol EK, Taştan H, et al. Research on the antioxidant, wound healing, and anti-inflammatory activities and the phytochemical composition of maritime pine (*Pinus pinaster* Ait). *J Ethnopharmacol* 2018;211:235-46.
- Shetty S, Udupa S, Udupa L. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* Linn in rats. *Evidence-Based Complement Altern Med* 2008;5:95-101.
- Mahoney N, Molyneux RJ, Campbell BC. Regulation of aflatoxin production by naphthoquinones of walnut (*Juglans regia*). *J Agric Food Chem* 2000;48:4418-21.
- Fernández-Agulló A, Pereira E, Freire MS, et al. Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Ind Crops Prod* 2013;42:126-32.
- Omidi Ghaleh Mohammadi M, Mirghazanfari SM. Investigation of Iranian pomegranate cultivars for wound healing components. *Eur J Transl Myol* 2019;29:22-6.
- Hakkim FL, Shankar CG, Girija S. Chemical Composition and Antioxidant Property of Holy

Effects of Persian walnut green husk on rat wound healing

Eur J Transl Myol 30 (1): 210-218, 2020

- Basil (*Ocimum sanctum* L.) Leaves, Stems, and Inflorescence and Their in Vitro Callus Cultures. *J Agric Food Chem* 2007;55:9109-17.
21. Rice-Evans C. Flavonoid Antioxidants. *Curr Med Chem* 2001;8:797-807.
 22. Fernández-Agulló A, Pereira E, Freire MS, et al. Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Ind Crops Prod* 2013;42:126-32.
 23. Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J Agric Food Chem* 1995;43:1813-9.
 24. Oliveira I, Sousa A, Ferreira ICFR, et al. Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food Chem Toxicol* 2008;46:2326-31.
 25. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;39:44-84.
 26. Khorshid F, Ali SS, Alsofyani T, Albar H. *Plectranthus tenuiflorus* (Shara) Promotes Wound Healing: In vitro and in vivo Studies. *Int J Bot* 2010;6:69-80.
 27. Sheeba M, Emmanuel S, Revathi K, Ignacimuthu S. Wound healing activity of *Cassia occidentalis* L. in albino Wistar rats. *Int J Integr Biol* 2009;8:1-6.
 28. Hagh-Nazari L, Goodarzi N, Zangeneh MM, et al. Stereological study of kidney in streptozotocin-induced diabetic mice treated with ethanolic extract of *Stevia rebaudiana* (bitter fraction). *Comp Clin Path* 2017;26:455-63.
 29. Pastar I, Stojadinovic O, Yin NC, et al. Epithelialization in Wound Healing: A Comprehensive Review. *Adv Wound Care* 2014;3:445-64.
 30. Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 2013;13:159.
 31. Boniakowski AE, Kimball AS, Jacobs BN, et al. Macrophage-Mediated Inflammation in Normal and Diabetic Wound Healing. *J Immunol* 2017;199:17-24.
 32. desJardins-Park HE, Foster DS, Longaker MT. Fibroblasts and wound healing: an update. *Regen Med* 2018;13:491-5.

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