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Efficacy of *Punica granatum L.* and *Matricaria chamomilla L.* herbal extracts on wound healing

Eficacia de los extractos herbales de *Punica granatum L.* y *Matricaria chamomilla L.* en la cicatrización de heridas

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ABSTRACT

Dermal injuries, which account for a significant portion of healthcare expenditures globally, occur in various forms due to the influence of various etiological factors, primarily trauma. For all these reasons, wound healing is among the most researched topics worldwide. This study aimed to investigate the effects of ointments obtained from *Punica granatum* L. (pomegranate) and *Matricaria chamomilla* L. (chamomile) extracts on secondary wound healing. 42 Sprague Dawley rats were used in the study. In the study, a total of six experimental groups were formed, each consisting of 7 rats: control group, healthy control group, *Punica granatum* treatment group, *Matricaria chamomilla* treatment group, Punica granatum and Matricaria chamomilla combination group (*Punica granatum* treatment + *Matricaria chamomilla* treatment), and the group receiving an application containing olive oil, beeswax, and natural vitamin E. An excisional wound model was created using a biopsy punch on the back of the subjects in all groups except the healthy control group. While ointments prepared from herbal extracts were applied to the rats in the *Punica granatum* treatment, *Punica granatum* treatment + *Matricaria chamomilla* treatment, and *Matricaria chamomilla* treatment groups, rats in the control group received 0.9 % NaCl, and rats in the olive oil, beeswax, and natural vitamin E group were treated with a mixture of olive oil, beeswax, and vitamin E without any herbal extracts. At the end of the study, it was determined that the *Matricaria chamomilla* treatment group had the highest mean in terms of wound closure percentage and wound perimeter change percentage, while the *Punica granatum* treatment group had the lowest mean ($P < 0.05$). Although the best groups in terms of histopathological scoring were the *Punica granatum* treatment and *Matricaria chamomilla* treatment groups, no statistical difference was observed between the groups ($P > 0.05$). In terms of oxidative stress parameters (except for superoxide dismutase), the treatment groups were statistically significantly closer to the healthy control group than to the control group. Considering all evaluation criteria, it was concluded that Matricaria chamomilla plant was more effective on wound healing than Punica granatum plant and was a more suitable option for clinical use.

Key words: Wound healing; Matricaria chamomilla; Punica granatum; rat; excisional wound model.

RESUMEN

Las lesiones dérmicas representan una parte significativa del gasto sanitario a nivel mundial y se producen por diversos factores etiológicos, principalmente el trauma. Por ello, la cicatrización de heridas es uno de los temas más investigados a nivel mundial. Este estudio tuvo como objetivo investigar los efectos de ungüentos obtenidos a partir de extractos de *Punica granatum* L. (granada) y *Matricaria chamomilla* L. (manzanilla) sobre la cicatrización de heridas por segunda intención. En el estudio se utilizaron 42 ratas Sprague Dawley. Se formaron un total de seis grupos experimentales, cada uno compuesto por 7 ratas: grupo control, grupo control sano, grupo de tratamiento con *Punica granatum*, grupo de tratamiento con *Matricaria chamomilla*, grupo de combinación *Punica granatum* + *Matricaria chamomilla* (*Punica granatum* + *Matricaria chamomilla*) y el grupo que recibió una aplicación que contenía aceite de oliva, cera de abeja y vitamina E natural. Se creó un modelo de herida excisional en la espalda de todas las ratas, excepto en el grupo control sano. Los grupos *Punica granatum*, *Matricaria chamomilla* y *Punica granatum* + *Matricaria chamomilla* recibieron los ungüentos con extractos herbales; el control recibió solución salina al 0,9 %, y el grupo que recibió la mezcla de aceite de oliva, cera de abejas y vitamina E sin extractos. Al final del estudio, el grupo *Matricaria chamomilla* presentó el porcentaje más alto de cierre de heridas y cambio de perímetro de la herida, mientras que el grupo *Punica granatum* tuvo los valores más bajos ($P < 0,05$). En cuanto a la puntuación histopatológica, los grupos *Punica granatum* y *Matricaria chamomilla* obtuvieron los mejores resultados, aunque sin diferencias significativas entre los grupos ($P > 0,05$). Los parámetros de estrés oxidativo, excepto la superóxido dismutasa, mostraron que los grupos tratados se acercaban más al control sano que al control. En conclusión, la Matricaria chamomilla demostró ser más efectiva que la Punica granatum en la cicatrización de heridas y se considera una opción más adecuada para uso clínico.

Palabras clave: Cicatrización de heridas; Matricaria chamomilla; Punica granatum; rata; modelo de herida excisional.

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INTRODUCTION

A wound is a condition in which the integrity of the skin and mucous membranes is impaired due to physical trauma or disease. Physical trauma such as crushing and sprains cause closed wounds where tissue integrity is not completely disrupted, while physical trauma such as cuts, punctures, and lacerations cause open wounds by completely disrupting tissue integrity [1 , 2 , 3]. Wound healing is a dynamic and complex biological process that includes inflammatory response, tissue formation and remodeling stages. Practices for the treatment and care of wounds provide the appropriate environment for these processes to occur within an acceptable timeframe [1 , 3 , 4].

The healing effects of herbal extracts on many health problems, especially wounds, have been thoroughly researched in Veterinary and Human Medicine, and their effectiveness has been accepted. Among the herbal extracts studied for their effects on wound healing are *Punica granatum* L. (pomegranate) and *Matricaria chamomilla* L. (chamomile) [5].

Punica granatum L. herbal extract contains polyphenols (tannins, punicalagin and ellagic acid), phenolic compounds (gallic acid) and triterpenoids (maslinic, ursolic acid and asiatic acid). While the gallic acid and tannins it contains have an astringent effect [6], polyphenols are known to stimulate dermal fibroblast proliferation, increase type I procollagen synthesis, and inhibit matrix metalloproteinase-1. *Matricaria chamomilla* L. plant extract has been reported to have positive effects on wound healing by inhibiting histamine release from leukocytes through its flavonoid content (quercetin and apigenin); reducing oxidative stress and increasing microcirculation through its coumarins; and supporting granulation tissue formation through its terpenoids, particularly bisabolol. Although studies investigating the effects of *Punica granatum* L. (pomegranate) and *Matricaria chamomilla* L. on wound healing exist, it is noteworthy that the number of these studies is limited, and some studies, particularly those reporting increased concentrations of *Punica granatum*, have slowed the rate of wound healing [5].

In this study, the effectiveness of *Punica granatum* L. (pomegranate) and *Matricaria chamomilla* L. (chamomile) plants on wound healing was determined and their usability for clinical applications was evaluated. The results of the study were evaluated in light of macroscopic findings such as the wound healing rate coefficient (WHRC), wound closure percentage (WCP), and wound perimeter change percentage (WPCP), as well as laboratory findings including histopathological and biochemical parameters. A healthy control group (HCG) was included in the study to serve as a reference for the evaluation of oxidative stress markers and histopathological scores. In addition, a group receiving olive oil + beeswax + vitamin E (OBEG) was incorporated to assess the potential effects of the excipients and carrier substances used in the preparation of the ointments and to ensure transparency regarding the formulation content.

MATERIALS AND METHODS

Study groups and wound model creation

The study sample consisted of 42 Spraque-Dawley rats (*Rattus norvegicus*) of 10 weeks of age. The rats were randomLy assigned to six groups of seven animals each. The study groups were the control group (CG), the healthy control group (HCG), the *Punica granatum* group (PG) (Geographical source: Fethiye region of Mugla, Türkiye), the *Matricaria chamomilla* group (MC) (Geographical source: Bayramic region of Canakkale, Türkiye), the *Punica granatum* + *Matricaria chamomilla* group (PG + MC), and the olive oil (Geographical source: Edremit region of Balikesir, Türkiye), + beeswax (Geographical source: Balikesir, Türkiye Botanical origin: *Olea europaea, Pinus* sp*., Quercus* sp.) + natural vitamin E group (OBEG). Following a 10-day (d) adaptation period, experimental wound models were created in rats in the CG, MC, PG, PG + MC, and OBEG groups.

Before wound creation, rats were administered intramuscularly at 10-minute (min) intervals, first 8 mg/kg xylazine hydrochloride, followed by 80 mg/kg ketamine hydrochloride. After anesthesia was achieved, the dorsal regions of the rats were shaved and disinfected, and circular wounds were created using 6 mm diameter biopsy punches (FIG.1).

FIGURE 1. Biopsy punch used to create the experimental wound model (a) and formation of the model (b).

Ointment preparation and applications

The plants to be used in the study were subjected to solvent extraction in methanol. The methanol was then removed using a rotavapor device (R-300 series, BÜCHI Labortechnik AG, Switzerland) to obtain herbal extracts. Herbal extracts were mixed with olive oil, beeswax, and vitamin E using the bain-marie method to obtain an ointment form. The composition of the ointments included 79 % olive oil, 10 % herbal extract herbal extract (*Punica granatum* and *Matricaria chamomilla*), 10 % beeswax, and 1 % vitamin E.

Although it varies depending on the specific formulations and the type of extract used, the inclusion of herbal extracts in oil-based semi-solid topical formulations is a frequently reported approach in the literature [7]. The ointments were applied to the PG, MC, and PG + MC groups three times a day for 10 d, and the wounds were left open without any dressing. The rats in the OBEG group were administered a mixture of olive oil, beeswax, and vitamin E, without any herbal extract. The wounds of the rats in the CG group were cleaned three times d with 0.9 % NaCl solution, whereas the rats in the HCG group received no intervention.

Macroscopic wound evaluation

The area and perimeter of the wounds were measured using Image J software from photographs taken on d 0, 3, 7, and 10. Using the wound area and perimeter, values such as the wound healing rate coefficient (WHRC), wound closure percentage

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(WCP), and wound perimeter change percentage (WPCP) were obtained and evaluated according to the formulas in TABLE I. The WCP and WHRC formulas used in this study represent simplified and more understandable adaptations of the wound healing assessment formulas described by Cukjati *et al.* [8]. The WPCP formula has not been found exactly as such in the literature by us and has not been derived from any source; it was developed for this study as a criterion for evaluating wound closure using simple mathematical calculations.

TABLE I
FORMULAS AND ABBREVIATIONS USED IN THE CALCULATION OF WOUND HEALING RATE COEFFICIENT, WOUND CLOSURE PERCENTAGE, AND WOUND PERIMETER CHANGE PERCENTAGE

	Formula	Explanation of the abbreviations in the formula
WHRC	$(A_0-A_t)/t$	A_0: Area of wounds on the first day of assessment A_t: The area of the wound after a certain number of days t: Number of days passed
WCP	$100-((A_t \times 100)/A_0)$	A_0: Area of wounds on the first day of assessment A_t: The area of the wound after a certain number of days
WPCP	$((P_0-P_t)/ P_0) \times 100$	P_0: The perimeter of the wounds on the initial assessment day P_t: Perimeter of the wound on a specific day

WHRC: Wound healing rate coefficient, WCP: Wound closure percentage, WPCP: Wound perimeter change percentage)

Histopathological evaluation

At the end of the study (d 10), the rats were euthanized by decapitation under anesthesia. For histopathological examination, skin tissue samples were fixed in 10 % neutral formalin and subjected to routine tissue processing. Subsequently, 5 µm sections were taken from the tissues embedded in paraffin blocks using a microtome (HM325, Thermo Scientific, Waltham, MA, USA), stained with hematoxylin and eosin, and prepared for examination. The preparations were examined under a light microscope (Leica Microsystems, Wetzlar, DM 750, Germany), and photographs were taken. Using a modified version of the method by Greenhalgh *et al.* [9], 10 randomLy selected areas along the wound line were examined under a 40x objective, and scored on a scale from 1 to 15 [10].

Areas containing a small number of inflammatory cells and granulation tissue were scored between 1 and 3; areas generally containing a high density of inflammatory cells, a small number of fibroblasts, blood vessels, and collagen fibers, and immature connective tissue were scored between 4 and 6. Areas with a high density of fibroblasts and collagen fibers, thick connective tissue growth, increased blood vessels, the beginning of epithelial formation, and a small number of inflammatory cells were scored between 7 and 9.

Areas consisting of fibroblasts and dense collagen fibers, vascular granulation tissue, and an epithelial layer partially and/or completely covering it were scored between 10 and 12. Scores between 13 and 15 were given to areas with well-developed epithelial tissue covering a mature connective tissue layer, and a reduced number of fibroblasts and blood vessels.

Determination of oxidative stress parameters

At the end of the experiment, all blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Plasma was collected by centrifuging by refrigerated centrifuge (NF NUVE NF800R, Türkiye) blood samples in EDTA tubes at 1630 G for 15 min. Malondialdehyde (MDA), a marker of lipid peroxidation, was measured in plasma.

For the measurement of Glutathione (GSH) and Glutathione Peroxidase (GSH-Px), whole blood was used. Blood samples with EDTA plasma separation were washed three times in saline (0.9 % NaCl). Then, erythrocyte Catalase (CAT) and Superoxide Dismutase (SOD) activities were assessed. All measurements were performed in duplicate.

Malondialdehyde levels, which are used to indicate damage to cell membranes and are the end product of lipid peroxidation, were measured in plasma using the spectrophotometric method (Thermo Scientific Genesys 10S UV-VIS, USA) established. This method is based on the spectrophotometric determination of the pink-colored complex formed by the reaction between MDA and thiobarbituric acid (TBA). 0.67 g of TBA was dissolved in 80 mL of 10 % perchloric acid and diluted to 100 mL with distilled water to prepare the TBA solution.

After preparing a 10 % trichloroacetic acid (TCA) solution, 3 parts TCA solution were mixed with 1 part TBA solution. Before analyzing the samples, a blank tube was prepared to detect any decrease in absorbance that might be caused by substances that could absorb at 532 nm. 0.25 mL of physiological saline was added to the blank tube, and 0.25 mL of plasma was added to the sample tubes. 2.25 mL of color reagent (TBA and 10 % TCA) was added to the test tubes. The tubes were sealed, mixed, and incubated at 100 °C for 20 min. After cooling under tap water, the tubes were centrifuged at 850 G for 10 min at + 4 °C, and the resulting supernatant was carefully collected using a pipette and its absorbance was read at 532 nm [11].

Glutathione levels, an important antioxidant, were determined by spectrophotometric detection of the yellow color resulting from the reaction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) with sulfhydryl groups in homogenates. A precipitating solution was prepared by dissolving 1.67 g of glacial metaphosphoric acid, 0.20 g of disodium EDTA, and 30 g of NaCl in 100 mL of distilled water. 42.60 g of Na2HPO4 was weighed and diluted to 1 L with distilled water to prepare 0.3 M Na2HPO4. 20 mg of DTNB was diluted to 100 mL with 1 % Na-citrate solution to prepare the DTNB solution (Ellman Reagent).

One (1 mL of distilled water was added to the blank tube, and 1 mL of blood samples was added to the sample tubes. 1.5 mL of precipitating solution was added to all tubes and centrifuged at 850 G for 30 min at + 4 °C. 0.25 mL of the filtrate from the top of the tubes was taken and transferred to clean tubes. 1 mL of 0.3 M Na2HPO4 and 0.125 mL of Elman's reagent were added. The test tubes were then mixed and read against a blank at a wavelength of 412 nm [12].

Catalase activity, which prevents cellular damage by breaking down hydrogen peroxide (H_2O_2) into water and oxygen, was determined by measuring the rate of this breakdown. A 30 mM H2O2 solution was prepared by diluting 0.34 mL of 30 % H2O2 to 100 mL with phosphate buffer. 8.90 g of Na2HPO4 (2H2O) and 6.81 g of KH2PO4 were weighed and dissolved in 1 L of distilled water.

The buffer was prepared by adding KH2PO4 to the Na2HPO4 (2H2O) solution at pH 7.0. Then, 2 mL of sample and 1 mL of phosphate buffer were added to a blank tube, and the instrument was zeroed relative to the blank. After adding 2 mL of sample and 1 mL of H2O2 solution to the sample tubes, the difference in absorbance at 0 and 30 seconds was determined [13].

Glutathione peroxidase activity was determined based on the enzyme's ability to convert reduced GSH to oxidized glutathione (GSSG) while reducing peroxides. The resulting GSSG was then reduced back to GSH via glutathione reductase (GR), consuming NADPH. The decrease in NADPH concentration was monitored spectrophotometrically to calculate GSH-Px activity. Tris buffer was prepared by weighing 121.14 g of Tris, dissolving it in distilled water to adjust the pH to 8.0, then adding 1.68 g of EDTA (5 mM EDTA) and completing the volume to 1 L. A 0.1 M GSH solution was prepared by weighing 0.31 g of GSH and dissolving it in 10 mL of distilled water.

A GR solution was prepared by dissolving 10 units of GR in each 1 mL of solution; 7.22 µl of GR was dissolved in 1 mL of distilled water. A 2 mM NADPH solution was prepared by dissolving 0.07 g of NADPH in 30 mL of distilled water. 7 mM t-BOOH was prepared by dissolving 10 µL of t-BOOH in 10 mL of distilled water. 100 µL of 1 M Tris-HCl buffer, 20 µL of 0.1 M GSH, 100 µL of 10 Units of GR, 100 µL of 2 mM NADPH, and 670 µL of distilled water were added to the blank tube, mixed, and pre-incubated at 37 °C for 10 min.

The spectrophotometer was zeroed against the prepared blank at a wavelength of 340 nm. 100 µL of 1 M Tris-HCl buffer, 20 µL of 0.1 M GSH, 100 µL of 10 Units of GR, 100 µL of 2 mM NADPH, 660 µL of distilled water, and 10 µL of sample were added to the sample tubes. The sample tubes were mixed and pre-incubated at 37 °C for 10 min. 10 µL of 7 mM t-BOOH was added to the tubes. The decrease in optical density at 0 and 2.5 min at a wavelength of 340 nm was determined [14].

Superoxide dismutase activity was determined based on the principle of converting superoxide radicals into the less harmful hydrogen peroxide. The removal of superoxide radicals in the presence of SOD inhibited the reduction of nitroblue tetrazolium (NBT). SOD activity was calculated based on the degree of inhibition in NBT reduction. A reaction solution was prepared by mixing 0.3 mM xanthine, 0.6 mM Na2EDTA, 0.15 mM NBT, 400 mM Na2CO3, and 1 g/L bovine serum albumin (BSA). Then, solutions of 167 U/L xanthine oxidase, 2 M (NH4)2SO4, and 0.8 mM CuCl2 were prepared.

Erythrocyte hemolysate, diluted 1/5, was mixed with an equal volume of chloroform/ethanol (3/5-V/V) and centrifuged at 2980 G for 45 min at + 4 °C. The clear supernatant was separated for SOD activity determination. Then, 0.1 mL supernatant, 2.85 mL reaction solution, and 0.05 mL xanthine oxidase were added to the sample tubes. In the blank tube, the same amount of deionized water was added in place of the supernatant. All samples were incubated at 25 °C for 20 min. CuCl2 (1 mL) was added to all tubes to stop the reaction. The absorbances of the samples and the blank against the reaction solution were measured at 560 nm using a spectrophotometer [15].

Statistical analysis

The data from the study were analyzed using the SPSS 22.0 software package. The normality of the data distribution was assessed based on histogram, coefficient of variation, skewness, and kurtosis values, Detrended Normal Q-Q Plot graphs, and Shapiro-Wilk test results. In data showing a normal distribution, the difference between groups was tested using one-way analysis of variance (One-Way ANOVA). When a significant difference was detected, the Tukey HSD test (if variances were homogeneous) and the Tamhane test (if variances were not homogeneous) were applied to determine between which groups the difference occurred.

For data not following a normal distribution, the difference between groups was analyzed using the Kruskal-Wallis test. When a significant difference was found, the Mann-Whitney U test was used for pairwise comparisons. The assumption of sphericity for macroscopic parameters related to wound healing was tested using Mauchly's test. Changes on days 0, 3, 7, and 10 were evaluated with Repeated Measures ANOVA and the Bonferroni post hoc test if the assumption of sphericity was met, and with a Greenhouse-Geisser correction of the Repeated Measures ANOVA and the Bonferroni test if the assumption of sphericity was not met. Numerical variables are presented as mean ± standard error, and the statistical significance level was accepted as $P < 0.05$ [16].

RESULTS AND DISCUSSION

The dermal injuries, which account for 4 % of global healthcare expenditures, heal through a biological process that involves stages such as hemostasis, inflammation, proliferation, and remodeling [1, 2, 17, 18, 19, 20, 21]. For faster and healthier wound healing, it is crucial that these stages occur in an appropriate environment [22]. Herbal raw materials can be used today in the treatment of medical conditions, including wound healing [3, 23]. *Matricaria chamomilla* L. (chamomile) or *Matricaria recutita* L. (German chamomile) has been used since ancient times as a medicinal plant for gastrointestinal disorders and skin conditions [24].

Scientific evidence explaining or demonstrating the effects of *Matricaria chamomilla*, a plant frequently used in Germany for the treatment of skin disorders, on wound healing is quite limited [24, 25, 26, 27, 28]. Punica granatum has been investigated in various studies for its wound healing potential, and different results have been reported depending on the concentrations used [5]. Nevertheless, extracts obtained from the vegetative (roots, stems, leaves) and generative (flowers, fruits) parts of the plant have been reported to exhibit antioxidant, anti-inflammatory, and antibacterial activities due to the presence of various phytochemical compounds [29, 30], and consequently, they are thought to potentially have beneficial effects on wound healing.

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Macroscopic findings

The statistical analysis results of within-group (time-dependent) and between-group (at the same time point) differences for the WHRC, WCP, and WPCP are presented in TABLE II.

TABLE II
STATISTICAL ANALYSIS OF WOUND HEALING RATE COEFFİCİENT, WOUND CLOSURE PERCENTAGE, AND WOUND PERIPHERY CHANGE PERCENTAGE VALUES ACCORDING TO GROUPS

	CG	PG	MC	PG + MC	OBEG	P
WHRC						
0-3. days	6.58 ± 1.04^{A}	3.32 ± 0.79	5.46 ± 1.20	3.68 ± 0.54	4.33 ± 0.86	0.097
3-7. days	2.76 ± 0.41^{B}	4.21 ± 0.76	3.80 ± 0.62	4.20 ± 0.75	2.95 ± 1.42	0.346
7-10. days	4.21 ± 0.48^{AB}	6.06 ± 0.50	4.31 ± 0.52	5.28 ± 0.84	5.10 ± 0.42	0.137
P	**0.012**	0.075	0.487	0.409	0.177	
WCP						
3. day	42.18 ± 4.83 ^{Aa}	19.80 ± 3.88^{Ab}	35.59 ± 6.97 ^{Aab}	24.17 ± 2.67 ^{Aab}	30.55 ± 5.43 ^{Aab}	**0.024**
7. day	67.52 ± 3.86^{B}	55.01 ± 5.53^{B}	69.62 ± 2.97 ^{B}	61.49 ± 6.72 ^{B}	60.15 ± 8.11 ^{B}	0.192
10. day	96.48 ± 0.65 ^{Cab}	93.63 ± 1.96^{Ca}	98.30 ± 0.45^{Cb}	97.60 ± 0.54 ^{Cab}	97.63 ± 0.51 ^{Cab}	**0.030**
P	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	
WPCP						
0-3. days	20.71 ± 4.41^{A}	11.25 ± 2.11^{A}	22.16 ± 3.73^{A}	14.09 ± 2.79 ^{A}	18.64 ± 2.36^{A}	0.114
3-7. days	26.94 ± 4.43^{A}	26.15 ± 4.89^{A}	29.90 ± 2.40^{A}	28.14 ± 5.80^{A}	23.66 ± 4.01^{A}	0.897
7-10. days	66.26 ± 3.38^{B}	63.30 ± 3.50^{B}	76.80 ± 3.79^{B}	73.83 ± 3.17^{B}	73.64 ± 3.53^{B}	0.053
0-10. days	80.89 ± 1.88^{Cab}	75.32 ± 3.88^{Ca}	87.41 ± 2.00^{Cb}	83.75 ± 2.46^{Cab}	84.01 ± 2.06^{Cab}	**0.035**
P	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	

WHRC: Wound Healing Rate Coefficient, WCP: Wound Closure Percentage, WPCP: Wound Periphery Change Percentage. Uppercase letters show the changes over time within each group. Lowercase letters show the differences between groups at each time point

When WHRC data within the same time period were evaluated, no statistically significant difference was found between the groups. Although not statistically significant, the WHRC value was found to be higher in the CG and MC groups between d 0-3, and in the PG and PG + MC groups between d 3-7 and 7-10.

At the end of the 10th d, it was observed that the mean values of the MC group was statistically significantly higher than the other groups in terms of WCP and WPCP ($P < 0.05$). When the mean WCP and WPCP values of the PG group were compared with those of the other groups, they were found to be statistically significantly lower, making it the group with the least improvement in terms of macroscopic findings. It was determined that the WCP and WPCP averages of the rats in the OBEG group were statistically significantly lower than those in the MC group and statistically significantly higher than those in the PG group (FIG.2).

When the intra-group time-dependent relationship of WHRC data was evaluated, it was determined that there was no statistically significant difference in any group except the CG group. It was determined that the WHRC averages of the rats in the CG group were statistically significantly higher between d 0 and 3 compared to the other d. When the intra-group time-dependent relationship of WCP data was evaluated, a statistically significant difference was observed in all groups on d 3, 7 and 10. When the intra-group time-dependent relationship of WPCP data was evaluated, it was determined that there was no statistical difference between d 0-3 and 3-7 in all groups, but both of these periods were statistically significantly lower than d 7-10.

FIGURE 2. Macroscopic view of wounds in rats in all groups on d 0, 3rd, 7th and 10th of the study: CG group (a), PG group (b), MC group (c), PG + MC group (d), OBEG group (e)

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Nayak *et al*. [24], in their study using three different wound models (excision, incision, and dead space) and administering *Matricaria recutita* aqueous extract in the drinking water of rats, reported that the wound closure rate was significantly higher compared to the control group. Niknam *et al*. [5], in their study investigating the effects of ointments containing different concentrations of *Punica granatum* L. (pomegranate) and *Matricaria chamomilla* L. (chamomile) extracts, as well as their combinations, on wound healing, reported that the wound closure rates in all treatment groups were significantly higher compared to the placebo group.

In the same study, it was reported that increasing the concentration of the active component enhanced the wound closure rate of *Matricaria chamomilla* L., whereas the wound closure rate of *Punica granatum* L. decreased. Niknam *et al*. [5] reported that, at d 14, the highest wound closure rate was observed in the group treated with 10 % *Matricaria chamomilla*, followed by the group treated with a combination of pomegranate and chamomile.

In this study, the observed decrease in wound closure rate with increasing concentrations of Punica granatum may be a result of dose-dependent biological effects. At low concentrations, the tannins present in *Punica granatum* may accelerate wound healing through their antioxidant and antimicrobial activities, whereas at higher concentrations, they may interact with cellular proteins, suppress fibroblast proliferation, and slow down epithelialization.

Similar to the findings of Niknam *et al.* [5], the decrease in wound closure rate observed with the ointment prepared from 10 % *Punica granatum* L. (pomegranate) extract in this study suggests that increasing the concentration of this plant may negatively affect wound healing.

Jarrahi [31] reported that Matricaria chamomilla accelerates wound healing and shows a promising potential for the treatment of burn injuries. In their study [31], the wound healing percentage in the group treated with *Matricaria chamomilla* exceeded 90 % on day 55, whereas in the present study, the WCP and WPCP averages in the MC group of rats exceeded 90 % by day 10. The difference between these two studies is thought to be attributable to protein denaturation in burn wounds, which may prolong the healing process.

Histopathological findings

The histopathological evaluation in this study determined that the HCG group showed a statistically significant difference compared to all other groups. Analysis of the mean histopathological scores revealed that the CG group exhibited lower values compared to the PG and MC groups, and higher values relative to the PG + MC group; however, these differences were not statistically significant (TABLE III, FIG. 3).

TABLE III
STATISTICAL ANALYSIS OF HISTOPATHOLOGICAL SCORES ACCORDING TO GROUPS

	HCG	CG	PG	MC	PG + MC	OBEG	P
Histopathological scores	15.00 ± 0.00[a]	10.38 ± 0.31[b]	11.33 ± 0.52[b]	10.99 ± 0.48[b]	10.27 ± 0.70[b]	10.72 ± 0.44[b]	< 0.05

FIGURE 3. Normal histological appearance of the skin, 40x, HxE (a, HCG group); increased connective tissue in the dermis and mild neovascularization, 100x, HxE (b, CG group); acanthosis in the epidermis, 200x, HxE (c, MC group); hair follicle formation in areas close to the hypodermis, 100x, HxE (d, PG group); single-layer epithelium formation in the wound area and increased connective tissue in the dermis, 100x, HxE (e, PG + MC group); healing observed at the wound line, 100x, HxE (f, OBEG group).

In the study by do Nascimento *et al.* [21] investigating the effects of specially designed bio-interactive membranes containing *Punica granatum* L. extract on wound healing, it was reported that these membranes were significantly more effective than standard gelatin-based membranes in terms of wound shrinkage, granulation tissue formation, collagen deposition and organization. This suggests that the tannins and phenolic compounds in Punica granatum may increase fibroblast proliferation through their antioxidant effect.

Nayak *et al*. [24] reported that the wound epithelialization rate was significantly higher in rats given the extract compared to the control group, in their study in which they created three different wound models (excision, incision and dead space) in rats and investigated the effect of adding *Matricaria recutita* aqueous extract to their drinking water on wound healing. Niknam *et al.* [5], in their study investigating the effects of ointments containing different concentrations of *Punica granatum* L. and *Matricaria chamomilla* L. extracts and their combinations on wound healing, reported that the epithelial volume, myofibroblast/fibroblast number and collagen fiber content in all treatment groups of rats were significantly higher than in the placebo group. Although no significant difference in epithelial volume was observed between the treatment groups, higher epithelial volumes were recorded in the groups treated with 5 % pomegranate, 5 % chamomile and 2.5 % pomegranate.

In addition, the highest collagen fiber content was reported in the group treated with 10 % *Matricaria chamomilla*. In the study conducted by Lukiswanto *et al.* [32], the effectiveness of *Punica granatum* on the healing of burn wounds was examined histopathologically; in the group treated with 10 % *Punica granatum* extract, regular and dense collagen deposition, complete and mature epithelialization, low inflammatory cell infiltration, and appropriate levels of angiogenesis were observed, while in the other treatment groups, these parameters were weaker or immature.

In this study histopathological evaluations revealed no statistically significant differences between the groups. However, wound tissue in rats in the PG and MC groups showed more mature epithelialization and regular collagen deposition compared to other groups, with scores closer to the HCG group. Although not statistically significant, these findings indicate that both herbal extracts histopathologically support wound healing compared to other groups.

Biochemical findings

When oxidative stress parameters were evaluated, it was found that in rats in the CG group, MDA levels were increased, while GSH levels and CAT and GSH-Px activities were decreased compared to the HCG group ($P < 0.05$ and $P < 0.001$). In rats in the PG, MC, and PG+MC groups, MDA levels were decreased, while GSH levels and CAT and GSH-Px activities were increased. Furthermore, while MDA levels and CAT and GSH-Px activities in the PG, MC, and PG + MC groups were similar to the averages of the HCG group, GSH levels were similar to HCG levels in all groups except the PG group. No significant difference was observed between the groups in terms of SOD activity ($P > 0.05$) (TABLE IV).

TABLE VI
STATISTICAL ANALYSIS OF OXIDATIVE STRESS PARAMETERS ACCORDING TO GROUPS

	MDA (nmol/mL)	GSH (µmol/mL)	CAT (k/g Hb)	GSH-Px (U/mg Hb)	SOD (U/g Hb)
HCG	7.56 ± 0.19[ab]	39.20 ± 0.32[a]	65.59 ± 1.68[a]	141.70 ± 2.73[a]	54.03 ± 0.90
CG	10.06 ± 0.22[c]	31.18 ± 0.72[c]	45.28 ± 1.82[b]	111.71 ± 3.36[b]	50.84 ± 1.85
PG	8.01 ± 0.28[b]	36.90 ± 0.69[b]	66.05 ± 1.49[a]	141.99 ± 1.67[a]	52.82 ± 1.47
MC	7.64 ± 0.21[ab]	38.64 ± 0.42[ab]	66.11 ± 1.08[a]	138.82 ± 1.25[a]	52.98 ± 1.76
PG+MC	7.06 ± 0.16[a]	40.49 ± 0.53[a]	71.27 ± 1.37[a]	149.06 ± 2.85[a]	53.30 ± 0.53
OBEG	7.68 ± 0.11[ab]	39.64 ± 0.22[a]	65.73 ± 1.19[a]	141.87 ± 2.60[a]	52.22 ± 0.50
P	< 0.001	< 0.05	< 0.001	< 0.001	-

Literature searches revealed no studies that directly evaluated the effects of *Punica granatum* L. and Matricaria chamomilla plants on wound healing through serum oxidative stress parameters. However, in a study by Niknam *et al.* [5], the antioxidant activities of methanol fractions of chamomile and pomegranate flowers were investigated, and it was reported that both plants have strong antioxidant capacity, but pomegranate showed a higher activity compared to chamomile.

In the present study, when serum oxidative stress parameters of rats were measured, a high antioxidant effect was observed in both pomegranate and chamomile groups. Interestingly, in the groups where pomegranate, which has the highest antioxidant capacity in the study by Niknam *et al.* [5], was applied, MDA levels were found to be highest, except for the control group. This may be due to the local application of the ointment prepared from *Punica granatum* and the relatively delayed wound healing in these animals compared to other groups.

CONCLUSION

In this study observed that the 10 % chamomile ointment accelerated wound healing, whereas pomegranate slowed it down. However, when evaluated in conjunction with the existing literature, it is believed that an ointment prepared and applied at lower concentrations of pomegranate enhances wound healing, with its antioxidant capacity playing a particularly significant role in this process. Considering all the data, this study concluded that *Matricaria chamomilla* was more effective in wound healing than the other groups and more suitable for clinical use.

Ethical statement

This study was approved by the Local Ethics Committee on Animal Experiments at Firat University (dated 05.04.2024, Session Number 23589).

Conflicts of Interest

The authors of this article declare that there are no conflicts of interest.

Author Contributions

Conceptualization of the study: EP and AOA; Preparation of herbal extracts and formulation of ointments: NT; Project implementation: EP, AOA, and ASO; Histopathological analyses: OO and MPO; Biochemical analyses: EK; Statistical analysis: EP, HS; Manuscript writing: EP and AOA.

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