Volume: 5 **Issue:** 1
Year: 2024
DOI: 10.53811/ijtcmr.1440406

International Journal of Traditional and Complementary Medicine Research

Publisher
Düzce University

ORIGINAL RESEARCH

Treatment of Burn Wounds with a Chitosan-Based Hydrogel Dressing Containing *Artemisia absinthium* L.: A Comprehensive In Vivo Study

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Received: 20.02.2024 Accepted: 16.04.2024

Abstract

Objective: Wormwood (*Artemisia absinthium* L.) is a valuable medicinal plant that has been used for the wound healing since ancient times. Hydrogel dressings are often preferred for wound care in treatment. In this study, the effects of chitosan-based hydrogel dressings containing wormwood were investigated in rats with a burn wound model for the first time.

Material-Method: Certain phenolic compounds in wormwood extracts were detected by LC-MS/MS and antioxidant activities were calculated using the DPPH. The antibacterial activity of the dressings was tested using the disk diffusion method. Tissues taken from 48 female Sprague-Dawley rats were histopathologically examined at days 3rd and 21st. Skin tissue Il-1α, Il-6, TNF-α and Il-10 levels were measured using ELISA. All data obtained from histopathological examination and cytokine levels were statistically evaluated.

Results: Fifteen phenolic compounds were quantitatively determined in wormwood extracts. The antioxidant activities of high-, medium-, and low-dose wormwood extracts were 91.1% ± 0.054, 89.6% ± 0.012 and 84.1% ± 0.02, respectively. The hydrogel dressings showed no antibacterial activity against *S. aureus* (ATCC 29213) or *P. aeruginosa* (ATCC 27853). Granulation tissue formation, collagen increase, and regular scar appearance were higher in all three wormwood groups. Wound contraction was completed and remodeling phase started at day 21st, especially in the high-dose wormwood treatment group. Tissue cytokine levels were determined in pg/ml in all groups.

Conclusion: It was determined that *A. absinthium* L. can promote wound healing through various mechanisms of action and shows immunomodulatory effects, and is appropriate for use as a wound dressing in the form of a chitosan-based hydrogel.

Keywords: Burn, Chitosan, Hydrogel, Wormwood, Wound Dressing

*This study presented in Konuralp Ulusal Tıp ve Sağlık Bilimlerinde Multidisipliner Çalışma Kongresi, 22-24 December 2023, Online.

INTRODUCTION

Burn injuries are a public health issue that has existed since the beginning of humanity and continues to be a major concern across the world. They originate from varying sources, including friction, cold, heat, radiation, chemical, or electrical[1]. Heat originated thermal burns are the most prevalent form of burn injury that may necessitate treatment in a burn center. They account for approximately 86% of all burn injuries and are caused by fire or flames, hot liquids, or contact with hot objects[2].

Burns are divided into four categories based on the extent of tissue damage. First-degree burns damage only the epidermis, which is the outermost layer of the skin. Second-degree burns are partial-thickness burns that affect the epidermis and underlying dermis to different levels. Third-degree burns are full-thickness injuries that damage the entire dermis. Fourth-degree, deep full-thickness burns are the most severe types of burns that destroy skin, nerves, bones and organs[3,4].

Burn healing is pathophysiologically similar to

cutaneous wound healing in that it involves inflammation, proliferation, and remodeling phases. Although the phases are the same for all wound types, the duration of each phase varies depending on the type of wound[5]. The inflammatory phase comprises homeostasis through clot formation and the initiation of the healing process by immune system activation. The migration of epithelial cells, formation of granulation tissue, angiogenesis, and the beginning of wound contraction occur during the proliferation phase. Lastly, in the remodeling phase, scar formation and tissue strengthening are observed.

Burn care includes multifaceted procedures with several components. The key components of this procedure include wound dressing, infection management, fluid balance, and surgical intervention. Covering the burn area has been practiced since ancient times for a variety of reasons, including prevention of contamination and physical damage, keeping the wound moist and promoting re-epithelialization, avoiding water and heat loss and lowering pain[6,7]. Chitosan, known for its homeostatic characteristics[8], contributes in the wound healing by affecting multiple mechanisms. Various chitosan-based hydrogel dressing formulations have function on different stages of wound healing, encouraging healing or attenuating the impact of negative factors. It has been shown to influence macrophage activity, promote cell proliferation and formation of granulation tissue[9,10].

Artemisia absinthium L. is a bitter-tasting, aromatic perennial bush-like herb that grows 40-120 cm tall and has silver green hairy divided leaves, and small yellow flowers[11]. Because of its unique bitter flavor, it was given the name absinthium in Ancient Greek, which means undrinkable. It is also known as wormwood because it has been used to cure intestinal worms since ancient times[12]. This species has been of great ethnopharmacological interest in several cultures. Its medical usage has been dated back to the Ebers Papyrus (1552 BC) in Egypt[13]. Wormwood is registered in certain pharmacopoeias and monographs, and widely used in folk medicine for different ailments including the gastrointestinal diseases, inflamed wounds[14], diabetes, hypertension, malaria, wounds[15], diarrhea, dysentery, stomachache, bacterial infections, boils, cuts, wound healing, erysipelas[16] and acne vulgaris[17,18].

There are limited studies focusing on the in vivo effects of *A. absinthium* L. on wound healing. Existing investigations on the wound healing activity of *A. absinthium* L. have tended to focus on excisional wound models rather than on experimental burn models. In present paper, we produced a chitosan-based hydrogel dressing containing wormwood extract and studied its effects on an experimental rat burn model. We aimed to develop a practical commercial wound dressing for burn wounds based on the outcomes of this study.

MATERIALS AND METHODS

Plant materials and extraction

Wormwood saplings were purchased from a local medicinal plant garden in Yalova, Turkey. The purchased plant was identified by a taxonomist in the herbarium of Düzce University, Turkey. Fresh leaves were harvested, washed with distilled water, and dried at room temperature. The dried leaves were converted into a fine powder using a mechanical grinder and stored in an airtight bag. Powdered plant materials were extracted in ethanol %70 respectively 20, 50, and 100 g/500 ml using a magnetic stirrer for 12 h at room temperature. All the samples were centrifuged, filtered, and removed solvents using a rotary evaporator at 60°C. The dried extracts were stored at +4˚C prior to use.

Quantitative analysis of phenolic compounds by LC-MS/MS

The hydroethanolic extract of wormwood was analyzed for 26 phenolic compounds using liquid chromatography-tandem mass spectrometry (LC-MS/MS; Shimadzu 8030). An Inertsil ODS-4 (2.1x50 mm, 2µm) column was used for chromatographic analysis. The column oven temperature was set at 40°C during the analysis. A binary gradient system was used with mobile phase A (ultrapure water + 0.1% formic acid) and mobile phase B (methanol + 0.1% formic acid), adjusted to 0-95% B (0-4 mins), 95% (4-7 mins), and 5% (7.01-12 mins). The flow rate was set at 0.400 ml/min and the injection volume was 10 µl. Calibration curves were drawn using at least four concentration points in triplicates for each standard. The extract containing 10 mg/100 ml of wormwood was passed through a 0.22 µm single-use filter and analyzed.

Determination of antioxidant activity

The antioxidant activity of wormwood hydroethanolic extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl; $C_{18}H_{12}N_5O_6$) free-radical scavenging method. It is based on mixing 1.8 ml of 0.1 mM DPPH solution prepared in ethanol with 0.2 ml of extract and incubating in the dark for 30 min. After incubation, the

absorbance was read using a spectrophotometer against a blank at 517 nm. Low, medium, and high doses (2%, 5%, and 10%) of wormwood extract were measured three times each. The antioxidant activity was calculated using the equation given below (Equation 1), using the average absorbance values obtained from each dose.

Antioxidant Activity % = 100 × $A_0 - A_s$ / A_0 (Eq. 1)

(A_0: Blank absorbance, As: Sample absorbance)

Preparation of chitosan-based hydrogels

Hydrogels were synthesized using low molecular weight chitosan (molecular weight 50-190 KDa, Sigma Aldrich). Chitosan with a final concentration of 2% (w/v) was stirred in a 1% acetic acid solution for approximately 4 h at room temperature until completely dissolved. Gelatin (240 bloom) was dissolved in distilled water at 40°C to a final concentration of 1% (w/v). Equal volumes (245 ml) of chitosan and gelatin solutions were added to the dry wormwood extract. Glycerol (5 ml) was used as the plasticizing agent and 2% glutaraldehyde (5 ml) was used for cross-linking. A total of 500 ml of mixture was cross-linked for 6 hours at room temperature by stirring. After cross-linking, prepared hydrogels were transferred to a Petri dish with a thickness of 6-10 mm and matured at +4˚C for 12 hours.

Testing antimicrobial activity

The antimicrobial activities of the hydrogels were tested against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) strains using the disk diffusion method. 6 mm diameter wells were opened on Mueller-Hinton agar with an agar driller. Bacterial suspensions fixed to 0.5 McFarland standard were spread on agar Petri dishes with a swab. Hydrogels containing three different doses of wormwood and the vehicle chitosan hydrogel were loaded into the wells. Hydrogels loaded into the wells were tested in liquid form, skipping the maturation step at +4˚C. For comparison, commercial antibiotic discs containing vancomycin (30 µg) and meropenem (10 µg) were used for *S. aureus* and *P. aeruginosa*, respectively. The prepared agar disks were incubated at 37˚C for 18-24 hours.

Burn model in rats

The animals included in the study were obtained from the Experimental Animals Application and Research Center of Düzce University (Düzce, Turkey), with permission from the local ethics committee. A randomized experimental design was carried out in six groups of eight animals each, with a total of 48 female 20-24 weeks old Sprague Dawley rats. The animals were weighed prior to anesthesia (ketamine 90 mg/kg, xylazine 10 mg/kg intraperitoneal injection), and the back of each rat was shaved once sedated. Each animal was injected with 2 ml of intraperitoneal saline to prevent dehydration. After antisepsis with povidone-iodine, a burn wound model was made with an iron seal measuring 2 cm × 2 cm on the backs of the animals. To create a third-degree full-thickness burn, the iron seal was exposed to open fire for 40 s and applied to the back of the animal for 20 s under mild pressure. The temperature of the iron seal was fixed at 150°C in each application and controlled by an infrared thermometer. The experimental groups were as follows:

1. Positive Control Group: Silverdin cream containing 1% silver sulfadiazine was applied topically to the animals once a day after the burn model.

2. Negative Control Group: The animals were modeled for burns, but no treatment was applied.

3. Vehicle Group: After burn model was created, the chitosan-based hydrogel without wormwood extract was applied topically once a day.

4. Low-Dose Wormwood Group: 2% wormwood extract in a chitosan-based hydrogel was applied topically once a day.

5. Medium-Dose Wormwood Group: 5% wormwood extract in a chitosan-based hydrogel was applied topically once a day.

6. High-Dose Wormwood Group: 10% wormwood extract in a chitosan-based hydrogel was applied topically once a day.

Histopathological examination

On the third day of the trial, 24 animals were sacrificed to assess acute outcomes, and on the 21st day, the remaining 24 animals were sacrificed to study chronic outcomes. Full-thickness tissue samples were removed from the treated burn areas. The collected tissue samples were divided into two equal parts, and the parts to be used for the analysis of cytokines were stored at -80°C. Tissue samples for histopathological examination were fixed in a 10% formaldehyde solution for at least 48 h. Tissue samples were fixed in paraffin blocks, sliced into 5 µm sections and stained with hematoxylin-eosin. Histopathological slides were examined blindly by a pathologist under light microscope at Düzce University Faculty of Medicine. Granulation tissue, giant cells, collagen increase, loss of skin

appendages, epidermal loss, and deterioration in collagen fibers were evaluated as 0 (Absent) or 1 (Present). Acute inflammation, chronic inflammation, fibrosis, epidermal regeneration, and eosinophil parameters were scored as 0 (None), 1 (Low), 2 (Medium), 3 (High).

Analysis of immunomodulator cytokines

The proinflammatory cytokines interleukin 1 alpha (IL-1α), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and the anti-inflammatory cytokine interleukin 10 (IL-10) were determined by ELISA (ELK Biotechnology, China). Tissue samples stored in a -80°C were brought to room temperature and lysed in 1 ml of 1X PBS solution with a homogenizer for 2 min. The supernatants obtained from centrifuged homogenates were separated for analysis. The ELISA was performed in line with the user manual instructions.

Ethical statement

This research was approved by the Düzce University Local Ethics Committe Presidency On Animal Testings with decisions number 2022/06/08.

Statistical analysis

The tissue cytokine levels analysis was performed using JASP 0.17 software. The Shapiro-Wilk test was used to determine the normal distribution of tissue IL-1α, IL-6, IL-10, and TNF-α levels. The homogeneity of variances was evaluated using Levene's test. The variation of dependent variables according to day and group factors was analyzed using Two-Way ANOVA. Tukey's test was applied for post-hoc analyses that included multiple comparisons, with the findings provided as mean and standard deviation. Results with $p<.05$ were considered statistically significant. Histopathological data were analyzed using the IBM SPSS Statistics version 26.0. Histopathological scores between the experimental groups were analyzed using Fisher's Exact Test with Monte Carlo Simulation. The simulation was repeated 10.000 times with a confidence interval of 99%, and the significance level was set at $p<0.05$.

RESULTS

Quantified phenolic compounds

A total of 26 specific phenolic compounds were quantitatively analyzed in the extract using LC/MS-MS. Of the 26 compounds analyzed, 15 were identified; the remaining 11 compounds could not be detected in the extract. Undetectable phenolic compounds are catechin hydrate, vanillic acid, hydroxybenzoic acid, protocatechic acid, salicylic acid, phlorizin hydrate, 2-hydroxy-1,4-naphthoquinone, naringenin, silymarin, kaempferol, alizarin. The retention times and amounts of detected compounds are listed in Table 1.

Table 1. Name, retention time, and amount of detected compounds

Compounds	RT (min)	Amount (µg/l)
Acetohydroxamic acid	0.406	315.55
Syringic acid	3.001	500.69
Thymoquinone	3.337	762.06
Resveratrol	3.366	717.06
Fumaric acid	0.809	3593.71
Gallic acid	1.278	421.76
Caffeic asit	2.836	120.71
4-hydroxycinnamic acid	3.489	37.31
Oleuropein	3.567	79.82
Myricetin	3.644	35.04
Ellagic acid	3.681	130.1
Quercetin	3.891	268.79
Butein	3.935	196.97
Luteolin	4.069	57.07
Curcumin	4.672	15.09

*RT: Retention time

Antioxidant activity

The highest antioxidant activity for the 0.1 mM DPPH solution was found in the high-dose extract containing 100 mg/ml wormwood as %91.1 ± 0.054. The medium-dose extract containing 50 mg/ml wormwood had an antioxidant activity of %89.6 ± 0.012, while the low-dose extract containing 20 mg/ml wormwood had an antioxidant activity of %84.1 ± 0.02.

Antibacterial activity

Hydrogels containing three dosages of wormwood extract and the vehicle chitosan hydrogel exhibited no antibacterial action against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). The antibacterial activity of the hydrogels was tested using the disk diffusion method, as illustrated in figure 1.

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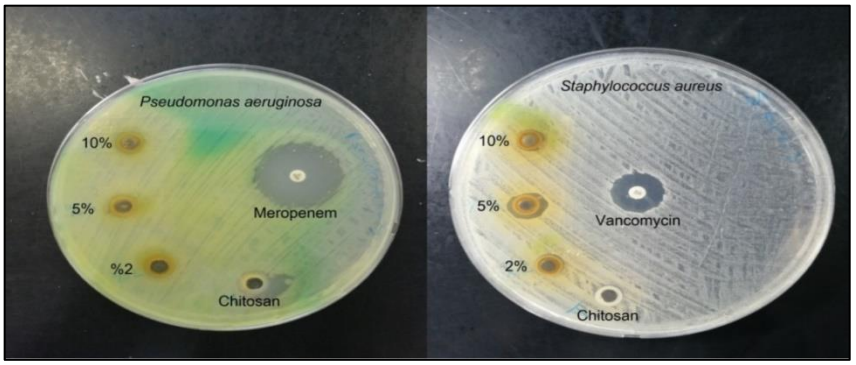

Figure 1. The antibacterial activity of the hydrogels against *S. aureus* and *P. aeurginos*

Histopathological examination

Histopathological changes between the 3rd and 21st days were used to evaluate wound healing. Statistical evaluation of histopathological data using Fisher's Exact Test with Monte Carlo simulation is shown in Table 2.

Table 2. Statistical distribution of histopathological scores in the experimental groups

Parameter	Day	p-value
Acute Inflammation	3rd	0.257
	21st	0.184
Chronic Inflammation	3rd	0.064
	21st	0.276
Fibrosis	3rd	0.131
	21st	**0.018***
Epidermal Regeneration	3rd	0.429
	21st	0.269
Granulation	21st	0.417
Giant Cell	21st	0.362
Loss of Skin Appendages	21st	1.000
Collagen Increase	3rd	0.626
	21st	0.123
Epidermal Loss	3rd	1.000
	21st	0.265
Deterioration in Collagen Fibers	3rd	0.135
	21st	0.097

*No significant findings were obtained from the parameters of acute and chronic inflammation, epidermal regeneration, granulation, giant cells, loss of skin appendages, collagen increase, epidermal loss, and deterioration of collagen fibers on the 3rd and 21st days. The findings of the fibrosis evaluation on the 3rd day were not significant, however the results obtained on the 21st day were. Granulation and giant cells were not seen in the histopathologically analyzed tissues on the 3rd day, and all samples had a loss of skin appendages. In any of the 48 tissues analyzed, no eosinophils were observed.

Results of cytokine levels

The proinflammatory cytokines IL-1α, IL-6, TNF-α, and the anti-inflammatory cytokine IL-10 were determined at pg/ml levels in tissue samples using ELISA. The average amounts of tissue cytokine levels in the groups depending on the day are shown in Table 3.

Table 3. The average amounts of tissue cytokine levels

Group	Day	IL-1α	IL-6	TNF-α	IL-10
Vehicle	3rd	227.8±104.9	375.2±234.9	545.8±229.9	543.0±235.4
	21st	588.6±192.4	844.6±465.2	684.9±42.0	589.7±241.1
Positive Control	3rd	520.0±117	232.1±150.7	503.2±130.9	358.4±102.9
	21st	497.7±155.3	632.7±411.4	681.6±18.1	463.0±195.1
Negative Control	3rd	606.8±93.5	312.8±34.5	507.6±156.5	388.3±131.7
	21st	581.3±38.2	487.2±240.4	610.8±42.2	454.7±118.6
Low-Dose	3rd	295.3±143.4	305.2±152.3	532.4±50.7	273.0±33.6
	21st	531.1±168.0	385.8±263.8	384.1±112.8	462.2±344.9
Medium-Dose	3rd	451.9±308.5	430.8±171.8	547.4±142.4	368.4±53
	21st	447.8±76.3	577.7±256.2	629.9±135.7	385.5±131.7
High-Dose	3rd	341.1±76.1	255.8±46.9	412.4±270.2	476.8±106.8
	21st	376.9±79.0	277.7±81.2	746.6±158.2	721.4±401.1

*Two-way ANOVA was used to analyze changes in cytokine levels based on group and day as the main effects, as well as interactions between them. Statistical significance was set at $p<.05$. The p-values obtained from the Two-Way ANOVA for tissue cytokine levels are shown in Table 4.

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International Journal of Traditional and Complementary Medicine Research

Publisher
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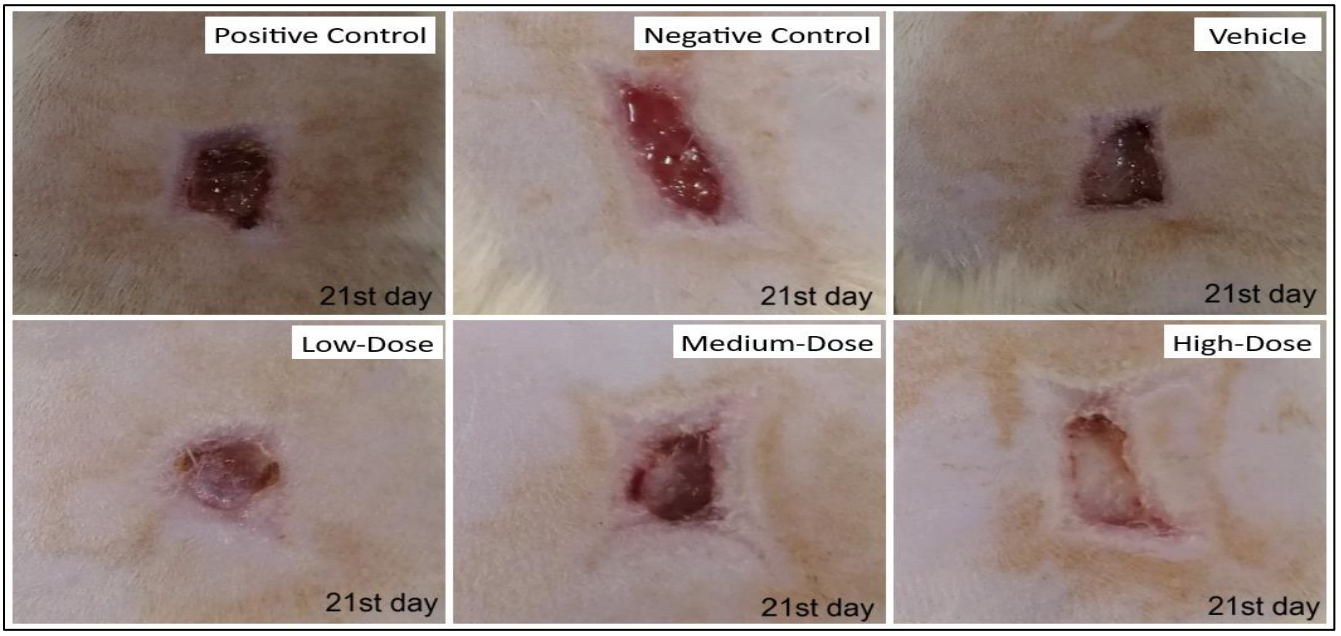

Figure 2. A comparative pictures of scar tissues between experimental groups
*Granulation tissue formation persists in the positive control, negative control, and vehicle groups. Wound beds continue to exude, are soft and edematous, and scar tissue has not fully developed. Granulation, on the other hand, was completed in all wormwood treatment groups. Scar tissue was developed in varying degrees, particularly in the high-dose wormwood group. Scar tissue has lessened in appearance and turned pale pink, indicating that the remodeling phase has begun.

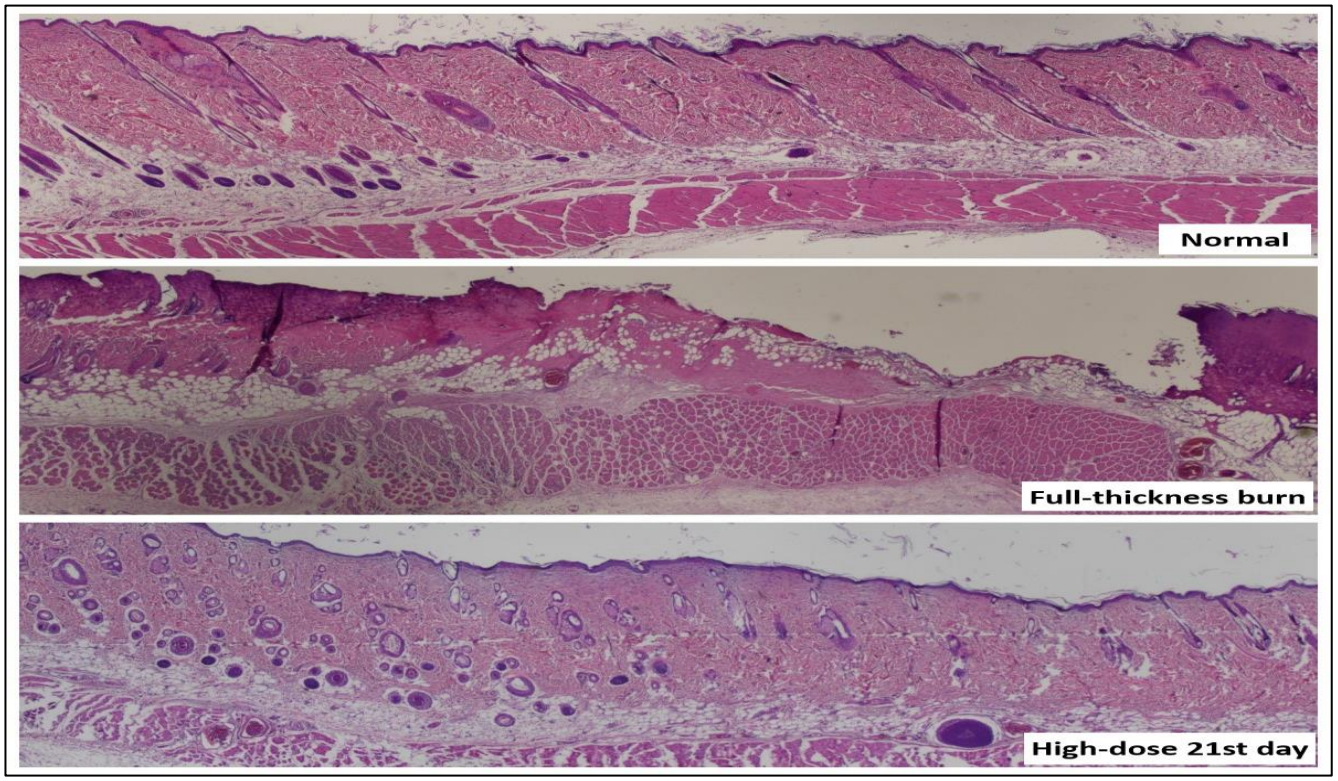

Figure 3. Normal, full-thickness burn and high-dose 21st day rat skin histopathological comparison
*****The epidermis and its outermost section the stratum corneum, dermis, hypodermis, adipose tissue, and muscle tissue are all present in normal rat skin. The appendages of the skin were whole, the pale pink collagen fibers were regular and filamentous, and the vascular systems were intact. The epidermis and skin appendages are destroyed in full-thickness burn. Vascular structures and muscle tissue were discovered to be severely ruptured. The integrity of the dermis, particularly collagen fibers, has deteriorated significantly. There was no acute or chronic inflammation observed in the high-dose wormwood group. Granulation and epidermal regeneration were increased and no epidermal loss was seen. There is also an increase in collagen fibers and blood vessels.

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Table 4. The statistical results of tissue cytokine levels from the Two-Way ANOVA

	IL-1α	IL-6	TNF-α	IL-10
Group	**0.033***	0.113	0.357	0.120
Day	**0.025***	**0.004***	**0.024***	0.068
Group × Day	**0.044***	0.392	0.068	0.866

*The main effects and the interaction of tissue IL-1α levels were statistically significant. While the day's main effects on tissue IL-6 and TNF-α levels were statistically significant, the interactions were not. As the main effect or the interaction, changing in tissue IL-10 levels were not statistically significant.

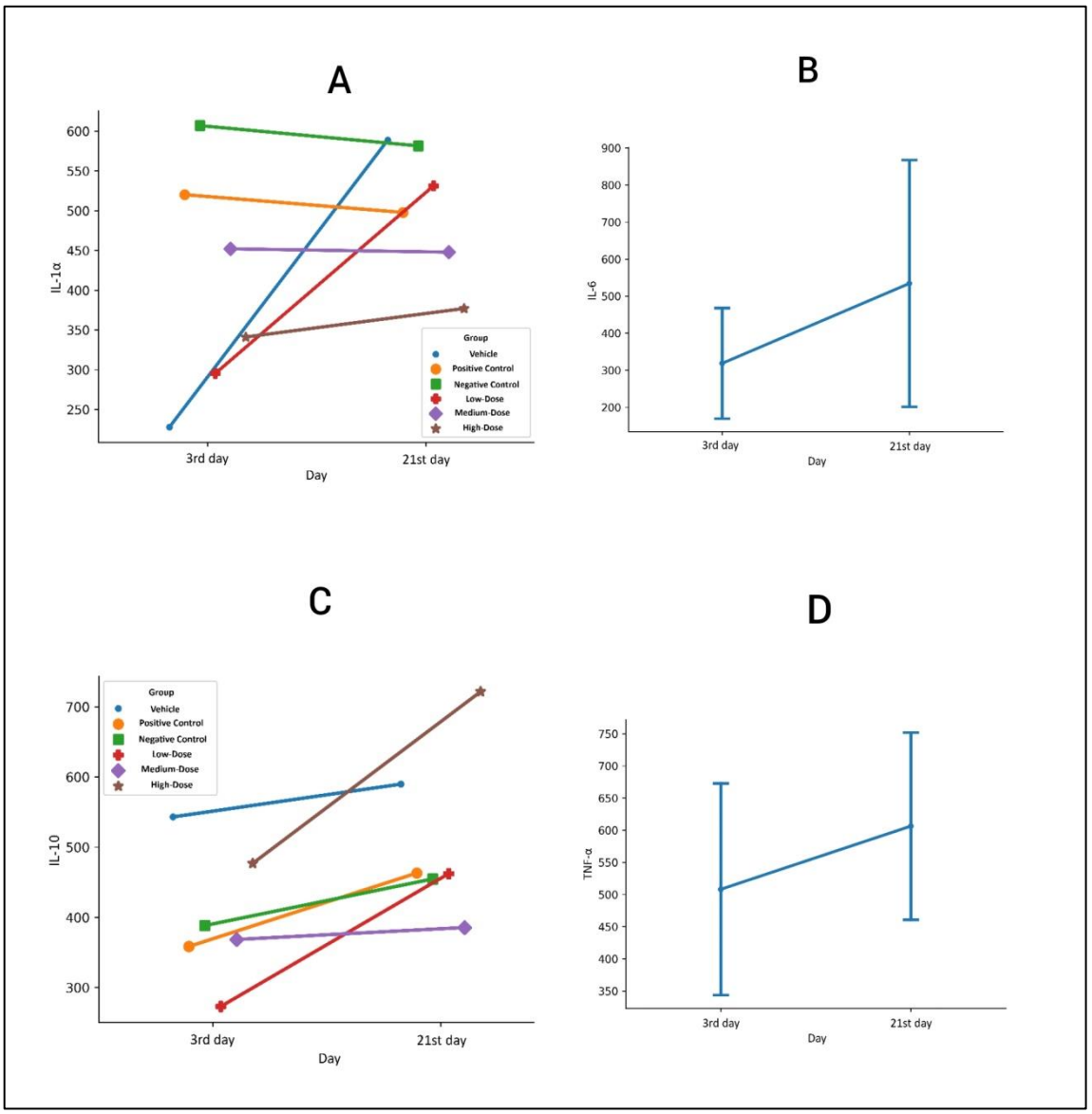

Figure 4. Time-dependent changes in tissue cytokine levels in the groups

***A.** When tissue IL-1α levels in the groups were evaluated, the changes on the 3rd and 21st days in the vehicle (p=0.001) and low-dose treatment groups (p=0.026) were statistically significant. Although not statistically significant, IL-1α levels decreased slightly over time in the positive control, negative control, and medium-dose treatment groups while increasing in the high-dose treatment group. **B.** The day-independent variable had a statistically significant effect on IL-6 levels. Multiple comparisons revealed that tissue IL-6 levels on the 3rd day were 318.702 ± 149.2 pg/ml, according to the post-hoc Tukey's test findings. Tissue IL-6 levels were determined to be 534.3 ± 332.9 pg/ml on the 21st day. **C.** Although not statistically significant, tissue IL-10 levels increased in all groups with various scales. **D.** According to the post-hoc Tukey's test findings of the day variable, tissue TNF-α levels were found to be 606.3 ± 145.3 pg/ml on the 3rd day and 508.1 ± 164.4 pg/ml on the 21st day.

DISCUSSION

Wound healing consists of several repair processes, including inflammation, epithelial regeneration, granulation, neovascularization, and wound contraction. Several new treatment techniques targeting various stages of wound repair have been tested in vitro and in vivo. The usage of plants containing bioactive phytochemicals from diverse chemical families is one of these techniques. Phytochemicals are known to affect repair processes through various mechanisms. These mechanisms include reducing oxidative damage due to their antioxidant and antibacterial effects, regulation of the immune system, and acceleration of tissue repair. Considering all of these, we conducted the first study on the effects of chitosan-based hydrogel containing wormwood on burn wound treatment.

Artemisia absinthium L. has been found in the current study to have strong antioxidant properties and a high phenolic component concentration. It was determined that chitosan-based hydrogels containing the extract of the plant can be synthesized and applied as a functional wound dressing on burn treatment. On the 21st day of treatment, considerable changes in wound appearance were observed in individuals with the similar histopathological evaluations. In the positive control, negative control, and vehicle groups, the wound sites remained edematous and exuded with no scab development. On the other hand granulation and scar tissues developed in the wormwood treatment groups. The color of the scar tissue paled and began to disappear, particularly in the high-dose wormwood group.

Moacã et al. (2019) reported that they detected chlorogenic acid, caffeic acid, p-coumaric acid, isoquercitrin, rutin, quercitrin, luteolin and apigenin in their study in which they analyzed ethanolic wormwood extract by LC-MS[19]. Lee et al. (2013) analyzed the methanolic extract by HPLC and found myricetin, quercetin and kemferol among flavanols; caffeic acid, p-, o-, m-coumaric acid and ferulic acid among hydroxycinnamic acid derivatives; gallic acid, protocatechic acid, β-resorcylic acid, vanillic acid and salicylic acid from hydroxybenzoic acid derivatives; rutin, vanillin, hesperetin, resveratrol, naringenin from other phenolic compounds[20]. Koyuncu (2018) reported that he detected coumarin, p-coumaric acid, caffeic acid, vanillic acid, salicylic acid, quinic acid, ferulic acid, chlorogenic acid, rosmarinic acid, protocatechuic acid, cinnamic acid, sinapinic acid, vanillin, malic acid, syringic acid, hesperetin, naringenin, rutin, quercitrin, apigenin, isoquercitrin, rhoifolin in wormwood aqueous methanol extract by LC-MS/MS[21].

In our study, wormwood extract was prepared by maceration in 70% ethanol and its effect on wound healing was investigated quantitatively and phytochemically using LC/MS-MS. Fifteen of the 26 phenolic compounds investigated in the extract were detected at µg/L level. Detected compounds caffeic acid (120.71 µg/l), 4-hydroxycinnamic acid (37.31 µg/l), syringic acid (500.69 µg/l), acetohydroxamic acid (315.55 µg/l), thymoquinone (762.06 µg/l), resveratrol (717.06 µg/l), curcumin (15.09 µg/l), myricetin (35.04 µg/l), gallic acid (421.76 µg/l), quercetin (268.79 µg/l), oleuropein (79.82 µg/l), butein (196.97 µg/l), fumaric acid (3593.71 µg/l), ellagic acid (130.1 µg/L) and luteolin (57.07 µg/l). When compared with the chromatographic analysis results given above, it was observed that caffeic acid, cinnamic acid, syringic acid, myricetin, gallic acid, quercetin, and luteolin compounds were similar. The phytochemicals detected may vary qualitatively and quantitatively owing to the different solvents, extraction techniques, and devices used in the studies.

Many studies have shown that *A. absinthium* L. has high antioxidant activity because of the polyphenols it contains, and as a result, it is an effective free radical scavenger. It has been suggested that this high antioxidant activity and free radical scavenging ability accelerates wound healing processes[22]. Minda et al. (2022) reported that aqueous ethanolic wormwood extract at a dose of 1 mg/ml showed approximately 87% antioxidant activity for 0.1 mM DPPH[23]. In another study where the extract obtained by a similar method was investigated; Moacã et al. (2019) calculated the amount of wormwood required for the IC_{50} value, which means scavenging half of the free radicals contained in 1 mM DPPH solution, as approximately 0.5 mg/ml[19].

In the study we conducted, high-dose extract containing 100 mg/ml wormwood versus 0.1 mM DPPH solution was 91.1 ± 0.054%; medium-dose extract containing 50 mg/ml wormwood 89.6% ± 0.012; low-dose extract containing 20 mg/ml wormwood showed 84.1 ± 0.02% antioxidant activity. Because of the different amounts of wormwood used in these studies, it is thought that there are different antioxidant activity values for the same amount of DPPH. Additionally, owing to its limited antioxidant capacity, it was concluded that

there is no linear relationship between the amount of wormwood and its antioxidant activity.

Several studies have shown that wormwood essential oils have strong antimicrobial and antifungal effects. However, different results have been reported for extracts prepared using various solvents and extraction methods. Erel et al. (2010) investigated the antimicrobial effects of wormwood methanolic Soxhlet extracts using the disk diffusion method. They reported that the extract did not show activity against *Staphylococcus aureus* (ATCC 6538/P), but showed antimicrobial activity (9 mm inhibition) against *Pseudomonas aeruginosa* (ATCC 27853)[24]. Erdogrul (2002) investigated the antimicrobial effects of wormwood extracts obtained by Soxhlet extraction using ethyl acetate, methanol, acetone, and chloroform on the *Staphylococcus aureus* (ATCC 25923) strain by the disk diffusion method. According to the study results, while no effect was observed in methanol, acetone, and chloroform extracts, antimicrobial activity (14 mm inhibition) was observed in the ethylacetate extract[25]. Hrytsyk et al. (2020) investigated the antimicrobial effects of wormwood, which they extracted with 70% ethanol with the help of fractional maceration, using the disk diffusion method. The study concluded that it showed antimicrobial activity on *Staphylococcus aureus* MS (9.37 ± 0.85 mm inhibition) and *Pseudomonas aeruginosa* (5.33 ± 0.54 mm inhibition)[26].

In our research, hydrogels containing low, medium, and high doses of wormwood extract prepared by simple maceration with 70% ethanol did not show antibacterial effects against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) strains. This may be due to the differences in the strains, tested dose amounts as well as the extraction method. Another plausible explanation is that chitosan-based hydrogels cannot disperse adequately due to their viscous nature.

Proinflammatory cytokines mediate critical cellular responses through biochemical increases and decreases. Sultan et al. (2020) investigated the effects of wormwood methanolic Soxhlet extract in a wound healing study on Wistar rats. They concluded that changes in serum IL-6 and TNF-α levels contributed to the wound healing process as immunomodulators[27].

In our study, tissue IL-1α, IL-6, IL-10, and TNF-α levels were determined at the pg/ml level by ELISA. There is no previous research on the determination of cytokine levels in skin tissue. Tissue IL-1α levels showed a statistically significant increase from day 3 to day 21 in the vehicle and low-dose groups. Although not statistically significant, there was a certain level of increase in the high-dose treatment group. There was a slight decrease in the medium-dose treatment, positive control, and negative control groups, although the difference was not statistically significant. In contrast, although not statistically significant between the groups, tissue IL-6 and IL-10 levels tended to increase over time in all experimental groups. Tissue TNF-α levels decreased over time in the low-dose treatment group but increased significantly in the remaining groups. We also concluded that the highest increase in tissue TNF-α level was observed in the high-dose treatment group. When these results are evaluated, it is thought that wormwood aqueous ethanol extract shows immunomodulatory effects on tissue cytokine levels and contributes positively to the wound healing process.

There are in vitro and in vivo wound healing studies on wormwood used for wound healing purposes in traditional medicine. Minda et al. (2022) investigated the effects of aqueous ethanolic wormwood macerate on the human keratinocyte cell line (HaCaT). According to the results of this study, wormwood stimulates more than 97% of keratinocyte migration and substantially contributes to wound closure[23]. In a study examining the effects of essential oil in the excisional wound model created on Wistar rats; they reported that the group treated with 10% wormwood essential oil improved better than the negative and positive control groups[28]. Another excisional wound study on Wistar rats concluded that 10% Soxhlet extract of wormwood strongly affects granulation and epithelialization formation and accelerates tissue repair[29].

The effects of wormwood on the in vivo burn wound model were investigated for the first time in this study. It was established that wormwood at different doses stimulates wound healing and is suitable for use as a wound dressing in the form of a chitosan-based hydrogel for burn. Wormwood treatment groups, especially high-dose, formed a more consistent scar tissue than the other treatment groups. On the 21st day, the wound had completely contracted, and the scar color began to disappear.

Although macroscopic and histopathological appearance of the treatment groups were superior, the negative control group also healed over time. Larger-scale studies or human trials might provide additional information regarding the efficacy and adverse effects of topical treatments containing

wormwood extract.

CONCLUSION

Burn wounds continue to be a significant public health issue, affecting millions annually with varying degrees of severity. Bioactive molecules contained in medicinal plants contribute to cutaneous wound healing by affecting various mechanisms such as inflammation, re-epithelialization, granulation tissue formation, angiogenesis, and fibrosis. They can help treat persistent burn wounds with secondary infections or when other topical therapies are ineffective or have negative side effects.

Artemisia absinthium L. is quite abundant in phenolic compounds and has a high antioxidant potential. The obtained phytochemicals affect repair processes through various mechanisms. Antioxidant potentials, reduction of oxidative damage, antimicrobial effects, regulation of the immune system, and acceleration of tissue repair can be considered as these mechanisms. The current study can lead detailed research of the biochemical and metabolic pathways accompanying wound healing processes in future studies. The effects of novel bioactive compounds belonging wormwood such as absinthin, anabsinthin, artabsin, and artemisinin on wound healing should be investigated in future studies. According to the results of further research, it will be obtain an important cost-effective wound care product.

Author contributions: Conceptualization: [MAydin, YO, NCC]; Methodology: [MAydin, YO, SKC, MAlpay]; Formal analysis: [MAydin, YO]; Investigation: [MAydin]; Resources: [MAydin]; Data curation: [MAydin, YO, SKC, MAlpay]; Writing-original draft: [MAydin]; Writing-review&editing: [YO]; Supervision: [NCC]; Project administration: [MAydin, NCC]; Funding acqusition: [MAydin, NCC]

Conflict of interest: The authors declare that they have no conflicts of interest.

Funding: This study was supported by research fund of Düzce University (BAP-2021.04.03.1220).

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