Turk J Pharm Sci 2024;21(2):95-103
DOI: 10.4274/tjps.galenos.2023.03746

ORIGINAL ARTICLE

# Determination of the Effects of Ankaferd Wound Dressing on the Wound Healing Process in Rats

Erhan ŞENSOY[1], Eda GÜNEŞ[2]*, Mehmet Okan ERDAL[3]

[1]Karamanoğlu Mehmetbey University Faculty of Medicine, Department of Midwifery, Karaman, Türkiye
[2]Necmettin Erbakan University Faculty of Tourism, Department of Gastronomy and Culinary Arts, Konya, Türkiye
[3]Necmettin Erbakan University Meram Vocational School, Department of Property Protection and Security, Konya, Türkiye

## ABSTRACT

**Objectives:** The effects of a composite nanofiber wound dressing material consisting of a polyvinylidene alcohol and polyvinylidene pyrrolidone polymer mixture with a hemostatic agent doped with Ankaferd Blood Stopper (ABS) on the healing of experimentally induced dermal wounds in rats were examined.

**Materials and Methods:** Rats were divided into 4 groups (n= 6). Histological material was examined on tissues taken from the wound site, whereas total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) analyses were performed on blood samples taken from the cardia. The material that was produced had hydrophilic properties, and both the ABS-doped and-undoped forms of the material positively affected wound healing.

**Results:** In the histopathological examinations, macroscopic evaluations revealed a statistically significant difference between the groups in terms of wound diameter, reepithelialization, and inflammation formation ($p$= 0.019). In parallel with wound healing and histological outcomes, TAS values increased in the ABS-doped groups, and TOS and OSI values decreased in the wound dressing groups ($p < 0.05$).

**Conclusion:** It was concluded that the ABS-dopped dressing did not have a negative effect on wound healing, it accelerated healing, and it could be used effectively and safely to treat skin injuries. However, further studies are needed to evaluate the clinical and histopathological benefits and potential adverse effects of wound dressings produced using ABS-doped polymers on wound healing.

**Keywords:** Ankaferd, nanofiber, wound, rat, oxidative stress index

## INTRODUCTION

A skin wound is an injury that compromises the integrity of the epidermis as a physical barrier, thus disrupting its normal anatomical composition and physiology.[1] Today, millions of people are injured due to various causes, and many hemostatic agents are used to stop bleeding, with different levels of action and duration of stopping bleeding. The Ankaferd Blood Stopper (ABS), which is used after surgery and is effective in many cases such as bleeding gums, is an antibacterial hemostatic agent without additives consisting of various ratios of dried roots and leaves of *Thymus vulgaris L., Glycyrrhiza glabra L., Vitis vinifera L., Alpinia officinarum Hance,* and *Urtica diocia L.,* which are plants that are used in traditional Turkish medicine.[2-4]

In the intermediate recovery step, hemostasis is a natural process consisting of the inflammation, proliferation, and maturation stages.[5,6] In this process, the general characteristics of the injured tissue should be well known, and tissue-specific treatment methods should be applied. The aim of these methods is to create an ideal environment for epithelial formation in the injured area by stimulating the inflammatory cells, platelets, and extracellular matrix involved in wound healing.[5] Wound dressings are among the treatment methods that can create an ideal environment for proliferation by mimicking the extracellular matrix, protect the wound against microorganisms and infections, and contribute to the healing process.

*Correspondence: egunes@erbakan.edu.tr, Phone: +90 506 491 34 80, ORCID-ID: orcid.org/0000-0001-7422-9375
Received: 30.01.2023, Accepted: 13.05.2023

Today, with the discovery of new-generation biopolymers and the development of novel production techniques, modern wound dressings are being produced as an alternative to conventional wound dressings.[7] Polyvinyl alcohol (PVA) and polyvinylidene pyrrolidone (PVP), which are non-toxic, bio-based, renewable, and sustainable polymers, are used in biomedical, pharmaceutical, and regenerative medicine.[8] They can be converted into biocompatible and biodegradable, water-retaining, water-soluble, and nanofiber forms.[9] As natural synthetic polymers, PVA and PVP are easy to process because they have controllable physical properties and mechanical strength, and so, they are widely used for wound dressings.[10-12] Electrospinning, which is a nanofiber production technique, is a preferred method aimed at producing nanodiameter fibers by the electric field effect of polymer-based gels, and it is preferred because fibers that are one hundred times smaller (with an average radius of 10 nm-500 nm) can be produced compared to those produced by the classical method.[13-15] It is stated that the products used in treatments with experimental practices reduce oxidative stress (TOS/TAS = OSI) by increasing the total antioxidant status (TAS).[16,17] OSI analysis is used to determine oxidative stress levels as an easy, precise, automated, and inexpensive method.

Although various polymers and methods are used in nanofiber production, there is no study in the literature in which ABS-doped PVA and PVP polymers were produced by the electrowinning method. The aim of this study was to investigate the healing process and biochemical effects of a medical textile product containing an ABS-added polymer that can stop bleeding and heal wounds quickly in a rat model with experimentally induced wounds. Scanning electron microscopy (SEM) and Fourier transform infrared spectrophotometry (FTIR) analyses were carried out to characterize ABS dopped and non-dopped ABS polymers in the production of the material.

## MATERIALS AND METHODS

The study was initiated by obtaining the approval of the ethics committee of Selçuk University, Experimental Medicine Research and Application Center (decision date: 25.01.2019 and decision number: 2019-2). All chemical materials used in the study were supplied by Sigma and Merck.

*Preparation of the nanofibers*

In this study, nanofibers were prepared to form a homogeneous wound dressing surface and provide a longer-lasting effect by the slow release of the drug additive. In addition, a control group was formed to examine the effects of the nanofiber surface with and without the drug additive and show the effects of the drug. The electrowinning process was performed for nanofiber wound dressing production.[12,18,19] Sterile gauze was used for fiber collection, and two different solutions were used as polymer solutions.[9] First, 10% by mass PVA (Mw: 72.000) in pure water was obtained by stirring at 70 °C for 1 h. Then, 10% PVP (MW: 58.000) in ethanol was prepared using a magnetic stirrer at room temperature for 30 min. These two polymer solutions were mixed at room temperature at a ratio of 3/4 PVA and 1/4 PVP to obtain a carrier polymer solution. 10 mL of the carrier polymer solution was used to make nanofiber without any additive. Taking 10 mL of the same polymeric solution, 1 mL of Ankaferd was added and nanofibers were produced. The unhopped fiber was collected on sterile gauze with a rotary collector at a speed of 400 rpm under the influence of an electric field of 1.7 kV/cm at a flow rate of 1 mL/h. The ABS-doped fiber was collected with a rotary collector at a speed of 400 rpm under an electric field of 1.9 kV/cm at a flow rate of 0.6 mL/h.

*Experiments on material characterization*

The experiments in this context were conducted at Necmettin Erbakan University, Science and Technology Research and Application Center (BITAM).

*SEM micrographs of polymers*

SEM was used to examine the morphology of the produced materials. The surface morphologies of the polymers were examined at magnifications of 1.000 and 5.000.

*FTIR analysis*

FTIR analyses were performed to determine the interaction between the drug mixture and the polymer structure.[20]

*Animal materials and treatments*

Twelve-week-old female Wistar albino rats with an average weight of approximately 295 g were used in this study. Female rats were preferred because the skin of females is thinner than that of males, and the lack of estrogen in males would negatively affect cutaneous wound healing.[21,22] The rats were housed without any water or feed restriction at room temperature in a 24 hours light-dark cycle.[23] The study was planned to include 24 rats, with four groups (n= 6 each) (Table 1).

After the groups were formed, the rats were administered 70 mg/kg ketamine and 10 mg/kg xylazine through the intraperitoneal (*i.p.*) route.[24,25] In each rat whose back area was shaved, a wound with a diameter of 15 mm was created using a biopsy needle. Group I was not treated. Group II received a single dose of local ABS in spray form. ABS-dopped wound dressing was used for group III, non-dopped ABS wound dressing was used for group IV (Figure 1), and the study was completed on day 14.[26]

*Histological evaluation*

The skin tissue samples were embedded in paraffin after routine tissue processing steps, and the samples were fixated in 10% formaldehyde for histological analyses.[27] Sections of paraffin blocks with thicknesses of 6 µm each were stained

**Table 1. Groups**

| Groups (n= 6) |
|---|
| Group I Control |
| Group II ABS local application |
| Group III ABS-doped wound dressing |
| Group IV ABS non-doped wound dressing |

ABS: Ankaferd blood stopper

with hematoxylin-eosin to determine their general histological structures.[28] The sections were examined at 40x magnification under a light microscope equipped with a digital camera (Nikon Eclipse, E-400 equipped with Nikon DS Camera Control Unit DS-L1 with DS Camera Head DS-5M), and digital images of the relevant areas were taken.[29-32]

*Scoring evaluation scale*

Wound scoring was performed as 0 (no inflammation), 1 (mild inflammation), 2 (moderate inflammation), and 3 (severe inflammation). Wound diameter was recorded in millimeters.[25,26] The scoring process was performed according to the evaluation scale shown in Table 2.

*Biochemical analysis*

On the last day of the study, 1.5 mL of blood was intracardially taken from each rat. After the blood samples were centrifuged for 3000 cycles at +4 °C for 10 min, the serum parts were separated. TAS and TOS measurements of sera were performed using commercial kits (Rel Assay Diagnostics). Using a spectrophotometer (Biochrom Libra S22), the absorbances of the samples were measured at 660 nm, and OSI was determined according to the standard reference.[33,34]

*Statistical analysis*

The Statistical Package for the Social Sciences (SPSS, version 21.0, IBM Corporation, Armonk, NY) software was used for all statistical analyses. One-way analysis of variance and the least significant difference method were used for pair-wise comparisons ($p < 0.05$). Statistical analysis was performed on the 14th day in the scoring of wound diameters.

## RESULTS

*Materials and characterization*

*SEM micrographs of polymers*

Using the SEM images, the average fiber diameters were calculated with the help of the "Fiji ImageJ for Windows V 1.8.0" program from National Institutes of Health images, and the plots shown in Figure 3 were obtained. The fiber diameters ranged from 400 nm to 2 µm. The average diameter of the unadulterated fibers was 1.066 µm, and the average diameter of the doped fibers was 0.974 µm. The determined average diameter values were sufficient to provide permeability.[35-37] While a mesh structure was detected at the points where the fibers overlapped on the surfaces of the doped fibers, no droplet or clump-like formation was observed on the surfaces (Figure 2).

*FTIR analysis*

In the FTIR spectra of the PVA-PVP polymer mixture, an O-H stretching vibration peak was observed at approximately 3290 $cm^{-1}$. The spectrum band corresponded to a C-H stress that occurred at a peak of asymmetrical stretching vibration of approximately 2862 $cm^{-1}$.[35,37] PVA and PVP had C=O groups showing a vibration band at about 1716 $cm^{-1}$. The C-N stretching vibration peak of the amine structure was observed at 1242 $cm^{-1}$. The spectrum bands for the C-O groups, which are

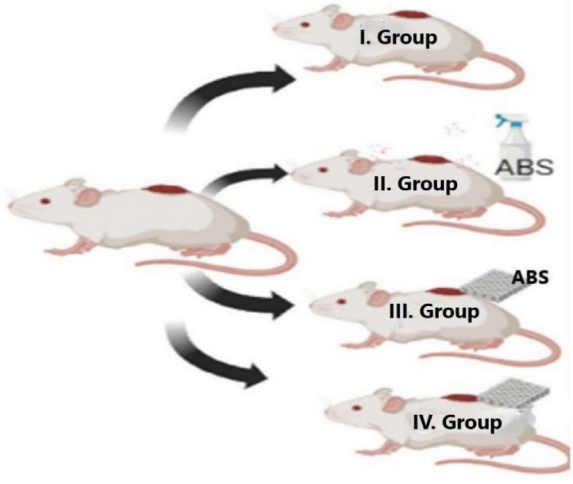


**Figure 1.** Stages of wound formation and treatment in rats
ABS: Ankaferd blood stopper

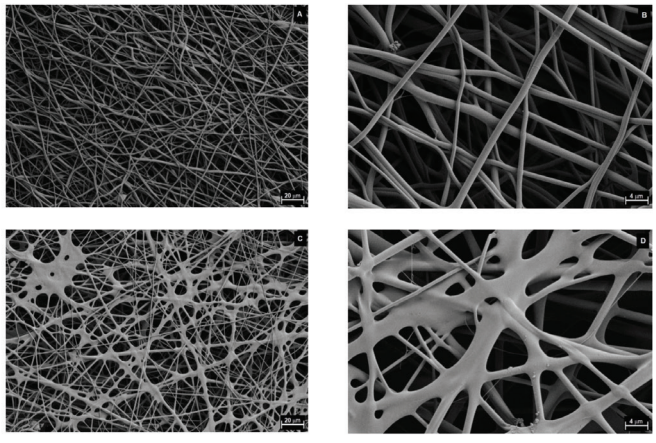

**Figure 2.** SEM micrographs of polymers, A) ABS non-doped (1000 x), B) ABS non-doped (5000 x), C) ABS-doped (1000 x), D) ABS-doped (5000)
SEM: Scanning electron microscopy, ABS: Ankaferd blood stopper

**Table 2. Scoring evaluation scale**

| Ulcer (unit) | Reepithelization (unit) | Inflammation (unit) |
|---|---|---|
| 0: None | 0: None | 0: None |
| 1: At 1/3 of the wound site | 1: At 1/3 of the wound site | 1: Lightweight |
| 2: At 2/3 of the wound site | 2: At 2/3 of the wound site | 2: Medium |
| 3: The entire wound site | 3: The entire wound site | 3: Severe |

acetyl groups, appeared at 1100 $cm^{-1}$. The plane for the C-H bend was outside the rings and formed an absorption band of approximately 720 $cm^{-1}$.[36] In the case of the ABS-dopped fibers, a soft formation of around 1645 $cm^{-1}$ was observed instead of the peak loss at 1716 $cm^{-1}$. Here, the oxygen band in the C=O group was transformed into a C=C stretching vibration by a free reaction. It was concluded that an oxidation reaction occurred by the binding of oxygen atoms in the ABS additive (Figure 4).

*Body weights*

It was determined that there was no statistically significant difference between the mean body weights of the rats in the groups ($p$= 0.643). The statistics of the test were found to be $F_{23}$= 0.497 ($p > 0.05$) (Table 3).

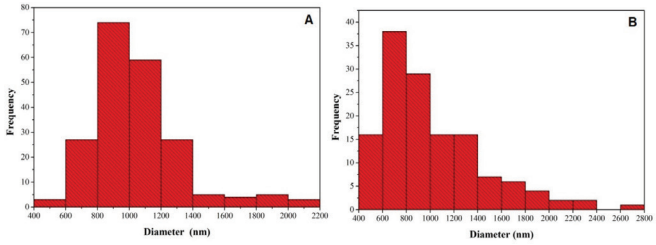


**Figure 3.** A) ABS non-doped and B) ABS-doped wound dressing fiber diameters (nm). Average values of ABS-doped (A) and ABS-undoped (B) fibers made with "Fiji ImageJ Program" depending on the density of the polymer

ABS: Ankaferd blood stopper

*Wound scores*

There was no loss of animals in the groups. In the evaluations of the median scores on the last day of the study, the average wound diameter was found to be reduced by half compared with the first day of the study in the rats in group I, whereas the recovery in group II was more limited. In group III, where the best healing process was observed, almost all wounds were closed, and there was even hair growth in the injured area. It was determined that the recovery rate in group IV was faster than that in groups I and II (Table 4). In the Tukey and Duncan tests, the test statistics were found to be $p$= 0.019, degree of freedom= 23, and F=4.144. When the wound diameters were compared, it was seen that the 1st and 4th groups were nearly similar. It was observed that the wound diameter in the second group did not decrease. It was determined that the wound diameter in the 3rd group decreased statistically significantly (wound diameter: group II > I > V > III; $p < 0.05$).

**Table 3. Average body weights of the rats**

| Groups (n= 6) | Average body weight (gr) ± SD | | |
|---|---|---|---|
| | First day | Day three | Last day |
| Group I | 283 ± 4.60 | 288 ± 4.58 | 270 ± 4.30 |
| Group II | 297 ± 3.34 | 295 ± 1.12 | 295 ± 4.39 |
| Group III | 286 ± 2.24 | 287 ± 5.31 | 267 ± 1.66 |
| Group IV | 292 ± 2.38 | 292 ± 3.39 | 287 ± 6.42 |

SD: Standard deviation

**Table 4. Values obtained from wound scores on different days of the study**

| Group (n= 6) | Day | Ulcer (mm)* | Reepithelization (mm)* | Inflammation (unit)* | Wound diameter (mm) |
|---|---|---|---|---|---|
| Group I | Day 0 | 3 | 0 | 0 | 15 x 15 |
| | Day 3 | 3 | 0 | 0 | 14 x 14 |
| | Day 7 | 2 | 2 | 0 | 10 x 10 |
| | Day 14 | 2 | 2 | 0 | 7 x 7 |
| Group II | Day 0 | 3 | 0 | 0 | 15 x 15 |
| | Day 3 | 3 | 0 | 0 | 12 x 12 |
| | Day 7 | 2 | 1 | 0 | 11 x 11 |
| | Day 14 | 2 | 1 | 0 | 10 x 10 |
| Group III | Day 0 | 3 | 0 | 0 | 15 x 15 |
| | Day 3 | 2 | 1 | 0 | 10 x 10 |
| | Day 7 | 1 | 2 | 0 | 6 x 6 |
| | Day 14 | 0 | 3 | 0 | 1 x 1 |
| Group IV | Day 0 | 3 | 0 | 0 | 15 x 15 |
| | Day 3 | 2 | 1 | 0 | 10 x 10 |
| | Day 7 | 2 | 2 | 0 | 7 x 7 |
| | Day 14 | 1 | 2 | 0 | 6 x 6 |

*Scoring was performed according to the "Scoring Evaluation Scale" specified in Table 2

There was no significant difference among the groups in the macroscopic wound measurements performed on day 3 (Figure 5, $p > 0.05$). In the evaluation made on the seventh day, brown-colored scab formation was observed in all groups. The wound healing rate was the highest in group III, and the healing rate in group IV was higher than those in groups I and II ($p < 0.05$). In the histopathological evaluations, reepithelization was found to occur at a high rate in groups III and IV, at a moderate rate in group I, and at a minimum rate in group II ($p < 0.05$). On the 14$^{th}$ day, the wounds were completely closed in all rats in group III, whereas the wounds of the rats in group IV were substantially closed. The wounds were partially closed in group I, and healing was more limited in group II (Figure 5). In summary, reepithelization occurred at the maximum level in group III, at a high level in group IV, at a moderate level in group I, and at the minimum level in group II (reepithelization rate: group III > IV > I > II; $p < 0.05$).

In groups III and IV, the borders of the epidermis, dermis, and hypodermis showed normal morphological features, and the densities of connective tissue and collagen in the dermis layer were sufficient, whereas the density of collagen in the other groups was insufficient. Wound healing was characterized by a decrease in the number of neutrophils and new vascularization (Figure 6). The ABS-dopped wound dressing accelerated healing, whereas the non-dopped ABS wound dressing had a limited effect on wound healing (Figure 2). In addition, it was determined that the wound healing rate in the group that was administered local ABS to the injured area was slower than that in the control group.

*Biochemical analysis*

The level of oxidation (TOS) was the highest in the control group. ABS was found to reduce oxidation (Table 5). The application of wound dressing with the ABS additive caused a significant decrease in TOS values (Table 5). In parallel with these results, the level of antioxidants (TAS) increased in groups IV and II, but the highest increase was observed in group III ($p < 0.05$) (the OSI ranking: group III > IV > II > I).

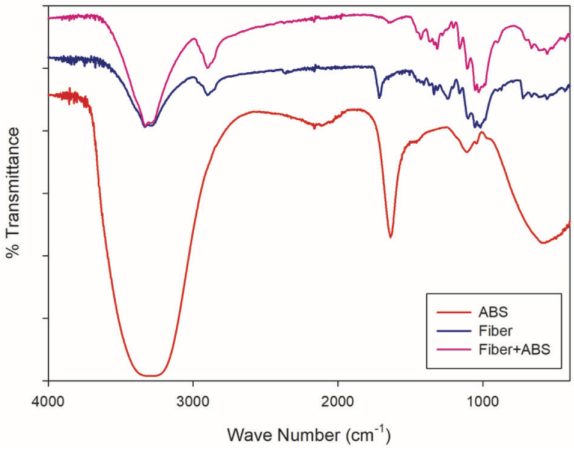


**Figure 4.** FTIR analysis chart

FTIR: Fourier transform infrared spectrophotometry

## DISCUSSION

Injuries caused by traumas, surgical operations, and burns can lead to serious health problems, ranging from disability to death. Wound dressing is a practical process that allows the wound to heal in a short time under hygienic conditions.[38] If bleeding occurs in the injured area, wound dressings containing hemostatic additive agents may be preferred.[39] In this study, the effects of wound dressings containing nanofibers with doping with ABS, which is a hemostatic agent, on wound healing were evaluated in macroscopic, histopathological, and biochemical terms.

Hydrophilicity is a sought-after feature for wound dressing materials.[35,36,40-42] PVA and PVP are biocompatible polymers with biodegradability and non-toxic properties[9]. The addition of silver


**Figure 5.** Change in wound diameters in the groups determined on the first (A) and last (B) days of the study

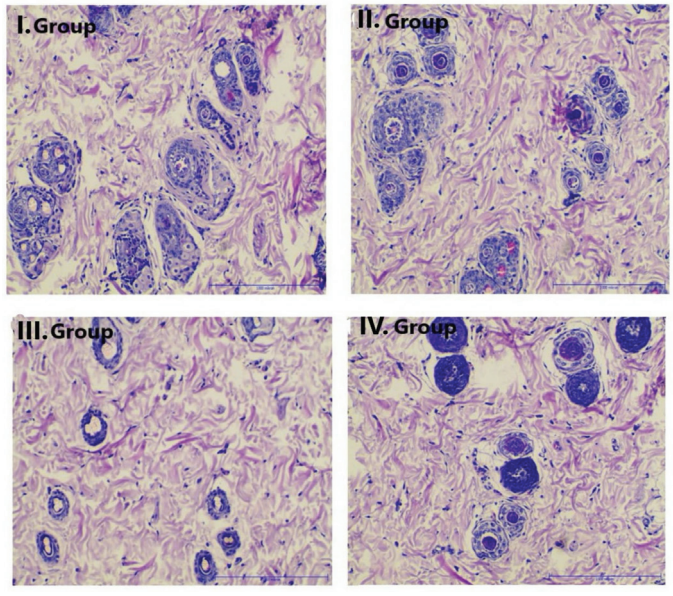


**Figure 6.** On the last day of the study, skin section samples belonging to different groups

(H-E staining, scale 100 µm x 40)

**Table 5. Comparison of serum TAS, TOS, and OSI values between the groups**

| Groups/ Parameters | TAS (mmol Trolox Equiv/L) *Avg. ± SD | TOS (μmol $H_2O_2$ Equiv/L) *Avg. ± SD | OSI (Arbitrary Unit) *Avg. ± SD | p |
|---|---|---|---|---|
| Group I | 1.01 ± 0.05[a] | 13.21 ± 1.80[a] | 1.31 ± 0.07a | 0.000 |
| Group II | 1.50 ± 0.15[b] | 13.00 ± 2.00[b] | 0.87 ± 0.14b | |
| Group III | 2.75 ± 0.01[c] | 11.10 ± 2.18[c] | 0.40 ± 0.13c | |
| Group IV | 1.65 ± 0.20[b] | 12.50 ± 1.25[b] | 0.76 ± 0.13b | |

*There are significant differences between different letters (a to c) in the same column ($p < 0.05$), Avg.: Average, SD: Standard deviation, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index

nanoparticles to PVA and PVP had a notable effect on wound healing in New Zealand white rabbits.[14] It was determined that the wound dressing used in this study was sufficient to provide the permeability of fiber diameters. Moreover, no droplet or lump-like formation was observed on the surface. Because of these properties, it is thought that the substance produced in this study would be suitable for use and may be a preferred method in case of injuries.

During the recovery process, wound diameter was examined for reepithelialization and inflammation formation. In the measurements made on the last day of the study, the mean wound diameter in the ABS-dopped wound dressing group was much smaller than that in the control group, whereas the mean wound diameter in the local ABS-treated group was higher than that in the control group. This situation was interpreted as the ABS-dopped wound dressing being effective in healing, and the local ABS application slowed down healing.

There are many studies in the relevant literature investigating the effects of ABS on wound healing. It was reported that ABS is an agent that accelerates recovery and is effective in accelerating the wound healing process in periodontal treatment.[43] Similarly, ABS is effective in trauma-related soft tissue defects that cannot be repaired by primary closure.[44] ABS was observed to accelerate the healing process in bone defects created in the rat tibia.[45] It was shown that ABS applied to wounds on the back skin of rats accelerated healing.[46] According to a similar study, ABS speeds up wound healing in rats during the early period.[47] ABS has also been reported to be effective in healing full-thickness skin wounds in rats.[39] It was seen to provide healing even in second-degree burns.[4] In a similar study, ABS had a positive effect on wound healing in rats.[27] It was also stated that ABS has a positive effect on wound healing in diabetic rats.[48] In contrast with the aforementioned results, ABS was not found to be effective in preventing postoperative intra-abdominal adhesions, and its use could be harmful because it caused abdominal organ damage.[49] In the same study, it was stated that ABS did not prevent adhesions but increased them on a macroscopic level.

Our findings supported previous results, and the ABS-dopped dressings in this study were effective in reducing the wound size from 15x15 mm to 1x1 mm, thus contributing to healing. Although our results were similar to those of other studies in the literature in which the positive effects of ABS on wound healing have been stated,[26,27] they differed from some others showing that ABS is not effective alone, and it even slows healing. It was thought that the amount of ABS used in each study, the diameter of the wound that is created, and the age and sex of the animals may be different. The cycle occurring in females may have affected the results of our study by affecting the wound healing process and every other process in the body.

Reepithelialization is a stage of healing that occurs during the proliferation phase and is regulated by growth factors. Measuring the level of re-epitheliazation is a frequently used method for the histopathological evaluation of the healing process.[44,45] It was stated that the level of epithelialization in the tibia tissue of the rat group in which ABS was used in the treatment was higher than that in the control group.[44] ABS is effective in full-thickness wound healing by accelerating proliferation in the epithelial tissues of rats.[39] In a similar study, ABS, which was used for treating rat palatal mucosal injuries, increased the level of reepithelialization.[50] Another similar study revealed that ABS contributed to epithelialization in a full-thickness skin wound model in rats.[26] According to our results, the rate of re-epitheliazation was the highest in the ABS-dopped dressing group (III), and it was limited in the local ABS group (I) (Figure 5, Table 4). It was considered that brown scab formation, which was observed more prominently in the ABS-dopped dressing group than in the other groups, occurred because of reepithelialization and was caused by the formation of an encapsulated protein network on the wound area. Consistent with our results, scab formation was observed in the wound area in similar studies using ABS.[51-53]

Inflammation is a reaction developed by the body in cases of tissue damage, exposure to certain chemicals, and infectious diseases. This situation, in which the substances taking part in the immune system are directed to the damaged tissue, is an important parameter used in evaluating wound healing.[26] While inflammation is not observed in the normal healing process, its severity may increase in necrotic conditions.[53] In wound treatment, interventions that prevent inflammation are preferred. The use of ABS prevented inflammation and necrosis in experimentally created bone defects in the tibia of rats.[45] It has been reported that ABS does not cause inflammation in experimentally induced full-thickness skin wound healing in rats.[39] According to another study, ABS prevented inflammation by inhibiting collagen destruction during the recovery period.[53,54] ABS reduced inflammation in a wound model created in rats.[27] Likewise, it was reported that the use of ABS

in rats with colonic anastomosis-induced collagen formation and increased anastomosis.[55] In a study that was conducted to prevent postoperative intra-abdominal adhesions using ABS, an increase in inflammatory reactions to fibrosis was observed in the groups using ABS.[49] In the evaluation made with the available data, it was determined that inflammation did not occur in any animal in our study, and this situation was compatible with most other studies in the literature.

OSI is a measure of oxidative stress. Oxidative stress occurs because of the overproduction of reactive oxygen species or when antioxidant protection is insufficient.[56] It has been reported that increased OSI values in many conditions, such as diabetes, wounds, burns, and aging, can be reduced using the antioxidant properties of various herbal extracts.[17,57-67] Our results showed that ABS, which is an herbal product, is effective against oxidative stress that occurs during the wound healing process, in line with the literature. The use of only dressing and ABS alone corresponded to a reduction in the increased stress by 1.5 times ($p < 0.05$). It was determined that the ABS-dopped dressing increased the TAS levels by approximately twice, while reducing the OSI values by 1/3 compared with the control (Table 5).

## CONCLUSION

The results showed that ABS alone did not contribute to wound healing, and the ABS-dopped wound dressing provided rapid and uneventful healing in the injured area. It was determined that local ABS application did not shorten the wound healing duration, and the ABS-dopped nanofiber dressing provided healing in the injured area in a much shorter time than the control group. Because it does not cause inflammation, ABS is a hemostatic agent with antiseptic and antimicrobial properties. Our results indicate that ABS-dopped dressings can be used safely, do not have any negative effects on wound healing, and can be an effective and safe product for use by shortening the healing process in skin wounds.

*Ethics*

**Ethics Committee Approval:** The study was initiated by obtaining the approval of the ethics committee of Selçuk University, Experimental Medicine Research and Application Center (decision date: 25.01.2019 and decision number: 2019-2).

**Informed Consent:** Not necessary.

*Authorship Contributions*

Concept: E.Ş., E.G., M.O.E., Design: E.Ş., E.G., Data Collection or Processing: E.Ş., E.G., Analysis or Interpretation: E.Ş., E.G., Literature Search: E.Ş., E.H., M.O.E., Writing: E.Ş., E.G., M.O.E.

**Conflict of Interest:** No competing interests declared.

**Financial Disclosure:** This research was supported within the scope of the project numbered 03-M-21, which was accepted by the Scientific Research Projects Commission of Karamanoğlu Mehmetbey University.

## REFERENCES

1. Gupta A, Kowalczuk M, Heaselgrave W, Britland ST, Martin C, Radecka I. The production and application of hydrogels for wound management: A review. Eur Polym J. 2019;111:134-151.
2. Beyazit F, Beyazit Y, Tanoglu A, Haznedaroglu IC. Ankaferd hemostat (ABS) as a potential mucosal topical agent for the management of COVID-19 syndrome based on its PAR-1 inhibitory effect and oestrogen content. Med Hypotheses. 2020;143:110150.
3. Zeki ÖC, Nenni M, Çelebier M, Öncül S, Ercan A, Süslü İ, Haznedaroğlu İC. Antitumor activity of Ankaferd Blood Stopper® on MCF-7 breast cancer: A proteomic approach to ascertain the mechanism of the action. J Herb Med. 2021;28:100449.
4. Ozcan EC, Gul M, Dundar S, Bozoglan A, Karasu N, Bal A, Gunes N, Bingul MB. Effects of local application of the ankaferd blood stopper on osseointegration in three different surface titanium implants. J Oral Biol Craniofac Res. 2021;11:524-528.
5. Satar NY, Akkoc A, Oktay A, Topal A, Inan K. Evaluation of the hemostatic and histopathological effects of ankaferd blood stopper in experimental liver injury in rats. Blood Coagul Fibrinolysis. 2013;24:518-524.
6. Gül Satar N, Cangül İT, Topal A, Oktay A, İnan K, Akgül MB. Effects of Ankaferd Blood Stopper (ABS) and topical tripeptide copper complex (TCC) on wound healing in rats: an experimental study. Kafkas Univ Vet Fac. 2014;20:545-551.
7. Ambekar RS, Kandasubramanian B. Advancements in nanofibers for wound dressing: A review. Eur Polym J. 2019;117:304-336.
8. AlAbdulaal TH, Almoadi A, Yahia IS, Zahra HY, Alqahtani MS, Yousef ES, Hussein KI, Jalalah M, Harraz FA, Al-Assiri MS. High optical performance of Gd2O3-doped PVA/PVP composite films for electronic and laser CUT-OFF filters. Optik. 2022;268:169741.
9. Sanz Espinar G, Universidad Autónoma de Madrid (España). https://riull.ull.es/xmlui/bitstream/handle/915/30978/C_22_%282022%29_28.pdf?sequence=1&isAllowed=y.cedille.;undefined;513-519. Epub 2022
10. Dhivya S, Padma VV, Santhini E. Wound dressings-a review. Biomedicine (Taipei). 2015;5:22.
11. Li H, Williams GR, Wu J, Lv Y, Sun X, Wu H, Zhu LM. Thermosensitive nanofibers loaded with ciprofloxacin as antibacterial wound dressing materials. Int J Pharm. 2017;517:135-147.
12. Chen K, Hu H, Zeng Y, Pan H, Wang S, Zhang Y, Shi L, Tan G, Pan W, Liu H. Recent advances in electrospun nanofibers for wound dressing. Eur Polym J. 2022;178:111490.
13. Alharbi HF, Luqman M, Khalil KA, Elnakady YA, Abd-Elkader O, Rady AM, Alharthi NH, Karim MR. Fabrication of core-shell structured nanofibers of poly (lactic acid) and poly (vinyl alcohol) by coaxial electrospinning for tissue engineering. Eur Polym J. 2018;98:483-491.
14. Alipour R, Khorshidi A, Shojaei AF, Mashayekhi F, Moghaddam MJM. Skin wound healing acceleration by Ag nanoparticles embedded in PVA/PVP/Pectin/Mafenide acetate composite nanofibers. Polym Test. 2019;79:106022.
15. Geetha K, Sivasangari D, Kim H, Murugadoss G, Kathalingam A. Electrospun nanofibrous ZnO/PVA/PVP composite films for efficient antimicrobial face masks. Ceram Int. 2022;48:29197-29204.
16. Taş A, Köklü S, Beyazit Y, Karaca G, Astarcı HM, Akbal E, Koçak E, Topçu G, Haznedaroglu IC. Percutaneous ankaferd injection to *in vivo* liver tissue in comparison to ethanol in an experimental rat model. Clin Res Hepatol Gastroenterol. 2011;35:549-553.

17. Topal A, Satar NYG, Cangul IT, Oktay MA, Inan K, Cecen G, Akarsu EP, Can H. Ankaferd blood stopper accelerates deep second degree burn wound healing in rats. Acta Vet Brno. 2018;87:261-267.

18. Kurakula M, Koteswara Rao GSN. Moving polyvinyl pyrrolidone electrospun nanofibers and bioprinted scaffolds toward multidisciplinary biomedical applications. Eur Polym J. 2020;136:109919.

19. Bozkaya O, Arat E, Gün Gök Z, Yiğitoğlu M, Vargel I. Production and characterization of hybrid nanofiber wound dressing containing *Centella asiatica* coated silver nanoparticles by mutual electrospinning method. Eur Polym J. 2022;166:111023.

20. Kustiati U, Wihadmadyatami H, Kusindarta DL. Dataset of Phytochemical and secondary metabolite profiling of holy basil leaf (*Ocimum sanctum* Linn) ethanolic extract using spectrophotometry, thin layer chromatography, Fourier transform infrared spectroscopy, and nuclear magnetic resonance. Data Brief. 2022;40:107774.

21. Cross SE, Naylor IL, Coleman RA, Teo TC. An experimental model to investigate the dynamics of wound contraction. Br J Plast Surg. 1995;48:189-197.

22. Dorsett-Martin WA. Rat models of skin wound healing: a review. Wound Repair Regen. 2004;12:591-599.

23. Ramsey A, Bailey S, Pollock D. Diurnal differences in mitochondrial bioenergetics is lost in bmal1 knockout rats. FASEB J. 2022;36.

24. Imani A, Rajani SF, Rakhshan K, Faghihi M, Nemati M, Parsazadegan T. The role of nitric oxide on the antiarrhythmic effects of ketamine/xylazine in a rat model of acute cardiac ischemia-reperfusion. Curr Res Physiol. 2022;5:302-311.

25. Connell AR, Hookham MB, Fu D, Brazil DP, Lyons TJ, Yu JY. Comparisons of $\alpha$2-adrenergic agents, medetomidine and xylazine, with pentobarbital for anesthesia: important pitfalls in diabetic and nondiabetic rats. J Ocul Pharmacol Ther. 2022;38:156-166.

26. Özbaysar Sezgin S, Saraç G, Şamdancı E, Şenol M. The effects of Ankaferd, a hemostatic agent, on wound healing. Turkderm - Turk Arch Dermatol Venereol. 2015;49:218-221.

27. Satar NY, Akkoc A, Oktay A, Topal A, Inan K. Evaluation of the hemostatic and histopathological effects of Ankaferd Blood Stopper in experimental liver injury in rats. Blood Coagul Fibrinolysis. 2013;24:518-524.

28. Gül M, Günay A. Effect of caffeic acid phenethyl ester and Ankaferd Blood Stopper® on palatal wound healing in the diabetic rats. SRM J Res Dent Sci. 2020;11:172-177.

29. Şensoy E, Öznurlu Y. Determination of the changes on the small intestine of pregnant mice by histological, enzyme histochemical, and immunohistochemical methods. Turk J Gastroenterol. 2019;30:917-924.

30. AL-Dahhan MAH, Al-Samawy ERM, Al-Kaisei BI, Jarad AS. Effect of synthetic colorants (Sunset yellow and Ponceau 4R) in some biochemical and histopathological parameters of albino rats. QJVMS. 2014;13:80-84.

31. Hassan MT, Mohamed HK, Hammad WA. Antihyperlipidemic activity of green coffee beans extract against Poloxamer407 toxicity in male albino rats. Egypt J Exp Biol (ZooL). 2020;16:65-72.

32. Elbanna K, Sarhan OM, Khider M, Elmogy M, Abulreesh HH, Shaaban MR. Microbiological, histological, and biochemical evidence for the adverse effects of food azo dyes on rats. J Food Drug Anal. 2017;25;667-680.

33. Alharbi N, Elobeid M, Virk P. Protective effect of quercetin treatment against cadmium-induced oxidative stress in a male rat model. Pakistan J Zool. 2019;51:2287-2296.

34. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37:277-285.

35. Özgün E, Sayılan Özgün G, Eskiocak S, Yalçın O, Süer Gökmen S. Effect of L-carnitine on serum paraoxonase, arylesterase and lactonase activities and oxidative status in experimental colitis. Turk J Bioch. 2013;38:145-153.

36. Ovington LG. Advances in wound dressings. Clin Dermatol. 2007;25:33-38.

37. Sarip MN, Noor MFHM, Ahmad Z, Shuhaime N, Dahan RM, Arshad AN, Ismail WIWW. Conductivity study of polyvinyl alcohol/polyvinyl pyrrolidone (PVA/PVP)-KOH coatings system. AIP Conference Proceedings. 2018.

38. Wang Z, Rong F, Li Z, Li W, Kaur K, Wang Y. Tailoring gas-releasing nanoplatforms for wound treatment: An emerging approach. J Chem Eng. 2023;452:139297.

39. Yıldız Gülhan P, Güleç Balbay E, Çelik M, Annakkaya AN, Arbak P. Fatigue frequency and related factors in patients with sarcoidosis. Turk Thorac J. 2019;20(Suppl 1):244.

40. Akalin C, Kuru S, Barlas AM, Kismet K, Kaptanoglu B, Demir A, Astarci HM, Ustun H, Ertas E. Beneficial effects of Ankaferd Blood Stopper on dermal wound healing: an experimental study. Int Wound J. 2014;11;64-68.

41. Münch AS, Simon F, Merlitz H, Uhlmann P. Investigation of an oleophobic-hydrophilic polymer brush with switchable wettability for easy-to-clean coatings. Eur Polym J. 2022;180:111629.

42. Maalej H, Maalej A, Bayach A, Zykwinska A, Colliec-Jouault S, Sinquin C, Marchand L, Ktari N, Bardaa S, Ben Salah R, Chamkha M, Boufi S, Nasri M. A novel pectic polysaccharide-based hydrogel derived from okra (Abelmoschus esculentusL. Moench) for chronic diabetic wound healing. Eur Polym J. 2023;183:111763.

43. Erçetin S, Haznedaroğlu İC, Kurt M, Onal İK, Aktaş A, Kurt ÖK, Göker H, Özdemir O, Kirazlı S, Fırat HC. Safety and efficacy of ankaferd blood stopper in dental surgery. UHOD. 2010;20:1-5.

44. Demirel ME, Mohamed SM, Ali YB. Soft tissue infection and delayed wound healing due to neglected animal bite: A case report. J Contemp Med. 2018;8:280-281.

45. Işler SC, Demircan S, Cakarer S, Cebi Z, Keskin C, Soluk M, Yüzbaşioğlu E. Effects of folk medicinal plant extract Ankaferd Blood Stopper on early bone healing. J Appl Oral Sci. 2010;18:409-414.

46. Aktop S, Emekli-Alturfan E, Ozer C, Gonul O, Garip H, Yarat A, Goker K. Effects of Ankaferd Blood Stopper and Celox on the tissue factor activities of warfarin-treated rats. Clin Appl Thromb Hemost. 2014;20:16-21.

47. Aktaş A, Er N, Korkusuz P, Zeybek D, Onur M, Tan G, Özdemir O, Karaismailoğlu E, Karabulut E. Ankaferd-induced early soft tissue wound healing in an experimental rat model. Türkiye Klinikleri J Med Sci. 2013;33:1344-1353.

48. Bulut E, Baş B, Altunkaynak BZ, Bekçioğlu B, Erdem Koç G, Gönülol E, Önger ME, Kaplan S. Efficacy of Ankaferd Blood Stopper on bone healing in diabetic rats: a stereological and histopathological study. Biotech Histochem. 2014;89:535-543.

49. Arkan B, Yılmaz D, Gökdere Çinar H, Uzun R. Clinical decision-making levels of nursing students and affecting factors. Cyprus J Med Sci. 2022;7:738-744.

50. Gül M, Günay A, Tanik A. An evaluation of the effects of caffeic acid phenethyl ester and Ankaferd blood stopper on secondary wound healing of oral mucosal tissue. Turk J Med Sci. 2020;50:248-257.

51. Kandemir O, Buyukates M, Kandemir NO, Aktunc E, Gul AE, Gul S, Turan SA. Demonstration of the histopathological and immunohistochemical effects of a novel hemostatic agent, Ankaferd Blood Stopper, on vascular tissue in a rat aortic bleeding model. J Cardiothorac Surg. 2010;5:110.
52. Tokgöz H, Karakaya K, Hanci V, Abduşoğlu M, Erol B, Türksoy O, Akduman B, Mungan NA. Protective value of a folkloric medicinal plant extract against mortality and hemorrhage in a life-threatening renal trauma model. Urology. 2010;75:1515.
53. Karaca G, Aydin O, Pehlivanli F, Kocael A, Pekcici R, Duymus E, Akgedik S, Guler O. Effect of ankaferd blood stopper in experimental peritoneal adhesion model. Ann Surg Treat Res. 2016;90:213.
54. Aydın BK, Altan E, Acar MA, Erkoçak ÖF, Ugraş S. Effect of Ankaferd blood stopper® on tendon healing: an experimental study in a rat model of Achilles tendon injury. Jt Dis Relat Surg. 2015;26:31-37.
55. Ekici U, Ferhatoğlu MF, Çitgez B, Uludağ M. Effects of folk medicinal plant extract ankaferd blood stopper on healing of colon anastomosis: an experimental study in a rat model. Med Bull Şişli Etfal Hosp. 2019;53:154-159.
56. Çetin S, Usta A, Ekici P, Dede S, Yüksek V. Sarcoptes ovis ile Enfekte Koyunlarda Serum Protein Fraksiyon Profili. Atatürk University Journal of Veterinary Sciences. 2020;15:70-75.
57. Oxidative Stress in Neurodegenerative Diseases. Metal-based Neurodegeneration. 2013:75-109.
58. Shang M, Zhao J, Yang L, Lin L. Oxidative stress and antioxidant status in women with gestational diabetes mellitus diagnosed by IADPSG criteria. Diabetes Res Clin Pract. 2015;109;404-410.
59. Hailat N, Al-Kahil S, Alkofahi A, Lafi S, Al-Ani F, Al-Darraji A, Bataineh Z. Effects of *Nigella sativa* extracts on antibody response of rats vaccinated with *Brucella Vaccine* (Rev-1). Pharm Biol. 1998;36:217-221.
60. Ahmad S, Beg ZH. Evaluation of therapeutic effect of omega-6 linoleic acid and thymoquinone enriched extracts from *Nigella sativa* oil in the mitigation of lipidemic oxidative stress in rats. Nutrition. 2016;32;649-655.
61. Inci A, Sarı F, Sarıkaya M, Yılmaz U, Coban M, Gul S, Akın O, Şahinturk Y, Yılmaz N. Sp445increased oxidative stress in diabetic nephropathy and relationship to soluble klotho levels. Nephrol Dial Transplant.. 2017;32(Suppl 3):273.
62. Kumandaş A, Karslı B, Kürüm A, Çınar M, Elma E. Comparison of the effects of zinc-silver cream and Nigella sativa oil on wound healing and oxidative stress in the wound model in rats. Ankara Univ Vet Fak Derg. 2020;67:33-40.
63. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. Evidence-Based Complementary and Alternative Medicine. 2019;2019:1-30.
64. Abdulahad D. Investigation of the erythrocyte fragility, hematological and antioxidant effects of oleander (*Nerium oleander* L.) Flower ethanolic lyophilized extract in diabetic rats. KSU J. Agric Nat. 2020;23;1495-1502.
65. Saraç H, Durukan H, Demirbaş A. Nutrient Concentrations and antioxidant activity of *Achillea millefolium* L. (Yarrow), one of the important medicinal plants. Turkish JAF Sci Tech. 2021;9:590-594.
66. Sarıtaş Z, Korkmaz M, Demirel HH, Bülbül A, Sarıtaş TB, Görücü F, Koç Y. Wound healing effect of Anzer origin propolis specimens on rats' intestinal incision. Ankara Univ Vet Fak Derg. 2022;69;91-97.
67. Sahin NE, Oner Z, Oner S, Turan MK. A study on the correlation between spleen volume estimated via cavalieri principle on computed tomography images with basic hemogram and biochemical blood parameters. Anat Cell Biol. 2022;55;40-47.