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RESEARCH ARTICLE

The Effects of Colloidal Silver Solution and Hypochlorous Acid on Wound Healing in the New Zealand Rabbit

Harun ÇINAR[1]*, Ahmet Gürol KALAYCI[2], Mustafa Doğa TEMİZSOYLU[1], Volkan İPEK[3], İbrahim Taha DANIŞMAN[1]

[1]Department of Veterinary Surgery, Faculty of Veterinary, Burdur Mehmet Akif Ersoy University, Burdur, Turkey
[2]Department of Accounting and Tax Applications, Yalvaç Vocational School, Isparta Applied Sciences University, Isparta, Turkey
[3]Department of Pathology, Faculty of Veterinary, Burdur Mehmet Akif Ersoy University, Burdur, Turkey

ABSTRACT

The aim of this study was to investigate and evaluate the effects of colloidal silver and hypochlorous acid solutions on wound healing. To evaluate the wound healing activity of colloidal silver solution and hypochlorous acid solutions, an in vivo excisional wound model was applied in New Zealand rabbits (n = 21). The rabbits were randomly divided into 3 groups with seven individuals in each group. Group 1 was used as a negative control and no treatment was applied. Groups 2 and 3 were treated with crystalline (hypochlorous acid) and colloidal silver, respectively. The treatments were applied topically (in the form of spray) to the wound area of each rabbit daily. Wound diameters were measured on days 4, 8 and 12 using calipers and histopathological examinations were performed on days 4, 8 and 12 of the treatment. In terms of wound closure, both hypochlorous acid and colloidal silver solutions showed comparable wound healing activity with the control group. In conclusion, hypochlorous acid and colloidal silver have a positive effect on dermal wound healing in rabbits.

Key Words: Rabbit, Hypochlorous acid, Colloidal silver, Wound healing

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Yeni Zelanda Tavşanında Kolloidal Gümüş Solüsyonu ve Hipokloröz Asitin Yara İyileşmesi Üzerine Etkileri

ÖZ

Bu çalışmanın amacı, kolloidal gümüş ve hipokloröz asit çözeltilerinin yara iyileşmesi üzerine etkilerinin araştırmak ve değerlendirmektir. Kolloidal gümüş çözeltisi ve hipokloröz asit solüsyonlarının yara iyileştirme aktivitesini değerlendirmek için Yeni Zelanda tavşanlarında (n = 21) in vivo ekzisyonel yara modeli uygulandı. Tavşanlar, her grupta yedi bireyden oluşacak şekilde rastgele 3 gruba ayrıldı. Grup 1 negatif kontrol olarak kullanıldı ve hiçbir tedavi uygulanmadı. Grup 2 ve 3 sırasıyla kristalin (hipokloröz asit) ve kolloidal gümüş ile tedavi edildi. Tedaviler yara bölgesine topikal olarak (sprey şeklinde), her tavşanın yara bölgesine günlük olarak uygulandı. Yara çapları 4, 8 ve 12. günlerde kumpas kullanılarak ölçülmüş ve tedavinin yine 4, 8 ve 12. günlerinde histopatolojik incelemeler yapılmıştır. Yara kapanması açısından, hem hipokloröz asit hem de kolloidal gümüş solüsyonları kontrol grubu ile karşılaştırılabilir yara iyileştirme aktivitesi göstermiştir. Sonuç olarak, hipokloröz asit ve kolloidal gümüş tavşanlarda dermal yara iyileşmesi üzerinde olumlu bir etki göstermektedir..

Anahtar Kelimeler: Tavşan, Hipokloröz asit, Kolloidal gümüş, Yara iyileşme

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ORCID ID; *HÇ: 0000-0003-4412-8949, AGK: 0000-0001-8249-6254, MDT: 0000-0002-3773-1304, Vİ: 0000-0001-5874-7797, İTD: 0009-0007-1288-5562*

*Corresponding author e-mail: hcinar@mehmetakif.edu.tr

INTRODUCTION

Wound is a condition in which the integrity of the multilayered flat epithelium is disrupted and loses its function due to various reasons (Robson et al, 2001). The wound may be superficial or deep enough to cover tendon, muscle, vessel, nerve, bone, and subcutaneous connective tissue (Alonso et al, 1996). Wound healing is a synchronised and complicated biological process that starts because of stimulation of many interconnected mechanisms (Gurtner et al, 2008). Wound healing stages generally consist of haemostasis, inflammation, cell proliferation and remodelling (Diegelmann and Evans, 2004). Injury can occur in all tissues and organs of the body and the healing process is similar in almost all of them. The healing process occurs with the cooperation of biological and immunological systems (Attinger et al, 2006). Wound products have a very important place in wound research and applications all over the world. These wound care products contribute to the natural healing process by protecting the tissue and activating cell production (Valenta et al, 2004).

Silver is a metal that has been encountered with different usage areas since ancient times (Serenella et al., 2019). In addition to wound dressings and creams in the medical field, silver is used in medical devices and in the coating of antibiotics (Jean-Yves et al., 2013). There are conclusive studies that the use of silver alloy indwelling catheters for short-term catheterisation will reduce the risk of catheter-induced urinary tract infections (Beattie M., 2011; Schumm et al., 2008). Silver generally has low toxicity and minimal risk when used in silver-approved medical applications (Lansdown, 2006). Until the invention of antibiotics, silver was used both for food preservation and as a good therapeutic agent. Today, silver is known to have antibacterial effect on nearly six hundred bacteria (Fung et al., 1996). Nanoparticle silver is obtained by separating silver metal into very small pieces in the order of nanometre. In this way, silver nanoparticles can be transported to very remote points in the living body through fluids. Silver has antibacterial effect as well as antiallergic effect. Ionic silver is formed by introducing silver into a liquid in the form of Ag + ions. This liquid is called colloidal in the sense of suspended. Since colloidal ionic silver atoms are positively charged, they are suspended in the liquid by repelling each other. This liquid can be applied to the skin or administered sublingually, by drinking or through the rectum to ensure absorption. Dose and concentration studies for intravenous applications are ongoing (Rosenblatt et al., 2009; Deery, 2009; Lancaster and Steady, 2010; Wesley, 2009). Silver and most silver compounds have oligodynamic effects and are toxic to bacteria, algae and fungi when used in vitro (Lansdown, 2006). The activity of silver compounds as antiseptics is such that the biologically active silver ion (Ag^+) irreversibly damages key enzyme systems in the cell membranes of pathogens (Lansdown, 2006).

Hypochlorous acid (HOCl) is a non-cytotoxic microbiocidal agent active against all bacterial, viral, and fungal human pathogens (Mekkawy and Kamal, 2014). HOCl can be formed by combining acidic chlorine oxide (Cl) with water. Stabilised in the form of a physiologically stable solution, HOCl is a naturally occurring molecule produced by neutrophils to destroy pathogens (Gold et al., 2019). It has an excellent bactericidal effect against various microorganisms due to its high oxidising capacity and is highly advantageous due to its practical applicability in healthcare institutions or food industry (Tsai et al., 2023).

The main features of hypochlorous acid in its mechanism of action are oxidation of sulfhydryl enzymes, oxidation of amino acids, chlorination of amino acids, inhibition of protein synthesis, loss of intercellular substance, decrease in nutrient uptake, decrease in oxygen uptake, oxidation of respiratory components, decrease in adenosine triphosphate production, DNA breakage and suppression of DNA synthesis. It causes rapid death of all bacteria, viruses and fungi and inactivation of prions. Due to these properties, resistance cannot be developed by microorganisms (Sakarya, 2019).

This study aims to investigate the effects of colloidal silver and hypochlorous acid solutions on wound healing.

MATERIALS and METHODS

Wound Healing Activity

Animals

In this study, 21 male New Zealand rabbits with an average body weight of 2000-2500 g, approximately 6-12 months of age, were selected to create the excision wound model. The care, feeding and biopsy procedures of the animals to be used in the study were supervised at Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Centre. Rabbits were housed individually in standard cages measuring 45×40×50 cm. The rabbits were kept under standard conditions including 12:12 h light-dark cycle, 50-70% humidity and 25°C ± 3°C temperature range. In terms of feeding, they were given unlimited access to standard feed and water. The study was conducted in accordance with the guidelines of the European Community Council Directive (2010/63/EU). Burdur Mehmet Akif Ersoy University Rectorate Animal Experiments Local Ethics Committee approved the study as stated in its decision dated 15 January 2020 and numbered 613.

Excision Wound Model

The excision wound model procedure in rabbits was adapted from the protocol established by Huang et al. (2019). Rabbits were anaesthetised using a combination of xylazine and ketamine (3-5 mg/kg xylazine and 50 mg/kg ketamine). While anaesthetised, the dorsal skin of each rabbit was shaved using an electric razor and then disinfected with topical povidone-iodine. Four full-thickness wounds, each 8 mm in diameter, were made on both the left and right side of the midline on the back of each animal.

The wounds created are not immobilized in any way. The best model of wounds that would normally occur in nature are those that are not immobilized. However, fixation of the wounds has advantages such as supporting the healing process, faster healing, and reducing the risk of infection. In fixed wounds with well-united epithelialization edges, healing begins almost immediately as there is no defect to be filled by granulation tissue. Such reasons may also support the use of the immobilized wound model.

Considering the healing phases of the wound, the days determined for the wounds were selected and evaluated. Since the repair phase usually starts 3 to 5 days after the injury, we chose day 4 as the first day to be evaluated for the wound. Wound contraction reduces wound size through cells in the granulation tissue. Wound contraction is associated with a complex interaction of extracellular matrix, cells and cytokines. Due to such factors, the full-thickness skin edges of the wound, from the periphery to the center, are pulled inward by contraction. This shrinks noticeably within 5 to 9 days after injury. Taking this timing into account, we chose day 8 as the second day to evaluate the wound. During the maturation phase of the wound, the strength of the wound is maximized due to changes in the scar as the wound heals. The most rapid gain in wound strength occurs between 7 and 14 days after injury, when collagen rapidly accumulates in the wound. Taking this into account, we chose day 12 as the third day to evaluate the wound.

Three biopsies were taken from three different wounds in each animal. At the end of the study, the fourth wound was left unbiopsied to assess the degree of wound closure.

Treatment Schedule

Rabbits were randomly divided into 3 groups with seven individuals in each group. Group 1 was used as a negative control and no treatment was applied. Groups 2 and 3 were treated with crystalline (hypochlorous acid) and colloidal silver, respectively. The treatments were applied topically (in the form of a spray) to the wound area of each rabbit daily. Biopsies for histopathological evaluation were taken on days 4, 8 and 12 under xylazine and ketamine induced anaesthesia. The complete healing of the right caudal wound facilitated the assessment of the level of wound closure. On day 12, the diameters of the right caudal wounds were measured using callipers. Wound areas were also determined with a caliper immediately after wound induction. The quantitative data and results obtained from this study were primarily based on caliper measurements, ensuring the accuracy and reliability of our findings.

Histopathology

After fixation in 10% neutral buffered formaldehyde solution, 4 μm thick sections were taken from the biopsy specimens. The sections were embedded in paraffin and stained with haematoxylin and eosin (H & E). Histopathologists evaluated inflammatory cell infiltration (both acute and chronic), angiogenesis, fibroblast maturation levels and the amount of granulation tissue in the injured area. Angiogenesis was scored as 0=absent, 1=mild (less than 5 vessels at 1 high magnification), 2=moderate (6-10 vessels at 1 high magnification) and 3=significant (more than 10 vessels at 1 high magnification). Inflammation was scored as 1=very mild (0-25 cells at 3 high magnification), 2=mild (25-50 cells at 3 high magnification), 3=moderate (50-75 cells at 3 high magnification) and 4=significant (75-100 cells at 3 high magnification) and evaluated according to this scoring. The amount of fibrosis and fibroblast maturation levels were also scored as 0=absent, 1=mild, 2=moderate and 3=significant. The scoring approach was consistent with previous literature (Gül Satar et al., 2017; Güzel et al., 2019). Fibroblast maturation was evaluated according to cellular morphology and arrangement in the tissue. Mature fibroblasts were identified by their elongated spindle shape, arranged in parallel arrays, and reduced cellular density compared to more proliferative regions of granulation tissue. In addition, the presence of a well-developed extracellular matrix was indicative of active collagen production and deposition by mature fibroblasts. This served as an important criterion for assessing fibroblast maturity (Broughton et al., 2006). Group comparisons were made separately for each biopsy timeline.

Statistics

Whether the statistical analysis methods met the parametric test assumptions was checked by Kolmogorov-Smirnov test. Chi-square analysis method was used for comparison of all groups. One-way analysis of variance (ANOVA) or Friedman test was used for the comparison of related groups, and one-way analysis of variance (ANOVA) and Tukey test or Kruskal Wallis-H test was used for the comparison of independent groups. A significance level of $p<0.05$ was accepted. IBM SPSS Statistics 22 package programme was used for the test.Macroscopic Observations

Wound diameters were measured macroscopically, and wound areas were calculated. The obtained data were evaluated statistically on the 4th, 8th, and 12th days. On days 4 and 8, a statistically significant difference was found in all 3 groups. On day 4, the colloidal silver group had the best wound healing (p = 0.012), followed by crystalline and control groups (p < 0.001 in both groups). On the 12th day, there was no statistical difference between the crystalline and colloidal silver groups (p= 0,910), while these two groups were statistically different from the control group (p< 0,001) and the healing rate was found to be better than the control group. Data of wound diameters after closure of wounds were given in Table 1.

Histopathological Findings

Biopsy specimens (8 mm in diameter) taken from different edges of the wounds of each rabbit on days 4, 8 and 12 with a punch biopsy tool were subjected to routine follow-up procedures for histopathological examinations after 10% formaldehyde fixation. Sections (5 µm) were stained with haematoxylin and eosin and evaluated under light microscope. Inflammatory cell counts and angiogenesis were evaluated to determine the inflammation and healing process during the healing phase of wound healing. For this purpose, inflammatory cells and vessels were counted in four high magnification fields (400x) from random areas in the epidermis or dermis under the crust from each skin section. Inflammatory cells were scored between 1-4 (0-25=1, 26-50=2, 51-75=3, >75=4). The same selected areas were analysed for the number of vessels and the numbers were noted. All histological sections were blindly evaluated by the same investigator. Minitab® 16.1.1 package programme was used to analyse the data obtained. After determining the normal distribution of the data by Ryan-Joiner normality test, One-Way Anova Tukey test was used to reveal the differences between the groups.

As a result of statistical analysis, a significant increase in angiogenesis was determined in the crystalline group on the 4th day compared to the control and silver groups. On the 8th and 12th days, no significant difference was observed between the groups. In terms of inflammatory cell score, there was no significant difference between the groups on the 4th and 8th days, while a significant increase was observed in the crystallin group compared to the silver group on the 12th day, but no significant difference was found between both groups and the control group (Figure 1). Statistical data were given in Table 2. In the light of these data, although crystallin application increases angiogenesis in the early period of wound healing, it may have a negative effect on wound healing because it increases inflammatory cell infiltration in the advanced process. It was concluded that silver nanoparticles may contribute to wound healing by suppressing inflammation in the later stages of wound healing without affecting angiogenesis.

Table 1. Data of wound diameters after closure of wounds according to groups in macroscopic examination (according to the πr^2 formula).

Animal / Groups	Crystalline 4. Day	Control 4.Day	Colloidal Silver 4. Day	Crystalline 8. Day	Control 8.Day	Colloidal Silver 8. Day	Crystalline 12. Day	Control 12.Day	Colloidal Silver 12. Day
Rabbit-1	78	113	28	50	96	13	3	28	20
Rabbit-2	50	113	28	13	78	13	13	64	3
Rabbit-3	78	154	50	50	96	13	20	50	50
Rabbit-4	113	113	28	50	78	28	3	13	3
Rabbit-5	78	50	38	28	28	28	7	38	13
Rabbit-6	113	78	28	64	113	28	0	3	0
Rabbit-7	50	113	50	7	13	7	0	28	3
Rabbit-8	78	113	28	13	28	13	1	28	20
Rabbit-9	50	113	50	20	64	13	1	38	1
Rabbit-10	78	78	38	38	28	20	7	50	7
Rabbit-11	50	78	50	28	50	20	0	28	1
Rabbit-12	50	78	28	20	38	13	3	28	3
Rabbit-13	50	113	50	28	78	28	1	28	0
Rabbit-14	50	78	38	20	28	1	3	20	1
Rabbit-15	50	78	50	28	64	20	3	20	7
Rabbit-16	78	113	28	13	50	7	3	38	3
Rabbit-17	50	78	50	38	78	13	1	20	1
Rabbit-18	78	64	50	38	64	20	7	13	3
Rabbit-19	28	28	20	28	50	28	1	20	1
Rabbit-20	50	133	78	50	96	20	0	3	1
Rabbit-21	50	28	20	28	50	3	3	13	0

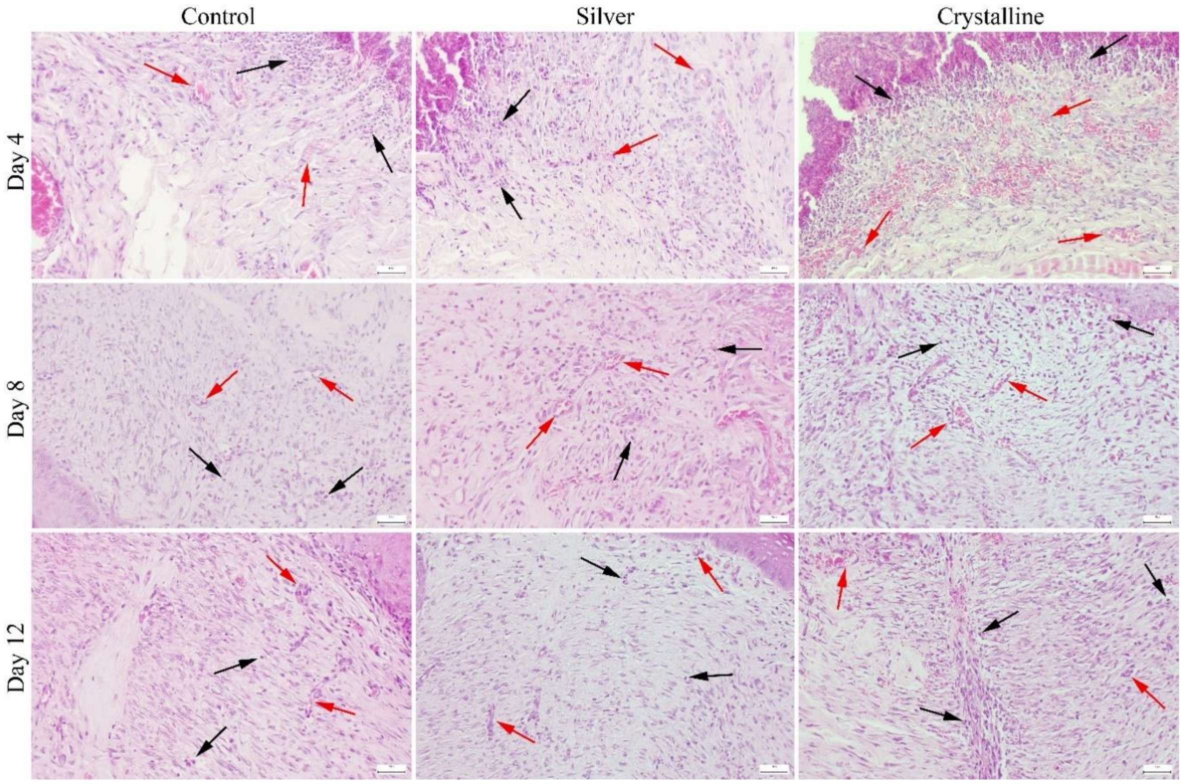

Figure 1: Appearance of angiogenesis (red arrows) and inflammatory infiltrations (black arrows) in the wound area on days 4, 8, and 12 in control, silver, and crystalline groups. H&E staining. Bars: 50 µm.

Table 2. Statistical data between groups for histopathological parameters.

Day	Group	Angiogenesis	Inflammation
4	**Control**	13.50 ± 1.84^{a}	3.333 ± 0.422^{a}
	Silver	12.17 ± 1.99^{a}	4.000 ± 0.000^{a}
	Crystallin	23.17 ± 1.99^{b}	3.833 ± 0.167^{a}
	P value	**0.002**	0.206
8	**Control**	20.83 ± 1.35^{a}	2.833 ± 0.543^{a}
	Silver	29.67 ± 1.78^{a}	3.167 ± 0.543^{a}
	Crystallin	27.33 ± 5.06^{a}	2.833 ± 0.543^{a}
	P value	0.163	0.883
12	**Control**	19.33 ± 2.39^{a}	1.833 ± 0.307 ^{ab}
	Silver	27.00 ± 2.99^{a}	1.000 ± 0.000 ^{a}
	Crystallin	22.00 ± 2.88^{a}	2.333 ± 0.422 ^{b}
	P value	0.172	**0.022**

a,b : The statistical difference between the means with different letters in the same row is significant.

DISCUSSION

Wound products are of great importance in wound-related research and applications. Wound care products contribute to the natural healing process by protecting the tissue and activating cell production (Valenta et al, 2004). In addition to wound dressings and creams, silver is used in medical devices and in the coating of antibiotics (Jean-Yves et al., 2013). Until the invention of antibiotics, silver was used both for food preservation and as a good therapeutic agent. Today, it is known that silver has antibacterial effect on nearly six hundred bacteria (Fung et al., 1996). Nanoparticle silver is obtained by separating silver metal into very small pieces in the order of nanometre. In this way, silver nanoparticles can be transported to very remote points in the living body through fluids. Silver has antibacterial effect as well as antiallergic effect. Ionic silver is formed by introducing silver into a liquid in the form of Ag + ions. This liquid is called 'Colloidal Silver' in the sense of suspended. Since colloidal ionic silver atoms are positively charged, they are suspended in the liquid by pushing each other. This liquid can be applied to the skin or administered sublingually, by drinking or through the rectum to ensure absorption. Dose and concentration studies for intravenous applications are ongoing (Rosenblatt et al., 2009; Deery, 2009; Lancaster and Steady, 2010; Wesley, 2009). Silver and most silver compounds have oligodynamic effect (Lansdown, 2006). The effectiveness of silver compounds as antiseptics is that the biologically active silver ion (Ag+) irreversibly damages key enzyme systems in the cell membranes of pathogens (Lansdown, 2006). Our study's focus on the healing efficacies of colloidal silver and crystalline silver treatments aligns with the rich history of silver's medicinal use, offering insights into its role within modern wound care paradigms.

In a seminal study by Pansara et al. (2020), the efficacy of a chitosan-based film, enriched with chitosan-stabilized silver nanoparticles (CH-AgNP-CHF), on promoting wound healing was meticulously assessed. This experimental investigation was conducted on 24 male Wistar rats, each inflicted with a standardized wound of dimensions 1.5 x 1.5 cm, subsequently infected with the Escherichia coli strain to simulate a bacterial infection scenario. The subjects were systematically divided into four distinct groups, comprising six rats each, to evaluate the therapeutic potential of various treatments. These included a control group treated with sterile gauze, a group receiving a chitosan solution devoid of nanoparticles (empty chitosan film) as a placebo, a group treated with the CH-AgNP-CHF4 film, and a final group administered with MSN-G, a commercial gel containing 0.002% nanocrystalline silver. The comparative analysis of wound healing efficacy from the initial day to the 21st day post-treatment revealed a hierarchical order of effectiveness, prominently showcasing the CH-AgNP-CHF4 film as superior, followed by MSN-G, the empty chitosan film, and the sterile gauze. Noteworthy observations were made by the 14th day, where the CH-AgNP-CHF4 film and MSN-G treatments had almost entirely facilitated wound healing, mirroring the findings from our concurrent study that indicated a rapid and near-complete healing by day 12 in wounds treated with colloidal silver.

Further detailed observations by Pansara et al. (2020) included wound closure rates (WCR) at various intervals. On the third day, CH-AgNP-CHF4 film demonstrated significantly higher WCR at 65.30%, in stark contrast to the lower percentages noted in other groups, which progressively increased by the 5th and 7th days, reaching near-complete closure by day 14, and achieving full closure by day 21 in the CH-AgNP-CHF4 film and MSN-G groups. These findings were corroborated by histopathological analyses, indicating keratinization in the CH-AgNP-CHF4 and MSN-G treated groups, suggestive of accelerated wound healing facilitated by silver's presence. Our study's outcomes align closely with those of Pansara et al., albeit with an emphasis on colloidal silver's pronounced effect on expedited wound healing observed as early as the fourth day. However, it was noted that while crystalline silver applications initially promoted angiogenesis, contributing positively to the early stages of wound healing, there could be potential adverse effects related to inflammatory cell infiltration in later stages. Nonetheless, it was concluded that silver nanoparticles might effectively enhance wound healing by modulating inflammation during the critical latter stages, without detrimentally impacting angiogenesis.

Masood et al. (2019) evaluated the effects of nanoparticle silver impregnated chitosan-PEG (polyethylene glycol) hydrogel application on wound healing in wounds created in diabetic rabbits. A total of two wounds of 20 mm in size were created on both sides of the midline on the back of the rabbits. In this way, 5 different groups were formed. In the 1st group (negative control), no treatment was applied to the rabbits. In the second group (positive control), the wounds were treated with nitrofurazone (0.2% w/w). Groups 3, 4 and 5 were treated with AgNP (Nanoparticle silver), chitosan - PEG hydrogel only and chitosan-PEG hydrogel containing AgNP, respectively. Wound sites were closed with a standard surgical dressing and re-dressed on days 4, 8 and 12. According to the results of the study, according to both macroscopic and histopathological findings, it was reported that wound healing was faster and more effective in the silver-containing groups compared to the negative control group. In our study, as in this study, colloidal silver was found to be faster and more effective in wound healing compared to the control group.

Hypochlorous acid (HOCl) is a non-cytotoxic microbiocidal agent active against all bacterial, viral, and fungal human pathogens (Mekkawy and Kamal, 2014). HOCl is formed by combining acidic chlorine oxide (Cl) with water. Stabilised in the form of a physiologically stable solution, HOCl is a naturally occurring molecule produced by neutrophils to destroy pathogens (Gold et al., 2019). It has an excellent bactericidal effect against various microorganisms due to its high oxidising capacity (Tsai et al., 2023). Kuwabara et al. (2018) created chronic wounds infected with Pseudomonas aeruginosa in diabetic mice and used HOCl solution for 12 days to prevent infection and wound treatment in these chronic wounds. In this study, a 1 full thickness wound was created on the back with a sterile 8 mm dermal punch biopsy tool and bacterial inoculation with Pseudomonas aeruginosa strain at a density of 1.0 × 106 CFU / ml was performed after the wound was created. As a result of this study, it was reported that cleaning the wounds with HOCl solution resulted in a slight delay in wound repair compared to the control group, no significant difference in histopathological examinations and a significant decrease in P. aeruginosa bioburden. Our study contrasted with this study, and it was found that HOCl solution accelerated wound healing compared to the control group.

Kuwabara et al. (2020) explored wound healing in diabetic mice with Pseudomonas aeruginosa infections, focusing on treatments with hypochlorous acid (HOCl) and a novel coating of nanoparticle silver (Ag NPs) with chitin-nanofibre sheets (CNFS). The research meticulously prepared the HOCl solution and nanoparticle silver, ensuring precise concentration and formulation for optimal wound healing assessment. Through creating standardized full-thickness wounds and treating these with varying combinations of HOCl, Ag NPs, and CNFS, the study meticulously evaluated the therapeutic efficacy over a 12-day period, comparing outcomes against a non-cleaned control group. The methodology involved daily treatment regimens and consistent monitoring of wound closure rates, providing a comprehensive dataset on the healing progression. Notably, the study found that all treated groups, except the non-cleaned control, showed significantly enhanced wound healing, with the combination of HOCl and CNFS/Ag NP standing out for its pronounced beneficial impact. This group exhibited superior healing, supported by both macroscopic assessments and histological examinations highlighting reduced granulation tissue formation—a key indicator of effective wound management. Our investigation aligns with Kuwabara et al.'s findings, particularly noting the accelerated wound healing facilitated by HOCl solution across multiple observation points. This congruence underscores the potential of HOCl, both alone and in conjunction with nanoparticle silver and CNFS, in promoting wound healing in challenging diabetic models infected with Pseudomonas aeruginosa. These results not only validate the therapeutic promise of these compounds but also contribute to the broader understanding of their mechanisms and applications in advanced wound care strategies.

CONCLUSION

In conclusion, our findings offer compelling evidence for the use of colloidal silver and hypochlorous acid as effective agents in the management of dermal wounds. By accelerating wound closure and enhancing the quality of wound repair, these agents hold significant promise for improving patient outcomes in wound care. Future research should focus on optimizing formulations and delivery methods to maximize therapeutic benefits, alongside exploring their effects in diverse wound types and clinical settings. Our study contributes to the growing body of literature advocating for the incorporation of these agents into wound management protocols, potentially setting new benchmarks in the field of regenerative medicine and wound care.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: HÇ contributed to the project idea. HÇ, AGK and MDT contributed design and execution of the study. HÇ and İTD contributed to the acquisition of data. HÇ and Vİ analysed the data. HÇ, Vİ and İTD drafted and wrote the manuscript. BS, ED and MK reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Burdur Mehmet Akif Ersoy University Reserch Animals Application Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University (MAKU-HADYEK, Ref No: 73/613, Tarih: 15/01/2020).

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