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Rodents

Effects of Drone Brood Homogenate on Wound Healing: An Experimental Study on Rats

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ABSTRACT

Background: Wound healing is one of the most interesting topics in orthopaedic surgery, and there are many studies on the factors and mechanisms affecting this process.

Objectives: To evaluated the macroscopic and histopathological results of drone larvae homogenate (DLH) on wound healing in a full-thickness wound model.

Methods: Thirty male wistar rats (6–8 weeks, 250 ± 50 g) were used. A uniform circular full-thickness wound of approximately 18.44 ± 1.45 (control), 19.02 ± 1.24 (silverdin), 19.37 ± 1.28 (DLH) mm^2 was excised on the back of each rat. They were divided into control (n: 10), silverdin (n: 10) and DLH (n: 10) groups. DLH, collected from the beehive for 3–7 days in late spring and ready for use after homogenization and lyophilization. Two mL of physiological saline, silver sulfadiazine 1% and DLH were applied to the control, silverdin and DLH groups, respectively, and a thin layer that completely covered the wound, and repeated every 2 days for all groups for 14 days. The condition of the lesions was observed every 2 days and the amount of contraction and granulation tissue formed in the lesion was recorded. The lesioned areas was examined histopathologically.

Results: There was no statistically significant difference in lymphocyte, fibroblast, scar thickness, polymorphonuclear leukocyte (PMNL), minivascular density (CD34) and transforming growth factor-β1 (TGF-β1) among the control, silverdin and DLH groups ($p = 0.771$, 0.434, 0.07, 0.396). The scar density of the DLH group was found to be higher than the control and silverdin groups ($p = 0.003$). The average wound diameter of the control group (6.87 ± 0.93 mm^2) on the 10th day was found to be higher than the silverdin (4.39 ± 1.15 mm^2) and DLH groups (4.16 ± 0.55 mm^2) ($p = 0.0001$).

Conclusions: DLH has a positive effect on wound healing, especially by ensuring early wound contraction and wound scar formation.

1 | Introduction

Wound healing is a well-organized repair process that occurs after surgical procedures and major orthopedic traumas and involves the complex interaction of many cell types, cytokines or chemical mediators, and the extracellular matrix (Martin and Nunan 2015; Greaves et al. 2013). Depending on various factors, disorders that may occur in this organization delay or make the healing process chronic. Chronic wounds present a significant problem for both patients and surgeons (Han and Ceilley 2017; Powers et al. 2016; Caldwell 2010).

Nowadays, the use of chemical and synthetic drugs in wound healing has become extremely popular, but due to the emergence of some deficiencies, limitations (hepatotoxicity vs. nephrotoxicity) and side effects (wound infections, ülcerations, allergic reactions), the search for treatment using natural substances and medicinal plants still continues (Vaghardoost et al. 2018; Herman and Herman 2023; Hwang et al. 2021). When the literature is examined, the application of natural substances and medicinal plants in wound healing has been available for many years, especially honeybee products are still used as a healing and preventive solution in modern medicine (Pasupuleti et al. 2017; Vukovic et al. 2018; Kim et al. 2017; Khalil et al. 2017). Honeybee products such as propolis, honey, bee venom and recently drone larvae homogenate (DLH), which is especially a popular product in the food diet, have been used in traditional medicine for the treatment of various diseases since ancient times, and it has also been shown by many scientific studies to have biological activities such as antimicrobial, antioxidant, antitumor, anti-inflammatory and antiulcer (Oryan et al. 2018; Sidor et al. 2021; Sawczuk et al. 2019). Apart from the fact that these products contain many different chemicals and are used by bees in the hive due to their antibacterial-antioxidant effects, which are versatilely used in the pharmaceutical, cosmetics, food industry and alternative medicine (Guiné et al. 2022; Peršurić and Pavelić 2021). Drone larvae are available in larger quantities as part of the food diet. It is used at increasing rates, especially due to its rich content of protein source, hormone, essential amino acids, vitamins D and C, potassium and phosphorus, and when the literature is examined, its effects, such as estrogenic, nephroprotective and hepatoprotective, have been shown in in vitro studies (Hamamci et al. 2020; Seres et al. 2014).

As is the case all over the world, wound problems can occur at increasing rates in our country due to high-energy traumas in the young and elderly population; these wounds can lead to loss of labour, permanent disabilities and have high treatment costs. In recent years, importance has been given to natural and local drug production in order to reduce costs in the pharmaceutical sector. In addition, due to the increasing interest in natural products, as in every field, the research on the use of natural products for wound healing or strengthening the wound structure has come to the fore in this field. Our hypothesis is that due to the rich molecular content of drone larva homogenate, it will accelerate the wound healing process and have a positive effect. In this study, we aimed to evaluate the results of the macroscopic and histopathological effects of DLH on wound healing in a full-thickness open wound model in rats.

2 | Materials and Methods

2.1 | Materials

In this study, to obtain drone larvae, ready-made honeycombs containing male bee eyes were placed in the hives in the early spring, when egg laying was high. Ready-made combs with drone eyes were removed from the hives after 7–8 days, and 3–5 days old drone larvae were collected into sterile containers with pumping extractor without wasting time and were transported to the laboratory in organ transport boxes without breaking the cold chain. Drone larvae placed in sterile petri dishes were frozen and stored at −20°C. The lyophilization process was carried out by freeze-drying method at low pressure at −50°C for 2–3 h, and it was ground with a mortar to powder and stored at +4°C in daily dosages to be applied (Sawczuk et al. 2022). When it was to be applied to the wound, it was mixed with an appropriate amount of pure water to obtain a creamy consistency.

2.2 | Animals

The rats were simple randomly divided into 3 groups, 10 in each group [1st group: control (physiological saline), 2nd group: silver sulfadiazine 1% (Silverdin) and 3rd group: DLH]. The rats were on average 6–8 weeks old, weighed 250 ± 50 g and were monitored for any signs of health problems for 15 days of adaptation. The animals, which had no health problems, were kept under constant conditions (23°C–25°C, 30%–70% humidity, light/dark cycle 12/12 h) throughout the study and were fed with rat chow and tap water. The anesthetic drug dose was calculated by weighing each rat with an electronic scale. As an anaesthetic, a combination of ketamine (Eczacıbaşı, İstanbul, Turkey) 50 mg/kg and xylazine (Bayer, İstanbul, Turkey) 10 mg/kg was administered intraperitoneally from the left groin area. After anaesthesia, the back areas of the rats were shaved and prepared with 10% povidone iodine (Batticon, ADEKA, Turkey), a full-thickness wound was created with surgical blade in each of the control, silver sulfadiazine (DEVA, İstanbul, Turkey) and DLH groups, by removing 18.44 ± 1.45, 19.02 ± 1.24 and 19.37 ± 1.28 mm^2 of skin, respectively (Figure 1a–c). No medication was applied to the control group, 2 mL saline (Polifarma, Tekirdağ, Turkey) was applied to the skin lesion, 2 mL silverdin was applied to the silver sulfadiazine group, and DLH (2 mL) was applied to the treatment group to form a thin layer that completely covered the wound, and this was repeated every 2 days for all groups for 14 days (Figure 2a–c). After the surgical procedures, fentanyl citrate (0.02 mg/kg, Polifarma, Tekirdağ, Turkey) was administered subcutaneously to the animals for analgesia for 3 days. The subjects were monitored under the supervision of a specialist veterinarian, with five in each cage. The diameters of the skin lesions of the rats in each group were measured for contraction monitoring and simultaneously photographed and recorded on the 0th–3rd–7th–10th–14th days after the surgical procedure. Autocad software (Autodesk, USA) was used for diameter measurement on photographs (Figure 3). The lesioned area of the rats sacrificed at the end of the 14th day was excised for histopathological examination and placed in 10% formaldehyde and kept for pathological examination. The paraffin-embedded samples were stained with haematoxylin–eosin and Masson's trichrome stain.

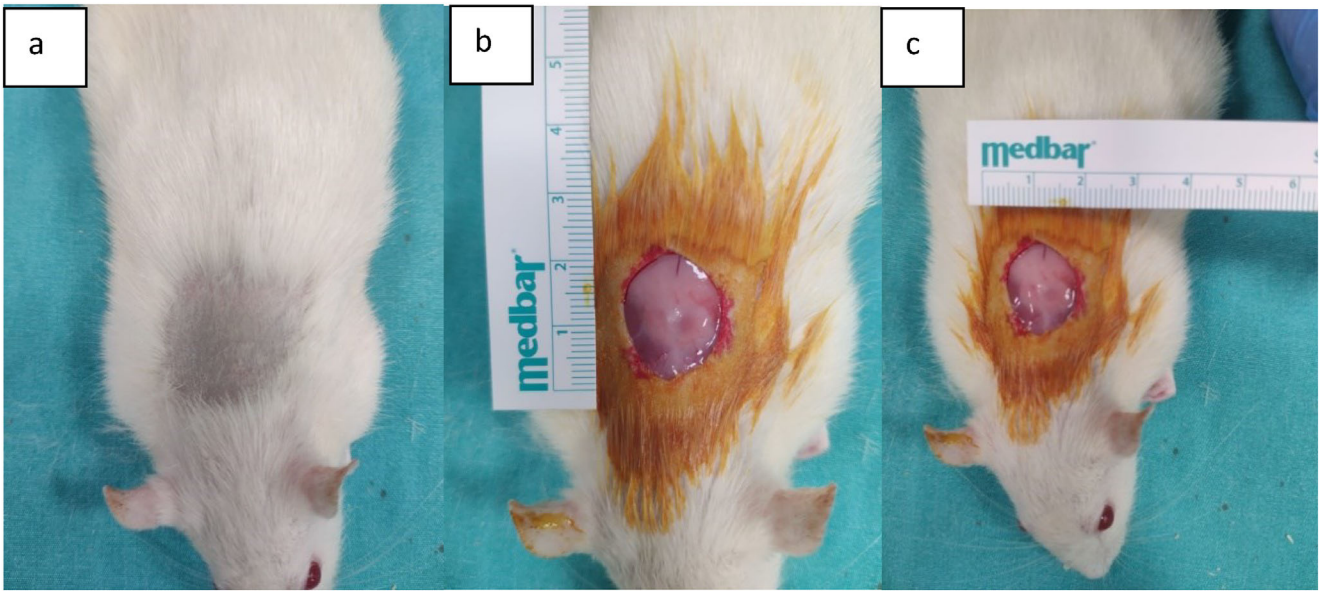

FIGURE 1 | (a–c) Shaving the back areas of the rats after anesthesia (a), creating a full-thickness wound by removing the skin after surgical preparation (b and c).

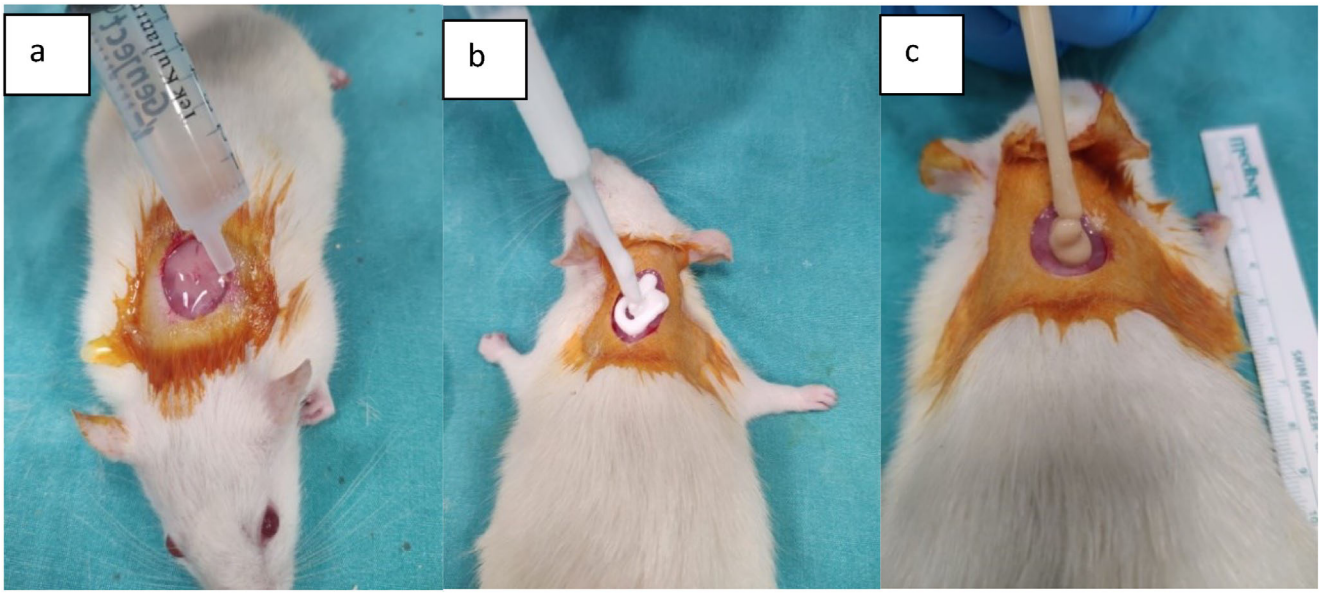

FIGURE 2 | (a–c) Application of physiological saline on the skin lesion of the control group (a), application of silverdin cream on the skin lesion of the silver sulfadiazine group (b), and application of DLH on the skin lesion of the treatment group (c).

All histological slides were reviewed by a pathologist (MG) who was blinded to experiment protocol. In addition, immunohistochemical staining was performed with CD31 (Biocare, 1/200) and transforming growth factor-β1 (TGF-β1) (Santa Cruz, 1/100) antibodies using the avidin biotin immunoperoxidase method. Lymphocyte density was categorized as mildly (1), moderately (2) and severely (3) (Figure 4). In addition, the number of fibroblasts in 1 mm^2 millimeter was measured (Figure 5). Numerical values were obtained by measuring of the scar thickness (Figure 6). After converting to black and white format of images from Masson's trichrome stain, scar densities were measured by ImageJ analysis program, as previously described (Figure 7) (Çalıskan et al. 2016). Vessel density of 1 mm^2 was obtained with CD31 dye. If TGF-β immunoexpression is focal (10% and below), score is 1, (10%–25%) score was given as 2, (25% and above) score was given as 3. Light intensity score was given as 1, moderate intensity score was given as 2, and severe intensity score was given as 3. These values were multiplied by each other to obtain the total score.

2.3 | Statistical Analysis

In this study, statistical analyses were performed with the NCSS (Number Cruncher Statistical System) 2007 Statistical

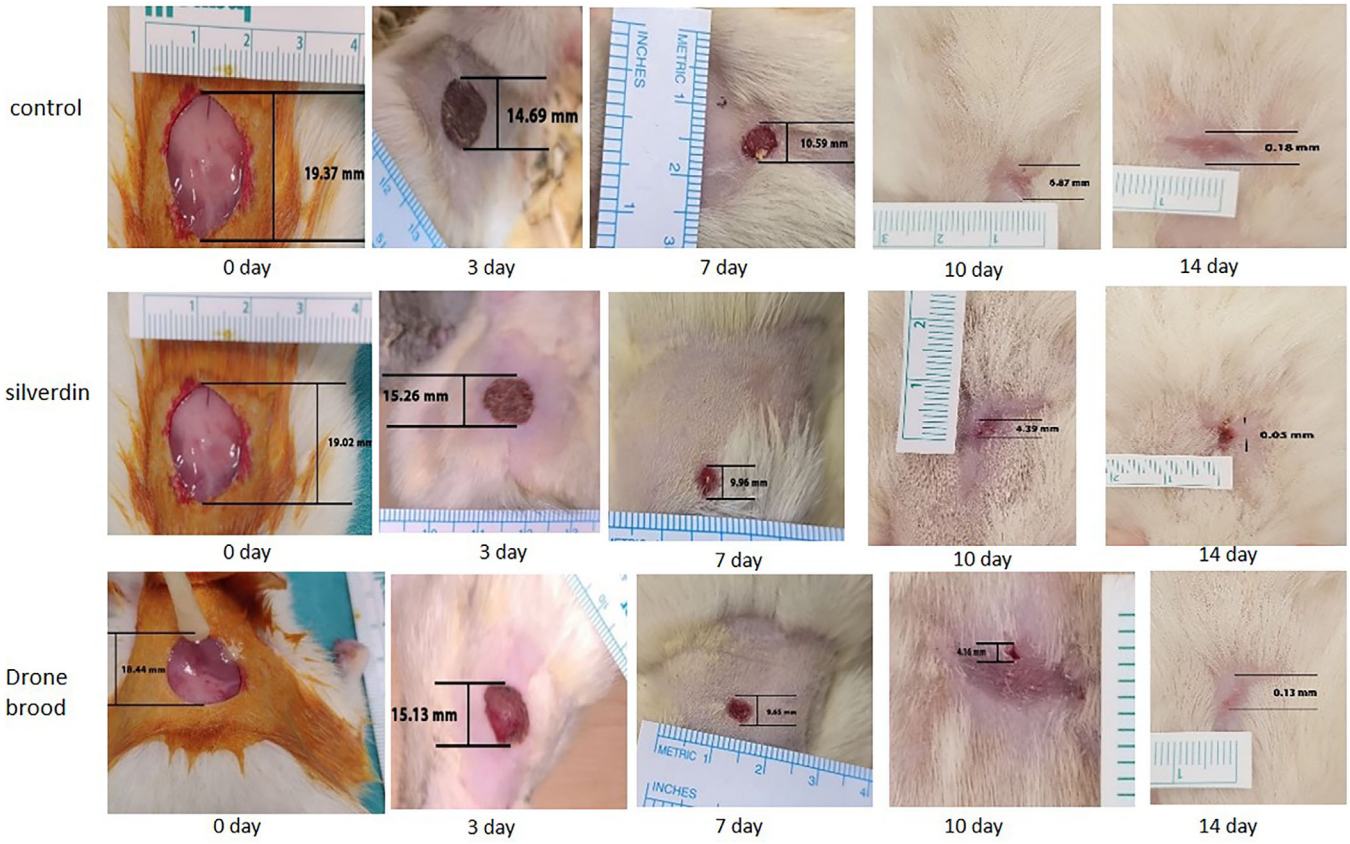

FIGURE 3 | Measurement of wound diameters of control, silverdin and DLH groups on Days 0, 3, 7, 10, 14 with the help of Autocad program.

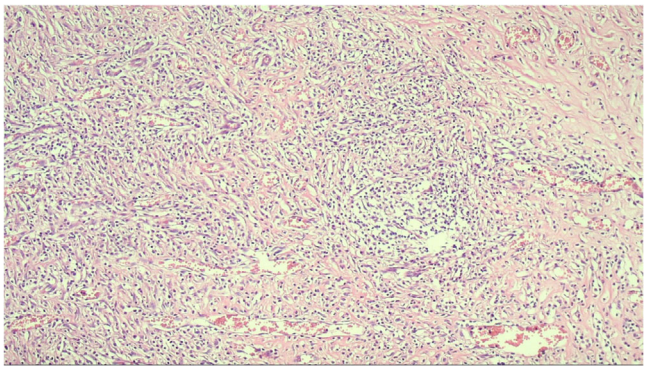

FIGURE 4 | Severely lymphocytic infiltration in scar area (H&E, ×200).

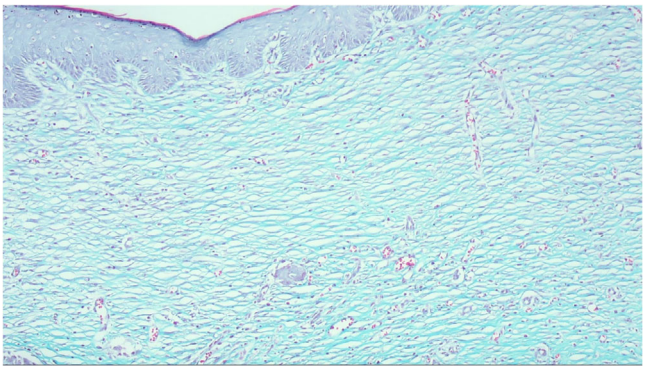

FIGURE 5 | Fibroblastic cells in scar area of a drone brood homogenate histopathology sample treated with DLH. (Masson's trichrome stain, ×200).

Software (Utah, USA) package program. In the evaluation of the data, in addition to descriptive statistical methods (mean, standard deviation, median, interquartile range), the distribution of variables was examined with the Shapiro–Wilk normality test. Paired one-way analysis of variance in time comparisons of normally distributed variables, Newman Keuls multiple comparison test for subgroup comparisons, One-way analysis of variance in comparisons between groups, Tukey multiple comparison test for subgroup comparisons, Kruskal Wallis test for intergroup comparisons of variables that do not show normal distribution, Dunn's multiple comparison test for subgroup comparisons, Chi-square test were used in comparisons of qualitative data. The results were evaluated at the significance level of $p < 0.05$.

3 | Results

No statistically significant difference was observed between the lymphocyte count distributions, fibroblast number averages and polymorphonuclear leukocyte (PMNL) distributions of the control (physiological saline), silver sulfadiazine 1% (Silverdin) and DLH groups ($p = 0.771$, 0.434 and 0.396). Additionally, no statistically significant difference were observed between the scar thickness averages of all groups ($p = 0.07$). A statistically significant difference was observed between the scar density averages of the control, silver sulfadiazine and DLH groups ($p = 0.004$). Although the scar density of the DLH group was statistically significantly higher than the control and silver sulfadiazine

FIGURE 6 | Measuring of the scar thickness in a control sample (Masson's trichrome stain, ×40).

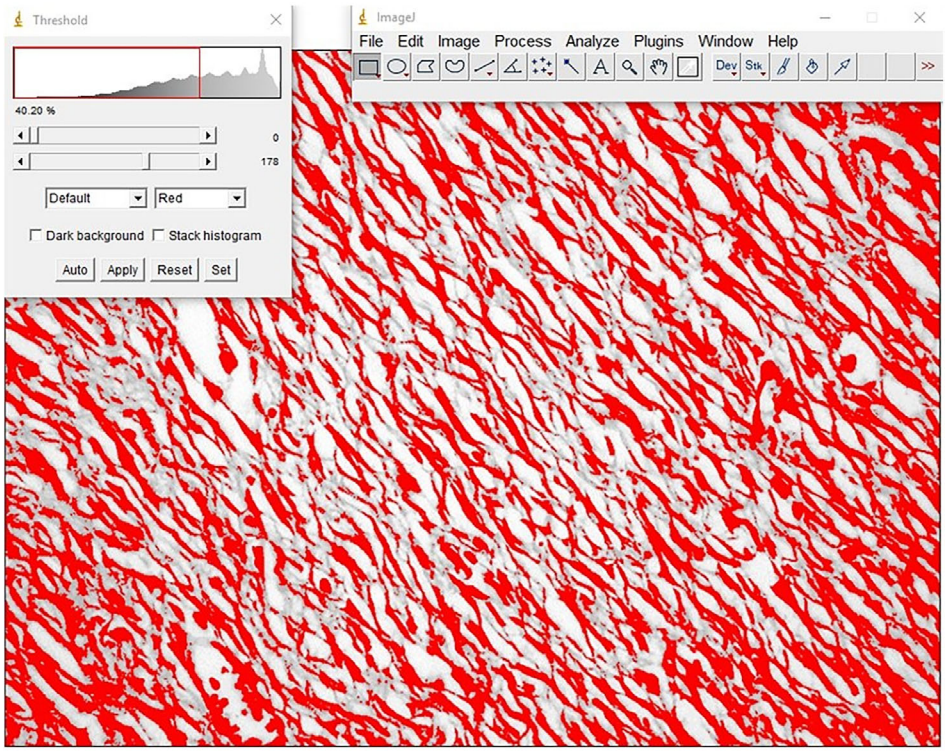

FIGURE 7 | Measuring of the scar density in a drone brood sample by ImageJ.

groups ($p = 0.003$), no statistically significant difference was observed among other groups ($p > 0.05$). No statistically significant difference was observed between the minivascular density (CD34) averages of all groups ($p = 0.323$). A statistically significant difference was observed among the TGF-β distributions of the control, silver sulfadiazine and DLH groups ($p = 0.0001$). But the presence of TGF-β heavy in the silver sulfadiazine group was found to be higher than in the control and DLH groups (Table 1).

No statistically significant difference was observed between the wound diameter averages of the control, silver sulfadiazine and DLH groups on Days 0, 3 and 7 ($p = 0.303$, 0.665 and 0.458). However, a statistically significant difference was observed among the 10th day wound diameter averages of all groups ($p = 0.0001$). The 10th day wound diameter average of the silver sulfadiazine and DLH groups was found to be statistically significantly lower than the 10th day wound diameter average of the control group ($p = 0.0001$). No statistically significant difference was observed among the average wound diameters of the silver sulfadiazine and DLH groups ($p = 0.840$). No statistically significant difference was observed between the 14th day wound diameter averages of all groups ($p = 0.510$) (Table 2, Figure 8).

TABLE 1 | Comparison of histopathological evaluation between control, silverdin and drone brood homogenate.

		Control		Silverdin		Drone brood		*p*
Lymphocyte	**No**	5	50.00%	5	50.00%	4	40.00%	0.771[b]
	Mild	4	40.00%	5	50.00%	4	40.00%	
	Moderate	0	0.00%	0	0.00%	1	10.00%	
	Severe	1	10.00%	0	0.00%	1	10.00%	
Fibroblast		1351.8 ± 197.45		1494 ± 346.86		1488.6 ± 258.22		0.434[a]
Scar thickness		1938.6 ± 461.41		1570.5 ± 418.78		1499.6 ± 424.53		0.07[a]
Scar density		35.1 ± 5.28		30.88 ± 10.2		42.94 ± 5.83		**0.004**[a]
PMNL	**No**	10	100.00%	9	90.00%	9	90.00%	0.396[b]
	Mild	0	0.00%	1	10.00%	0	0.00%	
	Moderate	0	0.00%	0	0.00%	1	10.00%	
Microvascular density (CD34)		65.3 ± 8.07		67.1 ± 10.16		61.2 ± 8.03		0.323[a]
TGF-*β*	**Mild**	8	80.00%	0	0.00%	0	0.00%	**0.0001**[b]
	Moderate	2	20.00%	3	30.00%	7	70.00%	
	Severe	0	0.00%	7	70.00%	3	30.00%	

Abbreviations: PMNL, polymorphonuclear leukocyte; TGF-*β*, transforming growth factor-*β*.
[a]One-way analysis of variance,
[b]Chi square test.

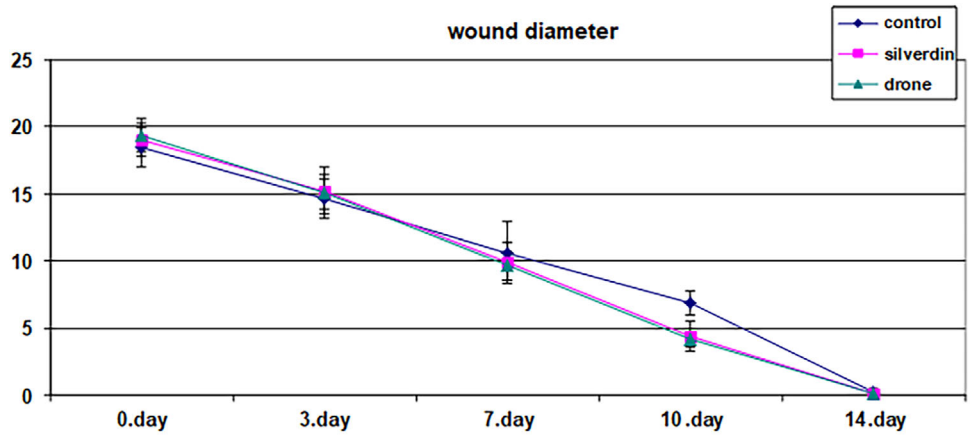

FIGURE 8 | Comparison of wound diameter between control, silverdin and DLH.

No statistically significant difference was observed between the 0–3 and 0–7 day wound diameter difference averages of the control, silver sulfadiazine and DLH groups ($p = 0.988$, 0.155). However, a statistically significant difference was observed between the 0th day and 10th day wound diameter averages of all groups ($p = 0.0001$).The 10th day wound diameter difference averages of the silver sulfadiazine and DLH groups were found to be statistically significantly higher than the 10th day wound diameter difference average of the control group ($p = 0.001$). No statistically significant difference was observed between the wound diameter difference averages of the silver sulfadiazine and DLH groups ($p = 0.211$). No statistically significant difference was observed between the wound diameter difference averages between Days 0 and 14 of all groups ($p = 0.260$) (Table 3). No rats died during the study period and no wound infection, allergic reaction or ulceration was observed in any rat.

4 | Discussion

Wounds that do not heal for a long time after major orthopedic surgeries are an important health problem that affects the quality of life of patients and also create very high health costs all over the world (Gantwerker and Hom 2011; Druecke et al. 2004). Many in vivo and in vitro studies have been conducted in the literature to develop more useful and effective dressings to accelerate the healing process and reduce the bacterial load in wounds (Oryan et al. 2018; Gantwerker and Hom 2011; Sağlam et al. 2024). Honeybee products (honey, bee venom, propolis, etc.), which have been used in the treatment of various diseases in traditional medicine for many years, have been widely tested in studies for their use as a healing and preventive solution in modern medicine since the end of the 20th century (Sawczuk et al. 2022; Sidor and Dżugan 2020).

TABLE 2 | Comparison of wound diameter between control, silverdin and drone brood homogenate.

Wound diameter (days)	Control (mm)	Silverdin (mm)	Drone brood (mm)	*p**
0	18.44 ± 1.45	19.02 ± 1.24	19.37 ± 1.28	0.303
3	14.68 ± 1.46	15.26 ± 1.71	15.13 ± 1.28	0.665
7	10.59 ± 2.31	9.96 ± 1.46	9.65 ± 1.06	0.458
10	6.87 ± 0.93	4.39 ± 1.15	4.16 ± 0.55	**0.0001**
14	0.18 ± 0.29	0.05 ± 0.16	0.13 ± 0.28	0.510
***p*‡**	**0.0001**	**0.0001**	**0.0001**	

Tukey multiple comparison test	*p*
Control/silverdin	**0.0001**
Control/drone brood	**0.0001**
Silverdin/drone brood	0.840

Newman Keuls multiple comparison test	Control	Silverdin	Drone
0 day/3 day	**0.0001**	**0.0001**	**0.0001**
0 day/7 day	**0.0001**	**0.0001**	**0.0001**
0 day/10 day	**0.0001**	**0.0001**	**0.0001**
0 day/14 day	**0.0001**	**0.0001**	**0.0001**
3 day/7 day	**0.0001**	**0.0001**	**0.0001**
3 day /10 day	**0.0001**	**0.0001**	**0.0001**
3 day /14 day	**0.0001**	**0.0001**	**0.0001**
7 day/10 day	**0.0001**	**0.0001**	**0.0001**
7 day/14 day	**0.0001**	**0.0001**	**0.0001**
10 day/14 day	**0.0001**	**0.0001**	**0.0001**

*One-way analysis of variance.

‡Paired one-way analysis of variance.

TABLE 3 | Comparison of wound diameter differences between control, silverdin and drone brood homogenate.

Wound diameter difference (days)		Control (mm)	Silverdin (mm)	Drone brood (mm)	*p*
0–3	**Mean ± SD**	3.76 ± 0.73817	3.76 ± 1.35745	4.24 ± 2.11198	0.988
	Median (IQR)	3.9 (3–4.4)	4.2 (2.38–4.85)	3.45 (2.43–6.85)	
0–7	**Mean ± SD**	7.85 ± 1.78715	9.06 ± 1.80505	9.72 ±.2.03076	0.155
	Median (IQR)	8.35 (5.6–9.43)	8.75 (7.73–10.05)	9.4 (8.53–11.38)	
0–10	**Mean ± SD**	11.57 ± 0.77896	14.63 ± 0.84334	15.21 ± 1.55095	**0.0001**
	Median (IQR)	11.65 (10.98–12.13)	14.4 (14.1–15.2)	15.2 (14.28–16.38)	
0–14	**Mean ± SD**	18.26 ± 1.34181	18.97 ± 1.28413	19.24 ± 1.30571	0.260
	Median (IQR)	17.9 (17.18–19.48)	18.95 (17.75–19.53)	19.3 (17.98–20.5)	

Dunn's multiple comparisons test	*p*
Control/silverdin	**0.001**
Control/drone brood	**0.001**
Silverdin/drone brood	0.211

Note: Kruskal Wallis test.

Abbreviations: IQR, interquartile range; SD, standard deviation.

In recent scientific studies, DLH, a honey bee product that has become popular and has an increasing importance in the food diet, has been shown to have antimicrobial, antioxidant and anti-inflammatory biological activities (Seres et al. 2014; Inci et al. 2023; Elashal et al. 2024; El-Wahed et al. 2024). Additionally, there are quite a few studies in the field of medicine investigating estrogenic activity, nephroprotective and hepatoprotective effects (Seres et al. 2013; Inandiklioglu et al. 2021; Doğanyiğit et al. 2020). In line with all these data, on the basis of the wound healing problems experienced in major orthopedic injuries, we applied DLH in an experimental full-thickness wound model, which has been shown to have antimicrobial, antioxidant and anti-inflammatory activities and is a popular food diet product.

In wound healing, the depth of the wound determines the amount of contraction and the source of keratinocytes used for re-epithelialization (Yannas et al. 2017). Contraction is an important part of wound healing, resulting in a reduction in wound size of up to 40%. In full-thickness wounds, contraction begins immediately after the injury and reaches its peak in the second week. It is a dynamic process in which collagen fibres synthesized by newly migrating fibroblasts form the connective tissue matrix. After that, fibroblasts differentiate into myofibroblasts, which are responsible for the traction force to pull the wound edges towards the wound centre, resulting in a gradual reduction of the wound area (Desmoulière et al. 2005). Although there was no significant difference in the number of fibroblasts in all groups in our study, the difference in the amount of wound contraction on the 10th day and the difference in wound diameter on the 0–10th day in the group to which we applied DLH were better than the control group.

Scar is one of the complex structures in the tissue regeneration and repair process in wound healing. It is a connective tissue change characterized by densely packed, oriented collagen fibrils in damaged tissue. The degree of fibrosis in wound healing varies according to organs, tissues and individuals (Druecke et al. 2004; Yannas et al. 2017). TGF-β is effective in the inflammatory and proliferative stages of wound healing and is a strong pro-inflammatory modulator and regulator of collagen, which is thought to be related to scar formation (Lichtman et al. 2016; Cowin et al. 2001; Vaughan et al. 2000). In our study, there was no significant difference in the average scar thickness of all groups, but the scar density in the DLH applied group was significantly higher than the other groups. When TGF-β distributions were evaluated, the heavy presence of TGF-β was more significant in the silver sulfadiazine group, which was the positive control group. Additionally, the presence of TGF-β medium was higher in number in the group treated with DLH. As in the literature, it has been stated that TGF-β is associated with scarring in some studies and that it has no effect in some studies (Cowin et al. 2001; Vaughan et al. 2000). In our study, it was concluded that it had no effect, as in the literature.

In the literature, we found only one experimental study similar to our study in which DLH was evaluated in wound healing (Andritoiu et al. 2021). In the study by Andritoiu et al., in which they evaluated the healing effect of ointments on the basis of honey bee products on cutaneous lesions, they compared honey, apilarnil (DLH), propolis and a mixture of all three, macroscopically and histopathologically. In this study where macroscopic wound diameter, re-epithelialization and wound contraction were evaluated, it was concluded that there was no difference among all groups. In addition, when evaluated histopathologically, it was stated that there was a similar appearance in terms of lymphocyte and fibroblast numbers, along with significant edema in the dermis, in all three models: incision, excision and thermal burn. As a result, it was emphasized that each of the propolis, honey and apilarnil-based formulations had a healing and re-epithelialization effect on three experimental models. When all three wound models were examined separately in the group treated with DLH -based ointment, it was stated that normal skin extensions were seen in the incision model on the 21st day, collagenization and associated lymphocytic leakage were present in the excision model, and dermal collagenization was observed in the thermal injury model (Andritoiu et al. 2021). In our study, unlike this study, we compared a control group using physiological saline in wound healing and a positive control group, silver sulfadiazine, whose effect has been previously proven. In our comparative experimental study in which we created a full-thickness skin defect and applied local DLH, we found that it increased scar density and wound contraction in early wound healing.

One of our limitations is that our experimental study is an animal study rather than a clinical study. The second is that the biochemical-molecular examination of the DLH we obtained was not carried out and different parameters indicating that wound healing was not examined histopathologically. In addition, the short follow-up period is also a shortcoming of the study.

In conclusion, wound healing is an excellent repair process that includes cell migration and proliferation, extracellular matrix synthesis, angiogenesis and remodelling. Disruptions that may occur in this repair process will cause major problems in the healing of acute and chronic wounds. When scientific studies investigating the most effective dressings in wound healing are evaluated, our experimental study shows that DLH can have a positive effect on wound healing by causing wound contraction and wound scar formation, especially in the early period. Drone larvae, which have become a popular part of the food diet in recent years, can be transformed into an effective apitherapeutic agent in wound healing. However, more animal studies are needed to determine the critical mechanisms of its pharmacological action and the appropriate amounts to be ingested to achieve optimal wound healing.

Author Contributions

Creating a hypothesis: Mehmet Arıcan and Meral Kekecoglu. Planning and organization: Tuğçe Çaprazlı and Yalçın Turhan. Material supply (biological, technical): Yalçın Turhan and Zekeriya Okan Karaduman. Data collecting: Tuğçe Çaprazlı, Zekeriya Okan Karaduman. Data analysis, statistics: Mehmet Arıcan, Meral Kekecoglu, Mehmet Gamsızkan. Article writing: Mehmet Arıcan.

Ethics Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Duzce University Animal Experiments Local Ethics committee of Duzce University Experimental Animal Research and Application Center (protocol code 2019/1/6 and date of

approval: 19 February 2019). Male Wistar rats used in the study were obtained from Duzce University Experimental Animal Research and Application Centre.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analysed during the current study are available from the corresponding author (Mehmet Arıcan) on reasonable request.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.70260.

References

Andritoiu, C. V., C. Lungu, M. Danu, et al. 2021. "Evaluation of the Healing Effect of Ointments Based on Bee Products on Cutaneous Lesions in Wistar Rats." *Pharmaceuticals (Basel)* 14, no. 11: 1146.

Caldwell, M. D. 2010. "Wound Surgery." *Surgical Clinics of North America* 90, no. 6: 1125–1132.

Çalıskan, E., M. Gamsızkan, G. Açıkgöz, et al. 2016. "Intralesional Treatments for Hypertrophic Scars: Comparison Among Corticosteroid, 5-Fluorouracil and Botulinum Toxin in Rabbit Ear Hypertrophic Scar Model." *European Review for Medical and Pharmacological Sciences* 20, no. 8: 1603–1608.

Cowin, A. J., T. M. Holmes, P. Brosnan, and M. W Ferguson. 2001. "Expression of TGF-Beta and Its Receptors in Murine Fetal and Adult Dermal Wounds." *European Journal of Dermatology* 11, no. 5: 424–431.

Desmoulière, A., C. Chaponnier, and G. Gabbiani. 2005. "Tissue Repair, Contraction, and the Myofibroblast." *Wound Repair and Regeneration* 13, no. 1: 7–12.

Doğanyiğit, Z., A. Okan, E. Kaymak, D. Pandır, and S. Silici. 2020. "Investigation of Protective Effects of Apilarnil Against Lipopolysaccharide Induced Liver Injury in Rats via TLR 4/HMGB-1/ NF-κB Pathway." *Biomedicine & Pharmacotherapy* 125: 109967.

Druecke, D., E. N. Lamme, S. Hermann, et al. 2004. "Modulation of Scar Tissue Formation Using Different Dermal Regeneration Templates in the Treatment of Experimental Full-Thickness Wounds." *Wound Repair and Regeneration* 12, no. 5: 518–527.

Elashal, M. H., A. A. Abd El-Wahed, M. A. Mohamed, et al. 2024. "Apilarnil Ameliorates Bisphenol A-Induced Testicular Toxicity in Adult Male Rats via Improving Antioxidant Potency and PCNA Expression." *Reproductive Toxicology* 125: 108570.

El-Wahed, A. A., S. A. M. Khalifa, B. Aldahmash, et al. 2024. "Exploring the Chemical Constituents and Nutritive Potential of Bee Drone (Apilarnil): Emphasis on Antioxidant Properties." *Chemistry and Biodiversity* 21: e202400085.

Gantwerker, E. A., and D. B Hom. 2011. "Skin: Histology and Physiology of Wound Healing." *Facial Plastic Surgery Clinics of North America* 19, no. 3: 441–453.

Greaves, N. S., K. J. Ashcroft, M. Baguneid, and A. Bayat. 2013. "Current Understanding of Molecular and Cellular Mechanisms in Fibroplasia and Angiogenesis During Acute Wound Healing." *Journal of Dermatological Science* 72, no. 3: 206–217.

Guiné, R. P. F., S. G. Florença, P. M. R. Correia, O. Anjos, C. Coelho, and C. A Costa. 2022. "Honey Bee (*Apis mellifera* L.) Broods: Composition, Technology and Gastronomic Applicability." *Foods* 11, no. 18: 2750.

Hamamci, M., Z. Doganyigit, S. Silici, et al. 2020. "Apilarnil: A Novel Neuroprotective Candidate." *Acta Neurologica Taiwanica* 29, no. 2: 33–45.

Han, G., and R. Ceilley. 2017. "Chronic Wound Healing: A Review of Current Management and Treatments." *Advances in Therapy* 34, no. 3: 599–610.

Herman, A., and A. P Herman. 2023. "Herbal Products and Their Active Constituents for Diabetic Wound Healing-Preclinical and Clinical Studies: A Systematic Review." *Pharmaceutics* 15, no. 1: 281.

Hwang, J. R., A. M. Cartron, and A. Khachemoune. 2021. "A Review of Nigella Sativa Plant-Based Therapy in Dermatology." *International Journal of Dermatology* 60, no. 12: e493–e499.

Inandiklioglu, N., Z. Doganyigit, A. Okan, E. Kaymak, and S. Silici. 2021. "Nephroprotective Effect of Apilarnil in Lipopolysaccharide-Induced Sepsis Through TLR4/NF-κB Signaling Pathway." *Life Sciences* 284: 119875.

Inci, H., E. Izol, M. A. Yilmaz, M. Ilkaya, Z. Bingöl, and I. Gülçin. 2023. "Comprehensive Phytochemical Content by LC/MS/MS and Anticholinergic, Antiglaucoma, Antiepilepsy, and Antioxidant Activity of Apilarnil (Drone Larvae)." *Chemistry and Biodiversity* 20, no. 10: e202300654.

Khalil, H., M. Cullen, H. Chambers, and M. McGrail. 2017. "Medications Affecting Healing: An Evidence-Based Analysis." *International Wound Journal* 14, no. 6: 1340–1345.

Kim, S., I. Hong, S. Woo, H. Jang, S. Pak, and S. Han. 2017. "Isolation of Abscisic Acid From Korean Acacia Honey With Anti-*Helicobacter pylori* Activity." *Pharmacognosy Magazine* 13, no. S2: S170–S173.

Lichtman, M. K., M. Otero-Vinas, and V. Falanga. 2016. "Transforming Growth Factor Beta (TGF-*β*) Isoforms in Wound Healing and Fibrosis." *Wound Repair and Regeneration* 24, no. 2: 215–222.

Martin, P., and R. Nunan. 2015. "Cellular and Molecular Mechanisms of Repair in Acute and Chronic Wound Healing." *British Journal of Dermatology* 173, no. 2: 370–378.

Oryan, A., E. Alemzadeh, and A. Moshiri. 2018. "Potential Role of Propolis in Wound Healing: Biological Properties and Therapeutic Activities." *Biomedicine & Pharmacotherapy* 98: 469–483.

Pasupuleti, V. R., L. Sammugam, N. Ramesh, and S. H Gan. 2017. "Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits." *Oxidative Medicine and Cellular Longevity* 2017: 1259510.

Peršurić, Ž., and S. K Pavelić. 2021. "Bioactives From Bee Products and Accompanying Extracellular Vesicles as Novel Bioactive Components for Wound Healing." *Molecules (Basel, Switzerland)* 26, no. 12: 3770.

Powers, J. G., C. Higham, K. Broussard, and T. J Phillips. 2016. "Wound Healing and Treating Wounds: Chronic Wound Care and Management." *Journal of the American Academy of Dermatology* 74, no. 4: 607–625. quiz 625–6.

Sağlam, S., Z. Orhan, M. Arıcan, et al. 2024. "Topical or Intravenous Administration of Tranexamic Acid Accelerates Wound Healing." *Joint Diseases and Related Surgery* 35, no. 1: 85–95.

Sawczuk, R., J. Karpinska, D. Filipowska, A. Bajguz, and M. Hryniewicka. 2022. "Evaluation of Total Phenols Content, Anti-DPPH Activity and the Content of Selected Antioxidants in the Honeybee Drone Brood Homogenate." *Food Chemistry* 368: 130745.

Sawczuk, R., J. Karpinska, and W. Miltyk. 2019. "What Do We Need to Know About Drone Brood Homogenate and What Is Known." *Journal of Ethnopharmacology* 245: 111581.

Seres, A. B., E. Ducza, M. Báthori, et al. 2013. "Raw Drone Milk of Honeybees Elicits Uterotrophic Effect in Rats: Evidence for Estrogenic Activity." *Journal of Medicinal Food* 16, no. 5: 404–409.

Seres, A. B., E. Ducza, M. Báthori, et al. 2014. "Androgenic Effect of Honeybee Drone Milk in Castrated Rats: Roles of Methyl Palmitate and Methyl Oleate." *Journal of Ethnopharmacology* 153, no. 2: 446–453.

Seres, A., E. Ducza, and R. Gáspár. 2014. "A Nyers heretej gesztagén hatásainak vizsgálata patkányban [Investigation of Gestagenic Effect of Raw Drone Milk in Rats]." *Acta Pharmaceutica Hungarica* 84, no. 2: 77–81.

Sidor, E., and M. Dżugan. 2020. "Drone Brood Homogenate as Natural Remedy for Treating Health Care Problem: A Scientific and Practical Approach." *Molecules (Basel, Switzerland)* 25, no. 23: 5699.

Sidor, E., M. Miłek, M. Tomczyk, and M. Dżugan. 2021. "Antioxidant Activity of Frozen and Freeze-Dried Drone Brood Homogenate Regarding the Stage of Larval Development." *Antioxidants (Basel)* 10, no. 5: 639.

Vaghardoost, R., S. G. Mousavi Majd, H. Tebyanian, et al. 2018. "The Healing Effect of Sesame Oil, Camphor and Honey on Second Degree Burn Wounds in Rat." *World Journal of Plastic Surgery* 7, no. 1: 67–71.

Vaughan, M. B., E. W. Howard, and J. J Tomasek. 2000. "Transforming Growth Factor-Beta1 Promotes the Morphological and Functional Differentiation of the Myofibroblast." *Experimental Cell Research* 257, no. 1: 180–189.

Vukovic, N. L., A. D. Obradovic, M. D. Vukic, D. Jovanovic, and P. M Djurdjevic. 2018. "Cytotoxic, Proapoptotic and Antioxidative Potential of Flavonoids Isolated From Propolis Against Colon (HCT-116) and Breast (MDA-MB-231) Cancer Cell Lines." *Food Research International* 106: 71–80.

Yannas, I. V., D. S. Tzeranis, and P. T. C So. 2017. "Regeneration of Injured Skin and Peripheral Nerves Requires Control of Wound Contraction, Not Scar Formation." *Wound Repair and Regeneration* 25, no. 2: 177–191.