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Research Article

Healing and Antimicrobial Effects of *Olea europaea* L. (Memecik olive oil) on Wounds in Rats

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ABSTRACT

This study evaluated the effectiveness of *Olea europaea* L. (Memecik olive oil) on wound healing in Wistar albino rats (n=63) were used to create an experimental back skin wound model. The subjects were divided into three groups: the control group, the olive oil group, and the drug (medication group). The study results revealed that the olive oil group exhibited faster wound healing and smaller wound areas, than the control and drug groups. Histopathological examination demonstrated a significant increase in epithelial tissue formation in the animals treated with olive oil. Olive oil positively affects wound healing by antimicrobial and antioxidant properties. The study also found that bacterial colonization increased on the second day in all groups, with the highest increase observed in the control group. However, bacterial counts were found to be below the initial levels in both the olive oil and drug groups on the 12th day, whereas it remained high in the control group. The bacterial inhibitory effect is thought to be related to the phenolic compounds in the treated group with olive oil.

Keywords: *Bacteria, epithelization, granulation, inflammation.*

Sıçan Yaralarında Olea europaea L. (Memecik zeytinyağı)'nin Antimikrobiyel ve İyileştirici Etkileri

ÖZET

Bu çalışma sıçanlarda *Olea europaea* L. (Memecik zeytinyağı) yara iyileşmesi üzerine etkinliğinin değerlendirildi. Çalışmada toplam 63 adet Winstar albino sıçan üzerinde deneysel sırt deri yara modeli oluşturulmuştur. Denekler kontrol grubu, zeytinyağı grubu ve ilaç grubu olmak üzere üç gruba ayrılmıştır. Çalışma sonucunda zeytinyağı grubunda kontrol ve ilaç grubuna kıyasla daha hızlı yara iyileşmesi ve makroskobik olarak daha küçük yara dokusu bulguları elde edilmiştir. Histopatolojik incelemede zeytinyağı grubunda epitel doku oluşumunda önemli bir artış olduğu belirlenmiştir. Zeytinyağının antimikrobiyel ve antioksidan özelliklere sahip olmasının yara iyileşmesi üzerinde olumlu bir etkiye sahip olabileceğini düşündürmüştür. Bu çalışmanın 12. gününde bakteri yükünün tüm gruplarda arttığı, en yüksek artışın da kontrol grubunda olduğu görülmüştür. Ancak 12. günde zeytinyağı ve ilaç grubunda bakteri yükü başlangıç seviyesinin altında, kontrol grubunda ise üzerinde bulunmuştur. Zeytinyağı ile tedavi edilen grupta görülen bakteri inhibisyon etkisinin, zeytinyağında bulunan fenolik bileşiklerle ilişkili olduğu düşünülmektedir.

Anahtar kelimeler: *Bakteri, epitelizasyon, granulasyon, inflamasyon.*

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Introduction

Olea europaea L. (Memecik olive) is an olive type from the Olea genus in the Oleaceae family that grows in the Mediterranean climate (Yanar, 2015). Memecik olive, which constitutes more than 50% of the tree population in the Aegean region, is the most important olive variety evaluated for oil and is widely grown in the provinces of Muğla, İzmir and Aydın in Türkiye (Ozturk et al., 2009). Memecik olive oil is a highly valuable product that constitutes 30% of the vegetable oil trade. It is obtained from the physical processing of Memecik olives (Ilyasoglu and Ozcelik, 2011).

Olive oil is produced by cold pressing the fruits of the *Olea europaea* tree. Türkiye ranks 5th in the world with an annual olive oil production of 120 thousand tons (Yanar, 2015). On the other hand, the Aegean region ranks 1st in Türkiye, accounting for 70% of the total production. (Ilyasoglu and Ozcelik, 2011). The constituents of the oil can be roughly divided into two groups: a major glycerol fraction and a minor unsaponifiable non-glycerol fraction (Tripoli et al., 2005). Olive oil has been shown to control the synthesis of cytokines, increase nitric oxide, and decrease the synthesis of prostaglandins and leukotriene B4 (Viola and Viola, 2009). Additionally, olive oil also has anti-inflammatory, antimicrobial, and antioxidant properties that are crucial for wound healing (Al-Waili et al., 2006). According to research, olive oil increases blood flow to all tissues and reduces inflammation, promoting wound healing (Nasiri et al., 2015).

The wound is a loss of skin function and integrity according to definitions given by physical (Imran et al., 2015). Both acute and chronic wounds fail to close within this time frame after a sluggish and disorganized healing process. Acute wounds heal within 7–10 days. Numerous factors, such as underlying illness, infection, protracted inflammation, medication use, and oxidative stress brought on by aging, have been linked to chronic wounds (Ibrahim et al., 2018).

In general, it is thought that natural remedies have fewer side effects than synthetic medications. Many natural products that have been traditionally used for wound healing, such as olive oil, and the use of herbal remedies is growing quickly (Panahi et al., 2012). Thus, this study aimed to investigate the effect of topical administration *Olea europaea* L. oil on wound healing in rats.

Materials and Methods

Animals

The study was conducted with the approval of the Muğla Sıtkı Koçman University Animal Experiments Local Ethics Committee under permit number 30.11.2021-45/21. A total of 63 Wistar albino rats with a live weight of 230-250 g were used in the study, with 21 animals in each group. The rats were provided by the Muğla Sıtkı Koçman University Milas Faculty of Veterinary Medicine Experimental Animals Application and Research Center. The rats were maintained in a 12/12 hour light/dark environment at a temperature of 22±2°C, with ad libitum access to food and water.

Experimental Design

Three groups were formed with 21 animals in each working group, making a total of 63 Wistar albino rats used. The created groups and applications are as follows:

1. Group (Control group): No medication or treatment was applied to the rats in this group from the time of wound formation.

2. Group (Olive oil group): *Olea europaea* L. (Memecik olive) olive oil was applied twice a day (0.1 ml) to the rats in this group until the day of sacrifice at the wound area.

3. Group (Drug group): The drug consisting of a nitrofurazone antibiotic pomade (Furacin®, Sanofi-Aventis, 0.2%, France) and centella asiatica (Madecasol® Bayer, 1%, Germany) mixed half was applied twice a day (0.1 ml) to the rats in this group until the day of sacrifice at the wound area.

Olea europaea L. (Memecik olive) Olive Oil

The olive oils used in the wound healing were obtained from the farm of Muğla Sıtkı Koçman University, Faculty of Milas Veterinary Medicine. For this purpose, oils from Memecik olives were preferred, which were cold-pressed during the harvest season (December-January) and were suitable for human consumption.

Wound Model and Measurements

Rats were anesthesized by administering 10 mg/kg Xylazine hydrochloride (Rompun®, Bayer 23.32 mg/ml, Germany) followed by 70 mg/kg Ketamin hydrochloride (Ketalar®, Parke-Davis, 50 mg/ml, Germany) injection intramuscularly. The dorsal area was shaved along a 3 cm line surrounding the intrascapular region. After the shaving area was disinfected with povidone-iodine (Batticon® Adeka, 10%, Türkiye) a full-thickness skin wound model was created using a 5 mm punch trephine. Following the operation, the wound site was measured in terms of length and width in mm until the day of sacrifice and documented by taking photographs. The photos were transferred to the "Image j" program in a computer environment to calculate the wound surface areas. Total wound healing was determined by looking at the difference in wound areas between day 2 and subsequent days. Treatment started simultaneously on day 0 in all groups.

Histopathologic Analyses

After the formation of the wound, rats were euthanized by cervical dislocation under general anesthesia on days 2, 5, 7, 10, and 15. In order to perform controlled histopathology, four rats were euthanized in each group on days 2, 5, 7, 10 and five rats were euthanized in day 15. For this purpose, the wound area and the surrounding full-thickness skin, including the panniculus layer, were removed from the body. The skin samples taken were fixed in a 10% formaldehyde solution for histopathologi-

cal examination. After fixation, the tissues underwent a tissue processing procedure consisting of an alcohol and xylene series, and then they were embedded in paraffin blocks. Sections with a thickness of 3 µm were transferred from the paraffin blocks to slides and stained with hematoxylin-eosin. Subsequently, microscopic examination was performed on these sections.

Collection and Analysis of Microbiological Swab Samples

After the formation of the wound, swab samples were taken from a 1 cm² wound area on days 0, 2, 6, and 12. The swab samples were sent to the microbiology laboratory for aerobic mesophilic live count. It was ensured that 12 hours had passed since the application of the positive control group and the olive oil group on the wound area before swabbing. Swab samples were taken from the wounds created in the control and experimental rat groups on days 0, 2, 6, and 12 and examined using the dilution method for bacterial counts. For this purpose, samples were taken by making smears on a 1 cm² area using swabs moistened with sterile physiological saline on wounds that had an average of 12 hours since the last dressing application. The swab samples were brought to the Microbiology Department Laboratory of Muğla Sıtkı Koçman University Milas Veterinary Faculty under cold chain. Dilutions were prepared up to 10^{-5} for total aerobic mesophilic live bacteria count in swab samples. Sterile physiological saline was used in the preparation of the dilutions. For this, the swabs brought to the laboratory were first placed in tubes containing 10 ml of sterile physiological saline and mixed using a vortex. This first dilution was prepared as a 10^{-1} dilution. Then, ten-fold dilutions were made with 1 ml transfers starting from the first dilution to tubes containing 9 ml of sterile physiological saline, and 0.1 ml from each dilution was taken and plated on previously prepared sterile Plate Count Agar (PCA, Biolife, Italy) culture media using the spread plate technique. Two replicates were performed for each dilution. The petri dishes where the plates were plated were incubated for 48 hours at 37 °C under aerobic conditions. Colony counts were performed on the petri dishes where growth occurred at the end of the incubation period. The results were evaluated by taking the averages of parallel studies.

Statistical Analysis

The statistical analysis of the data was performed using the SPSS® 26 statistical package program (Inc., Chicago, IL, USA). The normality of the variables was examined using analytical methods such as the Kolmogorov-Smirnov and Shapiro-Wilk tests. The homogeneity of variances was assessed using the Levene's test.

For the comparison of wound area and wound healing rate between groups, the One-way ANOVA test was used for normally distributed variables, while the Kruskal-Wallis test was used for non-normally distributed variables. In cases where significant differences were found among groups for normally distributed variables, pairwise post-hoc comparisons were performed using the Tukey test.

For histopathological examination, the Kruskal-Wallis test was used. Pairwise comparisons for non-normally distributed variables were performed using the Mann-Whitney U test and evaluated with Bonferroni correction.

Results with a P-value below 0.05 were considered statistically significant.

Results

Chemical Content of Olive Oil

Muğla Sıtkı Koçman University, Milas Veterinary Faculty, Department of Physiology laboratory conducted the measurement of the chemical composition of the olive oils used in wound healing using a gas chromatography device. Chemical laboratory analysis of Memecik olive oil, specific to the Milas district of Muğla, revealed the fatty acids, nutritional, and energy values of the oil. The analysis results represent the content of 100 ml of Memecik olive oil (Table 1).

Table 1. The chemical analysis of Memecik olive oil

Energy value	819 kcal/3367 Kj
Protein	0 g
Fat	91 g
Monounsaturated fatty acids	69 g
Polyunsaturated fatty acids	9 g
Saturated fatty acids	13 g
Trans fatty acids	0 g
Cholesterol	0 mg
Carbohydrates	0 g

Measurements of Wound Sizes

Results were based on the data obtained by measuring the photos taken every day from the formation of the wound until the day of sacrifice using the Image J program. Macroscopically, wound healing occurred faster in the olive oil group compared to the control and drug groups (Figure 1).

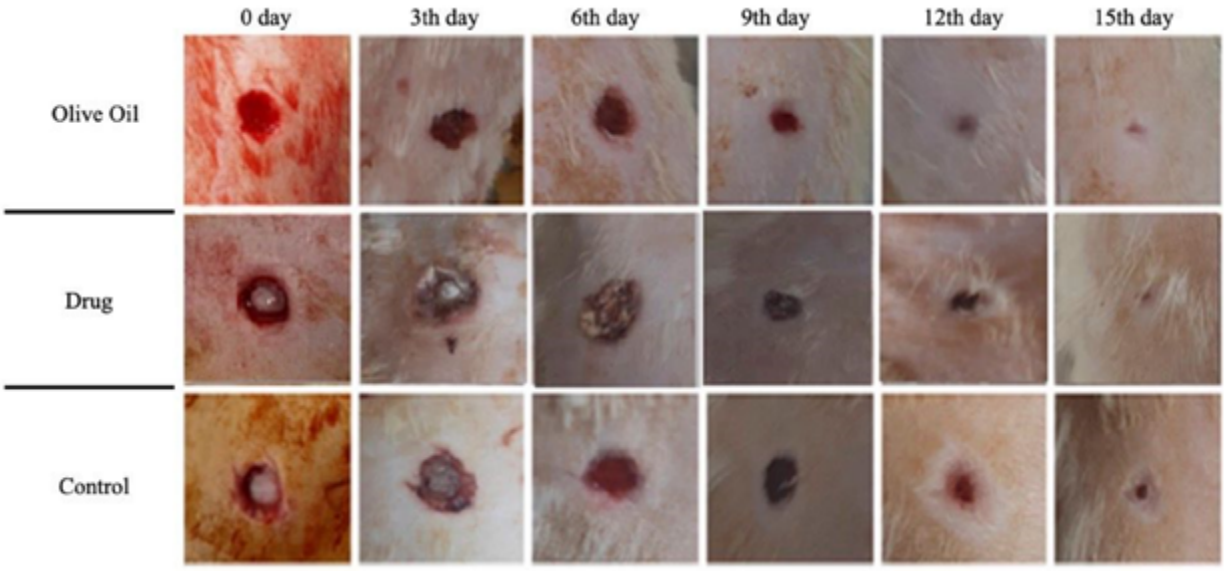

***Figure 1**: Wound healing by groups and days*

It can be observed that the olive oil group has a statistically significantly smaller mean wound area compared to the control and drug on all days. When looking at the P-values, statistically significant differences between the groups can be observed on all days (P<0.0001) (Table 2).

The group treated with olive oil demonstrated a notably higher rate of wound healing compared to the control and drug groups. This difference was statistically significant across all observed days. There are statistically significant differences between the groups on all study days (P<0.0001) (Table 3).

Histopathologic Results

In the histopathological examination conducted for epithelialization, it was determined that both the olive oil group and the drug group had higher values compared to the control group. As the days progressed, it can be observed that the average values of epithelialization parameters increased in all groups. On the 2nd and 5th days, the olive oil group showed higher average values in epithelialization parameters than the control and drug groups. However, these differences were not found to be statistically significant (P>0.05). On the 10th day, the olive oil group showed statistically significant differences in terms of epithelialization compared to the other two groups. On the 15th day, it showed statistically significant differences compared to the control group (P=0.014) (Table 4).

In terms of mononuclear cell infiltration, inflammatory infiltrations of varying degrees were observed in almost

Table 2. Wound areas on days 2, 5, 7, and 10 (mm^2)

Factors	2nd Day		5th Day		7th Day		10th Day	
	n	Mean±SE	n	Mean±SE	n	Mean±SE	n	Mean±SE
Olive oil	21	15.91±0.17[b]	17	9.62±0.32[b]	13	5.91±0.44[c]	9	0.34±0.22[c]
Drug	21	16.99±0.14[a]	17	11.92±0.29[a]	13	8.56±0.40[b]	9	5.71±0.43[a]
Control	21	17.17±0.14[a]	17	12.57±0.38[a]	13	10.27±0.33[a]	9	7.50±0.18[a]
P-value		<0.0001		<0.0001		<0.0001		<0.0001

[a-c]: Different letters in the same column are statistically significant (P<0.05). The values are given in mm^2(square millimeters).

Table 3. Total wound healing (%)

Factors	2nd Day		5th Day		7th Day		10th Day	
	n	Mean±SE	n	Mean±SE	n	Mean±SE	n	Mean±SE
Olive oil	21	18.96±0.89[a]	17	50.99±1.62[a]	13	69.90±2.23[a]	9	98.27±1.14[a]
Drug	21	13.46±0.71[b]	17	39.28±1.48[b]	13	56.37±2.04[b]	9	70.91±2.20[b]
Control	21	12.54±0.73[b]	17	35.97±1.96[b]	13	47.65±1.68[c]	9	61.80±0.91[b]
P-value		<0.0001		<0.0001		<0.0001		<0.0001

[a-c]: Different letters in the same column are statistically significant (P<0.05).

Table 4. Histopathologic evaluation

Histopathologic Parameters	n	Olive oil	Drug	Control	P-value
		Mean±SE	Mean±SE	Mean±SE	
2nd Day					
Epithelization	4	1.10±0.29	1.00±0.41	0.25±0.25	0.306
Inflammatory cell infiltration	4	2.25±0.25	1.50±0.29	2.24±0.48	0.253
Granulation tissue formation	4	0.50±0.29	1.50±0.29	0.25±0.25	0.051
5th Day					
Epithelization	4	1.25±0.25	1.00±0.41	0.50±0.50	0.357
Inflammatory cell infiltration	4	2.00±0.41	2.00±0.41	2.00±0.41	1.000
Granulation tissue formation	4	2.25±0.25	2.00±0.41	1.25±0.25	0.108
7th Day					
Epithelization	4	1.00±0.41	2.00±0.41	0.50±0.50	0.115
Inflammatory cell infiltration	4	2.00±0.41	0.75±0.25	2.25±0.48	0.064
Granulation tissue formation	4	1.50±0.64	2.00±0.41	1.00±0.41	0.360
10th Day					
Epithelization	4	2.75±0.25[ab]	3.00±0.00[a]	1.50±0.29[b]	0.014
Inflammatory cell infiltration	4	0.75±0.25	0.50±0.50	1.25±0.25	0.271
Granulation tissue formation	4	1.75±0.25	2.00±0.41	2.25±0.25	0.503
15th Day					
Epithelization	5	3.00±0.00[a]	3.00±0.00[a]	1.40±0.25[b]	0.001
Inflammatory cell infiltration	5	2.00±0.20	0.20±0.20	1.20±0.37	0.059
Granulation tissue formation	5	1.20±0.20[ab]	2.40±0.25[a]	1.00±0.32[b]	0.014

[a-b:] Different letters in the same line are statistically significant (P<0.05).

all cases in the control group, while they were detected at a milder level in the olive oil and drug groups. However, no statistically significant difference was found (P>0.05). In histopathologic examination was observed granulation tissue formation (star) in the olive oil group, epithelization and keratinization (arrow) in the drug group and severe inflammatory infiltration (star) in the control group (Figure 2).

In the olive oil group, the formulation of granulation tissue on the 15th day was found to be higher compared to the control group and lower compared to the drug group. This difference was statistically significant (P=0.014). Both the olive oil and drug groups exhibited significant formation of granulation tissue compared to the control group.

Microbiological Results

On the 0th day, the average bacterial counts from the inoculations in all experimental groups were found to be approximately similar. It was determined that the bacterial counts on the 2nd day had increased approximately tenfold in all experimental groups. On the 6th day, the lowest bacterial count was observed in the drug group, while the highest bacterial count was found in the control group. In the olive oil group, the bacterial count was approximately one and a half times lower than the control group. On the 12th day, bacterial counts in the drug and olive oil groups, except for the control group, were found to be at the levels observed on the 0th day (Table 5).

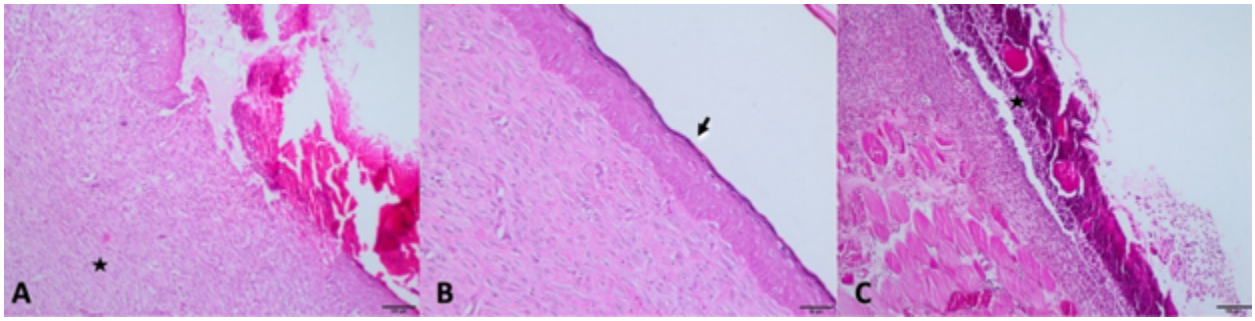

***Figure 2**. A- Granulation tissue (star) on olive oil group (12th day), B – Epithelization and keratinizatin (arrow) on drug group (12th day), C- Severe inflammatory infiltration (star) on control group (5th day)*

Table 5. Average values of live bacteria counts in units (cfu/cm^2)

Groups	0th Day	2nd Day	6th Day	12th Day
Drug	4.5x10^4	2.9x10^5	1.2x10^5	3.9x10^4
Olive oil	5.6x10^4	3.1x10^5	1.9x10^5	5.3x10^4
Control	5.9x10^4	3.5x10^5	3.2x10^5	1.7x10^5

cfu: colony forming unit.

Discussion

The healing process primarily consists of three different stages: inflammation, proliferation, and remodeling (Charles, 2007). Epithelialization, formation of granulation tissue, and collagen deposition are essential steps in the proliferation phase of wound healing (Broughton et al., 2006). The histopathological evaluation conducted in this study revealed a significant increase in epithelial tissue formation in animals treated with olive oil. Previous studies have indicated that the application of a physiological lipid to a wounded area of the skin accelerates healing by promoting homeostasis (Feingold, 2007). Similarly, the use of olive oil is also considered a lipid application, which has accelerated healing in terms of epithelialization in this study. Furthermore, the free radicals generated during the inflammatory process enhance the positive effect of olive oil on wound healing, thanks to its antioxidant properties (Massoud et al., 2022). The positive effects of olive oil on wound healing have been demonstrated in numerous studies (Rosa et al., 2014; Donato-Trancosa et al., 2016). Additionally, the statistically significant formation of granulation tissue in the olive oil group compared to the control group contributes to the beneficial effect of olive oil on wound healing. In the study conducted by Schanuel et al. (2019) on mice, experimental wounds were created and the mice were fed a diet rich in olive oil. As a result, it was found that the inflammatory response was low and collagen formation was high. These characteristics indicate that not only topical but also oral administration of olive oil has positive effects on wound healing. Similarly, Bayir et al. (2019) found regenerative effects of olive oil-based treatment in the histopathological evaluation of burn treatment, particularly in the dermis and epidermis layers, improving fibroblast activity and epithelial regeneration. These findings are consistent with the results of presented study.

Olive oil is a natural product that contains various phenolic compounds, which have antioxidative properties, positive effects on plasma lipoproteins, and antimicrobial activity (Lucas et al., 2011). One of the antimicrobial phenolic components found in olive oil is oleuropein, which is known to be effective against many bacterial pathogens (Sonmez and Gunes, 2018). Numerous in vitro studies have been conducted to investigate the antimicrobial effects of olive oil, and antibacterial effects have been observed against different microorganisms (Medina et al., 2006; Karaosmanoglu et al., 2010; Nazzaro et al., 2019). In a study by Medina et al. (2006), the antimicrobial activities of olive oil, corn oil, and sunflower oil were compared, and it was found that olive oil exhibited antimicrobial effects while the other edible oils did not. The highest antimicrobial activity was reported for olive oil. This phenomenon is associated with the phenolic compounds present in olive oil and the processing methods that may lead to a reduction in their content (Medina et al, 2006). Karaosmanoglu et al. (2010) examined Turkish extra virgin olive oils collected from different geographic regions and compared their phenolic compounds to those of refined olive oil, hazelnut oil, and canola oil in terms of their antimicrobial and antioxidant properties. They found that olive oils exhibited the highest antioxidant and antimicrobial effects. When reviewing the in vivo studies on the effects of olive oil on wound healing in the literature, along with studies using various modified olive oils (Pietrocola et al., 2018; Arozal et al., 2020), there were also studies focusing on the positive physiological properties of olive oil (Aguilera et al., 2004; Covas et al., 2006; Gonzalez-Correa et al., 2008). In this study, it was observed that bacterial colonization increased on the second day in all groups, with the highest increase seen in the control group. However, on the 12th day, bacterial counts were found to be below the initial load in both groups except for the control group. It is thought that the bacterial inhibitory effect in the olive oil group is due to the phenolic compounds present in olive oil (Medina et al., 2006; Bayram and Ozcelik, 2012).

Conclusion

In conclusion, it has been determined that olive oil is effective in wound healing, supports wound healing without the formation of granulation tissue at the histopathological level, and minimizes microbial growth, thus preventing the development of infection.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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