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Effect of oral use of various edible oils on wound healing in rats: randomized controlled experimental study

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ABSTRACT

There are various studies showing that oral supplements are shortening or facilitating effects on this process. Therefore, this study investigates the use of edible oils as supplements in the wound healing process. Of the 7 groups (control, *Hypericum perforatum* extracts in olive oil, olive, sesame, fish, black seed, sunflower), each has 8 Wistar Albino rats. In the experimental groups, 1.25 mL/kg oral oil was used for 10 days. Macroscopic images of the wound area were taken. Wound healing was evaluated by histological analysis. Collagen III, IL-6, TNF-α and TGF-ß1 density analyzes were performed on the tissue samples. According to macroscopic analysis, wound narrowing is higher in all groups on the 2nd and 4th days than the control group. Histopathological and immunohistochemical analyses of all experimental groups except sunflower oil group revealed better results than control group.

Keywords: *Hypericum perforatum* olive oil extract, sesame oil, black cumin oil, olive oil, sunflower oil, wound healing

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INTRODUCTION

The skin is the largest organ of the human body[1]. Because many patients endure skin injury, wound healing has a critical role in both daily life and healthcare[2,3]. The wound healing process, which consists of cell to cell and cell to matrix interactions, has 5 important steps; homeostasis and inflammation, granulation tissue formation, neovascularization, re-epithelialization, and remodeling[4].

Various studies have shown that malnutrition negatively affects the wound healing process[5]. Every step in the healing process ultimately depends on the circulating amino acids, lipids and carbohydrates[6]. The basal metabolic rate increases in the presence of a wound, which causes protein (muscle) breakdown and body dehydration[7]. For collagen synthesis, 1 kcal/g (collagen) is required and any change in the presence of precursor amino acids or energy substrate will affect collagen accumulation[8]. Lipids are one of the most important dietary components that contain the essential fatty acids and serve as carriers of lipid soluble vitamins[9]. Some researchers have shown that fatty acids are important substances in the wound healing process[10].

In the wound healing process, many modern and traditional treatment methods have been developed. Traditional treatments are cost effective and easy to access. Numerous nutrients obtained from herbal and animal sources have been used in research to accelerate or regulate wound healing. Some studies have suggested that fish oil may have positive effects on the wound healing process, but its ultimate benefit is still controversial[10-12]. It is also known that the effects of oleic acid on the wound healing process are dubious as well[13,14].

However, studies using *Hypericum perforatum* extract in olive (HPEO) oil for wound healing, which has been used among the folk for many years in order to cure various diseases such as gastritis, burns and bedsore[15-18], has shown that HPEO has huge potential on wound healing. It is stated that the anti-inflammatory effect and collagen accumulation of HP are effective in wound healing[19]. Olive oil is generally used in HP oil extract. Studies on olive oil reported that olive oil had positive effects on wound healing; therefore, the wound healing effect of the HPEO was synergistic[20,21].

Sesame oil has anti-inflammatory, antimicrobial and antioxidant activities[22]. Also, studies have shown that sesame oil can expedite wound healing and tissue regeneration in skin wounds, as it has a stimulating effect on fibroblast production[23]. The seeds of *Nigella sativa*, also known as black cumin, contain essential oils, proteins, alkaloids and saponins[24]. Some studies have evaluated the effects of *Nigella sativa* oil on wound healing, but controversy continues regarding its

use in the wound healing process[25,26]. In one study, it has been found that the oral use of olive oil has more positive effects than sunflower oil. In this study, patients who used olive oil for their burn wounds treatment, have higher levels of serum albumin, shorter hospitalization duration and shorter wound healing time[27]. The studies are limited in the literature showing that sunflower oil has an effect on wound healing. Nonetheless, the effect of sunflower oil has been investigated in the present study with the idea that sunflower oil may have positive effects due to the increasing energy need in the wound healing process.

In the literature, there are not any research that analyze the comparative effects of oral use of HPEO, olive oil, sesame oil, fish oil, *Nigella sativa* oil, and sunflower oil on skin wound healing. Therefore, this study aimed to analyze the effects of oral use of these oils on wound healing in rats with experimental wounds.

METHODOLOGY

Animals and Experimental Design

This animal experiment complied with the ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Also, all stages of this research have been approved by Istanbul Medipol University animal experiments ethics committee. This randomized controlled, single blind research was carried out on 56 healthy Wistar Albino male rats, weighing 300-350 ± 5 grams, in the Istanbul Medipol University Experimental Animal Breeding and Research Laboratory in Istanbul. During the experiment, the room temperature is 21 ± 2 °C, relative humidity is 40-60%, having ventilation system (change of 10-15/hour), inside the cage light intensity is 40 lux, the light period is 12 h light / 12 h dark and the noise level is below 85 dB. Throughout the study, all groups of rats were fed ad libitium with standard feed. After anesthesia, rats were placed in separate cages and divided into seven groups, with eight animals per group as follows: Group 1- control group; 1.25 ml/kg/day saline was given with oral gavage, Group 2: 1.25 ml/kg/day HPEO was given with oral gavage, Group 3: 1.25 ml/kg/day olive oil was given with oral gavage, Group 4: 1.25 ml/kg/day sesame oil was given with oral gavage, Group 5: 1.25 ml/kg/day fish oil was given with oral gavage, Group 6: 1.25 ml/kg/day black cumin oil was given with oral gavage, Group 7: 1.25 ml/kg/day sunflower oil was given with oral gavage. All oils and serum were given by physiological gavage and no anesthesia was applied during the gavage procedure.

Natural oils

Olive oil: Produced by the local people in Dikili, Izmir, Turkey

Sunflower oil: Vera® (Izmir, Turkey) brand oil was used.

HPEO: In this study, the flowers of St. John's Wort (*Hypericum perforatum*) plant which grown in the Bergama highlands of Izmir, were used. HP flowers collected from nature and then dried and powdered. Fifty g of HP was added to the glass bottle containing 500 ml of olive oil (olive oil used in the experimental group was used). The bottle was kept under the sun for 4 weeks, with 12 hours a day in the summer. It was observed that the red dye in the plant passes into olive oil[19].

Fish oil: Marincap® Capsules (500 mg) was used.

Black cumin oil: Karden® (Ankara, Turkey) brand oil was used.

Sesame oil: Karden® (Ankara, Turkey) brand oil was used.

It has been observed in the literature that the oils used in the experimental groups can be applied by oral gavage in a single dose of 0.5, 1, 1.25, 1.5, 2, 4 and 5 mL/kg/day [21, 28-31]. In order to determine the amount of fat used, the lowest HPEO dose found in the literature was taken as the basis [29]. 1.25 ml/kg/day was determined as the dose in all groups in order to avoid a difference in energy intake between the experimental groups. Fatty acid components in 100 grams of oils are shown in Table 1[32-34].

Table 1. The Fatty Acid Components in 100 grams of Sesame, Black cumin, Olive, Sunflower and Fish Oil

	Black cumin Oil (g)	Sesame Oil (g)	Olive Oil (g)	Sunflower Oil (g)	Fish Oil (g)
Total SFA (Saturated Fatty Acid)	11.6	15.53	15.583	10.382	
Total MUFA (Monounsaturated Fatty Acid)	27.7	42.31	68.373	30.917	
Total PUFA (Polyunsaturated Fatty Acid)	59.7	42.30	11.434	54.071	30
14:0 (Myristic acid)	0.1	0.01		0.067	
16:0 (Palmitic acid)	7.1	9.57	12.399	5.774	
17:0 (Margaric acid)		0.04	0.096		
18:0 (Stearic acid)	3.2	4.99	2.486	3.308	
20:0 (Arachidic acid)		0.63	0.459	0.249	
22:0 (Behenic acid)	0.6	0.10	0.143	0.717	
24:0 (Lignoceric acid)		0.05		0.268	
16:1 , n-7 (Palmitoleic acid)		0.11	0.755	0.105	
18:1 , n-9 (Oleic acid)	27.7	42.05	67.293	30.649	
20:1 , n-9 (Gadoleic acid)	0.2	0.15	0.325	0.163	
18:2 , n-6 (Linoleic acid)	59.2	42.04	10.812	53.985	
18:3 , n-3 (à- Linolenic Acid)	0.5	0.26	0.621	0.086	
20:5 n-3 (EPA- Eicosapentaenoic Acid)					18
22:6 n-3 (DHA- Docosahexaenoic Acid)					12

Surgery for excisional wound model

For general anesthesia in rats, 100 mg/kg ketamine HCl (Ketasol 10%, Richter Pharma, Austria) and 10 mg / kg Xylazine HCl (2% Rompun, Bayer, Istanbul, Turkey) were used. The stock solution was obtained by mixing 10 mg of xylazine HCl and 100 mg of ketamine into 10 mL of 0.9% isotonic sodium chloride solution. This prepared solution was given intraperitoneally (ip) in µL at 10 times body weight (grams)[35]. After general anesthesia, the back hair of the rats was shaved and washed with providon iodine solution. All procedures were performed aseptically. Full-thickness skin wounds were created using two excisional skin wounds, a punch biopsy tool with a diameter of 1 cm and 5 mm from the midline[36]. Each solution was administered by oral gavage each day until the rats were sacrificed on day 10. Flunixin 2.5 mg/kg/day was applied subcutaneously from the wound creation to the last day in order to reduce the pain of the rats.

Macroscopic evaluation of wound healing

Photographs were taken using a Sony digital camera (Sony Inc., Tokyo, Japan) on days 0, 2, 4, 6, 8 and 10 at a 90° angle to the wound surface in order to measure wound narrowing. Wound surface areas were calculated using an image analyzer (ImageJ.2.0 software, National Institutes of Health, Bethesda, MD) to evaluate the change in wound surface size during healing. This software was then used to analyze photographic results at 32 bit density. The software was used to export data in CSV format for custom analysis.

Histology: Light microscopic evaluation

One of the wound tissues in the back region was placed in neutral buffered formalin for light microscopic evaluation and was left to be fixed for 48 hours. Excess fixatives were removed by washing for 1 hour under running tap water. It was passed through ascending alcohol series (70%, 90%, 96%, 100%) for dehydration and made transparent with xylene. It was kept in paraffin in a 60 °C incubator oven for overnight and embedded in paraffin in ice molds the next day. In order to analyze general morphology with light microscope, microtome (Thermo Scientific, HM340E) sections taken approximately 5 µm thick, and stained with Hematoxylin-Eosin (H&E) (Empire Genomics, BPK 4088-2), Mason trichrome (Bio-optica BPK 2916). Sections were viewed with Nikon Eclipse (Nikon® Instruments Inc., United States).

Wound healing for each group was evaluated using the scoring system described by Geleano et al.[37]. According to this scoring system, the groups are scored between 1 and 4 under three headings: epidermal dermal regeneration, granula-

tion tissue thickness and angiogenesis.

For epidermal and dermal regeneration:

√ Poor epidermal organization in ≥ 60% of the tissue; 1 point
√ Incomplete epidermal organization in ≥ 40% of the tissue; 2
√ Moderate epithelial proliferation in ≥ 60% of the tissue; 3
√ Complete epidermal remodeling in ≥ 80% of the tissue; 4
For thickness of the granulation tissue:
√ Thin granulation layer; 1
√ Moderate granulation layer; 2
√ Thick granulation layer; 3
√ Very thick granulation layer; 4

For angiogenesis (only mature vessels were counted and identified by the presence of erythrocytes in the lumen):

√ ltered angiogenesis (one to two vessels/site) characterized by high degree of edema, hemorrhage, occasional congestion and thrombosis; 1
√ Few newly formed capillary vessels (3–4/site), moderate edema and hemorrhage, occasional congestion, intravascular fibrin deposition and absence of thrombosis; 2
√ Newly formed capillary vessels (5–6/site); 3
√ Newly formed and normal appearing capillary vessels (>7/site); 4

Immunohistochemistry

Sections taken 12 μm thick on positive charged slides with cryomicrotome (CM1950, Leica, Germany) and fixed in 4% PFA for 20 minutes. After washing with PBS and distilled water, they were incubated for 10 minutes at room temperature with 3% H2O2 to stop the endogenous peroxidase activity. Blocking solution (2% BSA containing 10% NGS) was dropped onto the sections and allowed to block for 1 hour at room temperature. Without washing, the sections were incubated with anti-collagen III (1: 100, cat # ab7778; Abcam), anti-IL-6 (1: 1000, cat # ab9324; Abcam), anti-TNFα (1: 1000, cat # ab6671) and anti-TGF-β1 (1:100, cat # sc-146, Santa Cruz) for overnight at +4 °C. The sections were washed 3 times for 5 minutes in PBS and then incubated for 10 minutes in biotinylated secondary antibody. After the sections were washed again 3 times for 5 minutes with PBS, incubated in streptoavidin peroxidase (Mouse and Rabbit Specific HRP (ABC) Detection IHC Kit, ab93677, Abcam) for 10 minutes. Conversion to brown in DAB chromogen (1.5 ml DAB substrate + 30 μl DAB chromogen) after washing with PBS was observed within 3 minutes. Then it was washed with PBS and nucleus staining was performed with Hematoxylin (Bio-

optica 60002). Slices were washed under running tap water for 10 minutes, and then sections were dehydrated in ascending alcohol series and covered with Biomount (Bio-optica 1611). Sections were imaged with Nikon Eclipse (Nikon® Instruments Inc., United States) at 4X magnification for semi-quantitative density analysis. Density analysis was performed using Image J (National Institutes of Health, Bethesda, MD, USA) software.

Statistical Analysis

All statistical analyses were performed using SPSS 24.0 software program. The results were expressed as means ± SD. For macroscopic wound healing assessment, light microscopic evaluation and immunohistological wound healing assessment the differences between the control group and experimental groups were analyzed by using Mann Whitney U test. Values for $p \leq 0.05$ were considered statistically significant.

RESULTS and DISCUSSION

Macroscopic wound healing

The calculated wound surface area values are given in Table 2 as percentage. For Day 0, all groups were considered 100%. In Figure 1, macroscopic images of the wound area which are photographed every two days, are given together.

Table 2. Calculated Wound Surface Areas

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Control	100.00	96.17±1.46	83.88±7.32	50.28±22.18	29.94±21.47	21.36±13.66
HPEO	100.00	78.83±5.99[a]	60.54±9.05[a]	40.46±9.98	24.95±13.49	12.05±8.29
Olive oil	100.00	87.31±2.26[a]	74.71±3.41[b]	42.24±3.16	24.39±5.40	12.87±4.55
Sesame oil	100.00	82.42±4.81[a]	62.05±12.33[b]	39.08±9.58	27.64±9.07	13.77±5.73
Fish oil	100.00	80.57±3.49[b]	70.30±4.05[b]	44.06±12.94	32.77±14.63	16.76±7.29
Black cumin oil	100.00	86.79±6.35[b]	74.22±8.57[c]	44.46±6.86	33.58±5.81	19.89±4.57
Sunflower oil	100.00	93.17±2.15[b]	72.47±8.33[c]	46.10±8.63	28.65±6.21	19.69±6.80

Values are percentage of open wound surface. Data are means ± SD. a : $p < 0.001$. b: $p < 0.005$. c: $p < 0.05$ vs. control group.

On the 2nd day, more wound closure was observed in all experimental groups ($p<0.005$), especially HPEO, Olive oil and Sesame oil groups ($p<0.001$) compared to the control group.

Although there was more wound closure in the control group on the 4th day compared to the 2nd day, even more wound closure was observed in all experimental groups. Highest one is HPEO group ($p < 0.001$) followed by the olive oil, sesame oil and fish oil groups ($p < 0.005$) and finally black cumin oil and sunflower oil groups ($p < 0.05$), compared to the control group.

On day 6, wound closure was higher in all experimental groups than the control group ($p > 0.05$). On the 8th day, wound closure was higher in all experimental groups than the control group except for the fish oil and black cumin oil groups ($p > 0.05$). When the 10th day was reached, wound closure was higher in all experimental groups than in the control group ($p > 0.05$).

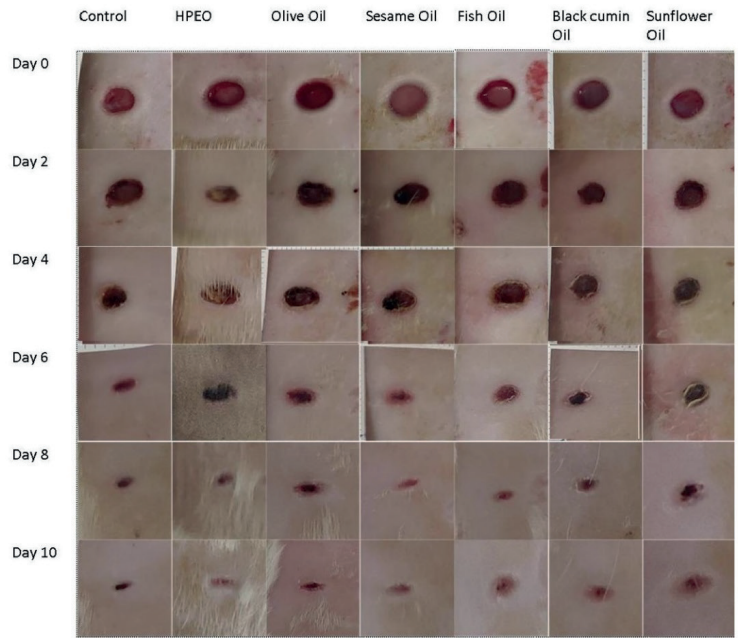

Figure 1. Macroscopic images of all groups on days 0, 2, 4, 6, 8 and 10

Examination of wound healing with Light microscopic evaluation

The effect of oral gavage applied oils on wound healing was stained with Mason trichrome stain for histopathological analysis. Sections were compared for epidermal regeneration, granulation tissue and angiogenesis (Fig. 2). The examination results of the groups, which are scored under three headings by light microscopic examination, are given in Table 3.

Table 3. Histological Wound Healing Scores

	Epidermal and Dermal Regeneration	Granulation Tissue	Angiogenesis
Control	2.2	2.4	2.6
HPEO oil	3.3[a]	3.3[b]	3.8[a]
Olive oil	3.4[a]	3.1[b]	3.3
Sesame oil	3.4[a]	3.6[b]	3.6[b]
Fish oil	3.2	3.0	3.0
Black cumin oil	3.6[b]	3.0	3.4
Sunflower oil	2.8	2.8	2.8

HPEO: *Hypericum perforatum* extract in olive. [a] $p < 0.01$, [b] $p<0.05$ vs. control group.

Considering the results, the control group showed lower scores in epidermal regeneration, granulation tissue and angiogenesis scoring than all experimental groups. Looking at the epidermal regeneration scores, HPEO, olive oil, sesame oil groups ($p <0.01$) and black cumin oil group ($p <0.05$) scored higher than the control. According to the granulation tissue scoring, HPEO, olive oil and sesame oil groups scored higher than the control ($p <0.05$). For angiogenesis scoring, HPEO group ($p <0.01$) and sesame oil group ($p <0.05$) scored higher than the control.

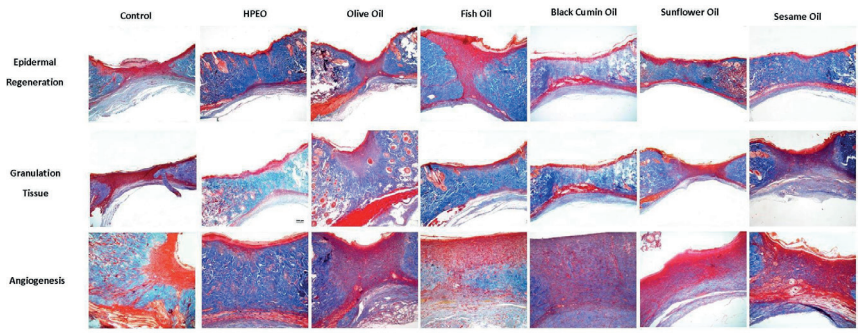

Figure 2. Mason trichrome staining in wound sections

Immunohistological examination results of scar tissue

In the wound healing model, the expression levels of collagen III, proinflammatory (TNF-α and IL-6) and anti-inflammatory (TGF- ß1) factors were compared with immunohistochemical methods (Fig. 3). According to the immunohistological analysis of the groups, the mean and standard deviation of densities measured with Image J program are given in Table 4. In this study, density analyzes of Collogen III, IL-6, TNF-α and TGF-ß1 were performed in the wound area.

According to Collagen III density, all experimental groups, mainly the HPEO group ($p < 0.001$), have higher density than the control ($p < 0.01$). When IL-6 density was examined, the densities of all experimental groups, except sunflower oil group, were lower than the control group. The difference in IL-6 density between control group and all experimental groups, except the olive oil and sunflower oil groups, was statistically significant ($p < 0.01$).

For TGF-ß1 density all experimental groups, except fish oil and sunflower oil groups, have higher density than the control group ($p < 0.01$). Actually, fish oil and sunflower oil groups have higher TGF-ß1 density than control, but this difference is not significant.

When TNF-α density was examined, a lower density than the control was observed in all experimental groups except for black cumin and sunflower oil groups ($p < 0.01$). TNF-α concentration was higher than control in black cumin oil and sunflower oil groups ($p > 0.05$).

Table 4. Immunohistology Density Measurements

	Collagen III		IL-6		TGF-ß1		TNF-α	
	Mean±SD	P	Mean±SD	P	Mean±SD	p	Mean±SD	p
Control	9.67±2.52		28.92±8.20		35.50±1.92		43.78±3.07	
HPEO Oil	48.49±3.33	<0.001	16.45±6.74	0.006	47.52±2.45	0.001	36.21±4.53	0.004
Olive Oil	38.34±7.83	0.001	22.17±3.40	0.051	46.51±3.21	0.001	38.74±1.50	0.001
Sesame Oil	39.36±6.80	0.001	12.34±7.63	0.002	46.33± 4.46	0.001	35.68±8.30	0.004
Fish Oil	18.31±4.67	0.003	11.09±4.92	0.005	38.67±3.81	0.149	35.55±4.05	0.003
Black Cumin Oil	23.41±3.11	0.001	12.89±3.01	0.002	44.18 ± 4.94	0.004	39.95±5.23	0.165
Sunflower Oil	24.45±7.35	0.001	30.49±3.91	0.432	38.60 ±3.66	0.073	40.77±6.39	0.432

HPEO: *Hypericum perforatum* extract in olive

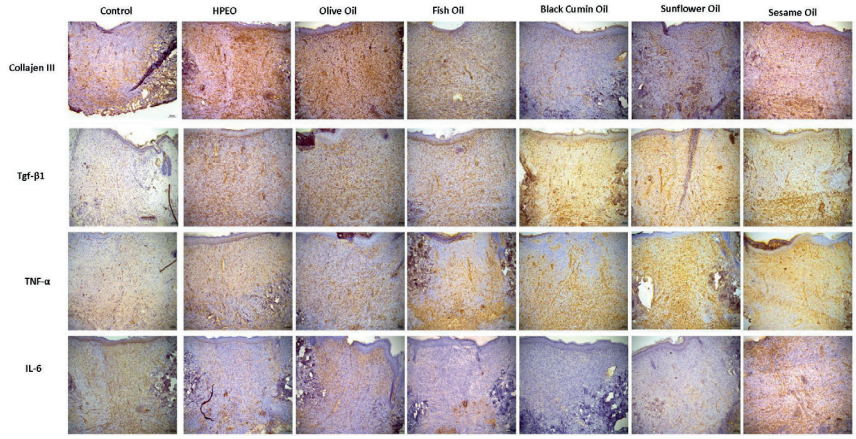

Figure 3. Immunohistochemical analysis of collagen III, proinflammatory factors (TNF-α and IL-6) and anti-inflammatory factor (TGF-β1) in wound healing sections (Magnification 4X)

The success ranking of groups at the end of experiment in analyzes

The success ranking of the experimental and control groups were given in Table 5. Although there was more wound closure in the experimental groups than the control group on days 2 and 4, there was no difference between the groups on the last day of the study (HPEO, olive oil, sesame oil, fish oil, sunflower oil, black cumin oil and control, respectively, from the most effective to the least effective. group).

Table 5. The success ranking of the experimental and control groups in analyzes

Wound healing	Most effective												Less effective
	Min												Max.
Epidermal and Dermal Regeneration	Black cumin	=	Olive	=	Sesame	=	HPEO	=	Fish	=	Sunflower	>	Control
Granulation Tissue	Sesame	=	HPEO	=	Olive	=	Black cumin	=	Fish	≥	Sunflower	>	Control
Angiogenesis	HPEO	=	Sesame	=	Black cumin	=	Olive	=	Fish	=	Sunflower	>	Control
Collogen III	HPEO	>	Sesame	=	Olive	>	Sunflower	=	Black cumin	=	Fish	>	Control
TGF-ß1	HPEO	=	Olive	=	Sesame	≥	Black cumin	≥	Fish	>	Sunflower	=	Control
	Min												Max.
Wound surface area day 10th	HPEO	=	Olive	=	Sesame	=	Fish	=	Sunflower	=	Black cumin	=	Control
IL-6	Fish	=	Sesame	=	Black cumin	≤	HPEO	≤	Olive	≤	Control	≤	Sunflower
TNF- α	Fish	=	Sesame	≤	HPEO	=	Olive	≤	Black cumin	=	Sunflower	≤	Control

HPEO: *Hypericum perforatum* extract in olive
=: Statististically smilar
≤: The previous and next groups are statistically at the same level of impact with each other. The mean of the previous group is smaller than the mean of the next group. However, at least two previous groups and the next group are statistically at different levels of impact.
≥: The previous and next groups are statistically at the same level of impact with each other. The mean of the previous group is bigger than the mean of the next group. However, at least two previous groups and the next group are statistically at different levels of impact.
<: The mean of the previous group is smaller than the mean of the next group.
>: The mean of the previous group is bigger than the mean of the next group.

When we examined the ranking of success level according to the epidermal regeneration score, it was seen that all experimental groups (black cumin oil, olive oil, sesame oil, HPEO, fish oil, sunflower oil, respectively) had similar effects with each other and were more effective than the control group ($p < 0.05$). According to the granulation tissue scoring, the effect levels of sesame oil, HPEO, olive oil, black cumin oil and fish oil groups are higher than the sunflower oil

and control groups ($p < 0.05$), but there is no difference between the effect levels of above-mentioned experimental groups. Fish oil and sunflower oil groups, which have no difference in effect levels, were more effective than the control group ($p < 0.05$). When analyzed in terms of angiogenesis score, while all experimental groups were more effective than the control group (HPEO, sesame oil, black cumin oil, olive oil, fish oil, sunflower oil and control groups, respectively) ($p < 0.05$), there was no difference between the effect levels of the experimental groups.

When the effect levels are examined according to Collagen III density, it is seen that all experimental groups are more effective than the control and also HPEO group is the most effective group ($p < 0.05$). Sesame oil and olive oil groups that come after HPEO are more effective than sunflower oil, black cumin oil and fish oil groups with the same effect level ($p < 0.05$). When TGF-ß1 density is examined, all groups are more effective than sunflower oil and control groups with similar effect levels ($p < 0.05$). The most effective HPEO, olive oil and sesame oil groups have a similar effect level, but sesame oil has a similar effect level with black cumin oil too. Black cumin oil has a similar effect with fish oil.

When IL-6 density was examined, fish oil, sesame oil, black cumin oil groups had a similar effect, but they were more effective than all other groups ($p < 0.05$). Respectively, black cumin oil with HPEO, HPEO with olive oil, olive oil with control and control with sunflower oil groups have similar effects. For TNF- α density success ranking fish oil and sesame oil groups have a similar effect and they are the most effective groups ($p < 0.05$). The least effective group is the control group ($p < 0.05$). HPEO and sesame oil groups have a similar effect. HPEO and olive oil groups show similar effects, and they are more effective than black cumin oil and sunflower oil groups.

In this study, rats with wounds on their backs were given 1.25 ml / kg of various oils (HPEO, olive oil, sesame oil, fish oil, black cumin oil and sunflower oil) for experimental groups and saline for control group for 10 days with oral gavage. To measure the effect of supplementation on wound healing, measurement of wound closure, histological wound healing examination, immunohistological analyzes were performed.

As can be seen in Table 5, there was no difference between the macroscopic images of the groups on the 10th day. As seen in Table 2, the effect levels of the experimental groups that were effective at the beginning (on the 2nd day) decreased over time (on the 4th day) and there was no difference between them and the control group on the last day of the study. Similarly, in a study which lavender oil was used as a treatment, lavender oil was seen to be effective in

wound closure at the beginning, but it was observed that there was no difference between the control group in the last 4 days[38]. In another research[39], while rapid wound closure was observed in the experimental groups at the beginning, no difference was found between the control group on the last day. In the research of Keskin et al.[4], when the histological wound healing score was examined, the Limonene and Fenchone groups were more effective than the control, while for wound surface areas, these groups did not differ from the control. As can be seen from these studies, although macroscopic imaging helps to examine the effect at first, it is not sufficient by itself. These results show that the experimental groups especially HPEO and sesame oil groups, initially accelerated the closure of the wound surface.

A study[40] concluded that sunflower oil provides an increase in granulation tissue and complete healing of the epidermis. In other studies[41-43], it was stated that sunflower oil was more effective in wound healing compared to control, and this may be due to the linoleic acid content. In our study, histological results were compatible with the literature, but when the immunohistological results were examined, it was seen that the sunflower oil group had high IL-6 and TNF-α in the wound tissue.

In the literature, it was reported that increased IL-6 and TNF-α were observed in the tissues of mice given sunflower oil[44]. In this study, although sunflower oil was generally more effective in wound healing than control, it was seen that it was less effective than other experimental groups. Like sunflower oil, the expected effect was not observed in the fish oil group. However, it was observed that the concentration of IL-6 and TNF- α in wound tissue was lower than all groups. Besides, it was determined that collagen-III density was lower than all groups except the control group and TGF-ß1 concentration was lower than all groups except control and sunflower oil groups.

In a study in which various oils were used[45], delays in epithelization and wound closure were observed in the fish oil supplement group. In our study, when the 10th day macroscopic wound closure was examined, there was no statistically significant difference between the groups. When we examined the histological wound healing of wound tissues, it was seen that the fish oil group was more effective than the control, but less effective than all experimental groups except the sunflower oil group. In a wound healing study using omega-3 fatty acids[46], it was found that EPA and DHA were not successful in wound healing contrary to what was expected. A similar result seen in our study was associated with the anti-inflammatory effect of EPA and DHA. Inflammation stimulates and aggravates early wound healing in the healing process so in the absence of inflam-

mation, wound healing may be delayed. In a review prepared by Komprda[47], it was seen in various studies that the wound healing effects of EPA and DHA are mixed (inconsistency): "decreased / increased collagen deposition, lower / higher counts of the inflammatory cells in the healing tissue."

Like fish oil, sesame oil has a high content of unsaturated fatty acids. High levels of polyunsaturated fatty acids can make the oil extremely susceptible to oxidation. However, sesame oil is extremely stable due to its antioxidant content (like polyphenols, carotene). Also, sesame oil contains 15.24 mg / kg of carotene which is the precursor of vitamin A. For healthy regeneration of the skin structure, vitamin A is essential. Sesame oil also shows antibacterial activity, which is an important feature in wound healing[48]. Sesame contains the most sesamin and sesamoline as fat soluble lignans compounds. These compounds have antioxidant and anticancer properties. Sesamin is protective against oxidative stress[49]. In a study[50] comparing the FRAP (Ferric Reducing / Antioxidant Power) values of sesame oil, olive oil and sunflower oil, it was concluded that 803 μmol /l, 153 μmol/l and 108 μmol/l, respectively. On the 15th and 31st days, while sesame and olive oil had the lowest oxidant value, it was observed that sunflower oil had the highest value. Sesame oil contains sesaminol, sesamolinol, sesamolin and sesamol which all have an antioxidant effect. This content reduces lipid oxidation and has a wound healing effect[51]. In a study, sesame ointment accelerated wound healing and stimulated fibroblast proliferation. In addition, sesame ointment has been found to be effective in angiogenesis. Also, it has been concluded that sesame ointment shortened the inflammation phase and promoted the proliferation phase[23]. As seen in our study, there were no difference between the sesame oil group and the fish oil group regarding TNF-α and IL-6 in scar tissue (Table 5). In addition, TGF-ß1 content was similar to HPEO group and olive oil group, probably due to the antibacterial properties of sesame oil. Also the collagen accumulation of this group was similar to the olive oil group and higher than all groups except HPEO group. In our study, it was also seen in the histological examination of the oils that Sesame oil was strikingly effective in wound healing among all oils. Olive oil has antioxidant properties due to its oleic acid, vitamin E and phenol compounds (tyrosol, hydroxytyrosol, oleuropein, 1-acetoxypinoresinol and flavonoid) content[52-54]. Donato-Trancoso et al.[54] have observed that olive oil application reduces the oxidative damage by reducing ROS (reactive oxygen products) and NO (Nitric Oxide) formation, thus reducing inflammation and necrosis, increasing wound closure and improving collagen deposition. In one study, supplementing the diet with olive oil instead of sunflower oil improved the healing of burns and reduced hospitalization in burn patients[27]. According to the study of Edraki et al.[52], infection

was seen in the control group but not in the olive oil group. This is thought to be due to the phenolic content of olive oil, such as secoiridoids (oleuropein and derivatives). In our study, we found that the collagen deposition in the wound tissue of the olive oil group was similar to the sesame oil group. Regarding the TGF-ß1 deposition, olive oil group was one of the highest in the experimental groups, and this result was consistent with the literature[53]. In addition, studies show that olive oil improves angiogenesis. In our study, the angiogenesis scoring of olive oil was better than the control group. In a study comparing olive oil and fish oil[53], it was stated that fish oil caused inflammation by increasing norepinephrine levels, but no inflammation was observed in the olive oil group due to the anti-inflammatory properties of it. This shows that olive oil is more effective in wound healing than fish oil. It can be a good wound healing supplement due to its content.

It is known that olive oil extract of *Hypericum perforatum* is traditionally used in wound healing[55]. Various studies have proven this effect[55-58]. This effect is thought to be the biological properties of the compounds in HP and the synergetic effect of the oil from which HP was extracted. HP contains Hypericin, which has antimicrobial, antiviral and anti-inflammatory properties[59-62]. The Hyperforin contained in HP contributes to wound healing by having antibacterial and antimalarial effects[63-66]. In addition, since quercetin is involved in the inhibition of monoamine oxidase A, it reduces the formation of free radicals and helps in wound healing[67]. HP contains glavanoids such as quarcitrin hyperosidei, rutin, kaempferol, biopigenin and amentoflavone[68]. As seen in our study, HPEO increased collagen and TGF-ß1 deposition. TNF-α level was similar to olive oil and IL-6 level was lower than olive oil. Only in the epidermal examination part of the histological wound healing examination, the HPEO group had a lower mean than the olive oil group. However, there was no difference between olive oil group and HPEO group in any histological wound healing score.

When black cumin oil was examined, it was seen that the results were more effective than the control, but not as successful as the sesame oil, HPEO and olive oil groups. In a study[69], HP oil cream and black cumin oil cream were used in wound healing. In this study[69], while the antioxidant properties of the black cumin group were higher, improvements were found in epithelization and granulation in the HP group. The benefits of black cumin oil actually come from its fatty acid and essential oil content. Black cumin oil has more than 30% fixed oil and 0.4%-0.45% volatile oil content. The biggest compound of this volatile oil content is thymokine (27.8-57.0%). Thymokine is followed by p-cymene, varvacrol, 4-terpineol, longifoline and t-anethole[26,32]. In addition, black cumin oil contains about 60% essential fatty acids[32]. Thymoquinone is stated to be

responsible for antioxidant, analgesic and anti inflamatory actions[26,32]. As seen in Table 1, black cumin oil contains 59.2% linoleic acid and 27.7% oleic acid[32]. It was stated that oleic acid prevented the formation of free radicals. However, the oleic acid content of black cumin oil is less than half of the content of olive oil. Therefore, black cumin oil may not be as effective as olive oil in the wound healing process. In addition, it is noteworthy that the linoleic acid content of sunflower oil and black seed oil are similar and the results we have found that they have similar affect levels on wound healing.

As a result of the present study clearly showed that orally used oil extract (HPEO) and oils (sesame oil, olive oil, black cumin oil, fish oil and sunflower oil) have wound healing effects. With the help of future studies, it will be acknowledged that the use of various oils as supplements in wound healing is important in terms of shortening the current treatment period and hospitalization.

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CONFLICT OF INTEREST

The authors declare no competing interest.

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