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# *Topical pomegranate (Punica granatum) extract cream accelerates wound healing by modulating VEGF and IL-10 expression in rats*

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**Abstract:** Wound healing is a complex biological process aimed at restoring the integrity and function of damaged tissues. The demand for effective and aesthetically satisfying wound repair has driven interest in natural therapeutic alternatives to synthetic drugs. *Punica granatum* (pomegranate) has been reported to possess significant wound healing properties. This study aimed to evaluate the effect of topical pomegranate extract cream on the expression of vascular endothelial growth factor (VEGF) and interleukin-10 (IL-10) in an excision wound model in rats. A post-test-only control group design was used with 48 male Wistar rats divided into 12 treatment groups. VEGF and IL-10 levels were measured on day 3 and day 7 after treatment. Statistical analysis using One-Way ANOVA and Tamhane's post hoc test showed significant differences in VEGF and IL-10 expression across treatment groups ($p = 0.000$), with the highest levels observed in the 20% pomegranate cream group on both days ($p < 0.05$). These findings indicate that topical pomegranate extract cream enhances wound healing by increasing VEGF and IL-10 expression, particularly at the 20% concentration.

**Keywords:** Pomegranate Extract Cream; Excision Wounds; VEGF Levels; IL-10 Levels.

## INTRODUCTION

A wound is a physical injury to one of the body's tissues that results in a discontinuity of the soft part of the body structure.[1] Aesthetically pleasing improvements are patient demands, including improved wound closure and infection control.[2] The anti-inflammatory cytokine *interleukin* 10 (IL-10) is an important regulator of tissue repair, controlling inflammation and scar formation.[3] Another important role of cytokines of *vascular endothelial growth* factor (VEGF) is to recruit other immune cells to the site of the wound.[4] Stimulates angiogenesis, collagen deposition, and epithelialization processes.[5] Wound care that is usually done with topical application is still an alternative because it is easy to use, and the price is cheap but the content can be considered a foreign object by the body, lowering collagen synthesis because it inhibits the growth of fibroblasts.[6] Alternatives using natural ingredients are needed to reduce the side effects of using synthetic drugs in wound healing, one of which is with pomegranate (*Punica granatum*), several studies report pomegranate extract significantly improving wound healing.[7,8] However, there are still few reports related to the effect of the use of pomegranate extract cream on the expression of VEGF and IL-10 in wounds, so research is needed.

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A retrospective analysis of Medicare beneficiaries in 2018 identified that 8.2 million people had injuries with or without infection. Medicare estimates for acute and chronic wound care range from $28.1 billion to $96.8 billion. The highest expenditure is for surgical wounds, followed by diabetic foot ulcers, with a higher tendency towards outpatient wound care costs than inpatient care. Rising healthcare costs, an aging population, awareness of the threat of intractable infections such as biofilms, and the continuing threat of diabetes and obesity around the world make wounds a major clinical, social, and economic challenge.[9]

Research shows that of all pharmaceutical products today, about 73%, contain ingredients derived from natural products.[10] Pomegranate dietary supplements were reported to suppress inflammatory cytokines, changes in plasma cytokines and Aβ, ATP, and inflammatory cytokines were investigated in the brains of transgenic rats, significant increases in levels of inflammatory cytokines IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, and TNF-α, Eotaxin activity decreased along with administration of dietary supplements containing pomegranate.[11] Pomegranates have the ability to be used in various therapies through different mechanisms.[8] Research with SPE (*standardized pomegranate extract*) accelerated the healing of second-degree burns, topical administration gave good results characterized by collagen density accompanied by complete and mature epithelium, low number of inflammatory cells, and angiogenesis.[12]

The potential of natural products in wound treatment has been reported in numerous studies, emphasizing products that have antioxidant, anti-inflammatory, and antimicrobial properties, such as alkaloids, saponins, terpenes, essential oils, and polyphenols from various plant sources.[13,14] In line with the above research, the potential of pomegranate extract in increasing the expression of VEGF and PDGF in the wound healing process on day 4. The results showed that the administration of pomegranate extract increased the expression of VEGF and PDGF in wounds after tooth extraction.[15] Molecules for wound repair are secreted by fibroblasts and other molecules present at the wound site. VEGF is an important cytokine that accelerates polarization, recruiting other immune cells to the site.[4] VEGF forms new blood vessels produced by various cells, one of which is macrophages.[15] Inhibition of VEGF production results in dysregulation of p38 *mitogen-activated protein kinase* (MAPK) activation as a signal response to stress, thereby activating various *pathways* that lead to inflammatory molecule gene expression, apoptosis dysregulation and cell migration inhibition in the angiogenesis process .[3]

Many herbal formulations have been reported to accelerate wound healing activity in excision wound models.[1] Pomegranate extract activity was observed by measuring increased expression of IL-10 which controls wound inflammation and scar formation.[9] VEGF as a marker of wound healing.[15] Topical administration of pomegranate extract to excision wound model rats is expected to accelerate wound healing by increasing the expression of VEGF and IL-10.

Several studies have explored the wound-healing potential of pomegranate in different models and formulations. Lukiswanto et al. (2019) demonstrated that standardized pomegranate extract accelerated second-degree burn healing in rats, characterized by increased collagen density, reduced inflammation, and angiogenesis[12]. While Rakhsandeh et al. (2022) reported that pomegranate seed oil reduced peritoneal adhesion through its antioxidant, anti-inflammatory, and antifibrotic activities.[16] Nirwana et al. (2017) observed that pomegranate fruit extract enhanced VEGF and PDGF expression in tooth-extraction wounds of guinea pigs, indicating stimulation of angiogenesis and tissue repair. In another context,[15] Essa et al. (2015) and Mehdi et al. (2022) showed that long-term dietary supplementation with pomegranate reduced systemic and neuroinflammation in transgenic mouse models.[11,17] These findings highlight that while pomegranate exhibits consistent anti-inflammatory and wound-healing properties across different systems, most studies have focused on oral

supplementation or non-excision wound models, with limited evidence regarding topical cream formulations and their direct effect on VEGF and IL-10 expression.

Therefore, this study aims to investigate the effect of topical pomegranate extract cream (10% and 20%) on VEGF and IL-10 expression in Wistar rats with excision wounds. This research is expected to provide new insights into the potential of pomegranate cream as a topical wound therapy, complementing existing studies on other formulations and wound models.

## MATERIAL AND METHOD

The research materials used pomegranate ethanol extract, alcohol 70%, 80%, paraffin, ketamine, Aquadest, Fine test ELISA kit Rat VEGF and fine test ELISA kit Rat IL-10.

This study is experimental with a *posttest only control group design*. The subject of the study was a male rat of the Wistar strain (*Rattus norvegicus*), aged 2-3 months with a body weight of 190-210 grams adapted for 7 days. The total number of subjects was 48 male wistar rats divided into 12 groups consisting of 4 rats each. Subjects were divided into examinations after day 3 of treatment and after day 7 of treatment. Animals were randomly allocated into experimental groups using a computer-generated randomization list. Investigators performing wound creation and treatment administration were different from those conducting ELISA analysis to minimize observer bias. This study obtained ethical approval from the Health/Medical Research Ethics Committee, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia (Ethical Clearance No. 147/IV/2024/Komisi Bioetik; issued 30 April 2024). All procedures followed institutional and national guidelines for the care and use of laboratory animals.

The group of healthy rats without excision wound treatment was given normal feed for 3 days (K1), the group of excision wound rats without treatment for 3 days (K2), the excision wound model mice were given a base cream for 3 days (K3), the excision wound model mice were given *bioplacenton* cream for 3 days (K4), the excision wound model mice were given a 10% pomegranate extract cream for 3 days (K5), and excision wound model mice and given 20% pomegranate extract cream for 3 days K6). On the 4th day, VEGF and IL-10 levels were checked. The group of healthy rats without excision wound treatment was given normal feed for 7 days (K7), the group of excision wound rats without treatment for 7 days (K8), excision wound model mice were given basic cream for 7 days (K9), excision wound model mice were given *bioplacenton* cream for 7 days (K10), excision wound model mice were given pomegranate extract cream 10% for a day (K11), and excision wound model rats and given 20% pomegranate extract cream for 7 days (K12). On the 8th day, VEGF-A and IL-10 levels in rat skin tissue homogenates were quantified by sandwich ELISA following the manufacturer's instructions and our laboratory SOPs. We used Rat IL-10 ELISA Kit (Elabscience, Cat. E-EL-R0016; analytical range 31.25–2000 pg/mL) and Rat VEGF-A ELISA Kit (Elabscience, Cat. E-EL-R2603; analytical range 31.25–2000 pg/mL). Optical density was read at 450 nm on a microplate reader, and concentrations were interpolated from the kit standard curves. Samples were run in duplicate; values above the top standard were re-assayed after appropriate dilution

The pomegranate sample was 1 kg, the part used was the pulp. The pulp is dried in the oven at a temperature of 50°C and pureed. The results are checked for moisture content with *moisture balance*, if the moisture content is below 10%, the drying results are considered good. The crushed pomegranates are then sifted with a sieve of 20 mesh. Then 500 grams of pomegranates were extracted using the maceration method with 70% ethanol solvent as much as 3,750 ml. Pomegranate simplicia powder is put into a dark-colored bottle separately. Simplisia is soaked using ethanol solvent for 5 days and occasionally shaken 3 times a day. After 3 days, it is filtered and the pulp is re-amacrated for 2 days with

70% ethanol as much as 1250 ml. The repetition was carried out three times. The collected filtrate is then thickened using a *rotary evaporator* at a temperature of 50ºC until a thick extract is obtained.

The mice that had been adapted for 7 days were anesthetized with a mixture of *ketamine* (60 mg/kgWB) and *xylazine* (20mg/WB), the surface of the skin that had been cleaned using *bovidon iodine* to avoid infection during wound making. The wound was made using a circular *biopsy punch* with a full thickness of 6mm. The next day, the rats were then given treatment according to their group. Topical treatment was given once a day for 4 days after making an excision wound model. Skin samples in the validation group were taken to make histological preparations by *hematoxilin eosin* (HE) staining and macroscopic observation of healing that occurred in the wound.

The preparation of pomegranate extract cream is carried out by preparing 50 grams of *vanishing cream* with a composition of *stearate acid, triethanolamine, glycerine, potassium hydroxide*, and *aquadest*. Heat water in a beacker glass, weigh 14.5 grams of *stearate acid* in a porcelain cup and place it over boiling water, stirring until it melts. Add 125mg *potassium hydroxide* sequentially then homogenize, add *Triethanolamine* 1.5 ml, *Glycerine 10 ml,* and *aquadest* 25ml until well mixed. The cream in 20 grams is made by weighing 0.6 grams of pomegranate extract then put in a mortar, adding enough *Tween* while homogenizing. Add 20 grams of *vanishing cream*, mix well until homogeneous, Pomegranate extract cream put in a pot.[18]

The concentrations of 10% and 20% pomegranate extract cream were selected based on previous evidence and preliminary observations. One experiment, gel formulations containing pomegranate peel extract showed strong antioxidant activity and favorable wound-healing properties, supporting the feasibility of topical peel-based preparations at mid-range concentrations [19]. Likewise, creams containing 5–10% pomegranate flower extract applied to burn wounds in rats achieved faster wound contraction and better collagen organization compared with standard silver sulfadiazine treatment [20]. Collectively, these findings suggest that concentrations in the 5–10% range are effective for promoting wound healing. Based on this evidence, our study used 10% as a reference dose and included 20% to further investigate possible dose-dependent effects while maintaining topical safety.

After the treatment, on the 3rd day in the K1-K6 group and on the 8th day in the K7-K12 group, Day 3 was chosen as the first observation point because it represents the peak of the inflammatory phase of wound healing, a stage dominated by macrophage infiltration and pro-inflammatory cytokine production. At this point, fibroblast migration and early angiogenesis are also initiated, marking the transition toward repair. In contrast, Day 7 corresponds to the proliferative phase, during which angiogenesis, fibroblast proliferation, collagen deposition, and re-epithelialization are most active. Previous studies describe the inflammatory phase as lasting for 1–3 days, followed by the proliferative phase beginning around day 4 and extending into the second week, thereby making these two time points appropriate for assessing VEGF and IL-10 as molecular markers of wound progression [21,22]

Prior to tissue collection, all Wistar rats were anesthetized and euthanized in accordance with ethical guidelines. The procedure started with incision on rats skin before tissue was taken in the injured part of the skin, using sterile scissors and tweezers. The tissue sample was cut and weighed, then the tissue was added with PBS (PH 7.4), the tissue sample was homogenized under cold conditions of 4ºC. Then centrifugation is carried out at a speed of 2000-3000 rpm, for 20 minutes. Centrifugation supernatants that have a lower specific weight are taken and used as test samples. Samples can be stored at -20ºC.

The skin tissue samples that have been obtained are then analyzed for VEGF and IL-10 levels using the ELISA method. ELISA VEGF and IL-10 analysis

is performed according to the procedures attached to the standard operating procedures for rat kit products. VEGF and IL-10 levels were analyzed using *a microplater reader* with a wavelength of 450nm.

All quantitative data were tested for normality (Shapiro–Wilk) and homogeneity of variance (Levene's test). One-way ANOVA was used to assess differences between groups, followed by appropriate post-hoc tests (Tamhane or LSD) depending on homogeneity results. This approach was chosen because it allows comparison across multiple groups and controls for type I error in multiple testing.

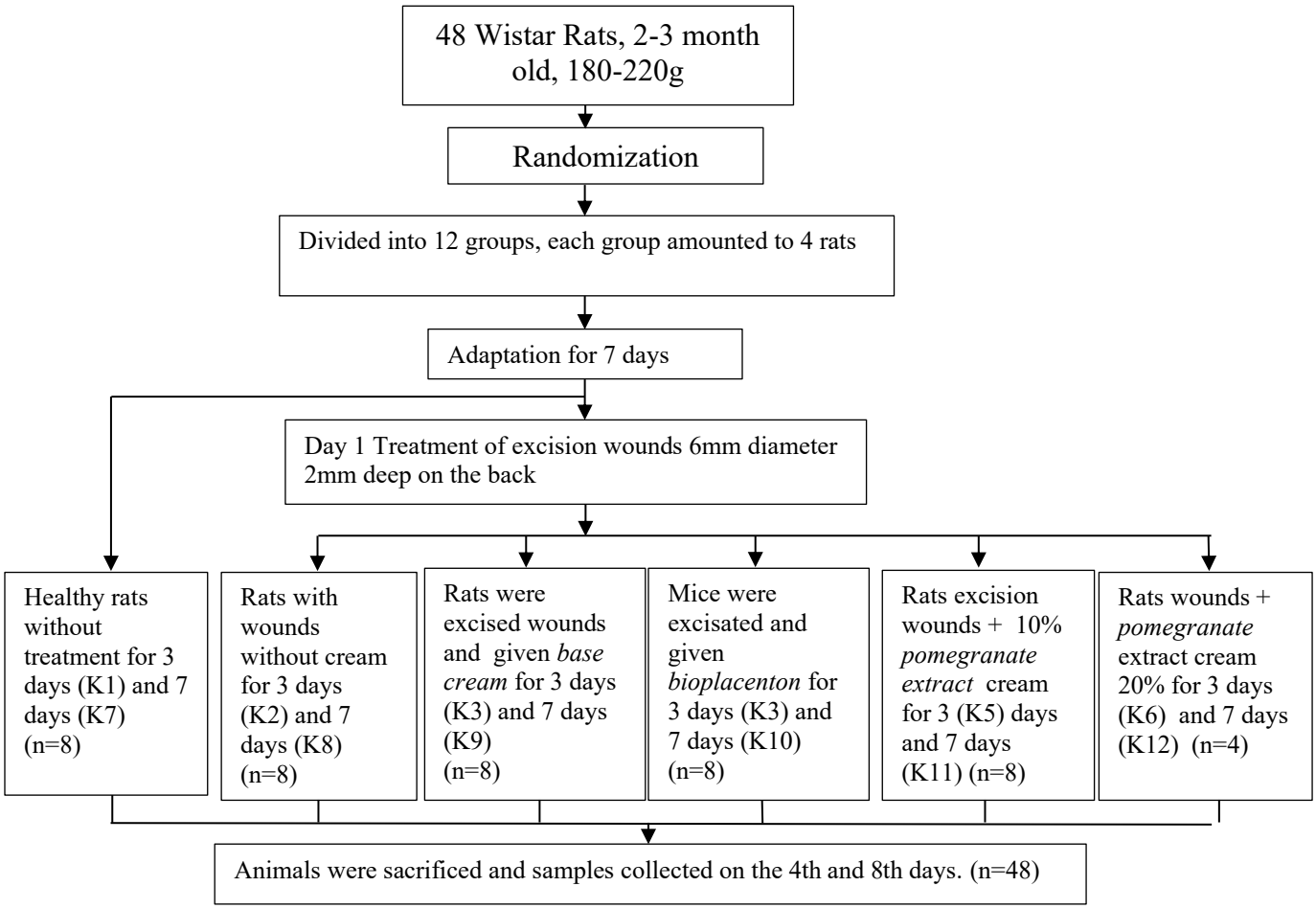


**Figure 1**. Animal group assignments and corresponding treatment timeline.

## RESULTS AND DISCUSSION

Macroscopic picture of the condition of excision wounds without cream treatment with a 20% dose of pomegranate extract cream treatment group in rat subjects after excision wounds on the first day as shown in the following figure.

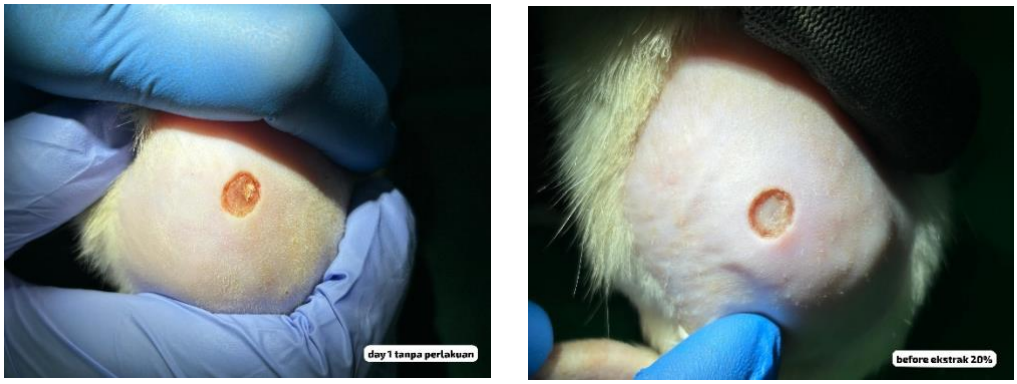


**Figure 2**. The first day of macroscopic treatment without treatment (left) and the 20% pomegranate extract treatment group (right)

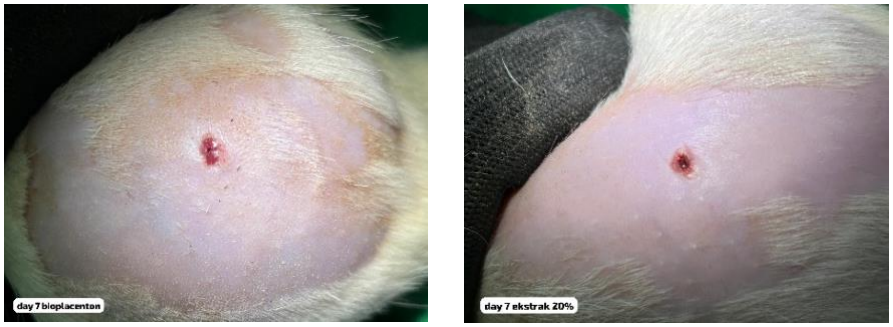


**Figure 3.** Macroscopic excision wound treatment group on day 7 treatment group with bioplacentone (left) and with 20% pomegranate extract (right)

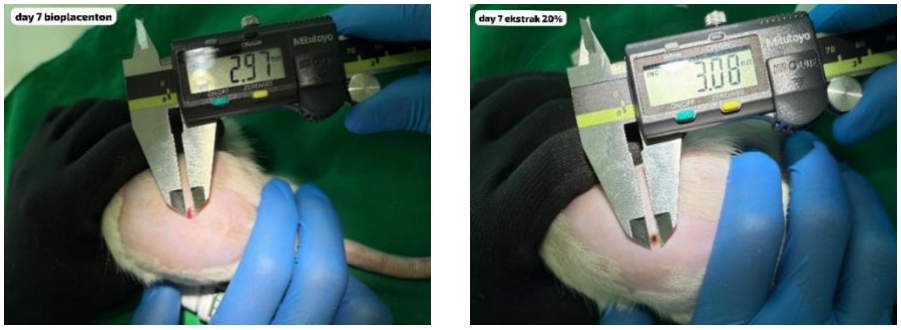


**Figure 4.** Measurement of wound healing diameter on day 7 of the treatment group with bioplacentone (left) and with 20% pomegranate

The macroscopic results of excision wound treatment on day 7 in figures 3 and 4 showed significant differences in the diameter of wound closure between groups, the excision wound treatment given 20% pomegranate extract cream (K6) macroscopic was almost equal to the excision wound group given bioplacenton. The diameter measurement of excision wounds given 20% pomegranate extract cream (K6) was 3.08mm in diameter, while in the excision wound group given bioplacenton, the measurement of wound diameter was 2.97mm. It can be concluded that the administration of 20% pomegranate extract cream is not much different from the diameter of wound healing using *bioplacenton*.

The results of the analysis of the average VEGF levels after the 3rd day of treatment in each research group group are shown in table 1.

**Table 1**. The average descriptive test of VEGF levels and *the one-way anova* test after the 3rd day of treatment

| Group | K1 Healthy Rats | K2 No inter-vention | K3 Base cream | K4 *Biopla centon* | K5 Dosage 10% | K6 Dosage 20% | *P value* |
|---|---|---|---|---|---|---|---|
| **VEGF pg/mL** | | | | | | | |
| Mean | 209.17 | 667.50 | 730.83 | 856.66 | 574.17 | 1020.0 | |
| SD | ±15.24 | ±43.24 | ±26.29 | ±8.61 | ±9.17 | ±58.56 | |
| *Shapiro-Wilk* | *0.691 | *0.392 | *0.369 | *0.972 | *0.650 | *0.259 | |
| *Levene Test* | | | | | | | 0.000 |
| *One way anova* | | | | | | | 0.000 |

Description: * *Shapiro-Wilk* = Normal (p>0.05)
* *Levene Test* = Homogen (p>0.05)
* *One way anova* = Significance (p<0.05)

The average VEGF level after day 3 treatment in the healthy group (K1) was 209.17 pg/mL which was the lowest result in VEGF levels compared to other groups, while the highest VEGF level in the 20% pomegranate extract cream group (K6) was 1020.00 pg/mL, When compared to the VEGF level in the group given *bioplacenton* 856.67 pg/mL, The results of VEGF levels in the K6 group experienced a significant increase in VEGF levels.

Based on table 1, it shows that the average VEGF level with *the Shapiro-Wilk* test obtained a normal distributed result (p>0.05) and the *Leuvene Test* test result has a heterogeneous data variation with a result of 0.000 (p>0.05). The results of the data were normally distributed and not homogeneous, a *one-way anova test* was carried out with a result of 0.000 (<0.05) showing that there was a significant difference between groups.

**Table 2**. The *Post hoc Tamhane test* of VEGF levels of rat skin tissue after excision wounds with the administration of pomegranate extract cream

| Group | K2 | K3 | K4 | K5 | K6 |
|---|---|---|---|---|---|
| **K1** | *0.001 | *0.000 | *0.000 | *0.000 | *0.001 |
| **K2** | - | 0.570 | *0.036 | 0.266 | *0.002 |
| **K3** | | - | *0.019 | *0.008 | *0.010 |
| **K4** | | | - | *0.000 | 0.146 |
| **K5** | | | | - | *0.007 |

The average comparison of VEGF levels was significantly different when compared to the excision wound group that was given a 20% dose of pomegranate extract cream compared to other groups, only the group that was given *bioplacentone* treatment was not significantly different (statistically similar) using *the Post hoc tamhane* test with the average VEGF level higher in the 20% dose group.

**Table 3**. The average descriptive test of VEGF levels and *the one-way anova* test after the 7th day of treatment

| Group | K7 Healthy Rats | K8 No inter-vention | K9 Base cream | K10 *Biopla centon* | K11 Dosage 10% | K12 Dosage 20% | *P value* |
|---|---|---|---|---|---|---|---|
| **VEGF Level pg/mL** | | | | | | | |
| Mean | 245.00 | 425.00 | 265.84 | 350.84 | 451.67 | 1315.0 | |
| SD | ±6.38 | ±10.36 | ±46.22 | ±15.24 | ±5.77 | ±193.23 | |
| *Shapiro-Wilk* | *0.272 | *0.855 | *0.430 | *0.691 | *0.195 | *0.110 | |
| *Levene Test* | | | | | | | 0.000 |
| *One way anova* | | | | | | | 0.000 |

The average VEGF level after day 7 treatment in the healthy group (K7) was 245.00 pg/mL which was the lowest result in VEGF levels compared to the treatment group after other days 7, while the highest VEGF level in the 20% dose of pomegranate extract cream group (K12) was 1315.00 pg/mL, All treatment groups when compared to the K12 group had a significant increase in VEGF levels.

Based on table 3, the average VEGF levels with *the Shapiro-Wilk test* were obtained with normal distributed results (p>0.05) and the results *of the Leuvene Test* had heterogeneous data variations with a result of 0.000 (p>0.05). The results of the data were distributed normally and non-homogeneously, a *one-way anova test* was carried out with a result of 0.000 (<0.05) showing that there were significant differences between groups. Significant differences between treatment groups, then a *Post hoc tamhane test* was carried out to determine the most influential dose comparison after the 7th day of treatment.

**Table 4.** *Post hoc Tamhane test* of VEGF levels of rat skin tissue after excision wounds with the administration of pomegranate extract cream after the 7th day of treatment

| Group | K8 | K9 | K10 | K11 | K12 |
|---|---|---|---|---|---|
| **K7** | *0.000 | 1.00 | *0.003 | *0.000 | *0.023 |
| **K8** | - | 0.071 | *0.006 | 0.106 | *0.039 |
| **K9** | | - | 0.358 | 0.054 | *0.016 |
| **K10** | | | - | *0.005 | *0.030 |
| **K11** | | | | - | *0.043 |

The average comparison of VEGF levels was different between the excision wound groups that were given a dose of 20% pomegranate extract cream compared to other groups, the administration of 20% pomegranate extract cream increased VEGF levels significantly from day 3 of treatment and increased until day 7 of treatment.

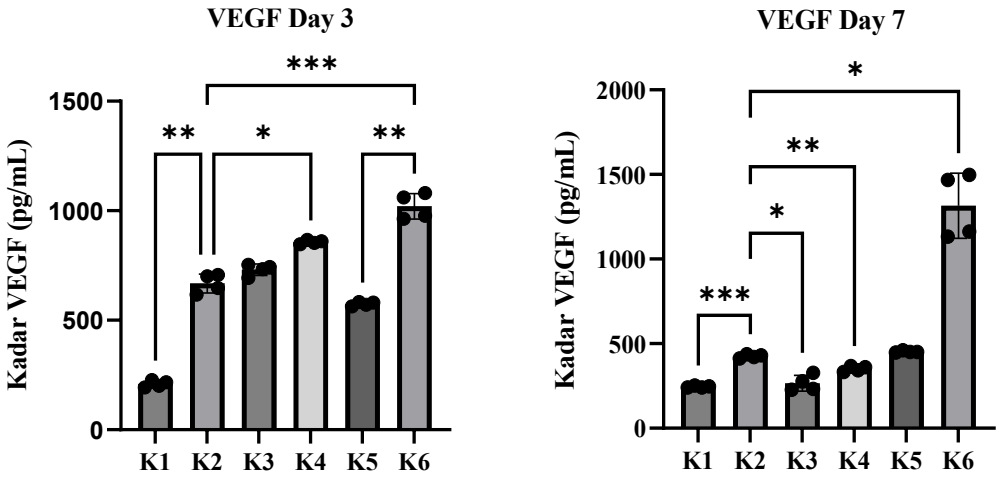


**Figure 1.** The average of VEGF levels on day 3 after treatment compared to day 7 after treatment; *Symbols indicate statistical significance compared with control groups: *p < 0.05, **p < 0.01, **p < 0.001

Comparison of the average VEGF level after the 3rd day of treatment with the 7th day of treatment showed a decrease in VEGF levels in the K2, K3, K4 and K5 groups in contrast to K6 who experienced an increase in VEGF levels on the 7th day of treatment with a 20% dose of pomegranate extract cream.

**Table 5.** The average descriptive test of IL-10 levels and *the one-way anova* test after the 3rd day of treatment

| Group | K1 Healthy Rats | K2 No inter-vention | K3 Base cream | K4 *Biopla centon* | K5 Dosage 10% | K6 Dosage 20% | *P value* |
|---|---|---|---|---|---|---|---|
| **IL-10 levels pg/mL** | | | | | | | |
| Mean | 196.61 | 300.52 | 323.56 | 519.81 | 809.02 | 958.05 | |
| SD | ±42.53 | ±51.17 | ±41.16 | ±28.71 | ±23.10 | ±39.82 | |
| *Shapiro-Wilk* | *0.441 | *0.433 | *0.581 | *0.540 | *0.543 | *0.880 | |
| *Levene Test* | | | | | | | 0.332 |
| *One way anova* | | | | | | | 0.000 |

The average IL-10 level after day 3 treatment in the healthy group (K1) was 196.61 pg/mL which was the lowest IL-10 level compared to other groups, while the highest IL-10 level in the 20% pomegranate extract cream group (K6) was 958.05 pg/mL.

Based on table 5, it shows that the average IL-10 level with *the Shapiro-Wilk* test obtained normal distributed results (p>0.05) and the *Leuvene Test* test results have homogeneous data variations with a result of 0.332 (p>0.05). The results of the data were distributed normally and homogeneously, a *one-way anova test* was carried out with a result of 0.000 (<0.05) showing that there was a significant difference between the groups.

**Table 6**. The *Post hoc LSD* test of IL-10 levels of rat skin tissue after excision wounds with the administration of pomegranate extract cream after the 3rd day of treatment

| Group | K2 | K3 | K4 | K5 | K6 |
|---|---|---|---|---|---|
| **K1** | *0.001 | *0.000 | *0.000 | *0.000 | *0.000 |
| **K2** | - | *0.001 | 0.413 | *0.000 | *0.000 |
| **K3** | | - | *0.000 | *0.000 | *0.000 |
| **K4** | | | - | *0.000 | *0.000 |
| **K5** | | | | - | *0.000 |

The average comparison of IL-10 levels was different between the excision wound groups that were given a 20% dose of pomegranate extract cream compared to other groups, it can be concluded that the administration of pomegranate extract cream has a significant effect on the increase in IL-10 levels with the highest increase in the 20% dose of pomegranate extract cream.

**Table 7.** The average descriptive test of IL-10 levels and *the one-way anova* test after the 7th day of treatment

| Group | K7 Healthy Rats | K8 No inter-vention | K9 Base cream | K10 *Biopla centon* | K11 Dosage 10% | K12 Dosage 20% | *P value* |
|---|---|---|---|---|---|---|---|
| **IL-10 levels pg/mL** | | | | | | | |
| Mean | 220.25 | 445.29 | 444.98 | 603.04 | 618.44 | 631.52 | |
| SD | ±49.26 | ±49.38 | ±42.85 | ±34.74 | ±25.10 | ±19.57 | |
| *Shapiro-Wilk* | *0.654 | *0.631 | *0.324 | *0.720 | *0.560 | *0.163 | |
| *Levene Test* | | | | | | | 0.050 |
| *One way anova* | | | | | | | 0.000 |

The average IL-10 level after day 7 treatment in the healthy group (K7) was 220.25 pg/mL which was the lowest IL-10 level compared to other groups, while the highest IL-10 level in the 20% pomegranate extract cream group (K12) was 631.52 pg/mL,

Based on table 7, it shows that the average IL-10 level with *the Shapiro-Wilk* test obtained a normal distributed result (p>0.05) and the *Levene Test* test

result had a homogeneous data variation with a result of 0.050 (p>0.05). The results of the data were distributed normally and homogeneously, a *one-way anova test* was carried out with a result of 0.000 (<0.05) showing that there was a significant difference between the groups.

**Table 8.** The *Post hoc LSD* test of IL-10 levels of rat skin tissue after excision wounds with the administration of pomegranate extract cream after the 7th day of treatment

| Group | K8 | K9 | K10 | K11 | K12 |
|---|---|---|---|---|---|
| **K7** | *0.001 | *0.000 | *0.000 | *0.000 | *0.000 |
| **K8** | - | *0.001 | 0.413 | *0.000 | *0.000 |
| **K9** | | - | *0.000 | *0.000 | *0.000 |
| **K10** | | | - | *0.000 | *0.000 |
| **K11** | | | | - | *0.000 |

The average comparison of IL-10 levels was significantly different between the excision wound groups after the 7th day of treatment who were given pomegranate extract cream compared to other groups, it can be concluded that the administration of pomegranate extract cream has a significant effect on the increase in IL-10 levels.

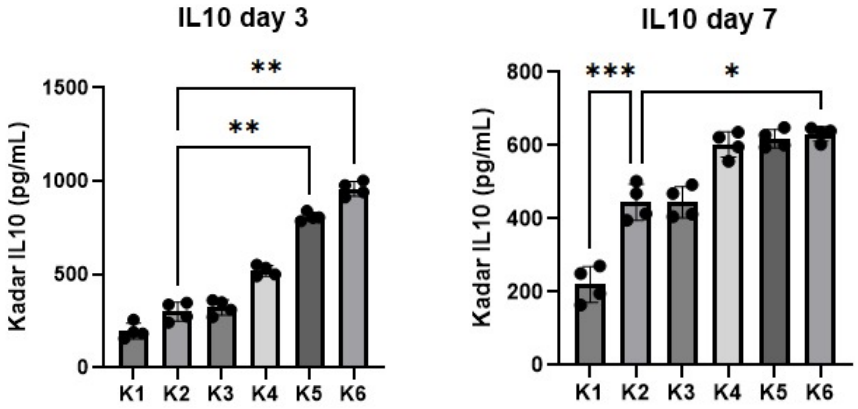


**Figure 2.** The average of IL-10 levels at 3 days after treatment compared to day 7 after treatment ; *Symbols indicate statistical significance compared with control groups: *p < 0.05, **p < 0.01, **p < 0.001

Comparison of the average IL-10 levels after the 3rd day after the treatment compared to the 7th day after the treatment showed an increasing trend of IL-10 levels in all treatment groups, the administration of 20% pomegranate extract cream had the highest average IL-10 levels compared to other groups, followed by the 10% pomegranate extract cream and excision wound treatment with *Bioplacenton*.

Correlation analysis demonstrated a significant positive association between IL-10 and VEGF expression across treatment groups. On day 3, IL-10 and VEGF levels were strongly correlated ($r = 0.82$, $p < 0.001$). On day 7, the correlation remained significant though slightly weaker ($r = 0.69$, $p = 0.002$). These results indicate that increased VEGF expression during wound healing is accompanied by elevated IL-10, suggesting coordinated roles of pro-angiogenic and anti-inflammatory signalling.

Wounds always begin with a phase of homeostasis that involves draining the lymphatic system and stopping bleeding, a highly integrated wound healing phase begins with the recruitment of pro-inflammatory cytokines-cytokines that cause inflammation. The inflammatory phase begins for 1-3 days, then the

proliferation phase lasts for 4-14 days, and the remodeling phase lasts for 14 to 1 year.[23]

Various cell strains and their products work together to heal skin wounds, which is an important physiological process. The recovery of wounds caused by local aggression begins in the inflammatory stage. These improvements include the replacement of specialized structures caused by collagen deposition and regeneration, which occurs in tandem with the process of cell proliferation and differentiation that runs posteriorly through cell tissues.[24]

The most important wound healing process is the transition from the inflammatory phase to the proliferation phase.[25] The transition from M1 macrophages to M2 macrophages is an important regulator of the reparative phase. Wound reepithelial requires regulated, guided, at least partially, proliferation, and differentiation of keratinocytes through growth factor production (VEGF).[22]

The results of the study showed that the effect of giving pomegranate extract cream could significantly increase VEGF levels. The administration of pomegranate extract cream increased VEGF levels on day 3 of treatment and lasted until day 7 after treatment, a decrease in VEGF levels occurred in the K7, K8, K9, K10 and K11 groups after day 7 treatment. The intervention with the administration of 20% pomegranate extract cream was effective in increasing VEGF levels on day 3 after treatment and continued to increase until day 7 after treatment. Adequate VEGF levels are important for proper wound healing.[26] This is followed by a period of strong cell proliferation, deposition and remodeling of the extracellular matrix (ECM), and ultimately scar formation.[26]

this findings are in line with previous research on the wound-healing potential of pomegranate. Lukiswanto et al. reported that topical administration of standardized pomegranate extract improved collagen density and angiogenesis in burn wounds,[12] while Nirwana et al. demonstrated increased VEGF and PDGF expression in tooth-extraction wounds treated with pomegranate extract.[15] Similar effects have also been described for other plant-based treatments such as Aloe vera and Centella asiatica, which are known to enhance angiogenesis and wound closure.[27,28] These comparisons emphasize that polyphenol-rich herbal formulations act through overlapping mechanisms, but the present study provides new evidence specifically for cream-based pomegranate extract, which is less commonly investigated compared to gels or oral preparations.

Angiogenesis and the formation of new blood vessels from existing blood vessels, are the main features of the healing phase of proliferation. This process causes a temporary increase in the number of blood vessels at the site of injury. The delivery of oxygen and nutrients from these new blood vessels is an important part of the repair process, and damage to angiogenesis is often associated with delayed wound healing. Various growth factors, cytokines, and lipid mediators produced in response to injury can stimulate angiogenesis.[26,29]

Macrophages release inflammatory cytokines that enhance the inflammatory response by recruiting and activating additional leukocytes. Macrophages are also important for decontaminating wounds from microbes and clearing apoptosis cells, thus paving the way for the resolution of inflammation and the beginning of the proliferation phase of wound healing. As the microenvironment changes at the base of the wound, macrophages undergo a phenotypic transition to an anti-inflammatory, regulatory, and reparative state, which stimulates keratinocytes, fibroblasts, and endothelials. cells to encourage tissue regeneration.

Many important pathways in the transition of M1 to M2 macrophages, PTEN reduction expands the activation of *protein kinase B* (AKT) which reduces inflammation and enhances anti-inflammatory states such as IL-10.[22] IL-10 will be produced to reduce or dampen inflammation, being able to trigger regenerative tissue repair that will regulate anti-inflammatory pathways in the wound healing process of the skin.[30]

One of the intracellular signaling pathways controlling the phenotypic switch involves p38 and miR-21. Within macrophages, miR-21 plays an important role in the transition from inflammatory to anti-inflammatory phenotype. The p38/Jun stress pathway is activated by PAMPs and DAMPs, causing the secretion of inflammatory cytokines, such as IL-1, TNF, and IL-6. Induce miR-21, one of the targets is PTEN. The reduction of PTEN expands the activation of AKT which turns off the inflammatory expression and improves the anti-inflammatory state. Fibroblasts and keratinocytes show a response to an increase in miR-21 that differs from macrophages and stimulates migration. This is very important for the initiation of tissue formation granulation and wound closure. miR-21 is involved in the cessation of inflammation in the wound healing phase and the initiation of the proliferative repair phase, In the macrophage phase M2 will activate anti-inflammatory cytokines and *growth factors* (IL-10 and VEGF).[22]

The results of IL-10 level analysis showed that the administration of pomegranate extract cream had a significant effect on the increase in IL-10 levels with the highest increase in the pomegranate extract cream group at a dose of 20%. Pomegranate extract cream that increases antioxidant activity also reduces inflammation in wounds During the inflammatory phase, Increases IL-10 levels which accelerates wound healing.[31] The antioxidant components in pomegranate extract donate electrons to free radicals, thereby neutralizing and producing relatively stable free radicals that can detoxify ROS.[31] This study proves that the use of pomegranate extract cream increases IL-10 and VEGF levels which accelerate wound healing.[32]

Nevertheless, several limitations should be acknowledged. This research was conducted in rats, which may not fully represent wound healing dynamics in humans. Histopathological confirmation of tissue changes such as collagen density and epithelial thickness was not performed, limiting the ability to link molecular results with tissue-level outcomes. In addition, the observation period was relatively short (7 days), so the long-term effects on remodeling and scar formation remain unknown.

Future studies should therefore expand to clinical trials to validate these findings in humans, as well as explore different formulations such as gels, hydrogels, or serums that may enhance topical delivery. Investigating the synergistic potential of pomegranate with other herbal extracts may also provide novel strategies for wound management.

In conclusion, this study strengthens the evidence that pomegranate extract promotes wound healing by upregulating VEGF and IL-10, supporting both angiogenesis and anti-inflammatory repair pathways. Compared to previous work, it adds novelty by demonstrating dose-dependent effects of cream formulations, highlighting their potential as a safe, natural alternative for wound therapy.

## CONCLUSION

The administration of pomegranate extract cream (*Punica Granatum*) had an effect on increasing the expression of VEGF in the skin tissue of wistar rats after excision wounds. The administration of pomegranate extract cream (*Punica Granatum*) had an effect on increasing the expression of IL-10 in the skin tissue of wistar rats after excision wounds. The administration of pomegranate extract cream (*Punica granatum*) had an effect on the expression of VEGF and IL-10 in the skin tissue of wistar rats after excision wound between the treatment groups compared to the control group.

## AUTHORS' CONTRIBUTIONS

**Citra Dewi Hartati**; Data curation, Investigation, Writing-Original draf; Visualization **Titiek Sumarawati**: Supervision, Reviewing; **Agung Putra:** Reviewing, Supervision, Conceptualization.

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## DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

## DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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