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Pomegranate extract cream enhances FGF and reduces IFN-γ in excision wounds of wistar rats

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Abstract: Wounds impose significant social and financial burdens, and effective healing requires regulation of growth factors and cytokines. Fibroblast growth factor (FGF) accelerates proliferation, angiogenesis, and tissue repair, whereas interferon-γ (IFN-γ) negatively affects the healing process. This study aimed to evaluate the effect of pomegranate (Punica granatum) extract cream on FGF and IFN-γ levels in excision wound model rats. A true experimental design with post-test only control groups was conducted using 48 Wistar rats divided into 12 groups, with evaluations on days 3 and 7. Treatments included base cream, Bioplacenton, and pomegranate extract creams at 10% and 20% concentrations. FGF and IFN-γ levels were measured by ELISA and analyzed with Kruskal–Wallis and one-way ANOVA. The results showed significantly higher FGF levels in the 20% pomegranate extract group compared to controls on both day 3 (1353.42 pg/mL, p=0.000) and day 7 (1565.00 pg/mL, p=0.000), while IFN-γ levels were significantly reduced across treatment groups, with the lowest levels in the 20% extract group (p=0.001). These findings indicate that pomegranate extract cream enhances wound healing by upregulating FGF and downregulating IFN-γ expression, with the 20% concentration showing the most effective response, comparable to Bioplacenton.

Keywords: Pomegranate extract cream; excision wounds; FGF levels, IFN-γ levels.

INTRODUCTION

An excision wound is an open wound caused by the cutting of the skin surface tissue due to scratches by sharp objects of varying depths.[1] Wounds itself have a huge social and financial impact, affecting the quality of life of millions of people.[2] consequently Wound healing has gained increasing attention in clinical and biomedical research.[3] it is a Complex processes that occur through regeneration or reconstruction of damaged tissues.[4] Faster healing is associated with increased expression of *Fibroblast growth factor* (FGF).[5] which Triggers cell proliferation and migration, promotes angiogenesis, regulates inflammation, protects cells from apoptosis, and stimulates protease expression.[6] Meanwhile, IFN-γ contributes negatively to the healing process of skin wounds.[7] Examining these two cytokines together provides important mechanistic insight, because FGF represents a pro-healing mediator while IFN-γ acts as a pro-inflammatory inhibitor.

Healing applications using topical applications with their good efficacy and tolerability are still an option. However, it is reported to have quite serious allergic effects, redness, dry, inflamed skin to peeling.[8] The use of herbal alternatives may

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reduce adverse effects associated with synthetic drugs by choosing herbal medicines made from natural ingredients as an alternative to minimize side effects. particularly plant-based formulations rich in antioxidants[9,10] Recent studies have emphasized the strong wound-healing potential of herbal therapies including pomegranate, Aloe vera, and Centella asiatica, which act via antioxidant and anti-inflammatory mechanisms.[11,12] The use of medicinal plants that have antioxidants such as the high phenolic content of pomegranate extract (*Punica granatum*) is reported to accelerate healing and improve the wound repair process.[13,14] Previous studies have shown that pomegranate extract accelerates wound contraction.[15] and increases expression of growth factors such as FGF-2 and TGF-β in dental wound models, [16, 17] but its specific effect on the balance between FGF and IFN-γ in excision wound models has not yet been reported. This represents an important research gap, as modulation of both factors simultaneously could provide a clearer understanding of how herbal therapies influence the molecular dynamics of wound healing.

Incidents and injury burden are one of the main factors in healthcare problems.[18] Wound care with different etiologies makes up a large part of the total healthcare budget. An estimated 1.5–2 million people in Europe suffer from acute or chronic injuries. These injuries are treated both in hospitals and in treatment clinics.[19] More than 8.2 million people suffer injuries in the U.S. with treatment costs ranging from $28.1 to $96.8 billion. The global wound care market size was valued at USD18.4 billion in 2018 and is expected to grow at a compound annual growth rate (CAGR) of 3.9% from 2019 to 2026.[18] Chronic wounds are the biggest burden on our health care and cost more than $25 billion a year and affect more than 6.5 million people in the U.S. alone.[20] The physical, mental, and social consequences of wounds further highlight the urgency of more effective and safer treatments.[19]

Research on various types of plants that are considered to have medicinal properties that are beneficial to human health has been carried out a lot. Pomegranate has been used traditionally and is believed to increase medicinal activity. Pomegranate extract has anti-inflammatory properties, has anti-bacterial, anti-oxidant, anti-cancer, and antifungal properties.[16] Plant extracts are more widely used in the dermatology, hair, and cosmetic industries than ever before.[14] The study used the basic ingredients of *Punica Granatum Peel Extract* ointment at doses of 10% and 15% b/b. The group that received 15% b/b extract ointment had a faster wound closure rate and a higher percentage of wound contraction. *Punica granatum* methanol extract has proven to be a promising wound healing agent.[17] Recent experimental work also demonstrated that pomegranate peel extract ointment enhanced collagen deposition and wound closure in burn wound models, supporting its potential as a topical therapy.[21,22]However, no previous studies have investigated the topical application of pomegranate extract cream on excision wound healing in Wistar rats with specific focus on FGF and IFN-γ modulation.

The development of *skin care* clinics with wound care as an effort to avoid the appearance of signs of aging, by slowing down and preventing the aging process, in order to increase confidence and always want to look attractive and youthful,[23] Increasing interest in using natural products, many plants have very important properties that play a role in the wound healing process. Plants are more potent healers because they promote repair mechanisms in a natural way. Therapy using plant extracts not only speeds up the healing process but also maintains aesthetics.[9] Pomegranate extract, which comes from the fruit part, is known to have anti-inflammatory and antioxidant properties that can help in the wound healing process.[24] including ellagic and gallic acids, which have been shown to reduce oxidative stress and regulate cytokine expression during wound repair.[12,22] These bioactivities may contribute to faster resolution of inflammation and enhanced tissue regeneration.

Wound healing is biologically precise and programmatic, through phases of hemostasis, inflammation, proliferation, and remodeling.[3] The normal response

to wound healing is a series of events that begin with injury.[4] Neutrophils are the first infiltration cells that appear within 24 hours of injury and are necessary for the body's defense response. Prolonged neutrophil infiltration is involved in the degradation of collagen through the production of proteinases such as MMP. Collagen synthesis by fibroblasts is induced by TGF-β and inhibited by IFN-γ.[7] However, no prior study has evaluated the topical application of pomegranate cream on FGF and IFN-γ expression in excision wound models. Therefore, this study aims to investigates the effect of pomegranate extract cream (Punica granatum) on excision wound healing in Wistar rats, with the hypothesis that it reduces inflammation by lowering IFN-γ levels while accelerating repair by increasing FGF levels.

MATERIAL AND METHOD

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

This research is classified as a *true experimental study using a* posttest-only control group *design* design conducted in April-May 2024 at the *Stem Cell and Cancer Research* Laboratory (SCCR) Semarang, Central Java. The subject in this study is a 2–3-month-old Wistar white rat with a weight ranging from 190-210 grams. The number of subjects was 48 rats divided into 12 groups, namely the normal group (K1) which was a healthy control, K2 the excision wound group that was not given treatment, the K3 the excision wound group that was given base cream (vanishing cream composed of stearic acid, triethanolamine, glycerin, borax, aquadest, and tween, applied topically once daily at a dose of 0.2 g).

The cream had a smooth, homogeneous consistency, was easily spreadable, and had a stable emulsion without phase separation during the study period. Quality control included macroscopic evaluation for color, odor, and homogeneity, and the preparation was stored at room temperature in sterile containers to prevent contamination. the K4 excision wound group that was given *Bioplacenton,* the K5 excision wound group that was given pomegranate cream 10%, and the K6 excision wound group that was given pomegranate cream 20%, the K1-K6 group for IF-γ level examination on the 4th day. Normal group (K7) which is a healthy control, K8 excision wound group that is not given treatment, the K9 the excision wound group that was given base cream, K10 excision wound group that is given *Bioplacenton,*[25] K11 excision wound group given pomegranate cream 10%, and K12 excision wound group given pomegranate cream 20%. K6-K12 group for FGF level check.

Validation of the excision wound model was performed macroscopically through standardized wound size and photographic documentation. topical concentrations of 10% and 20% pomegranate extract cream were selected based on prior evidence of efficacy at similar levels. Notably, a study using a 10% and 20% pomegranate extract cream were selected based on prior evidence of efficacy at similar levels. Notably, a study using a 10% (wt/wt) methanolic gel of *Punica granatum* peel extract significantly enhanced wound contraction and collagen content in excision wounds in Wistar rats compared to lower concentrations and controls.[26]

To explore whether a higher dose could yield enhanced biological activity, the 20% concentration was also included. Together, these doses allow the examination of both standard effective concentrations and potential dose-dependent responses. All Rats were randomly allocated into experimental groups using a computer-generated randomization sequence. All treatments were administered by a laboratories operator not involved in outcome measurement. operator performing ELISA assays and macroscopic wound assessments were blinded to group allocation to minimize bias.

Pomegranate Extract

The pomegranate sample was 2 kg, the part used was the pulp. The sample was dried in an oven at a temperature of 50°C and mashed The result was a moisture balance check, if the moisture content was below 10%, the drying result was 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. Then the simplicia 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 50°C until a thick extract is obtained.

Preparation of test animal subjects

The mice that had been adapted for 7 days were anesthetized with a mixture of ketamine (60 mg/kgbb) and xylazine (20mg/bb), the surface of the skin that had been cleaned using *povidone-iodine* to avoid infection during wound making. The wound was made using circular *punch biopsy* excision with a diameter of 6 mm. The next day, the rats were then given treatment according to their group. Topical treatment was given once a day after the creation of a wound model on the back of the rats. Skin samples in the validation group were taken to make histological preparations using the paraffin method with *hematoxylin* and *eosin* (H&E) staining.

Preparation of pomegranate extract cream preparations

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 melted. Add 125mg *of potassium hydroxide* in sequence then homogenize, add *Triethanolamine* 1.5 ml, *Glycerine10 ml,* and *aquadest* 25ml until well mixed. The making of cream in 20 grams is done 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.[27]

Sampling of rat skin tissue of excision wounds

After the treatment, on the 4th day of the K1-K6 group and on the 8th day of the K7-K12 group, rat skin tissue was taken. Previously, all Wistar rats were terminated first by anesthesia on the rats. Make a tissue incision in the injured part of the skin, using scissors and tweezers. The tissue sample is cut, then the tissue is added with PBS (pH 7.4). Then the tissue samples were homogenized in cold temperature conditions of 4°C. Next, centrifuge at a speed of 2000-3000 rpm, for 20 minutes. Then supernatants resulting from *centrifuges* that have lower specific weights are taken and used as test samples. If the sample will be stored first, then the sample can be stored at a temperature of -20°C, Excision wounds were created on the dorsal skin of each rat using a sterile biopsy punch with a standardized diameter. Validation of the wound model was performed macroscopically by monitoring wound size and appearance through photographic documentation at baseline and during treatment. This method has been widely used in rodent wound-healing studies and is considered reliable for evaluating topical interventions.

Examination of FGF and IF-γ levels using the ELISA method

The skin tissue samples that have been obtained are then analyzed for FGF and IF-γ levels using the ELISA method. Levels of IFN-γ and FGF in skin tissue homogenates were measured using commercial ELISA kits according to the manufacturer's instructions. Rat IFN-γ (Interferon Gamma) ELISA Kit (Cat: E-EL-R0009, detection range: 31.25–2000 pg/mL) and Rat bFGF/FGF2 (Basic

Fibroblast Growth Factor) ELISA Kit (Cat: E-EL-R0091, detection range: 15.63–1000 pg/mL) were obtained from Elabscience (Wuhan, China). All samples and standards were run in duplicate, and intra- and inter-assay coefficient of variation (CV) values were <10% and <12%, respectively, in accordance with the manufacturer's specifications.

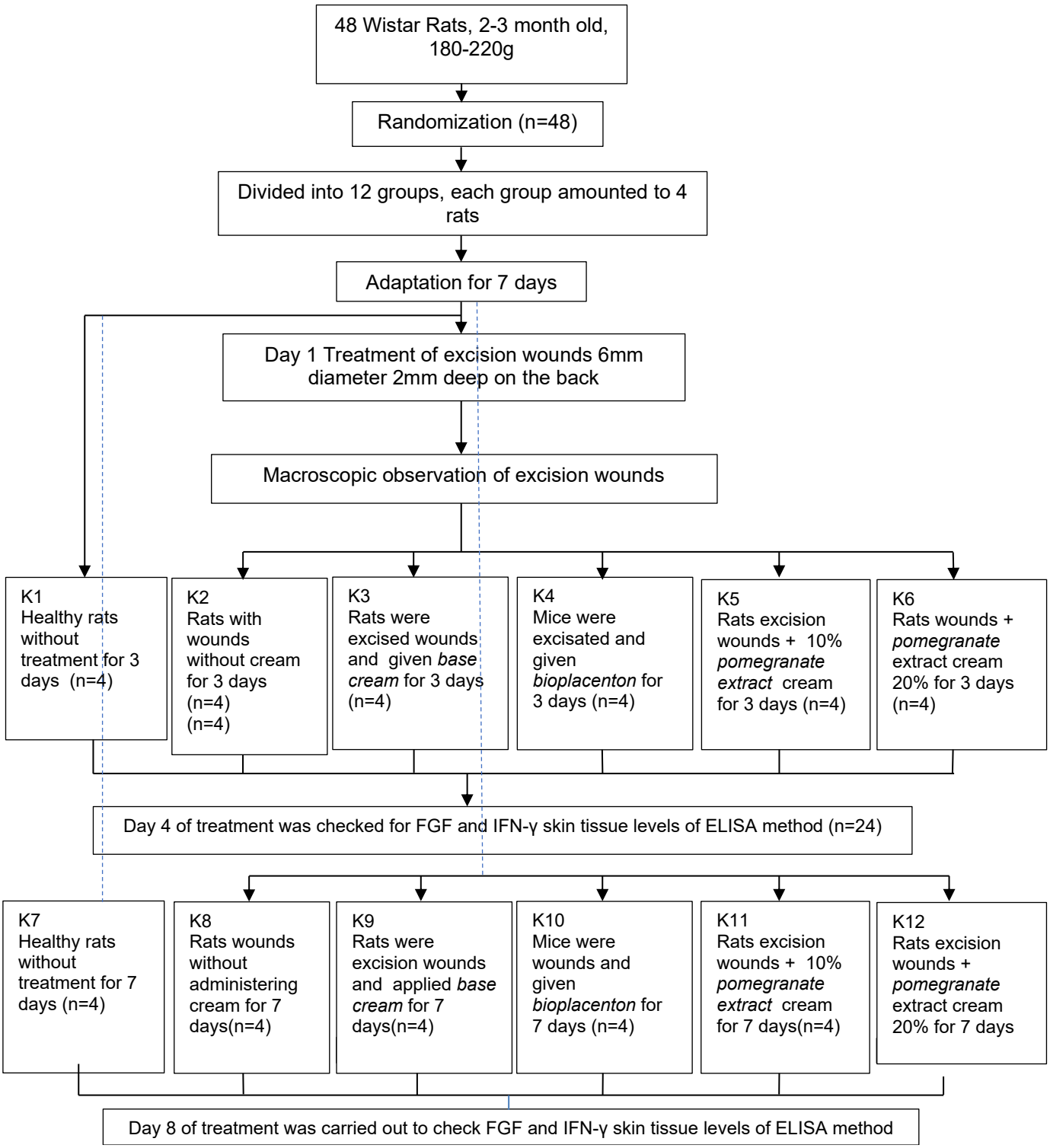

Figure 1. Diagram of animal group assignments with treatment sequence.

RESULTS AND DISCUSSION

Results

The macroscopic results (figure 1 and 2) of the treatment on day 3 showed no significant difference in wound closure in each group and the diameter of excision wounds was not different, in contrast to the macroscopic results of the treatment group on day 7 showing an acceleration of wound healing and closure, the excision wound group given 20% pomegranate extract cream (K12) macroscopic was equal to the excision wound group given bioplacenton. Excision wound group given extract cream 10% pomegranate (K11) also showed accelerated healing and wound closure compared to the group without treatment (K8) and those given *base cream* (K9).

The results of the test and the average analysis of FGF levels after the 3rd day and 7 days of treatment in each group of research subjects are shown in table 1. On day 3, the average FGF level in the healthy control group (K1) was 542.50 pg/mL, the lowest among all groups, while the highest level was found in the 20% pomegranate extract cream group (K6) at 1353.42 pg/mL. Compared with the Bioplacenton group (K4: 1190.00 pg/mL), the K6 group still showed higher FGF levels.

While On day 7, the average FGF level in the healthy control group (K7) was 578.33 pg/mL, again the lowest result, whereas the highest level was observed in the 20% pomegranate extract cream group (K12) at 1565.00 pg/mL. Similar to day 3, the FGF level in the 20% extract group exceeded that of the Bioplacenton group (K10: 684.17 pg/mL).

Based on table 1, it is shown The mean FGF level was lowest in the healthy control (K1: 542.50 ± 15.24 pg/mL; 95% CI 518.25–566.75) and highest in the 20% extract group (K6: 1353.42 ± 58.68 pg/mL; 95% CI 1260.05–1446.79) (Table 1). The Kruskal–Wallis test showed a significant difference among groups ($H = 20.92$, $df = 5$, $p < 0.001$, $\varepsilon^2 = 0.87$, very large effect). Pairwise comparisons indicated that K6 had significantly higher FGF levels than untreated controls (K2: 1000.83 ± 43.24; 95% CI 932.03–1069.63), with no overlap in CIs, confirming a robust treatment effect.

Table 1. Mean FGF Levels and Non-Parametric Statistical Analysis in Rats with Excision Wounds After Pomegranate Extract Cream Treatment on Day 3 and Day 7.

Group	Treatment	Day 3 FGF Level (pg/mL) Mean ± SD	Shapiro–Wilk *p*	Day 7 FGF Level (pg/mL) Mean ± SD	Shapiro–Wilk *p*
K1 / K7	Healthy rats	542.50 ± 15.24	0.690	578.33 ± 6.38	0.272
K2 / K8	Wound control (no intervention)	1000.83 ± 43.24	0.392	758.33 ± 10.36	0.855
K3 / K9	Base cream	960.00 ± 201.55	0.030	599.17 ± 46.22	0.430
K4 / K10	Bioplacenton®	1190.00 ± 8.60	0.972	684.17 ± 15.24	0.691
K5 / K11	Pomegranate extract cream (10%)	907.50 ± 9.17	0.650	784.99 ± 9.17	0.194
K6 / K12	Pomegranate extract cream (20%)	1353.42 ± 58.68	0.262	1565.00 ± 177.24	0.008

Note: Levene's test:
Day 3: $p = 0.001$ | Day 7: $p = 0.004$
Kruskal–Wallis test:
Day 3: $p < 0.001$ | Day 7: $p < 0.001$

Significant differences between treatment groups, then the *Mann Whitney test* was carried out to determine the most influential dose. The results *of Mann Whitney*'s test showed significant results in the group of excision wounds that were not treated (K2) compared to the 10% pomegranate dose cream (K5) with a value of 0.021 ($p<0.05$). The excision wound group that was not treated (K2) was compared to the 20% pomegranate dose cream (K5) with a value of 0.021 ($p<0.05$). It can be concluded that the administration of pomegranate extract cream at a dose of 20% after excision wound on day 3 significantly increased FGF levels

While According to Table 1, on day 7 the lowest mean FGF level was recorded in the healthy control group (K1: 578.33 ± 6.38; 95% CI 568.18–588.48), whereas the highest was observed in the 20% extract group (K6: 1565.00 ± 177.24; 95% CI 1282.97–1847.03). The Kruskal–Wallis test indicated significant differences among groups ($H = 21.79$, $df = 5$, $p < 0.001$, $\varepsilon^2 = 0.91$, very large effect). Notably, the confidence interval for K6 did not overlap with those of K2 (758.33 ± 10.36; 95% CI 741.84–774.82) or K4 (684.17 ± 15.24; 95% CI 659.92–708.42), confirming that 20% extract cream yielded substantially higher FGF levels. Follow-up Mann–Whitney analysis further showed significant differences between the untreated group (K2) and both the 10% extract (K5, $p = 0.020$) and 20% extract (K6, $p = 0.020$) groups. These findings indicate that topical administration of 20% pomegranate extract cream significantly enhanced FGF expression by day 7 post-excision

It showed FGF significantly elevation of FGF levels in the 20% pomegranate group suggests that bioactive compounds in the extract accelerated the proliferative phase of healing by stimulating fibroblast activity and vascular growth. Compared with Bioplacenton, which is a standard pharmaceutical agent, the higher FGF levels in the 20% group imply that the antioxidant and polyphenol components of pomegranate may provide additional support to cellular proliferation and extracellular matrix formation. The The maintenance of high FGF expression until day 7 also indicates that the extract not only initiates early fibroblast activation but sustains the remodeling process, which is vital for long-term wound strength and closure. This finding highlights the potential of pomegranate extract as a botanical therapy with comparable or even superior efficacy to conventional agents in promoting tissue regeneration.

The results of the test and the analysis of the average IF-γ levels after the 3rd day 7rd day of treatment in each group of research subjects are shown in table 2 as follows.

Table 2. Mean IFN-γ Levels and One-Way ANOVA Results in Rats with Excision Wounds After Pomegranate Extract Cream Treatment on Day 3 and Day 7

Group	Treatment	Day 3 IFN-γ (pg/mL) Mean ± SD	Shapiro–Wilk *p*	Day 7 IFN-γ (pg/mL) Mean ± SD	Shapiro–Wilk *p*
K1 / K7	Healthy rats	237.89 ± 9.65	0.537	567.54 ± 46.28	0.133
K2 / K8	Wound control (no intervention)	551.41 ± 20.85	0.655	191.18 ± 62.11	0.951
K3 / K9	Base cream	667.55 ± 40.47	0.508	409.70 ± 10.84	0.611
K4 / K10	Bioplacenton®	300.50 ± 80.35	0.890	193.56 ± 46.85	0.840
K5 / K11	Pomegranate extract cream (10%)	350.72 ± 9.43	0.384	461.18 ± 19.60	0.375
K6 / K12	Pomegranate extract cream (20%)	313.23 ± 8.29	0.714	409.36 ± 21.64	0.850

Note: Levene's test (homogeneity of variance):
Day 3: $p = 0.004$ | Day 7: $p = 0.004$
One-way ANOVA:
Day 3: $p = 0.001$ | Day 7: $p = 0.001$

On day 3, the lowest IF-γ level was found in the healthy control group (K1: 237.89 pg/mL), whereas the highest was observed in the base cream group (K3: 667.55 pg/mL). Among the treated groups, the Bioplacenton group (K4: 300.50 pg/mL) showed the lowest IF-γ level compared with the others. By day 7, the highest mean IF-γ level was again observed in the healthy control (K7: 567.54 pg/mL), while the lowest was recorded in the untreated wound group (K8: 191.18 pg/mL). Across all treatment groups, IF-γ levels remained lower than those of the healthy controls.

Based on Table 2, the average IF-γ levels in the day-3 groups were normally distributed ($p > 0.05$) but showed non-homogeneous data variation (Levene's test $p = 0.004$). Accordingly, a parametric one-way ANOVA was performed and showed a significant difference among groups ($p = 0.001$).

similarly, in the day-7 groups, the average IF-γ levels were normally distributed ($p > 0.05$) but again demonstrated non-homogeneous data variation (Levene's test $p = 0.004$). A one-way ANOVA also revealed significant group differences ($p = 0.001$).

Significant differences between treatment groups, then a *Post hoc Tamhane test* was carried out to determine the most influential dose. The average result of IF-γ of *the Post hoc Tamhane test* is as shown in the following table 3:

Table 3. Results of *the Post hoc Tamhane* test of IF-γ levels of rat skin tissue after excision wounds with the administration of pomegranate extract cream

3rd days of treatment						
Group	**K1**	**K2**	**K3**	**K4**	**K5**	**K6**
K1	-	*0.001	*0.002	0.975	*0.001	*0.001
K2		-	0.074	0.090	*0.001	*0.002
K3			-	*0.012	*0.008	*0.004
K4				-	*0.000	1.000
K5					-	*0.016
7rd days of treatment						
Group	**K1**	**K2**	**K3**	**K4**	**K5**	**K6**
K1	-	*0.002	0.072	*0.001	0.054	*0.041
K2		-	0.073	1.000	0.050	0.051
K3			-	*0.028	1.000	1.000
K4				-	0.014	0.013
K5					-	1.000

*Description: * Means p<0.05*

The results of *the Post hoc Tamhane test* on 3rd days showed The highest IF-γ level was found in the base cream group (K3: 667.54 ± 40.47; 95% CI 603.14–731.94), while the lowest was in the healthy control (K1: 237.88 ± 9.65; 95% CI 222.52–253.24). One-way ANOVA revealed significant differences ($F(5,18) = 76.85$, $p < 0.001$, $\eta^2 = 0.95$, very large effect). Post hoc Tamhane tests showed that IF-γ was significantly reduced in the 10% (K5: 350.72 ± 9.43; 95% CI 335.71–365.73) and 20% (K6: 313.23 ± 8.29; 95% CI 300.04–326.42) extract groups compared with untreated controls (K2: 551.41 ± 20.85; 95% CI 518.23–584.59). The lack of overlap between CIs highlights the strength of the effect.

While The results of the Post hoc Tamhane On day 7, the lowest IF-γ level was in the untreated control (K2: 191.18 ± 62.11; 95% CI 92.35–290.01), while the highest was in the healthy group (K1: 567.54 ± 46.28; 95% CI 493.90–641.18). ANOVA showed significant group differences ($F(5,18) = 56.15$, $p < 0.001$, $\eta^2 = 0.94$, very large effect). Post hoc analysis indicated no significant difference between K2 and the 10% (K5: 461.18 ± 19.60; 95% CI 429.99–492.37) or 20% (K6: 409.36 ± 21.64; 95% CI 374.93–443.79) extract groups, as their CIs broadly overlapped.

However, the healthy group (K1) showed consistently higher IF-γ levels than all wound groups. Its CI (493.90–641.18) did not overlap with the CIs for K2 (92.35–290.01), K4 (193.56 ± 46.85; 95% CI 119.01–268.11), or K6 (374.93–443.79), indicating a statistically and biologically meaningful elevation in IF-γ in healthy skin compared with wounded skin, regardless of treatment.

It showed that Pomegranate extract cream reduced IF-γ during the acute inflammatory phase (day 3), but by day 7. The marked reduction of IFN-γ in the 10% and 20% pomegranate extract groups on day 3 suggests that the extract suppressed excessive inflammatory signaling during the acute phase, thereby preventing tissue damage caused by prolonged inflammation. This is consistent with the known antioxidant and immunomodulatory effects of pomegranate polyphenols, which can inhibit NF-κB activation and reduce cytokine production. By day 7, IFN-γ levels converged across groups, reflecting the normal course of wound healing where inflammation naturally subsides. The finding that treatment groups showed lower IFN-γ than untreated wounds at both time points indicates that pomegranate extract facilitated a faster resolution of inflammation, allowing earlier entry into the proliferative and remodeling phases of healing. This dual action suppressing pro-inflammatory signals while supporting growth factor expression underscores the therapeutic relevance of pomegranate extract

Discussion

Wound healing is a complex, multistage process involving inflammation, proliferation, and remodeling, regulated by the interplay of cytokines, growth factors, and extracellular matrix components. The present study demonstrated that topical application of pomegranate (Punica granatum) extract cream significantly enhanced fibroblast growth factor (FGF) expression, particularly at the 20% concentration, and modulated interferon-γ (IF-γ) dynamics in excision wounds of Wistar rats.

The increase in FGF observed with 20% extract on both day 3 and day 7 suggests accelerated fibroblast activation, angiogenesis, and tissue remodeling. FGF family proteins, particularly FGF2 (bFGF), are known potent mitogens that stimulate keratinocyte proliferation, collagen deposition, and angiogenesis, thereby expediting wound closure .[28–30] Pomegranate's effect on wound repair is consistent with multiple recent reviews and experimental studies showing that Punica granatum extracts accelerate tissue repair and enhance growth factor–mediated processes in skin models.[31,32]

It could Mechanistic interpreted as upregulation of FGF in treated wounds likely reflects a combination of direct and indirect effects of pomegranate polyphenols (e.g., punicalagin, ellagic acid, anthocyanins). These compounds can enhance fibroblast function and viability, promoting synthesis of extracellular matrix components and paracrine growth factor release (including FGF family members).[33,34] Additionally, by reducing oxidative stress, polyphenols preserve cellular signaling fidelity during the proliferative phase, permitting more robust angiogenesis and collagen deposition.[32]

This Comparable wound healing effects have been reported with other botanicals. For instance on other study like study on *Centella asiatica* particularly its triterpenes such as asiaticoside has been shown to stimulate fibroblast proliferation and collagen synthesis in vitro [35]. Similarly, curcumin from *Curcuma longa* accelerates wound repair by enhancing fibroblast migration, collagen deposition, angiogenesis, and epithelialization during the proliferative phase [36]. *Aloe vera* also demonstrates robust effects on growth factor modulation; it enhances bFGF and TGF-β1 expression in fibroblasts and upregulates multiple factors (e.g., KGF-1, IGF-1, VEGF) and pathways (PI3K/Akt, MAPK) critical for cell proliferation and migration [37]. These parallels underscore the potency of antioxidant-rich phytochemicals in modulating growth factor pathways and

supporting tissue regeneration. Collectively, these comparisons indicate that pomegranate extract shares mechanistic pathways with other natural wound-healing agents but may provide stronger antioxidant-driven stimulation of fibroblast activity at higher concentrations.

In relations to its effect for inflammation, pomegranate extract reduced IF-γ expression during the acute inflammatory phase (day 3), aligning with its reported antioxidant and immunomodulatory effects [38,39]. IF-γ, secreted predominantly by activated T cells and NK cells, plays a central role in macrophage activation, leukocyte recruitment, and amplification of inflammatory cascades [40,41]. Pomegranate polyphenols inhibit pro-inflammatory signaling such as NF-κB and related MAPK pathways, thereby decreasing transcription of pro-inflammatory cytokines (including IFN-γ in certain contexts) and limiting excessive neutrophil/macrophage-mediated tissue damage [32,42] Moreover, several studies indicate that pomegranate-derived compounds promote macrophage polarization from a pro-inflammatory M1 phenotype to a reparative M2 phenotype, which favors resolution of inflammation and supports the remodelling phases of healing.

By day 7, however, differences between wound groups diminished, and suppression of IF-γ was less evident. This temporal pat tern is consistent with the natural course of acute inflammationan early, rapid cytokine response followed by resolution and transition to proliferation and suggests that pomegranate extract acts mainly to moderate the early inflammatory peak rather than completely suppress basal immune activity.[31]

The combination of early anti-inflammatory modulation (downregulated IFN-γ, NF-κB inhibition, M1 to M2 shift) together with enhanced proliferative signaling (upregulated FGF, improved fibroblast function) provides a plausible biological explanation for the accelerated wound closure observed with 20% pomegranate cream in our model. [32,33]

Despite these promising findings, several limitations must be acknowledged. First, pre-treatment baseline levels of FGF and IF-γ were not assessed, which limits the ability to quantify relative changes within individual subjects. Second, no histological scoring of wound tissue was performed, precluding direct correlation between molecular changes and morphological healing outcomes. Third, while our ELISA data demonstrate molecular modulation, we did not directly measure NF-κB activity, macrophage phenotype markers (e.g., CD86/CD206), or oxidative stress biomarkers (e.g., MDA, SOD), which would strengthen mechanistic claims. fourth, the study was conducted in a rodent model, and extrapolation to human wound healing requires caution due to differences in skin architecture and immune response. translational studies and formulation optimization (vehicle, dose, stability, skin penetration) are needed before clinical application can be recommended Future studies should incorporate histological analyses, pre- and post-treatment comparisons, and clinical trials to confirm translational relevance. , also needed to incorporated immunohistochemistry for macrophage and fibroblast markers, oxidative stress assays, and, if possible, pathway-specific readouts (NF-κB, MAPK phosphorylation).

In summary, pomegranate extract cream, especially at 20%, effectively enhanced FGF expression and modulated IF-γ during early wound healing, suggesting synergistic antioxidant and proliferative mechanisms. Compared with established natural wound-healing agents such as Curcuma longa, Centella asiatica, and Aloe vera, pomegranate shows comparable or superior effects in promoting tissue repair. These findings highlight its potential as a phytopharmaceutical intervention for wound management, warranting further mechanistic and clinical investigation.

CONCLUSION

The administration of pomegranate extract cream (*Punica Granatum*) had an effect on increasing FGF levels in the skin tissue of wistar rats after excision wounds. The administration of pomegranate extract cream (*Punica Granatum*) had an effect on reducing IF-γ levels in the skin tissue of wistar rats after excision wounds. The administration of pomegranate extract cream (*Punica Granatum*) had an effect on FGF levels and IF-γ levels of skin tissue of wistar rats after excision wounds between treatment groups compared to the control group.

AUTHORS' CONTRIBUTIONS

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

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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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APPENDIX

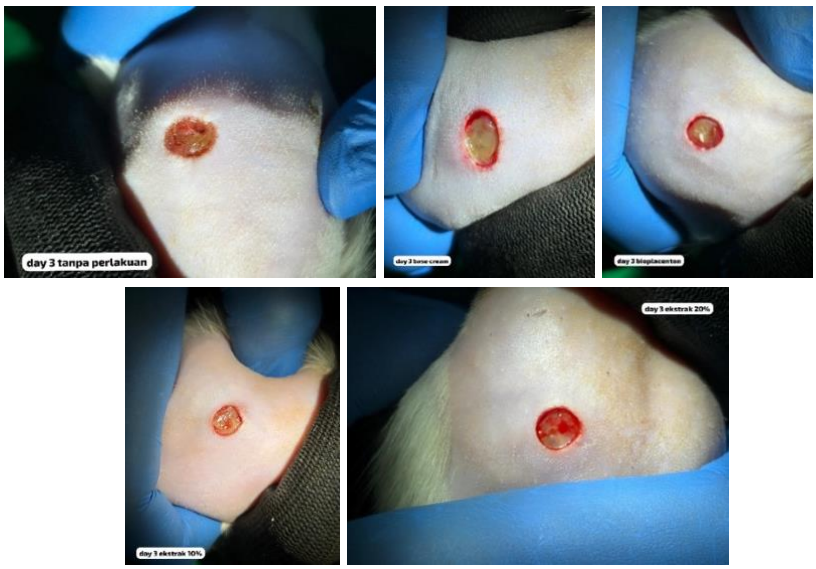

Figure 1. Macroscopic appearance of excision wounds in Wistar rats after treatment on day 3 and day 7.
Note: *Representative images from each group are shown (K1 = Healthy rats; K2 = Excision wound without treatment; K3 = Base cream; K4 = Bioplacenton; K5 = 10% pomegranate extract cream; K6 = 20% pomegranate extract cream). Wound area reduction was more pronounced in the Bioplacenton (K4) and 20% pomegranate extract (K6) groups by day 7. Scale bars = 5 mm.*

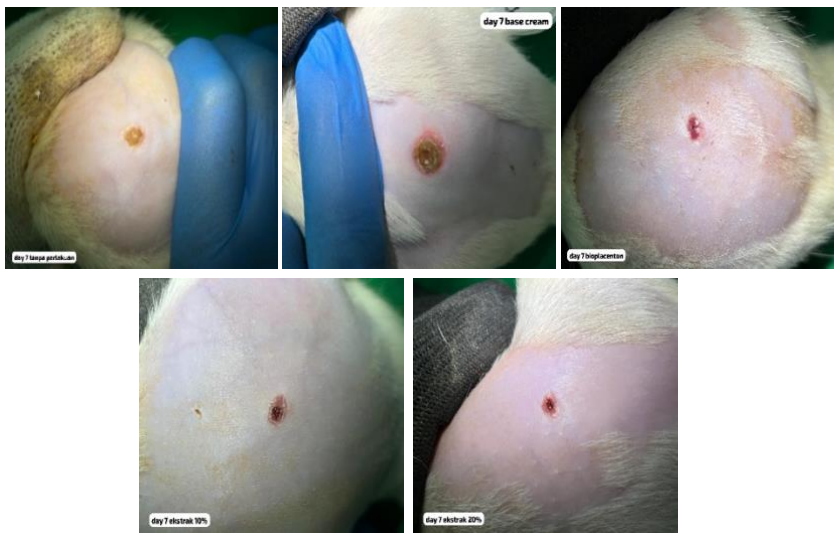

Figure 2. Macroscopic appearance of excision wounds in Wistar rats after treatment on day 3 and day 7.
Note: Representative images from each group are shown (K7 = Healthy rats; K8 = Excision wound without treatment; K9 = Base cream; K10 = Bioplacenton; K11 = 10% pomegranate extract cream; K12 = 20% pomegranate extract cream). Wound area reduction was more pronounced in the Bioplacenton (K4) and 20% pomegranate extract (K6) groups by day 7. Scale bars = 5 mm.

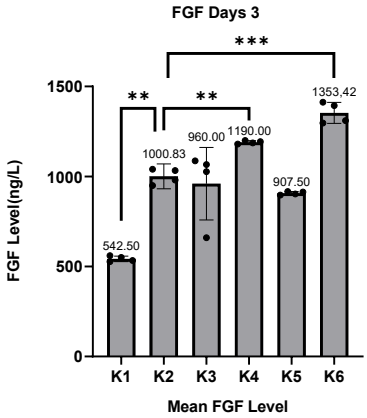

Figure 3. FGF levels (pg/mL) in rat skin tissue on day 3 after excision wound treatment (n = 4 per group).

Note: *Data are presented as mean ± SD . Groups: K1 = Healthy rats; K2 = Excision wound without treatment; K3 = Base cream; K4 = Bioplacenton; K5 = 10% pomegranate cream; K6 = 20% pomegranate cream. FGF levels were significantly increased in the 20% extract group compared with untreated wounds (p < 0.05).*

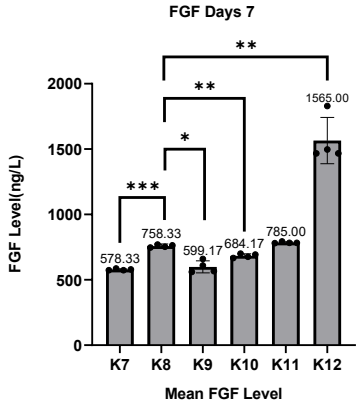

Figure 4. FGF levels (pg/mL) in rat skin tissue on day 7 after excision wound treatment (n = 4 per group).

Note: *Data are presented as mean ± SD . Groups: K7 = Healthy rats; K8 = Excision wound without treatment; K9 = Base cream; K10 = Bioplacenton; K11 = 10% pomegranate cream; K12 = 20% pomegranate cream. FGF levels were significantly increased in the 20% extract group compared with untreated wounds (p < 0.05).*

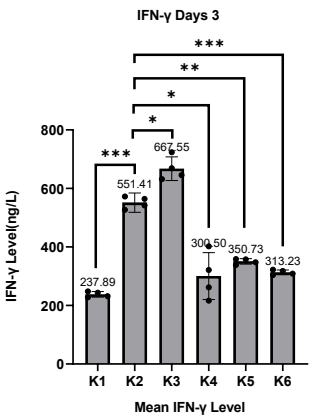

Figure 5. IFN-γ levels (pg/mL) in rat skin tissue on day 3 after excision wound treatment (n = 4 per group).

Note: *Data are presented as mean ± SD. Groups: K1 = Healthy rats; K2 = Excision wound without treatment; K3 = Base cream; K4 = Bioplacenton; K5 = 10% pomegranate cream; K6 = 20% pomegranate cream. Both 10% and 20% pomegranate extract cream significantly reduced IFN-γ levels compared with untreated wounds (p < 0.05).*

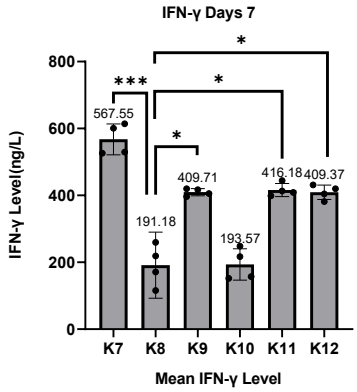

Figure 6. IFN-γ levels (pg/mL) in rat skin tissue on day 7 after excision wound treatment (n = 4 per group).

Note: *Data are presented as mean ± SD with 95% CI. Groups: K7 = Healthy rats; K8 = Excision wound without treatment; K9 = Base cream; K10 = Bioplacenton; K11 = 10% pomegranate cream; K12 = 20% pomegranate cream. No significant reduction in IFN-γ was observed at day 7 compared with controls, though values remained lower in treatment groups than untreated wounds.*