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*Effect of Pomegranate (*Punica granatum*) extract cream on PDGF and IL-1 levels in a rat excision wound model*

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Abstract: Wound healing requires special attention through proper wound closure and infection control. Evaluating wound healing outcomes is essential to minimize complications. Natural products with antioxidant, anti-inflammatory, and antimicrobial properties have the potential to support wound healing at various stages. One such product is pomegranate (*Punica granatum*), which has been reported to accelerate tissue repair and reduce wound size. This study aimed to investigate the effect of pomegranate extract cream (*Punica granatum*) on PDGF and IL-1 levels in rats with excision wounds. An in vivo experimental study was conducted using 48 Wistar rats divided into 12 treatment groups, with sample collection performed on day 3 and day 7 after treatment. PDGF and IL-1 levels were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. A significant difference in mean IL-1 levels was found using one-way ANOVA ($p = 0.000$; $p < 0.05$). Post hoc Tamhane analysis showed that the 20% pomegranate extract cream had the most significant effect in reducing IL-1 levels ($p = 0.000$). For PDGF, no significant effect was observed on day 3 (Kruskal-Wallis test, $p = 0.397$), while on day 7, a significant difference in PDGF levels was found between treatment groups (Kruskal-Wallis test, $p = 0.010$). The 20% pomegranate extract cream significantly increased PDGF levels compared to the bioplacenton group. In conclusion, topical application of pomegranate extract cream increases PDGF levels and reduces IL-1 levels in excision wounds, with the 20% concentration showing the most effective results.

Keywords: IL-1 Levels; PDGF Levels; Pomegranate Cream; Excision Wounds.

INTRODUCTION

Fast and aesthetically satisfying wound healing is a patient's demand, consideration of wound healing results is very important to minimize the risk.[1] Wound healing requires better special care in the form of wound closure and infection control.[1] Recent advances in the field of cellular and molecular biology have broadened the understanding of the biological processes involved in wound repair, tissue regeneration and accelerated healing.[2] Specifically, interleukin 1 (IL-1) has an important role in the acute inflammatory process, namely stimulating the release of other inflammatory mediators.[3] Platelet-derived growth factor (PDGF) helps coordinate the various stages of wound healing to ensure efficient healing after a wound has occurred.[4] Basic skincare can play a role in protecting the skin's protective function, controlling inflammation and promoting natural healing.[1] The potential of natural products in wound treatment has been reported in many studies, products that have antioxidant, anti-inflammatory, and antimicrobial properties, from various plant sources can interact in different stages of the wound healing process.[5–8] One of them is pomegranate (*Punica granatum*) which

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improves the repair process and reduces the area of wounds.[8] Pomegranate extract in wound healing related to molecular parameters is still few reported, one one recent systematic review of preclinical pomegranate extract studies in chronic wound healing stated found no in vivo research simultaneously evaluating both IL-1 suppression and PDGF elevation, and existing in vivo studies typically assess only one biomarker (e.g., PDGF or VEGF) without addressing IL-1 modulation[9].

On other instance, a study in *Cavia cobaya* (Guinea pig) showed that topical pomegranate fruit extract enhances PDGF (and VEGF) expression in post-extraction wounds; however, it lacked any measurement of IL-1 modulation,[10] highlighting the single-biomarker focus of past work furthermore shown more in-depth research is needed

Data from the Indonesian Ministry of Health (2016) shows that there are 41,034 cases of minor injuries or outpatient treatment and around 3,922 cases of hospitalization with serious injuries due to disasters. Antibacterial, anti-inflammatory, and antioxidant drugs can speed up wound healing.[11]

Previous research with pomegranate extract can accelerate wound healing, During the inflammatory stage in the first three days of injury, the wound healing effect is related to the degradation of cytokine activity released by macrophages; TNF-α, IL-1, PDGF and TGF-β. Furthermore, in the proliferation stage, PDGF and TGF-β will be secreted immediately after injury.[12] In line with the study, the wound healing activity isolated using the excision wound and incision wound models, the highest wound healing power observed was 201.83 ± 4.98 for the 10% BB punicalagin ointment isolated in the incision wound model. In addition, in the excision wound model, the highest wound reduction was observed on day 15 of 88 ± 0.78, which was optimal compared to the standard of wound reduction of 92 ± 0.91. As a result, pomegranate powder methanol extract can be used as a powerful phytoconstituent and wound healing ingredient.[13] Despite these findings, no published study to date has evaluated the topical use of a 20% pomegranate extract cream on excision wounds in Wistar rats while simultaneously quantifying IL-1 suppression and PDGF elevation this forms the novelty of the present research.

Wound healing should be optimized for the skin to heal quickly without scarring, the healing process varies from one procedure to another, and from one part of the body to another, each wound requires special care.[1] Wounds that cannot be healed are a challenge for clinicians and bear a lot of burden for patients. Wound epithelialization is an important component of wound repair.[14] IL-1 is involved in the regulation of immune and inflammatory responses, and plays a role in regulating the growth and differentiation of cells in the body.[3] Meanwhile, PDGF stimulates the proliferation and migration of important cells such as fibroblasts which play a role in the formation of connective tissue and accelerate the healing process.[4] 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.[15]

A number of Indonesian plants have the potential to be explored as cosmetics.[16] The use of plant extracts as ingredients in making cosmetics is increasingly in demand by consumers who are gradually starting to care about environmentally friendly products. Some of the phytochemical content of pomegranates includes polyphenols, flavonoids, anthocyanosides, alkaloids, lignans, and tri-terpenes. *Punica granatum* and its components have been clinically applied and have many pharmacological effects. Clinical trials of the therapeutic activity of *Punica granatum* are proven in fighting inflammation, however, the molecular mechanisms underlying the reaction of pomegranate extract still need to be further analyzed in depth.[17] This study, therefore, aims to investigate the effect of topical administration of 10% and 20% pomegranate extract cream on IL-

1 and PDGF levels in Wistar rats with an excision wound model, to determine its potential as a natural, effective wound-healing agent.

MATERIAL AND METHOD

The materials used in the study were pomegranate ethanol extract, ketamine, aquadate, alcohol 70%, 80%, paraffin, Fine test ELISA kit Rat PDGF, and Fine test ELISA kit Rat IL-1.

This study is experimental using a posttest only control group design conducted during April-May 2024 at the SCCR Laboratory, Faculty of Medicine, Unissula Semarang, the study approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang (No. 138/IV/2024/Komisi Bioetik). The research sample consisted of 48 male wistar rats aged 2-3 months randomly distributed among group treatment with a body weight of 180-220 grams divided into 12 groups, consisting of 4 rats in each group. Subjects were divided into examination after treatment day 3 and after treatment day 7 treatment.

Normal group (K1) healthy rats without treatment for 3 days, sham group (K2) excision wound rats without smearing cream for 3 days, negative control group (K3) excision wound rats and given base cream for 3 days, positive control group (K4) excision wound rats and given bioplacenton for 3 days, treatment 1 (K5) excision wound rats and given 10% pomegranate extract cream for 3 days, and treatment 2 (K6) rats with excision wounds and given 20% pomegranate extract cream for 3 days. Skin tissue samples were taken on day 4 to check for PDGF and IL-1 levels.

The examination after the 7th day of treatment consisted of normal group (K7) healthy rats without treatment for 7 days, sham group (K8) excision wound rats without smearing cream for 7 days, negative control group (K9) excision wound rats and given base cream for 7 days, positive control group (K10) excision wound rats and given bioplacenton for 7 days, Treatment 1 (K11) of excision wound rats and given 10% pomegranate extract cream for 7 days, Treatment 2 (K12) of excision wound rats and given 20% pomegranate extract cream for 7 days. Skin tissue samples were taken on day 8 to check for PDGF and IL-1 levels.

The pomegranate sample was 2 kg, the part used was the pulp. The sample was dried in an oven at a temperature of 50^{o}C and mashed, the result was a moisture content check with moisture balance, 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. 500 grams of pomegranates were extracted using the maceration method with a 70% ethanol solvent of 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 then filtered and the pulp is re-aacerated 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^{o}C until a thick extract is obtained.

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 bioplacenton to avoid infection during wound making. The wound was made using circular punch biopsy excision with a full thickness of 6 mm. The next day, the rats were then given treatment according to their group. Topical treatment was given once a day for 7 days after UV B irradiation. Skin samples in the validation group were.

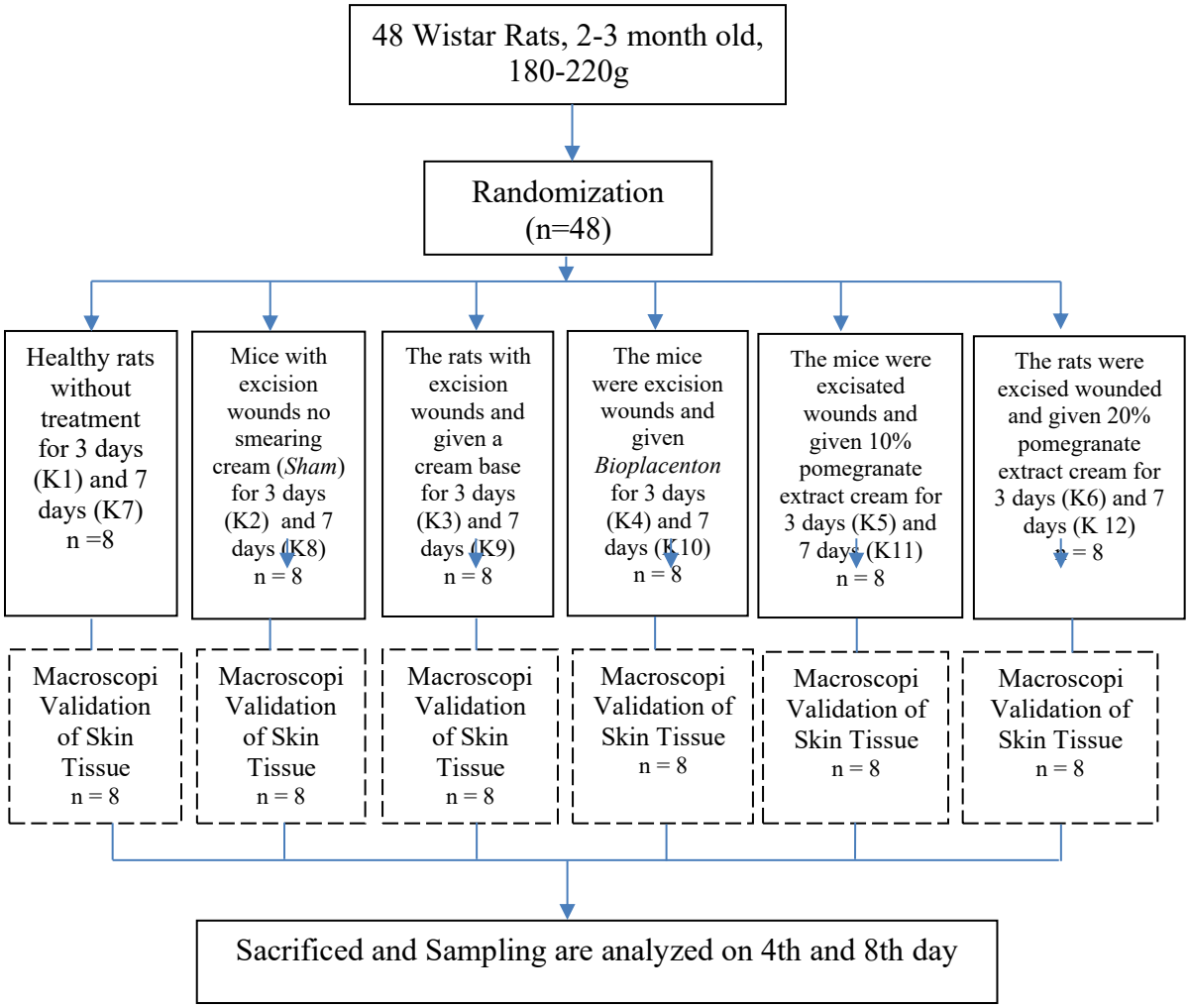


Figure 1. Layout of animal group assignments and corresponding treatment timeline.

Prepare the vanishing cream in 50 grams with the composition (Stearate acid, Triethanolamine, Glycerine, Borax, and aquadest). Heat water in a bekerglas, then put 14.5 grams of stearate acid in a porcelain cup and place it on boiling water, stirring until it melts. Add Borax 125 mg sequentially then homogenize, add Triethanolamine 1.5 ml, Glycerine 10 ml, and aquadest 25 ml until well mixed. The cream in 20 grams is done by weighing 0.6 grams of sweet peel extract then put in a mortar, adding enough Tween to be homogenized. Add 20 grams of vanishing cream, mix well until homogeneous, Pomegranate extract cream put in a pot.[18]

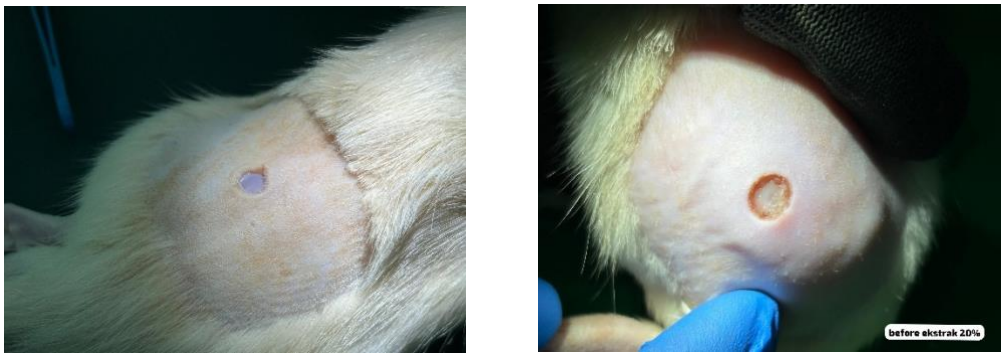
After the treatment, on the 4th and 8th days, tissue collection was carried out. Previously, all Wistar rats were terminated first by anesthesia on the rats. Make a tissue incision in the injured part of the skin, using scissors and tweezers. The tissue sample was cut and weighed, then the tissue was added with PBS (pH 7.4). Then the tissue samples are homogenized (destroyed) in cold conditions, 4°C. Next, setrifuss at a speed of 2000-3000 rpm, for 20 minutes. Then supernatants, namely centrifugation substances that have a lower specific weight, are taken and used as test samples. If the sample is to be stored first, then the sample can be stored at -20°C.

The skin tissue samples that have been obtained are then analyzed for PDGF and IL-1 levels using the ELISA method. ELISA analysis of PDGF and IL-1 were perform using commercial ELISA kits and Rat IL-1 ELISA Kit withThe intra-

assay coefficient of variation (CV) was <8% and the inter-assay CV was <10%. Each well contained 100 µL of sample or standard solution, and all measurements were performed in quadruplicate (4×) to ensure accuracy and reproducibility. Positive and negative controls supplied by the manufacturer were included in each assay. Optical density was measured at 450 nm using a microplate reader and concentrations were calculated from a standard curve.

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 figure 2.



(A) (B)

Figure 2. Excision wounds without treatment (A) and Excision wounds with treatment (B)

The macroscopic picture of excision wound treatment after the 7th day of treatment showed differences in wound area diameter, the group of untreated excision wounds with a wound diameter of 6.06 mm, the group that was given base cream with a wound diameter of 7.00 mm, the group that was given bioplacenton 2.97 mm, the group that was given 10% pomegranate extract cream with a wound diameter of 4.21 mm, and the group that was given 20% pomegranate extract cream with a wound diameter of 3.08 mm. The administration of pomegranate extract accelerates the closure of the wound area close to the diameter by the administration of bioplacenton, as shown in the following figure 3.

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

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

Group	K1 Healthy Rats	K2 *Sham*	K3 Cream Base	K4 Biopla centon	K5 Dosage 10%	K6 Dosage 20%	*P value*
IL-1 Level pg/mL							
Mean	14.08	171.30	147.22	171.68	120.87	118.26	
SD	±0.72	±5.48	±4.41	±1.23	±1.09	±6.86	
Shapiro-Wilk	*0.513	*0.789	*0.10	*0.233	*0.406	*0.161	
Levene Test							0.000
One way anova							0.000

Desciption :**Saphiro Wilk* = Normal (p>0.05)
**Levene Test* = Homogen (p>0.05)
**One way anova* = Significance (p<0.05)

The average IL-1 level was carried out by the Shapiro-Wilk test, the average result of IL-1 levels was normally distributed (p>0.05) and the data homogeneity test with the Leuvene Test test had non-homogeneous data variations with a result of 0.000 (p>0.05). The results of the data that were normally distributed and not homogeneous, conducted by the One-way anova test with a result of 0.000 (<0.05) showed that there was a significant difference in IL-1 levels between the treatment groups. Based on table 1, it was shown that the average results of IL-1 levels after day 3 treatment in the healthy group (K1) were 14.08 pg/mL, the sham group (K2) was 171.30 pg/mL, the base cream (K3) group was 147.22 pg/mL, the bioplacenton group (K4) was 171.68 pg/mL, the 10% dose group (K5) was 120.87 pg/mL and the 20% dose group (K6) was 118.26 pg/mL. IL-1 levels were lowest in the healthy rat group (K1) and IL-1 levels were highest in the bioplacenton group (K4).

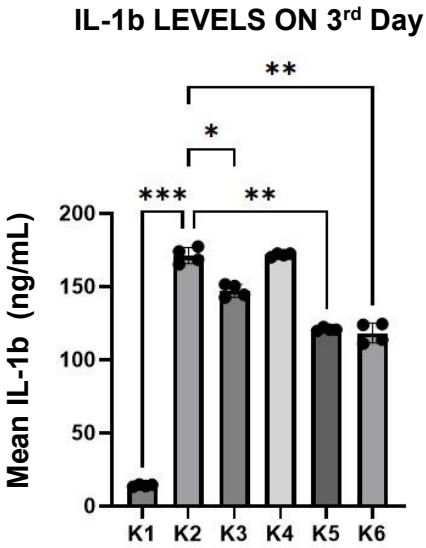


Figure 4. Mean ± standard deviation of interleukin-1 (IL-1) levels (pg/mL) in rat skin tissue after 3 days of treatment (n = 4 per group). Treatment groups: (a) K1 = Healthy rats, (b) K2 = Sham (wound without treatment), (c) K3 = Cream base, (d) K4 = Bioplacenton®, (e) K5 = Pomegranate extract cream 10%, and (f) K6 = Pomegranate extract cream 20%. Statistical analysis using one-way ANOVA ($p < 0.001$), followed by post hoc Tamhane test.

Comparison between treatment groups showed significant differences between the K1 group compared to all treatment groups that experienced increased IL-1 levels, The K2 group without intervention showed a significant difference in IL-1 levels reduction with the 10% extract cream treatment group and the 20% group (K5 and K6).

Significant differences between treatment groups were then evidenced by the Post hoc tamhane test to determine the most influential dose comparison. The results of the Post hoc tamhane test are shown in the following table 2:

Table 2. The Post hoc Tamhane test of IL-1 levels of rat skin tissue after excision wound after the 3rd day of treatment.

Group	K2	K3	K4	K5	K6
K1	*0.000	*0.000	*0.000	*0.000	*0.001
K2	-	*0.009	1.000	*0.003	*0.000
K3		-	*0.013	*0.012	*0.012
K4			-	*0.000	*0.006
K5				-	1.000

The average comparison of IL-6 levels in the healthy rat group was significantly different when compared to all excision wound treatment groups. The treatment group that was given a 20% dose of pomegranate extract cream showed

the most significant difference with the lowest average IL-6 levels compared to other treatment groups. It can be concluded that the administration of pomegranate extract cream has the effect of reducing IL-6 levels on the 3rd day after treatment.

Table 3. The average descriptive test of IL-1 levels and the one-way anova test after the 7th day of treatment

Group	K1 Healthy Rats	K2 *Sham*	K3 Base Cream	K4 Biopla centon	K5 Dosage 10%	K6 Dosage 20%	*P value*
IL-1 Level pg/mL							
Mean	53.68	159.59	202.17	88.17	187.36	68.67	
SD	±11.17	±5.20	±4.81	±7.64	±10.03	±14.80	
Shapiro-Wilk	*0.275	*0.731	*0.343	*0.087	*0.473	*0.270	
Leuvene Test							*0.300
One way anova							0.000

Description :**Saphiro Wilk* = Normal (p>0.05)
**Leuvene Test* = Homogen (p>0.05)
**One way anova* = Significance (p<0.05)

The average IL-1 level was carried out by the Shapiro-Wilk test, the average result of IL-1 levels was normally distributed (p>0.05) and the data homogeneity test with the Leuvene Test test had a homogeneous data variation with a result of 0.300 (p>0.05). The results of the data that were distributed normally and homogeneously, conducted by the One-way anova test with a result of 0.000 (<0.05) showed that there was a significant difference in IL-1 levels between the treatment groups.

Based on table 3, it was shown that the average result of IL-1 levels after day 7 treatment in the healthy group (K1) was 53.68 pg/mL, the sham group (K2) was 159.59 pg/mL, the base cream (K3) group was 202.17 pg/mL, the bioplacenton group (K4) was 88.17 pg/mL, the 10% dose group (K5) was 187.36 pg/mL and the 20% dose group (K6) was 68.67 pg/mL. IL-1 levels were lowest in the healthy rat group (K1) and IL-1 levels were highest in the base cream group (K3).

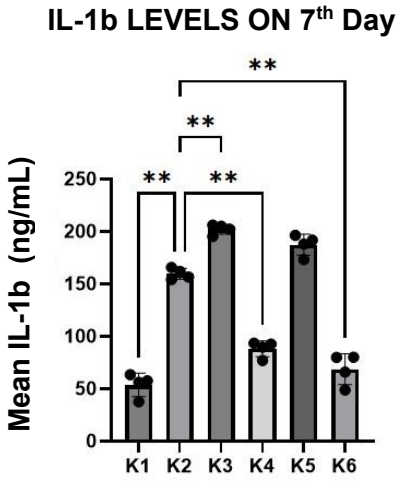


Figure 5. Mean ± standard deviation of interleukin-1 (IL-1) levels (pg/mL) in rat skin tissue after 7 days of treatment (n = 4 per group). Treatment groups: (a) K7 = Healthy rats, (b) K8 = Sham (wound without treatment), (c) K9 = Cream base, (d) K10 = Bioplacenton®, (e) K11 = Pomegranate extract cream 10%, and (f) K12 = Pomegranate extract cream 20%. Statistical analysis using one-way ANOVA ($p < 0.001$), followed by post hoc LSD test.

The comparison between the treatment groups showed a significant difference in the healthy rat group compared to all treatment groups that experienced increased IL-1 levels, The 20% extract cream treatment group showed the lowest average IL-1 level compared to the group with bioplacentone administration or other treatment groups.

Significant differences between treatment groups were then evidenced by the Post hoc LSD test to determine the most influential dose comparison. The results of the Post hoc LSD test are shown in the following table 4:

Table 4. Post hoc LSD test results of IL-1 levels of rat skin tissue after excision wound after treatment on the 7th day

Group	K2	K3	K4	K5	K6
K1	*0.000	*0.000	*0.000	*0.000	*0.001
K2	-	*0.009	1.000	*0.003	*0.000
K3		-	*0.013	*0.012	*0.012
K4			-	*0.000	*0.006
K5				-	1.000

Description * Means p<0,05

The average comparison of IL-1 levels in the healthy rat group was significantly different when compared to all excision wound treatment groups. The treatment group that was given a 20% dose of pomegranate extract cream showed the most significant difference with the lowest average IL-6 levels compared to other treatment groups. It can be concluded that the administration of pomegranate extract cream has the effect of reducing IL-6 levels on the 7th day after treatment.

Table 5. The average descriptive test of PDGF levels and the Kruskal Wallis test after the 3rd day of treatment

Group	K1 Healthy Rats	K2 *Sham*	K3 Cream Base	K4 Biopla centon	K5 Dosage 10%	K6 Dosage 20%	*P value*
PDGF Level pg/mL							
Mean	5.40	4.63	5.85	6.49	5.81	6.06	
SD	±0.48	±2.01	±1.29	±1.49	±1.29	±0.23	
Shapiro-Wilk	*248	*0.792	*0.212	0.044	*0.062	*0.994	
Leuvene Test							*0.300
Kruskal Wallis							0.397

Description: * *Shapiro-Wilk* = Normal (p>0.05)
* *Kruskal Wallis* = Significance (p<0.05)

Based on table 5, it was shown that the average results of PDGF levels after the 3rd day of treatment in the healthy group (K1) were 5.40 pg/mL, the sham group (K2) was 4.63 pg/mL, the base cream group (K3) was 5.85 pg/mL, the bioplacenton group (K4) was 6.49 pg/mL, the 10% dose group (K5) was 5.81 pg/mL and the 20% dose group (K6) was 6.06 pg/mL. The PDGF level was lowest in the sham group (K2) and the highest PDGF level was in the bioplacenton group (K4).

The average results of PDGF levels were carried out by the Shapiro-Wilk test, and the results of normal distributed data (p>0.05) were obtained in the K1, K2, K3, K5, and K6 groups but not normally distributed in the K4 group p=0.044 (p>0.05) The results of the data that were not normally distributed were then carried out by the Kruskal Wallis non-parametric test p=0.397 (<0.05) showed that there was no significant difference in PDGF levels between the treatment groups.

The comparison between the treatment groups showed insignificant differences as shown in figure 5 below:

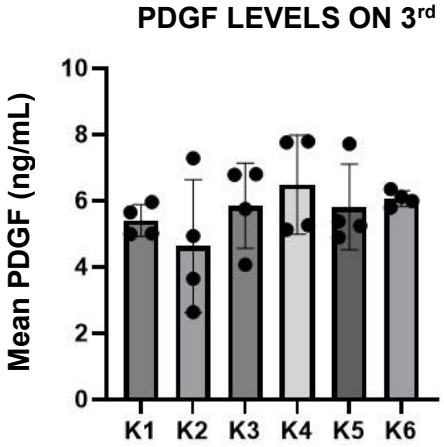


Figure 6. Mean ± standard deviation of platelet-derived growth factor (PDGF) levels (pg/mL) in rat skin tissue after 3 days of treatment (n = 4 per group). Treatment groups: (a) K1 = Healthy rats, (b) K2 = Sham (wound without treatment), (c) K3 = Cream base, (d) K4 = Bioplacenton®, (e) K5 = Pomegranate extract cream 10%, and (f) K6 = Pomegranate extract cream 20%. Statistical analysis using the Kruskal–Wallis test (H = 5.16, df = 5, p = 0.397).

Even though the Kruskal–Wallis test indicated no statistically significant difference in PDGF levels between groups (H = 5.16, df = 5, p = 0.397, ε^2 = 0.009, very small effect), a follow-up exploratory, non-confirmatory inspection of post-hoc Dunn–Bonferroni pairwise comparisons was performed to better understand potential trends in the data. This inspection suggested that the 10% dose group (K5) and the 20% dose group (K6) tended to have higher PDGF levels than the sham group (K2). The estimated rank–biserial correlations were r_rb = −0.37 for K2 vs K5 and r_rb = −0.78 for K2 vs K6, indicating moderate and large effect magnitudes, respectively, in the direction of higher PDGF for the treatment groups. For descriptive context, the 95% confidence interval for mean PDGF in K5 was 3.76 to 7.86 pg/mL and for K6 was 5.68 to 6.45 pg/mL, compared to 1.43 to 7.83 pg/mL for K2. These trends should be interpreted with caution as they are not statistically significant and are intended only to guide future hypothesis driven research.

Table 6. The average of test of PDGF levels and the Kruskal Wallis test after the 7th day of treatment

Group	K7 Healthy Rats	K8 No Inter-vension	K9 Cream Base	K10 *Biopla centon*	K11 Dosage 10%	K12 Dosage 20%	*P value*
PDGF Level pg/mL							
Mean	7.47	7.32	7.89	6.89	8.17	9.15	
SD	±1.26	±0.19	±0.16	±0.59	±0.09	±0.91	
Shapiro-Wilk	0.016	*0.168	*0.644	*0.276	*0.708	*0.379	
Kruskal Wallis							*0.010

Description: * *Shapiro-Wilk* = Normal (p>0.05)
* *Kruskal Wallis* = Significance (p<0.05)

The average results of PDGF levels were carried out by the Shapiro-Wilk test, and the results of normal distributed data (p>0.05) were obtained in the K2, K3, K4, K5 and

K6 groups but not normally distributed in the K1 group p=0.016 (p>0.05) The results of the data that were not normally distributed were then carried out by the Kruskal Wallis non-parametric test p=0.010 (<0.05) showed that there was a significant difference in PDGF levels between the treatment groups.

Based on table 6, it was shown that the average results of PDGF levels after day 7 treatment in the healthy group (K7) were 7.47 pg/mL, the sham group (K8) was 7.32 pg/mL, the base cream group (K9) was 7.89 pg/mL, the bioplacenton group (K10) was 6.89 pg/mL, the 10% dose group (K11) was 8.17 pg/mL and the 20% dose group (K12) was 9.14 pg/mL. The lowest PDGF levels were in the bioplacentone group (K10) and the highest PDGF levels were in the 20% dose group (K12). Comparison between treatment groups as shown in the following figure of graph 6:

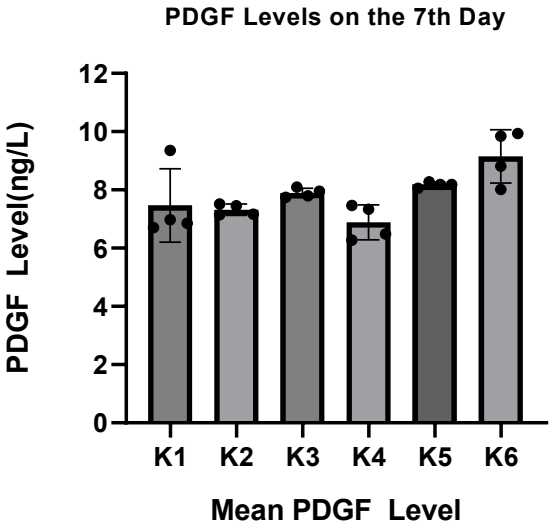


Figure 7. Mean ± standard deviation of platelet-derived growth factor (PDGF) levels (pg/mL) in rat skin tissue after 7 days of treatment (n = 4 per group). Treatment groups: (a) K7 = Healthy rats, (b) K8 = Sham (wound without treatment), (c) K9 = Cream base, (d) K10 = Bioplacenton®, (e) K11 = Pomegranate extract cream 10%, and (f) K12 = Pomegranate extract cream 20%. Statistical analysis using the Kruskal–Wallis test (p = 0.010), followed by Mann–Whitney test.

Table 7. The Mann Whitney test of PDGF levels of rat skin tissue after excision wound after day 7 treatment

Group	K2	K3	K4	K5	K6
K1	1.000	1.000	1.000	0.768	0.214
K2	-	1.000	1.000	0.683	0.186
K3		-	1.000	1.000	1.000
K4			-	0.140	*0.029
K5				-	1.000

Description * Means p<0.05

The comparison of the average PDGF levels of the group with the bioplacentone treatment was significantly different from the 20% dose group, different from the other treatment groups had no difference. The average PDGF level at the 20% dose was significantly increased when compared to the bioplacenton treatment group.

The comparison of the average PDGF levels of the group with bioplacentone treatment was significantly different from the 20% dose group, while no significant difference was observed between the other treatment groups. The average PDGF level at the 20% dose was significantly increased when compared to the bioplacenton treatment group.[19] This finding indicates that high-dose

pomegranate extract cream may provide a stronger stimulatory effect on growth factor production during the proliferative phase of wound healing, which is essential for optimal tissue repair the transition from the inflammatory phase to the proliferation phase is an important phase in wound healing.[20] The reparative phase is affected by the migration of macrophages M1 to M2. Regulated keratinocyte proliferation, migration, and differentiation, at least partially, controlled by growth factor production for wound reepithelial.[21] PDGF, in particular, acts as a chemoattractant for fibroblasts and smooth muscle cells, accelerating granulation tissue formation and neovascularization.[22]

The results showed a progressive decrease in IL-1 levels on the 3rd day after treatment with pomegranate extract cream with a further decline observed by the 7th day. The analysis carried out after day 3 describes the inflammatory condition of the excision wound where the inflammatory phase lasts for 1-3 days, Therefore, assessing IL-1 a key pro-inflammatory cytokine during this window is highly relevant to evaluate the anti-inflammatory effects of the treatment by using a pomegranate extract cream at a dose of 20% is effective in reducing IL-1 levels until day 7 which remained significantly lower than IL-1 levels in the bioplacenton group. This aligns with previous studies demonstrating that polyphenols in pomegranate, such as punicalagin and ellagic acid, can suppress IL-1 expression by downregulating NF-κB signaling.[23,24] as rapid change in the inflammatory mediators produced in the wound, shown IL-1 quickly takes on an important role during the early phases of wound healing. Our findings are consistent with Kumar et al. (2022), who observed a ~40% reduction in IL-1 after topical application of punicalagin in a rat model of excisional wounds[25]. The effect of IL-1 on the onset of excess scarring and keloid formation should also be monitored.[26]

The increase in IL-1 levels in the treatment group of pomegranate extract cream at a dose of 10% on the 7th day may be explained by inter-individual variability, environmental stressors, or genetic predisposition in the rats, which could trigger secondary inflammatory responses. IL-1 plays an important role in the acute inflammatory process by inducing the production of prostaglandins, TNF-α, IL-6, and IL-8, as well as other inflammatory mediators.[3] IL-1 also a potent regulator of adaptive immune responses, including increased regulation of MHC (major histocompatibility complex) molecules and stimulation of T cells. The IL-1 cytokine, one of the main mediators in the inflammatory response, has a variety of biological effects on other cells, including T and B cell activation, immune cell proliferation, and the production of cytokines and other mediators. A variety of pathological disorders, including some autoimmune diseases, cancer, rheumatoid arthritis, atherosclerosis, chronic inflammation, and autoimmune diseases, can lead to increased IL-1 expression.[27,28] Targeting IL-1 in wound management has therefore been proposed as a strategy to minimize chronic inflammation and improve healing outcomes.[29]

The analysis of PDGF levels after the 3rd day of treatment did not show a significant difference between the treatment groups because PDGF activity remains relatively low during the inflammatory phase. However, on the 7th day coinciding with the mid-proliferative phase PDGF levels were significantly higher in the 20% pomegranate extract cream group compared to the bioplacenton group. Examination of PDGF levels in the proliferation phase lasting from 4-14 is considered appropriate to be analyzed on the 7th day after treatment, gradual observation needs to be carried out to determine the peak of PDGF levels until the 14th day. The results of this study are in line with research that observes the activity of pomegranate extract with immunohistochemical methods in wounds with tooth extraction marks experiencing an increase in PDGF expression which is an indicator of the wound healing process.[30] Furthermore,some research reported that pomegranate extract not only upregulates PDGF but also modulates angiogenesis-related factors, potentially improving wound vascularization.[31]

The proliferation (division and growth) and migration (movement) of important cells, including fibroblasts, keratinocytes, and endothelial cells which aid in the formation of connective tissue and accelerate the healing process, are often stimulated by PDGF. In addition, PDGF stimulates the production of collagen, an important element in the development of wound structures. To guarantee effective healing of wounds or damages, PDGF helps coordinate the various stages of wound healing.[4] These coordinated effects may explain the superior PDGF response observed in the high-dose pomegranate group, suggesting a dose-dependent mechanism that warrants further exploration in chronic wound models, including diabetic ulcers. [32]

This study did not observe and monitor the onset of scars and keloid formation against the parameters analyzed, Future studies should include long-term follow-up, scar quality assessment using validated scales, and testing alternative delivery systems such as gels or nanoparticle based formulations[33] could further optimize the bioavailability and stability of pomegranate bioactives in topical wound care.

CONCLUSION

The administration of pomegranate extract cream (*Punica granatum*) demonstrated a significant positive effect on wound healing in rats with excision wounds, primarily through modulation of inflammatory and proliferative biomarkers. At a dose of 20%, the cream effectively reduced interleukin-1 (IL-1) levels by the 3rd day of treatment and maintained this reduction through the 7th day, indicating a sustained anti-inflammatory effect during the critical transition from the inflammatory to the proliferative phase of healing. Concurrently, the 20% dose significantly increased platelet derived growth factor (PDGF) levels by the 7th day, surpassing even the standard bioplacenton treatment, suggesting enhanced fibroblast recruitment, collagen synthesis, and angiogenesis. These findings support the dose dependent efficacy of pomegranate extract cream, with the 20% formulation showing optimal outcomes. Overall, *Punica granatum* extract cream holds promise as a natural, effective topical agent for accelerating wound repair by simultaneously suppressing pro-inflammatory mediators and stimulating growth factor production. However, the study is limited by it relatively short observation period, which did not extend beyond the 7th day to capture the complete wound healing cycle, the absence of histopathological or immunohistochemical confirmation of molecular findings; the lack of long-term scar quality assessment; and the use of only a single animal model without testing in chronic or infected wound conditions. Future research should address these limitations, explore alternative delivery systems for enhanced bioavailability, and assess the clinical applicability of this formulation in human wound care.

AUTHORS' CONTRIBUTIONS

Anggun Permata Sari: Visualization, Data curation , Investigation, Conceptualization, Writing-Original draf; *Agung Putra*: Supervision, Reviewing; *Titiek Sumarawati*: Reviewing, Validation, 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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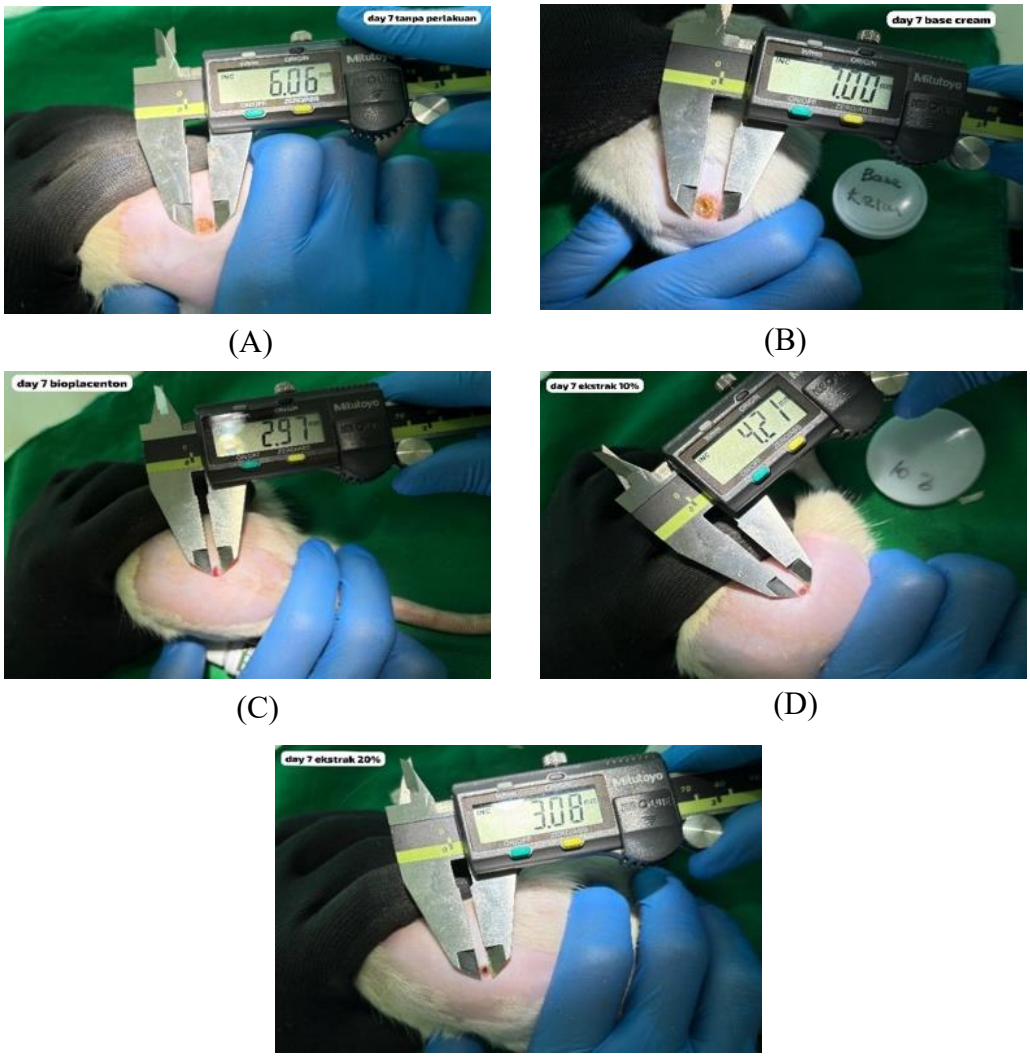

Figure 3. Macroscopic appearance of excision wounds on day 7 in different treatment groups (Wound healing diameter on day 7 of the untreated group (A), base cream group (B), bioplacenton group (C), 10% extract cream (D), and 20% extract cream (E))