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Pomegranate extract cream modulates TGF-β and IL-6 in wistar rats with excision wounds

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Abstract: Wound healing is a complex, multifactorial process, and disruptions at any stage can delay recovery. Pomegranate peel extract has been traditionally used to accelerate wound healing, with topical applications of natural products preferred due to their safety and availability compared to synthetic agents. This study evaluated the effects of pomegranate extract cream (*Punica granatum*) on TGF-β and IL-6 levels in Wistar rats with excision wounds. An in vivo experimental design was applied to 48 rats divided into 12 groups, with analyses performed on day 3 and day 7. TGF-β and IL-6 levels were measured using the enzyme-linked immunosorbent assay (ELISA). The results showed a significant reduction in mean IL-6 levels and a significant increase in mean TGF-β levels on both day 3 and day 7 (One-Way ANOVA, $p<0.05$). The 20% pomegranate extract cream demonstrated the greatest effect, reducing IL-6 and enhancing TGF-β expression, with outcomes comparable to bioplacenton treatment on day 7. These findings suggest that pomegranate extract cream may promote wound healing by modulating inflammatory and reparative molecular mediators.

Keywords: Pomegranate cream, Excision wounds, IL-6 levels, TGF-β levels.

INTRODUCTION

Wounds are a multiphase process that involves a complex response to injury to the skin. Changes in any of these phases can hinder wound healing. Identification and optimization of risk factors can be modified in wound management [1]. Wound healing includes a variety of cellular and biochemical reactions including inflammation, neovascularization and collagen deposition. Transforming Growth Factor Beta (TGF-β) signaling has a major role in keratinocyte function, wound re-epithelial regulation, and TGF-β pathway in acute wound healing and epithelialization. It has been proven that TGF-β is essential for epidermal homeostasis as well as for every stage of wound healing [2]. The nuclear-mediated inflammatory response of nuclear factor-kappaB (NF-κB) causes skin inflammation, releasing pro-inflammatory cytokines such as Interleukin-6 (IL-6) [3,4]. In the wound healing process, the IL-6 cytokine is very important [5]. Reduced inflammation is associated with faster wound healing rates [6]. Pomegranate peel extract is used to accelerate wound healing [7]. By utilizing topical applications using natural ingredients is an option because there are very few side effects and the availability is easy, compared to synthetic ingredients [8]. However, despite extensive research on wound healing, there remains a lack of studies that specifically evaluate molecular parameters such as IL-6 and TGF-β—following topical application of pomegranate extract in excision wound models.

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More than 90 percent of people in developed countries believe that medicinal plants are reliable [9,10]. As many as 32% of fruit peels are used in the production of medicines, followed by seeds (22%) and flowers (20%), whole plants (9%), shoots (7%), root bark (6%), seed and root peels (6%), and whole plants (9%) [7]. Knowledge of pharmaceutical herbs adds new horizons in the world of medicine. Herbal medicines are more cost-effective and safer compared to synthetic medicines [7]. Pomegranate has been used in traditional medicine to cure many diseases [7,11]. Pomegranate is used to treat parasitic diseases, seeds and peels of the fruit to treat diarrhea, pomegranate flowers to treat diabetes, bark, and roots to stop bleeding and heal wounds. Similarly, its leaves are used to control inflammation and treat indigestion [12].

Several studies have proven that *P granatum* improves the wound repair process. Nayak et al. assessed that the process of repairing pomegranate skin wounds showed a 95% reduction in wound area reported in animals treated with the extract compared to 84% in the control group [10]. In a study, methanol extract P granatum ointment was used at 10% and 15% b/b to determine the wound healing effect on a rat incision wound model. The result is the same as nitrofurazone ointment. The wound contraction activity of 10% and 15% P. granatum ointment is 97.8% and 98.4%, respectively, methanol extract of pomegranate is proven to have the potential to be a wound healing agent [7]. Identifying the optimal concentration of pomegranate extract for topical wound treatment is clinically relevant, as varying doses may differentially influence the balance between inflammation resolution and tissue regeneration. An inappropriate dose may result in prolonged inflammation, delayed epithelialization, or excessive fibroplasia, ultimately affecting the quality and speed of healing. Despite growing evidence that *Punica granatum* possesses wound-healing potential, existing studies have primarily examined single concentrations or have focused on macroscopic outcomes without assessing key molecular mediators. No published research has directly compared the effects of two concentrations of pomegranate extract cream (10% and 20%) on both a pro-inflammatory cytokine (IL-6) and a pro-reparative growth factor (TGF-β) in an excision wound model. This study addresses that gap by providing, for the first time, a comparative analysis of these molecular markers, aiming to clarify dose-dependent effects and inform the formulation of evidence-based herbal wound therapies.

IL-6 plays a central role in acute inflammation and is necessary for rapid wound healing. Released early in response to injury, IL-6 induces the release of pro-inflammatory cytokines from macrophages, keratinocytes, endothelial cells, and stromal cells that live in tissues. IL-6 has also been found to induce leukocyte chemotaxis in wounds. As inflammation develops, IL-6 signals are responsible for switching to the reparative environment. Regulation of wound healing is very important, improper pro-inflammatory signals can cause wounds to take longer to heal and risk infection [13]. Several growth factors are involved in wound healing, one of which is TGF-β, exerting pleiotropic effects on wound healing by regulating cell proliferation, differentiation, extracellular matrix and modulating immune responses [14].

Pomegranate rind extract (PRE) has a significant anti-inflammatory effect on ex vivo skin, ensuring that PRE regulates COX-2 in the active epidermis [11]. The study used pomegranate standardized extract (PSE) with 40% ellagic acid processed into ointment. SPE 10% accelerates the healing of second-degree burns in wounds. Pomegranate standardized with 40% ellagic acid is a promising herb for healing skin burns [9]. Pomegranate has the potential as a new approach to improving inflammatory diseases and pain related to various skin conditions (Houston et al., 2017). Therefore, this study aims to fill the identified gap by evaluating, for the first time, the comparative effects of 10% and 20% pomegranate extract cream on IL-6 and TGF-β expression in excision wound models, to better understand its dual role in modulating inflammation and promoting tissue repair.

MATERIAL AND METHOD

Research materials used pomegranate ethanol extract, 70%, 80% alcohol, paraffin, ketamine, Aquaades, Fine test ELISA kit Rat TGF-β and using fine test ELISA kit Rat IL-6.

This study is experimental research with a posttest only control group design. The subject of the study was a 2–3-month-old Wistar rat with a body weight of 190-210 grams that was adapted for 7 days. The total number of subjects was 48 male wistar rats divided into 12 groups, consisting of 4 rats in each group. The subjects were divided into 6 groups of examination after the 3rd day of treatment and 6 groups after the 7th day of treatment.

The examination after the 3rd day of treatment consisted of a normal group (K1) of healthy rats without treatment for 3 days, a *sham* group (K2) of excision wound rats without smearing cream for 3 days, a negative control group (K3) of excision wound rats and given a base cream for 3 days, a positive control group (K4) of excision wound rats and given bioplacenton for 3 days, Treatment 1 (K5) of excision wound rats and given 10% pomegranate extract cream for 3 days, and treatment 2 (K6) of excision wound rats and given 20% pomegranate extract cream for 3 days. Skin tissue samples were taken on day 4 to check for TGF-β and IL-6 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 TGF-β and IL-6 levels.

All animal procedures were approved by the Komisi Bioetika Penelitian Kedokteran/Kesehatan, Fakultas Kedokteran, Universitas Islam Sultan Agung Semarang (No. 139/IV/2024). The study was conducted at the Laboratorium Stem Cell and Cancer Research (SCCR), Semarang, Jawa Tengah, between April–May 2024. All procedures complied with the principles outlined in the Declaration of Helsinki and the National Guidelines for Health Research Ethics.

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 balance check, if the moisture content was below 10%, the drying result was considered good. Crushed pomegranates. Then it is sifted with a sieve of 20 mesh size. 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, 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^{o}C until a viscous extract is obtained.

The research subjects were 48 male rats of the Wistar strain (*Rattus norvegicus*), 2-3 months old, with a body weight of 190-210 grams, which were “Animals were randomly assigned to groups using a random number generator into 12 groups, each amounting to 4 rats. 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 in diameter. The next day, the rats were then given treatment according to their group. Topical treatment is given once a day for 7 days after the excision wound. The treatment of excision wounds was

validated macroscopic for observation and measurement of the diameter of the acceleration area at wound closure.

After the treatment, on the 8th day, a tissue was taken . Previously, all Wistar rats were terminated first by anesthesia on the rats while Blinding was applied during data collection and analysis to minimize bias 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 in cold conditions at a temperature of 4°C. Next, setrifugation is carried out at a speed of 2000-3000 rpm, with a time of 20 minutes. Centrifugation substrates that have lower specific weights are taken and used as test samples.

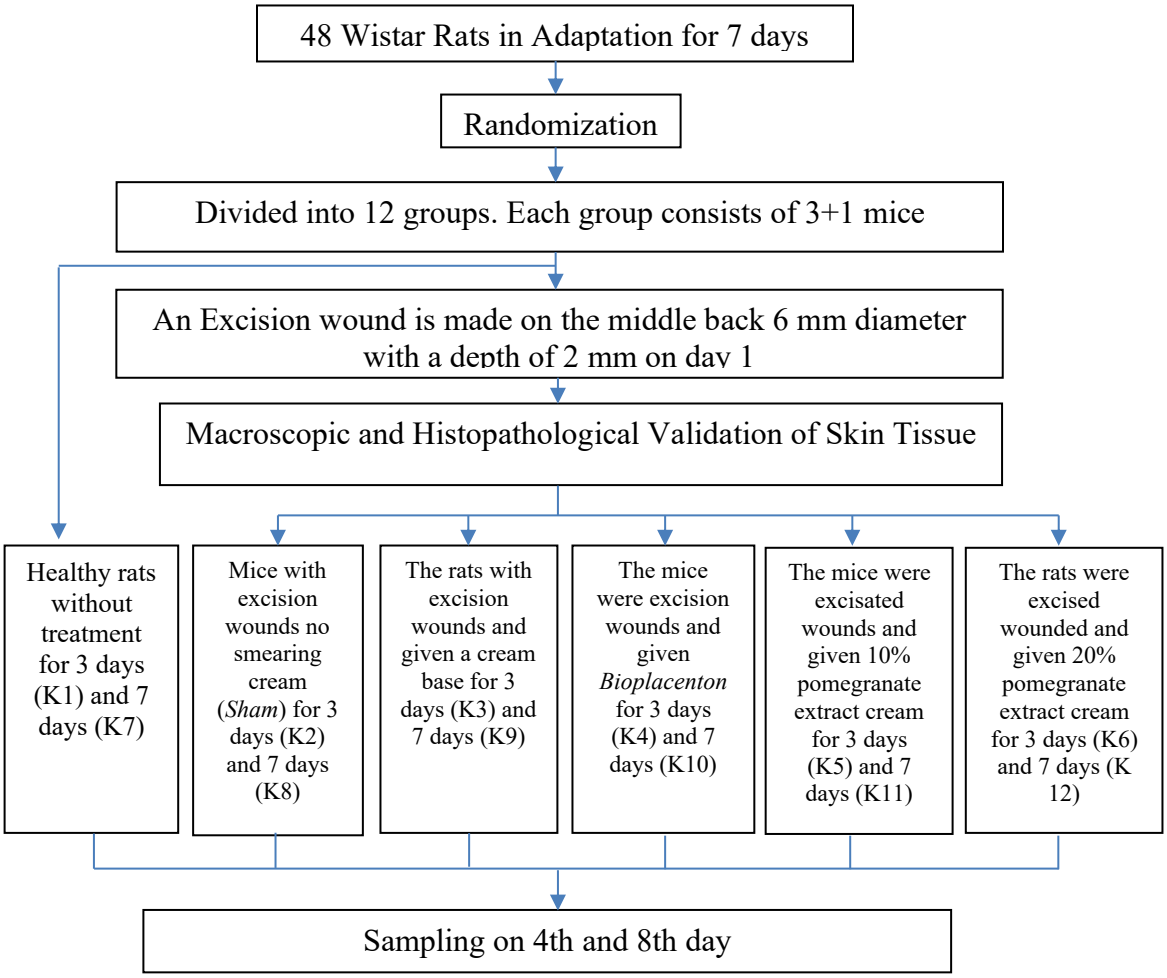
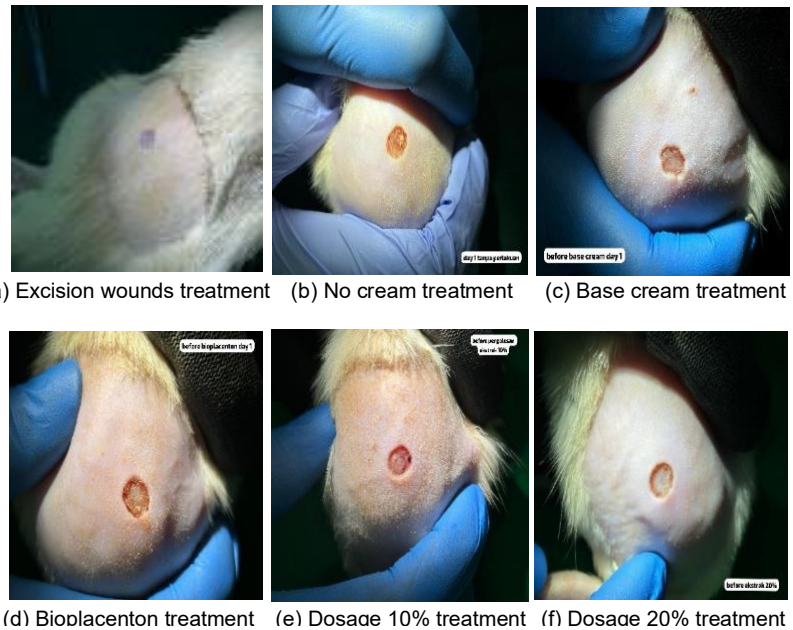


Figure 1. Animal grouping and treatment timeline

The skin tissue samples that had been obtained were then analyzed for TGF-β and IL-6 levels using the ELISA method. The analysis of the TGF-β and IL-6 ELISA was performed according to the procedure attached to the product. Analysis of TGF-β and IL-6 levels using *a microplater reader* with a wavelength of 450nm. Data from the sample were analys for normality using the Shapiro-Wilk test, followed by one-way ANOVA. Post-hoc comparisons were performed using LSD or Tamhane's T2 depending on the Levene's test results.

RESULTS AND DISCUSSION

A general macroscopic picture of the excision wound treatment conditions in each group on the first day as shown in figure.

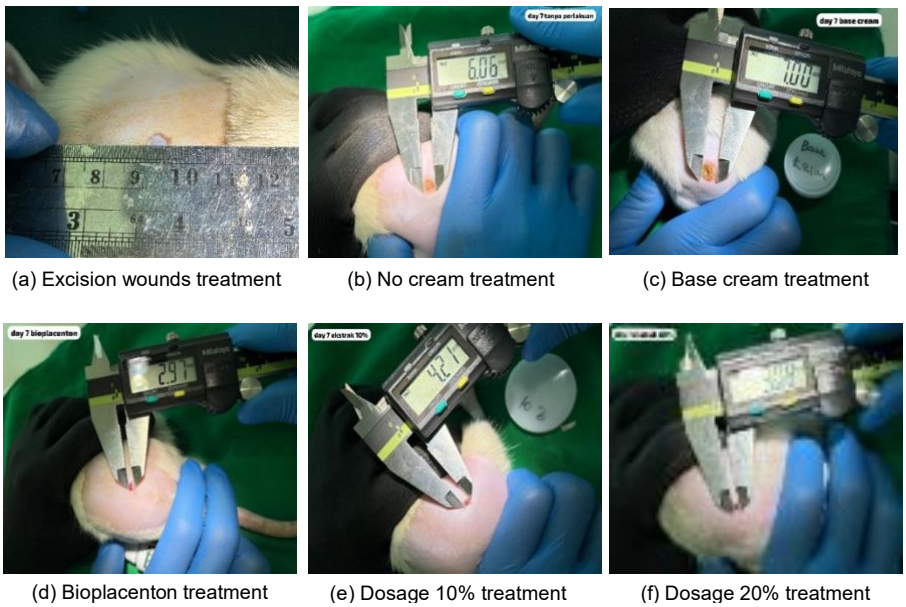


(a) Excision wounds treatment (b) No cream treatment (c) Base cream treatment

(d) Bioplacenton treatment (e) Dosage 10% treatment (f) Dosage 20% treatment

Figure 1. Macroscopic condition of excision wound treatment in each group on 1st dayThe

Macroscopic picture of the measurement of excision wound area after the 7th day of treatment showed differences in the group of excision wounds without treatment picture (b) with a wound diameter of 6.06 mm, the group given base cream picture (c) with a wound area diameter of 7.00mm, the group given bioplacenton picture (d) with a wound area diameter of 2.97mm, the group given pomegranate extract cream 10% picture (e) with a wound diameter of 4.21mm, and the group that was given a 20% pomegranate extract cream (F) with a wound diameter of 3.08mm. Macroscopic administration of 20% pomegranate extract accelerates the closure of wound area close to diameter with the administration of bioplacenton on the 7th day after treatment, as shown in figure 2.



(a) Excision wounds treatment (b) No cream treatment (c) Base cream treatment

(d) Bioplacenton treatment (e) Dosage 10% treatment (f) Dosage 20% treatment

Figure 2. Macroscopic conditions of excision wound treatment in each group on 7th day

The results of the analysis of the average IL-6 levels after the 3rd day of treatment in each group are shown in table 1.

Table 1. The average descriptive test of IL-6 levels and the one-way anova test after day 3 treatment

Group	Healthy Rats K1	Rats *Sham* K2	Cream Base K3	Biopla centon K4	Dosage 10% K5	Dosage 20% K6	*P value*
IL-6 Level pg/mL							
Mean	87.73	372.59	295.29	196.15	141.89	136.20	
SD	±7.94	±48.93	±50.85	±18.23	±17.64	±14.75	
Shapiro-Wilk	*0.567	*0.894	*0.823	*0.671	*0.423	*0.883	
Levene Test							0.043
One way anova							*0.000

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

The average results of IL-6 levels were carried out by the Shapiro-Wilk test obtained results in all normally distributed groups (p>0.05) and the data homogeneity test with the Leuvene Test had non-homogeneous data variants with a result of 0.043 (p>0.05). The results of the data were distributed normally and non-homogeneously, a one-way anova test with a result of 0.000 (<0.05) showed that there was a significant difference in IL-6 levels between the treatment groups on the 3rd day after the treatment.

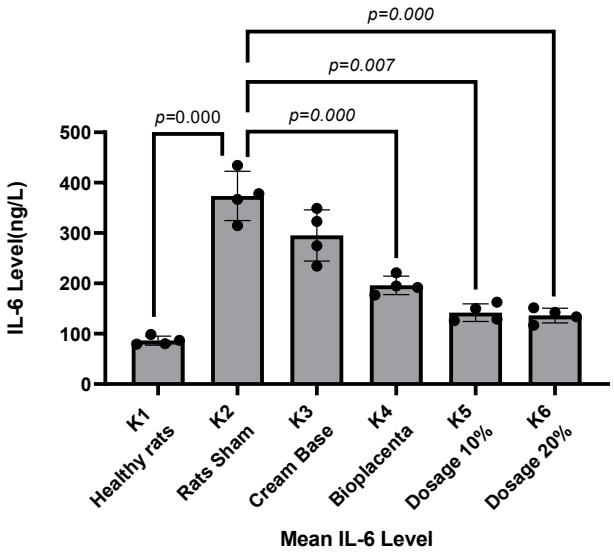


Figure 3. Mean IL-6 levels between groups after day 3 treatment

Comparison between treatment groups showed significant differences between K1 groups compared to all treatment groups with an average increase in IL-6 levels, The K2 *sham* group showed a significant difference in the average decrease in IL-6 levels with the 10% extract cream treatment group (K5) and the 20% (K6) group.

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

Table 2. Post hoc tamhane test of IL-6 levels of rat skin tissue after excision wound after day 3 treatment

Group	K2	K3	K4	K5	K6
K1	*0.000	*0.002	0.283	*0.005	0.957
K2	-	*0.023	*0.000	*0.007	*0.000
K3		-	*0.018	0.600	*0.001
K4			-	*0.052	0.261
K5				-	*0.005

Description: * Means p<0,05

The average comparison of IL-6 levels in the rat group without cream treatment (K2) was significantly different when compared to the 10% dose treatment group (K5) 0.051 (p<0.05) and the treatment group given a 20% (K6) pomegranate extract cream dose 0.051 (p<0.05). The group (K5) showed the most significant difference with the lowest average IL-6 levels compared to the other treatment groups. It can be concluded that the administration of pomegranate extract cream has the effect of lowering IL-6 levels on the 3rd day after treatment.

Table 3. The average descriptive test of IL-6 levels and the one-way anova test after day 7 treatment

Group	Healthy Rats K1	Rats *Sham* K2	Cream Base K3	Biopla centon K4	Dosage 10% K5	Dosage 20% K6	*P value*
IL-6 Level pg/mL							
Mean	288.20	599.66	475.09	343.77	448.23	285.48	
SD	±69.76	±59.54	±118.08	±49.62	±32.73	±66.45	
Shapiro-Wilk	*0.761	*0.149	*0.242	*0.972	*0.375	*0.471	
Levene Test							*0.228
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 results of IL-6 levels were carried out by the Shapiro wilk test, the results of the normal distributed average data (p>0.05) and the data homogeneity test with the Leuvene test had a homogeneous data variant of 0.228 (p>0.05). The results of the data were distributed normally and homogeneously, a one-way anova test with a result of 0.000 (<0.05) showed that there was a significant difference in IL-6 levels between the treatment groups.

Based on table 3, it was shown that the average result of IL-6 levels after day 7 treatment in the healthy group (K7) was 288.20 pg/mL, the *sham* group (K8) 599.65 pg/mL, the base cream group (K9) 475.09 pg/mL, the bioplacenton group (K10) 343.77 pg/mL, the 10% dose group (K11) 448.22 pg/mL and the 20% dose group (K12) 285.47 pg/mL. IL-6 levels were lowest in the healthy rat group (K12) and IL-6 levels were highest in the *sham* group (K8).

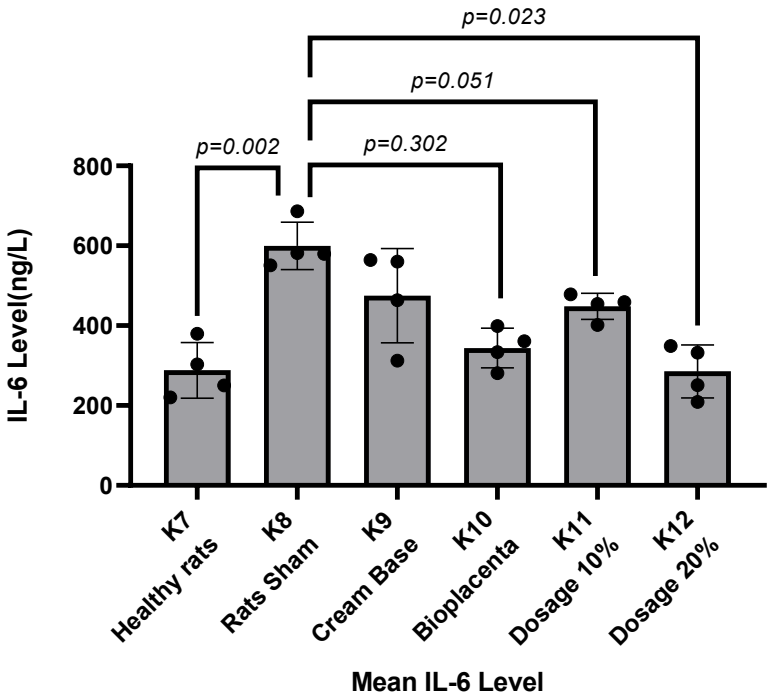


Figure 4. Mean IL-6 levels between groups on day 7 after treatment

The comparison between the treatment groups showed significant differences in the healthy rat group compared to all treatment groups that experienced an average increase in IL-6 levels, The 20% extract cream treatment group showed the lowest average result of IL-6 levels, the same as the treatment group with bioplacentone with a Post hoc LSD test score of 0.000 (<0.05). It can be concluded that the administration of pomegranate extract cream is effective in reducing IL-6 levels the same as the treatment with the administration of bioplacentones on the 7th day after treatment, as attached in the following table 4.

Table 4. The post hoc LSD test of IL-6 levels of rat skin tissue after excision wound after day 7 treatment

Group	K2	K3	K4	K5	K6
K1	*0.002	0.051	0.550	*0.020	*0.343
K2	-	0.856	0.302	*0.051	*0.023
K3		-	0.942	0.838	0.401
K4			-	1.000	1.000
K5				-	0.969

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 a 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 TGF-β levels and the one-way anova test after day 3 treatment

Group	Healthy Rats K1	Rats *Sham* K2	Cream Base K3	Biopla centon K4	Dosage 10% K5	Dosage 20% K6	*P value*
TGF-β Level pg/mL							
Mean	473.65	210.57	280.38	357.78	347.88	388.36	
SD	±31.95	±45.24	±61.39	±78.08	±30.89	±46.75	
Shapiro-Wilk	*0.831	*0.760	*0.819	*0.309	*0.985	*0.421	
Levene Test							0.012
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 level of TGF-β was carried out by the Shapiro-Wilk test, which obtained normal distributed results (p>0.05) and the data homogeneity test with the Leuvene Test test had non-homogeneous data variations with a result of 0.012 (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 the average TGF-β level between the treatment groups. Based on table 5, it was shown that the average results of TGF-β levels after the 3rd day of treatment in the healthy group (K1) were 473.65 pg/mL, the *sham* group (K2) was 210.57 pg/mL, the base cream (K3) group was 280.38 pg/mL, the bioplacenton group (K4) was 357.78 pg/mL, the 10% dose group (K5) was 347.88 pg/mL and the 20% dose group (K6) was 388.36 pg/mL. The lowest TGF-β levels were in the *sham* group (K2) and the highest TGF-β levels were in the 10% pomegranate extract cream group (K5).

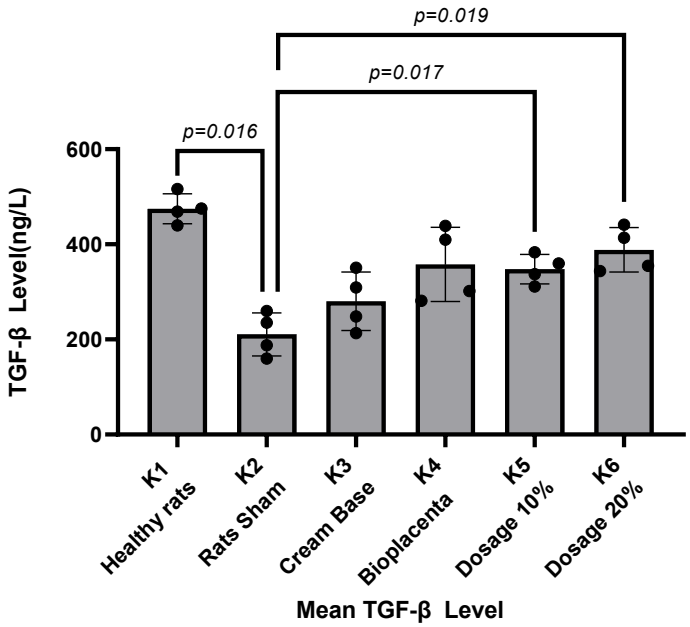


Figure 5. Mean TGF-β levels between groups on day 3 after treatment

Table 6. Post hoc tamhane test of TGF-β levels of rat skin tissue after excision wound after day 3 treatment

Group	K2	K3	K4	K5	K6
K1	*0.016	*0.049	*0.005	*0.064	*0.042
K2	-	0.654	0.043	*0.017	*0.019
K3		-	0.303	0.084	0.084
K4			-	0.076	*0.037
K5				-	1.000

The average comparison of TGF-β levels in the healthy rat group was significantly different when compared to all K1, K2, and K4 groups. The treatment group that was given a 20% dose of pomegranate extract cream showed the highest average increase in TGF-β levels compared to other treatment groups. It can be concluded that the administration of pomegranate extract cream has the effect of increasing TGF-β levels on the 3rd day after treatment.

Table 7. The average descriptive test of TGF-β levels and the one-way anova test after day 7 treatment

Group	Healthy Rats K7	Rats *Sham* K8	Cream Base K9	Biopla centon K10	Dosage 10% K11	Dosage 20% K12	*P value*
TGF-β Level pg/mL							
Mean	1158.27	237.15	302.11	901.76	744.61	1022.57	
SD	±258.69	±12.89	±51.77	±73.06	±91.77	±98.53	
Shapiro-Wilk	*0.804	*0.378	*0.890	*0.769	*0.051	*0.827	
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 TGF-β level was carried out by the Shapiro-Wilk test, which obtained a normal distributed result (p>0.05) and the data homogeneity test with the Leuvene Test had a non-homogeneous 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 in TGF-β levels between the treatment groups.

Based on table 7, it was shown that the average results of TGF-β levels after day 7 treatment in the healthy group (K7) were 1158.27 pg/mL, the *sham* group (K8) 237.15 pg/mL, the base cream group (K9) 302.11 pg/mL, the bioplacenton group (K10) 901.77 pg/mL, the 10% dose group (K5) 744.61 pg/mL and the 20% dose group (K6) 1022.57 pg/mL. The lowest TGF-β levels were in the *sham* group (K2) and the highest TGF-β levels were in the healthy rat group (K7).

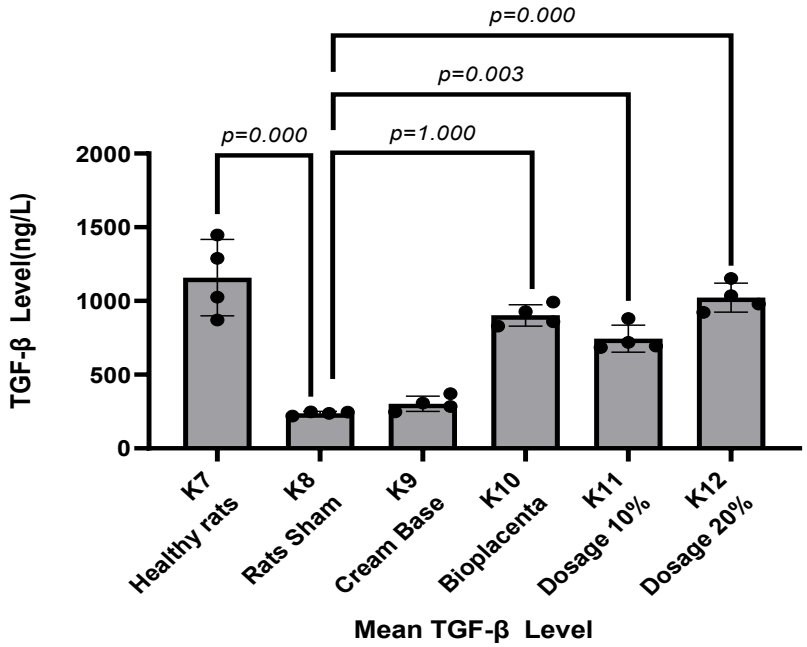


Figure 6. The average TGF-β levels between treatment groups after day 7

Table 8. TGF-β tissue level post hoc tamhane test rat skin after excision wound after day 7 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

Description: * Means p<0,05

The average comparison of TGF-β levels in the healthy rat group (K7) was significantly different when compared to all excision wound treatment groups. The treatment group given pomegranate extract cream showed the most significant difference with the highest average TGF-β levels compared to other treatment groups. It can be concluded that the administration of pomegranate extract cream has the effect of increasing TGF-β levels on the 7th day after treatment.

The results of the macroscopic study with the administration of 20% pomegranate extract accelerated the closure of the wound area close to the diameter with the group given bioplacenton. Wounds that begin with the homeostasis phase, include draining the lymphatic system and preventing bleeding. Then, the inflammatory phase lasts for 1 to 3 days, and the proliferation phase lasts from day 4 to day 14 [15]. Observations on the transition from the inflammatory phase on day 3 after treatment and the proliferation phase on day 7 after treatment are considered appropriate for analysis on IL-6 and TGF-β parameters.

The results showed that the effect of reducing IL-6 levels on the 3rd day after treatment, the administration of pomegranate extract cream at a dose of 20% had the lowest effect compared to other groups. Examination on the 7th day after treatment also showed a decrease with the administration of pomegranate extract cream equal to the treatment group with bioplacenton. In line with the study using pomegranate peel extract, wound healing activity was tested in rats using an excision wound model. The animals given the extract showed a reduction in the

wound area, the wound given the extract turned out to be epithelialized faster compared to the control [10].

The transition from the inflammatory phase to the proliferation phase is a very important phase in wound healing [16]. Acute inflammation is largely mediated by IL-6, which is also necessary for wound healing. Proinflammatory cytokines are released by tissue macrophages, keratinocytes, endothelial cells, and stromal cells when IL-6 is released at the beginning of the response to injury. It was also found that IL-6 causes leukocytes to migrate towards the wound. IL-6 signaling is in charge of triggering the transition to a reparative environment as inflammation increases [17 18]. The inflammatory response is essential in wound closure, and disruption to the IL-6 signaling pathway can inhibit wound healing. Therefore, the role of IL-6 in the healing of skin wounds is very important. Since M1 macrophages are IL-6 expressionists on wounds, IL-6 regulates the polarization of M2 by inducing the production of IL-4 Th2 and IL-4R cells in macrophages. M2 macrophages in particular are major secretaries of proliferative cytokines such as TGF-β [19]. The enhanced efficacy of 20% pomegranate extract compared to 10% may be related to its higher concentration of bioactive polyphenols, particularly ellagic acid, punicalagin, and anthocyanins, which exert potent anti-inflammatory effects via COX-2 inhibition and NF-κB pathway suppression. [20-22] This higher antioxidant capacity can limit oxidative stress-induced damage to fibroblasts and keratinocytes, thus facilitating faster transition from inflammation to proliferation. [23, 24]

The average results of TGF-β levels with the administration of pomegranate extract cream had an increased effect on the 3rd day after the treatment and lasted on the 7th day after the treatment. The *sham* group (K8) and the base cream group (K9) experienced an average decrease in TGF-β levels on the 7th day after treatment, but experienced an increase in the other groups (K10, K11 and K12), The increase in TGF-β levels in the treatment of 20% pomegranate extract cream showed the most effective increase compared to the bioplacenton group which concluded that the administration of 20% pomegranate extract cream against TGF-β levels was better than that of bioplacenton. This is consistent with the role of TGF-β in promoting fibroblast-to-myofibroblast differentiation, stimulating collagen deposition, and enhancing angiogenesis[25,26]. These processes are essential for robust granulation tissue formation, and phytochemicals in pomegranate extract may enhance TGF-β signaling through both Smad-dependent and non-Smad pathways. [27,28]

The proliferation phase that lasted for 4-14 days, namely on the 7th day after the treatment, showed better results than the 3rd day after the treatment. 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 [29]. TGF-β affects the processes of cell death, migration, differentiation, and proliferation pleiotropically [30]. The TGF-β signaling pathway has a major impact on cell differentiation. Signals associated with cell proliferation are activated by TGF-β [31]. However, literature also reports that prolonged or excessive TGF-β activity can lead to fibrosis or hypertrophic scarring [15,26] highlighting the need for careful modulation of this pathway.

Contradictory findings exist, as some studies using lower concentrations of pomegranate extract or different wound models have reported minimal improvement in healing[32,33]. Such discrepancies may arise from variations in extraction method, storage stability of phytochemicals, vehicle formulation, and wound type. Furthermore, the absence of histological evaluation, molecular pathway profiling, or longer observation in this study means that the complete remodeling phase and potential scar formation could not be assessed. These factors, along with the lack of dose ranging studies beyond 20% and absence of

human trials, should be addressed in future research to better define optimal formulation and clinical relevance.

Early detection of wounds, as well as intervention using pomegranate extract creams can be a promising substitute or alternative, Nevertheless, translating these findings into clinical use will require well controlled human studies to confirm safety, efficacy, and optimal dosing regimens, supported by mechanistic evaluations to understand long-term tissue outcomes.

CONCLUSION

The administration of pomegranate extract cream (*Punica granatum*) had an effect on the increase in TGF-β levels in rats after excision wounds. Pomegranate extract cream (*Punica granatum*) had an effect on reducing IL-6 levels in rats after excision wounds.

AUTHORS' CONTRIBUTIONS

Indah Roswita Sari ; Data curation , Investigation, Conceptualization, Writing-Original draf; **Titiek Sumarawati**: Reviewing, Supervision; **Agung Putra**: Validation, Supervision, , Reviewing.

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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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