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Effect of Pomegranate Extract Topical Cream (*Punica granatum*) on SOD and TNF-α Levels in Wistar Rats Excision Wound Model

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ABSTRACT

Wounds cause damage or loss of skin tissue components. The body will respond and trigger the wound healing process. Neutrophils will trigger the pro-inflammatory mediator, Tumor Necrosis Factor alpha (TNF-α) which is useful for starting inflammation. Providing exogenous antioxidants, such as those found in pomegranates, is very important. Pomegranate extract contains antioxidants such as flavonoids, alkaloids, tannins, triterpenes and saponins which have the potential to be a new treatment for inflammation caused by various skin disorders. Pomegranate extract significantly improves the wound healing process. However, an in-depth analysis of pomegranate extract cream which can accelerate the healing of excision wounds and influence SOD and TNF-α levels needs to be carried out, so that the results of this research can be a new alternative that supports topical wound treatment. An in-vivo experimental study was conducted with 48 Wistar rats, divided into 12 groups. The analysis was performed in two stages: six groups were analyzed after 3 days of treatment, and the remaining six groups were analyzed after 7 days of treatment. Data were tested using one-way ANOVA. Pomegranate extract cream reduced TNF-α levels, as shown by the One-Way ANOVA test ($p=0.001$, $p<0.05$). On day 3 after treatment, pomegranate extract cream had the same effect as the bioplacenton group. There was no significant difference between groups in SOD levels on day 3 after treatment, as indicated by the One-Way ANOVA test ($p=0.565$, $p>0.05$). However, on day 7 after treatment, there was a significant difference in SOD levels between groups, as shown by the One-Way ANOVA test ($p=0.037$, $p<0.05$). Pomegranate extract cream reduces TNF-α levels to the same extent as bioplacenton on day 3 after treatment and increases SOD levels in excision wounds on day 7 after treatment.

Keywords: Pomegranate extract cream, Excision wounds, TNF-α levels, SOD levels

INTRODUCTION

Excision wounds are a type of wound caused by tissue being cut due to a scratch from a sharp object. Wounds result in damage or loss of skin tissue components (Almadani *et al.*, 2021). The body will respond and trigger the wound healing process (Cahya *et al.*, 2020). Neutrophils will trigger the pro-inflammatory mediator Necrosis factor alpha (TNF-α) which is beneficial for initiating inflammation (Huang *et al.*, 2021). Pomegranate as a source of antioxidants, has the potential to be a new treatment for inflammation caused by various skin disorders (Houston *et al.*, 2017). Antioxidant enzymes have a role for every wound healing process (Kurahashi & Fujii, 2015). Excessive reactive oxygen species (ROS) are cytotoxic, redox balance must be strictly controlled to achieve normal wound healing. ROS produced by some oxidase and mitochondrial respiration must be reduced mainly by antioxidant enzymes such as Superoxide dismutase (SOD) (Kurahashi & Fujii, 2015). The provision of exogenous antioxidants is important, Pomegranate fruit is special because it is one of the fruits of heaven which contains antioxidants, flavonoids, alkaloids, tannins, triterpenes and saponins(Mansur *et al.*, 2022).

Pomegranate extract can accelerate wound healing, so this research focuses on observing SOD and TNF-α. The use of traditional medicines and plants in treating wounds not only accelerates healing but also reduces the financial burden for patients (Nema *et al.*, 2013). With a broader understanding of traditional practices, there is an appreciation and benefits for more people around the world (Shedoeva *et al.*, 2019).

Research reports using pomegranate extract and 40% ellagic acid in ointment form. A dose of 10% pomegranate extract accelerates the healing of second-degree burns, (Lukiswanto *et al.*, 2019). According to data from the Indonesian Ministry of Health (2016), there are around 3,922 cases of hospitalization with serious injuries due to disasters, and 41,034 cases of minor injuries or outpatient treatment. Anti-inflammatory, antibacterial, and antioxidant medications can help speed up the wound healing process (Shaygannia *et al.*, 2016). Wound healing goes through several phases, namely the hemostasis

phase, the inflammatory phase, the proliferation phase and the remodeling phase (Almadani *et al.*, 2021). In epithelialization, epithelial cells proliferate and spread throughout the wound surface. Wound contractions occur when myofibroblasts contract. Platelets release growth factors and other cytokines (Nema *et al.*, 2013). The optimal ROS level varies in each wound healing process. Therefore, each antioxidant enzyme needs to be improved, as needed to lower ROS levels in each wound healing process (Kurahashi & Fujii, 2015).

Research reports on the potential use of pomegranate in the field of dermatology (Lukiswanto *et al.*, 2019). Plant extracts are more widely used in the cosmetic industry than ever before, their properties in the field of dermatology are based on scientific research on pomegranates (Shaygannia *et al.*, 2016). The research conducted by Nema with this excision wound model concluded that methanol extract ointment concentrations (10% and 15%) showed significant wound healing activity. This is shown by a significant increase in the rate of wound contraction and an increase in the epithelialization of excision wounds from pomegranate (Nema *et al.*, 2013). The research conducted by Nema with this excision wound model concluded that methanol extract ointment concentrations (10% and 15%) showed significant wound healing activity. This is shown by a significant increase in the rate of wound contraction and an increase in the epithelialization of excision wounds from pomegranate (Mahdi *et al.*, 2018).

Oxidative stress as well as inflammation activate the NF-κB complex, which in turn is related to the cellular redox state (Zhang *et al.*, 2021). NF-κB actively translocates to the nucleus and activates the target gene (Zhang *et al.*, 2020). NF-κB induces the expression of inflammatory cytokines such as TNF-α, the NF-κB pathway also has antioxidant functions and targets such as Superoxide dismutase (SOD) (Zhang *et al.*, 2021). SOD is a very important antioxidant defense against oxidative stress in the body. This enzyme acts as a good therapeutic agent against diseases mediated by reactive oxygen species (ROS). SOD is used in cosmetics and personal care products as an anti-aging and antioxidant ingredient due to its ability to reduce free radical damage to the skin, thereby preventing wrinkles, fine lines, and age spots, as well as aiding wound healing, protecting against UV rays, and reducing other signs of aging (Younus, 2018).

Pomegranate extract significantly improves the wound healing process (Mahdi *et al.*, 2018). However, a new alternative to speed up topical wound treatment using pomegranate extract cream by observing SOD and TNF-α levels requires further analysis.

METHODS

This study uses experimental research using a post test only control group design which was carried out in April-May 2024 at the Stem Cell and Cancer Research Laboratory (SCCR) Semarang, Central Java. The subject of the study was a male rat of the Wistar strain aged 2-3 months with a body weight of 190-210 grams which was adapted for 7 days. The total number of subjects was 48 male wistar rats divided into 6 groups, each of which amounted to 5 rats.

Normal group (K1) was healthy rats without treatment for 7 days, *sham* group (K2) excision wound rats were given excision wound rats for 7 days, negative control group (K3) excision wound rats were given base cream for 7 days, positive control group (K4) excision wound rats were given bioplacentone for 7 days, treatment 1 (K5) excision wound rats were given pomegranate extract cream 10% for 7 days, and treatment 2 (K6) rats with excision wounds 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.

The data results in the study were carried out a normality test with the Shapiro Wilk test and a data homogeneity test with the Levene test, Possible descriptive results obtained. The data produced were normal and homogeneous (P>0.05), so a different test was carried out for the One Way Anova test and continued with the Post Hoc LSD test to find out the differences between each group. The data produced were normal and not homogeneous (P>0.05).

RESULTS

The results of the analysis of the average TNF-α levels in each group on the 3rd day after treatment are shown in table 1 as follows:

Table 1
Results of 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)	*Base Cream* (K3)	*Biopla Centon* (K4)	Dosage 10% (K5)	Dosage 20% (K6)	*P Value*
TNF-α Level pg/mL							
Mean	192,43	505,95	622,09	255,04	305,27	267,77	
SD	±9,64	±48,93	±40,47	±80,34	±9,42	±8,29	
Shapiro-Wilk	*0,536	*0,894	*0,507	*0,894	*0,384	*0,714	
Leuvene Test							0,004
One way anova							*0,001

Description: * *Shapiro-Wilk* = Normal (p>0,05)
* *Leuvene Test* = Homogen (p>0,05)
* *One way Anova* = Significance (p<0,05)

The average results of TNF-α levels were carried out by the Shapiro-Wilk test, the results were obtained 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 p=0.004

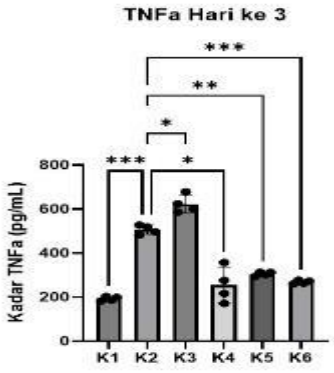

Figure 1. Average Graph of TNF-A Levels

(p>0.05). The results of the data were normally distributed and not homogeneous, then a one-way anova test was carried out with a result of p=0.001 (<0.05) showing that there was a significant difference in TNF-α levels between groups.

The results showed that the average TNF-α level in the healthy group (K1) was 192.43 pg/mL, the *sham* group (K2) was 505.95 pg/mL, the base cream (K3) group was 622.09 pg/mL, the bioplacenton group (K4) was 255.04 pg/mL, the 10% dose group (K5) was 305.27 pg/mL and the 20% dose group (K6) was 267.77 pg/mL. The comparison between the groups showed significant differences between groups (K1) compared to others. The average increase in TNF-α levels in the group of excision wounds without intervention (*sham*) was the most significant and the lowest decrease was in the excision wound group given bioplacenton (K4), then sequentially decreased in the dose of 20% pomegranate extract cream (K6) and 10% dose (K5).

Significant differences between groups were carried out by the Post hoc tamhane test to find out the comparison between the most influential groups. The results of the comparison of the average TNF-α levels between the groups showed a significant difference, the excision wound group given bioplacentone (K4) compared to the excision wound group given a 20% dose of pomegranate extract cream (K6) did not differ significantly. It can be concluded that the administration of pomegranate extract cream at a dose of 20% reduced TNF-α levels the same as the excision wound group that was given bioplacenta on the 3rd day after treatment. As shown in the table of Post hoc tamhane test results in table 2.

Table 2
Results of The Tamhane Post Hoc Test Day 3 After Treatment of Tissue TNF- A Levels

Group	K2	K3	K4	K5	K6
K1	*0,001	*0,001	0,794	*0,001	*0,001
K2	-	*0,042	*0,042	*0,001	*0,001
K3		-	*0,007	*0,002	*0,002
K4			-	0,910	1,000
K5				-	*0,011

Description: * Means p<0,05

The results of the analysis of the average TNF-α levels in each group on the 7th day after treatment are shown in table 3.

Table 3
Results of The Average Descriptive Test of TNF-α LEVELS and The 7th Day of The One-Way Anova Test After Treatment

Group	Healthy Rats (K7)	Rats *Sham* (K8)	*Base cream* (K9)	*Biopla centon* (K10)	Dosage 10% (K11)	Dosage 20% (K12)	*P value*
TNF-α Level pg/mL							
Mean	145,73	476,64	398,34	148,11	370,72	363,90	
SD	±62,10	±57,44	±48,14	±46,84	±19,59	±21,63	
Shapiro-Wilk	*0,951	*0,277	*0,775	*0,340	*0,374	*0,850	
Leuvene Test							0,004
One way anova							*0,001

Description: * *Shapiro-Wilk* = Normal (p>0,05)
* *Leuvene Test* = Homogen (p>0,05)
* *One way Anova* = Significance (p<0,05)

The average results of TNF-α levels were carried out by the Shapiro-Wilk test, the results were obtained 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 p=0.004 (p>0.05). The results of the data were distributed normally and non-homogeneously, then a one-way anova test with a result of 0.001 (<0.05) showed that there was a significant difference in the average TNF-α levels between groups.

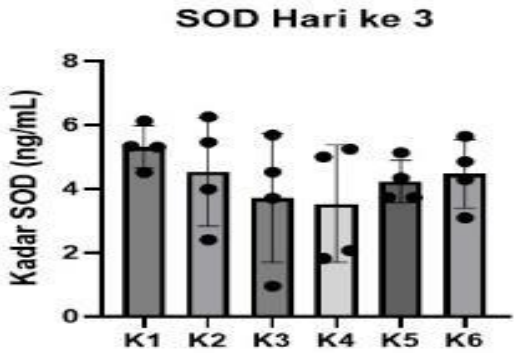

Figure 2. Average Graph of TNF-A Levels

The results showed that the average TNF-α level in the healthy group (K7) was 145.73 pg/mL, the *sham* group (K2) was 476.64 pg/mL, the base cream group (K3) was 398.34 pg/mL, the bioplacenton group (K4) was 148.11 pg/mL, the 10% dose group (K5) was 370.72 pg/mL and the 20% dose group (K6) was 363.90 pg/mL. The comparison between groups showed an average increase in TNF-α levels in the non-intervention excision wound group (*sham*) (K2), base cream group (K3), 10% dose group (K5) and 20% dose group (K6) on day 7 after treatment, but the group given bioplacenton (K4) and healthy rat group (K7) did not experience an average increase in TNF-α levels.

Significant differences between groups were carried out by the Post hoc tamhane test to find out the comparison between the most influential groups. The results of the comparison of the average TNF-α levels between the groups showed a significant difference, the excision wound group given bioplacenton (K4) had the most effective effect on the 7th day after treatment compared to other groups with the average TNF-α level comparable to the healthy rat group. As shown in the table of Post hoc tamhane test results in table 4.

Table 4
Results of The Post Hoc Test Day 7 After Treatment of The Average TNF-α Levels

Group	K8	K9	K10	K11	K12
K7	*0.002	*0.008	1.000	*0.024	*0.025
K8	-	0.527	*0.001	0.184	0.154
K9		-	*0.003	*0.007	*0.007
K10			-	*0.007	1.000
K11				-	1.000

Description: * Means p<0,05

The results of the analysis of the average SOD levels in each group on the 3rd day after treatment are shown in table 5 as follows:

Table 5
Results of The Average Descriptive Test of SOD Levels and The One-Way Anova Test on Day 3 After Treatment

Group	Healthy Rats (K1)	Rats *Sham* (K2)	*Base cream* (K3)	*Biopla centon* (K4)	Dosage 10% (K5)	Dosage 20% (K6)	*P value*
SOD Levels ng/mL							
Mean	5,31	4,52	3,71	3,53	4,23	4,46	
SD	±0,66	±1,69	±2,01	±1,83	±0,66	±1,07	
Shapiro-Wilk	*0,734	*0,821	*0,678	*0,094	*0,248	*0,948	
Leuvene Test							*0,098
One way anova							0,565

Description: * *Shapiro-Wilk* = Normal (p>0,05)
* *Leuvene Test* = Homogen (p>0,05)
* *One way Anova* = Significance (p<0,05)

The average results of SOD levels were carried out by the Shapiro-Wilk test obtained results in all normal distributed groups (p>0.05) and the data homogeneity test with the Leuvene Test test had a homogeneous data variant with a result of p=0.098 (p>0.05). The results of the data were distributed normally and homogeneously, then a one-way anova test was carried out with a result of p=0.565 (<0.05) showing that there was no significant difference in SOD levels between groups.

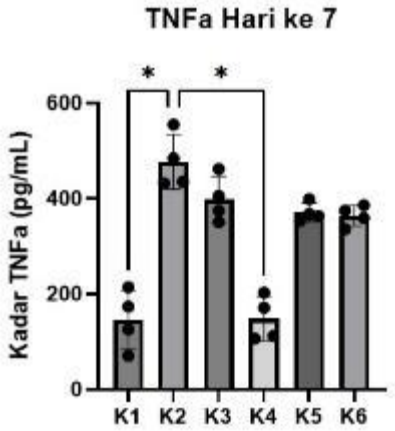

Figure 3. Average Graph of 3rd Day Sod Rates After Treatment

The results showed that the average SOD level in the healthy group (K1) was 5.31 ng/mL, the *sham* group (K2) was 4.52 ng/mL, the base cream group (K3) was 3.71 ng/mL, the bioplacenton group (K4) was 3.53 ng/mL, the 10% dose group (K5) was 4.23 ng/mL and the 20% dose group (K6) was 4.46 ng/mL. The comparison between the groups showed insignificant differences between all groups. The results of the analysis of the average SOD level in each group on the 7th day after treatment are shown in table 6 as follows:

Table 6
Results of The Average Descriptive Test of Sod Levels And The One-Way Anova Test on Day 7 After Treatment

Kelompok	Tikus sehat (K7)	Tikus *Sham* (K8)	*Base cream* (K9)	*Biopla centon* (K10)	Dosis 10% (K11)	Dosis 20% (K12)	*P value*
Kadar SOD ng/mL							
Mean	2,69	4,61	4,70	4,77	4,79	5,53	
SD	±1,16	±0,83	±0,70	±0,80	±0,877	±1,81	
Shapiro-Wilk	*0,903	*0,926	*0,867	*0,899	*1,000	*0,516	
Leuvene Test							0,043
One way anova							*0,037

Description: * *Shapiro-Wilk* = Normal (p>0,05)
* *Leuvene Test* = Homogen (p>0,05)
* *One way Anova* = Significance (p<0,05)

The average results of SOD levels were obtained by the Shapiro-Wilk test 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 p=0.043 (p>0.05). The results of the data were distributed normally and non-homogeneously, then a one-way anova test was carried out with a result of 0.037 (<0.05) showing that there was a significant difference in the average SOD level between groups.

The results showed that the average SOD level in the healthy group (K1) was 5.31 ng/mL, the *sham* group (K2) was 4.52 ng/mL, the base cream group (K3) was 3.71 ng/mL, the bioplacenton group (K4) was 3.53 ng/mL, the 10% dose group (K5) was 4.23 ng/mL and the 20% dose group (K6) was 4.46 ng/mL. Comparisons between groups showed insignificant differences between all groups.

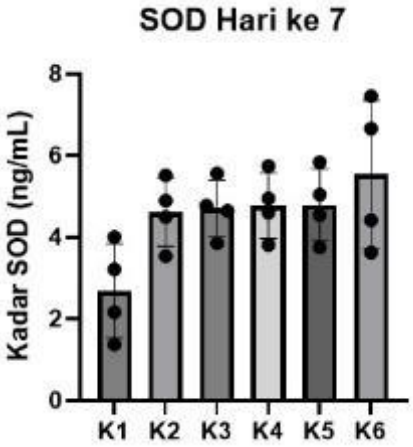

Figure 4. Average Graph Of 7-Day SOD Rates After Treatment

The results showed that the average SOD level in the healthy group (K7) was 2.68 ng/mL, the *sham* group (K2) was 4.61 ng/mL, the base cream group (K3) was 4.70 ng/mL, the bioplacenton group (K4) was 477 ng/mL, the 10% dose group (K5) was 4.79 ng/mL and the 20% dose group (K6) was 5.53 ng/mL. The comparison between groups showed an average increase in SOD levels in the excision wound group given a 20% dose of pomegranate extract cream (K12), the lowest average SOD level in the healthy rat group. The K8, K9, and K10 groups had different average levels, to see the significant differences between the groups, a Post hoc tamhane test was carried out to find out the comparison.

The results of the average comparison of SOD levels between groups showed significant differences, the dose of 20% pomegranate cream showed the highest average increase in SOD levels on the 7th day after treatment, as shown in the results of the Post hoc tamhane test in the following table 7:

Table 7
Results of The Post Hoc Test Day 7 After Treatment Of The Average SOD Levels

Group	K8	K9	K10	K11	K12
K7	*0,035	0,285	*0,038	*0,037	*0,026
K8	-	0,257	0,973	0,986	0,196
K9		-	0,271	0,263	0,866
K10			-	0,985	0,201
K11				-	0,201

Description: * Means p<0,05

Macroscopic observations in rat subjects after excision wounds show accelerated healing and wound closure as shown in the following figure:

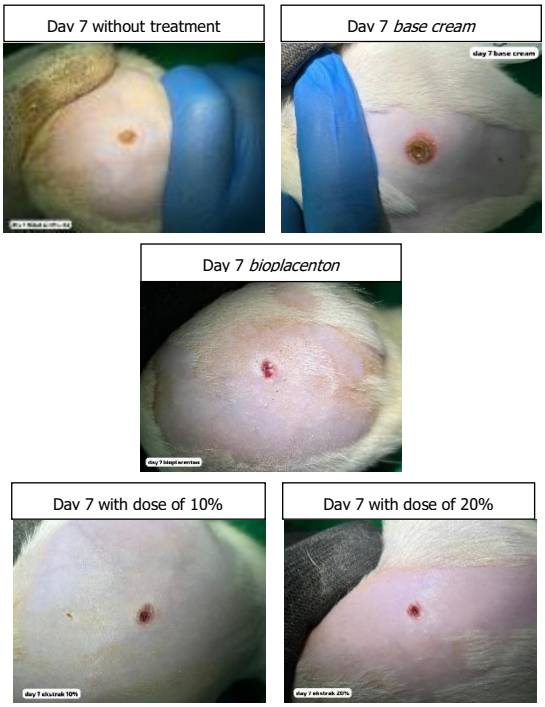

Figure 5. Macroscopic Conditions of Excision Wound Treatment In Each Group on The Seventh Day

The macroscopic picture of the treatment group on day 7 showed the acceleration of wound healing and closing, the excision wound group given 20% pomegranate extract cream had a wound diameter of 3.08mm macroscopic close to the group given bioplacenton with a wound area diameter of 2.97mm. The excision wound group that was given pomegranate extract cream with a wound diameter of 4.21mm, also showed an acceleration of wound healing and closure compared to the group without treatment (K2) with a wound diameter of 6.06 mm and the base cream (K3) with a wound diameter of 7.00mm. It can be concluded that the macroscopic picture of the excision wound area after the 7th day with the administration of pomegranate extract at a dose of 20% accelerates the closure of the wound area close to the diameter with the administration of bioplacenton.

DISCUSSION

Wound healing begins with the formation of granulating tissue such as fibrovascular tissue consisting of fibroblasts, collagen, and the formation of blood vessels. The vascular component is highly dependent on angiogenesis, new blood vessels begin to appear on the third day after the occurrence of the wound, will provide nutrients and mediators for the wound healing process. The role of macrophages is crucial in the subsequent process. Macrophages will produce various kinds of cytokines, one of which is TNF-α which is an important cytokine in the wound healing process. Macrophages also release growth factors and other substances to initiate and accelerate the formation of granulated tissue formations (Pereira *et al.*, 2016).

The results showed that the average increase in TNF-α levels in the group of excision wounds without intervention (*sham*) was the most significant and the lowest decrease was in the group of excision wounds given bioplacenton, then sequentially decreased in the dose of pomegranate extract cream of 20% and 10%. The administration of a 20% dose of pomegranate extract cream reduced TNF-α levels equal to the excision wound group given bioplacenta on the 3rd day after treatment. The comparison between groups showed an average increase in TNF-α levels in the group of excision wounds without intervention (*sham*) (K8), the base cream group (K9), the 10% dose group (K11) and the 20% dose (K12) group on the 7th day after treatment, but the group given bioplacenton (K10) of the healthy rat group (K7) did not experience an average increase in TNF-α levels.

TNF-α is one of the pro-inflammatory cytokines that plays a role in the singnaling of the inflammatory process. TNF-α is produced by macrophages and activated by lymphocyte T cells, antigens, Natural Killer (NK) cells, and Mast cells in the acute phase. TNF-α acts to induce and control inflammation. TNF-α plays an important role in protecting wounds from infection, inducing the proliferation of fibroblasts, keratinocytes and hair follicle regeneration. Macrophages peaked on day 3, the increase went straight with the increase in TNF-α. This theory is proven because there is a significant increase in TNF-α expression on day 3 (van Loo & Bertrand, 2023).

The average results of SOD levels between groups showed a non-significant difference on day 3 after treatment, but on day 7 after treatment, the results of the comparison of the average SOD levels between groups showed a significant difference, the dose of 20% pomegranate cream showed the highest average increase in SOD levels on day 7 after treatment.

High ROS levels continuously damage the structure of proteins, lipids, and DNA and activate cell death pathways, resulting in irreversible cell damage, malfunction, and death of organisms. Primarily expressed in the artery

walls, SOD3 is an SOD isoenzyme that increases the bioavailability of nitric oxide in addition to clearing superoxide. Nitric oxide activity increases and vascular contractile homoeostasis is increased when superoxide levels are lowered because SOD3 restricts the rapid interaction of nitric oxide with superoxide to produce peroxynilite. Perivascular inflammation is triggered by the loss of SOD3. The dermis and epidermis both contain SOD3 (Fujiwara *et al.*, 2016).

Antioxidants are produced by the body as a protective measure against reactive oxygen species (ROS). Molecules known as antioxidants prevent oxidation even at very low concentrations (Andarina & Djauhari, 2017). Antioxidants work in oxidative processes through a variety of ways, including as a binder of metal ions, adsorbing peroxyl lipid radicals, repairing oxidative damage, and trapping free radicals enzymatically or chemically. Antioxidants neutralize ROS and stabilize free radicals to stop oxidation by attracting or releasing electrons (Ikrima *et al.*, s.a.).

Enzymatic antioxidants found in the skin include catalase, glutathione peroxidase (GSH peroxidase), and superoxide dismutase (SOD) (Andarina & Djauhari, 2017). Skin cells create ROS in response to exogenous and endogenous chemicals, and excessive levels of ROS can damage cells. When ROS impacts the MAP signaling pathway, NF-kB and AP-1 (protein-1 activator), two transcription factors, are activated. stimulates the inflammatory response, enhances procollagen synthesis by blocking TGF-β/SMAD signaling, and enhances collagen breakdown through matrix degeneration and MMP production (Altobelli *et al.*, 2020; Gęgotek & Skrzydlewska, 2015).

Inflammation and cell-mediated immune responses are related to TNF-α signaling. TNF-α signaling regulates cellular processes such as immunomodulation, leukocyte trafficking, apoptosis, and anti-microbial effects (Bhat *et al.*, 2018). The proliferation of M1 macrophages is stimulated by TNFa, activation of TLR, IL-1, and interferon. Effective decontamination of the wound results in the end of the inflammatory phase and the beginning of the reparative stage, which is characterized by the proliferation of keratinocytes and fibroblasts. The regulation of the reparative phase is largely governed by the transition of M1 macrophages to M2-like macrophages and the various roles of AMP. It takes controlled proliferation, migration, and differentiation of keratinocytes at least partially aided by growth factor synthesis and AMP for wound reepitheliation to occur (MacLeod & Mansbridge, 2016). The deposition and regeneration of collagen, which deals with the process of cell proliferation and differentiation in the posterior region of cell tissue, promotes repair, which requires the replacement of certain components (Eming *et al.*, 2007).

ROS as a partially reduced oxygen metabolite that has a strong oxidation ability, damaging cells at high concentrations but at low concentrations (exact concentrations are still undetermined), ROS serves complex signaling functions. ROS is detrimental, as it oxidizes protein and lipid cell constituents and damages DNA. At physiological concentrations, ROS functions as a signaling molecule that regulates the growth of cell adhesion to other cells, differentiation, aging, and apoptosis (Mittal *et al.*, 2014).

Excessive ROS production can further lead to protein dysfunction, abnormal cellular interactions, deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) damage, and cell apoptosis. ROS also has the ability to regulate the formation of blood vessels (angiogenesis) at the wound site and optimal blood perfusion to the wound healing area. ROS plays a role in host defense through phagocytes that induce ROS blasts into pathogens present in wounds, thereby causing their destruction, and during this period, excessive leakage of ROS into the surrounding environment causes further bacteriostatic effects (Dunnill *et al.*, 2017).

Antioxidants overcome these limitations by inhibiting the oxidation of molecules and restoring normal physiological levels of ROS. However, these antioxidants are hampered by low bioavailability and bioactivity when administered directly to the wound. The incorporation of antioxidants into biomaterials has significant potential in designing wound healing therapies. The provision of antioxidants is important, one of which is sourced from pomegranates (Nayak *et al.*, 2013). Pomegranate extract significantly improves the wound healing process (Mahdi *et al.*, 2018). Free radicals can be inhibited or reduced with antioxidants that act as scavengers against oxidative damage. The antioxidant component of pomegranate extract donates one electron to free radicals, neutralizes them and creates relatively stable free radicals, so that it can detoxify ROS (Arief *et al.*, 2018). This study proves that the use of pomegranate extract cream increases antioxidant activity by reducing inflammation by lowering TNF-α levels and increasing SOD levels which accelerate wound healing. Promotes wound healing and tissue remodeling (Katoh, 2019). SOD accelerates the process of network repair and network regeneration (Farooq *et al.*, 2021).

This study did not treat the dose of pomegranate extract cream by increasing the dose more to its molecular parameters, the next study can be carried out on chronic wound conditions with longer treatment by observing TNF-α and SOD levels. Factors that affect the results such as environmental, genetic, and nutritional factors can be a concern to avoid bias in research.

CONCLUSION

The administration of pomegranate extract cream (*Punica Granatum*) decreased the TNF-α levels of skin tissue of wistar rats after excision wounds. The administration of pomegranate extract cream (*Punica Granatum*) increased the SOD level of skin tissue of wistar rats after excision wounds.

SUGGESTION

Analysis of pomegranate extract cream with greater variations and concentrations to see the reaction to TNF-α levels and SOD levels as well as other molecular

parameters. Future research could be extended to all parameters with different experimental designs.

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