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Effect of Topical Glutathione in Wound Healing of Deep Dermal Burn Injury Male Rats (*Rattus Novergicus*) Wistar Strain

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ABSTRACT

Background:Burn injury have significant morbidity and mortality rates. Tissue around burn injury frequently damaged as a secondary wound (secondary thermal injury). There is no clear consensus of second degree burns treatment, as many variability of wound care materials. Glutathione is one of the antioxidants that can minimize the effects of oxidative stress by increasing intracellular Glutathione and accelerating wound healing by increasing the capacity of fibroblast contraction.
Aim: The study seeks to examine the effect of topical Glutathione on the healing process of deep dermal burns using male Wistar rats (*Rattus Novergicus*) as the experimental subjects.
Material and Methods: This research is a true experimental study using post test only control group design. Thirty-two rats were treated for burns on the backs (dorsum) by attaching heated iron plate at 100°C. The samples were divided into 2 groups of 16 rats in treated group with topical Glutathione application, and 16 rats in control group with topical placebo. The area of epithelialization and microscopic observation of fibroblasts, collagen and neovascularization was carried out on 5th day (D-5) and 12th day (D-12) studies.
Results:Obtained in microscopic increased fibroblasts maturation, collagen deposition, neovascularization and macroscopic re-epithelialization in group of rat samples applied by topical Glutathione in D-5 and D-12 studies and obtained significant differences compared with rat groups that were applied topical placebo.
Conclusion: Topical Glutathione can accelerate wound healing of deep dermal burns in male rats (Rattus Novergicus) wistar strain.

Keywords:Burn Injury, Burns, Deep dermal, Glutathione, Rats wistar strain (*Rattus Novergicus*)

1. Introduction

Burn injuries are a critical form of trauma that warrant careful consideration. A burn injury refers to harm inflicted on the skin or tissues as a result of heat or another form of sudden trauma (International Society of Burn Injuries, 2016). Burns can occur when the skin or tissues sustain damage from exposure to hot liquids (scalds), solid objects (contact burns), fire, radiation, electricity, or chemicals. Globally, burn injuries are linked to substantial rates of morbidity and mortality in conjunction with other types of injuries (Peck & Toppi, 2020).

According to the World Health Organization (WHO), there are an estimated 11 million individuals worldwide who suffer from burn injuries annually. In the United States, fire-related burns accounted for 41% of cases, as reported by the American Burn Association (ABA) National Burn Repository in 2019 (Jeschke et al., 2020). Over the period of 2018-2020, Dr. Soetomo General Hospital treated 307 patients with burn injuries. The primary cause of burn injuries at this hospital in Surabaya from 2017 to 2020 was fire, making up 56% of cases (Burn Unit Data, Dr. Soetomo General Hospital Surabaya, 2020). Thermal burns, which include scald burns (65%), contact thermal burns (15%), and fire-related burns (20%), are the most common type of burn injury according to other sources (Zor et al., 2009). Among burn injuries, those classified as deep partial thickness (second-degree burns) are the most frequently encountered (Schwarze et al., 2008).

The tissues surrounding burn injuries often suffer damage as secondary thermal injuries. Established treatment procedures exist for superficial burns (second-degree) and deep dermal burns (third-degree). However, there is no clear consensus on the treatment of second-degree burns (Atiyeh et al., 2005), leading to a wide range of available wound care materials. The effectiveness of products used for the treatment of partial-thickness thermal burns remains uncertain (Buz et al., 2016). One commonly used treatment for burn management is the topical antibiotic Silver sulfadiazine (SSD). However, SSD has several limitations, including limited penetration depth into the wound, inhibited wound contraction, slow and incomplete epithelialization, hypersensitivity, and ineffectiveness against certain microorganisms (Hussain & Ferguson, 2006).

Various research suggests utilizing topical vitamin C/Ascorbic acid in managing second-degree burns. Vitamin C, a water-soluble micronutrient, functions as a significant antioxidant essential for wound healing, infection prevention, inflammation mitigation, and promotion of epithelialization (Haddadi et al., 2020). Nevertheless, challenges exist, including the instability of vitamin C compounds, limited penetration into dermal layers despite adequate dosages, and decreased availability due to UV exposure (Telang, 2013).

Glutathione is an antioxidant that has the ability to reduce the impact of oxidative stress by boosting intracellular Glutathione levels and promoting wound healing through improved fibroblast contraction capabilities (Kopal et al., 2007). The incorporation of Glutathione in the treatment of burn wounds is anticipated to enhance cellular defenses against subsequent thermal injuries (Underdown, 2013). According to a study carried out by Kopal et al. in 2007, a reduction in inflammatory responses was noted on the 5th day, along with the development of new granulation tissue and extracellular matrix. By the 12th day (D-12), complete epithelialization and full wound healing were observed.

Burn injuries result from harm to the skin or other organic tissues due to heat, radiation, electricity, friction, or chemical exposure (WHO, 2021). Most burn injuries occur as a result of heat from scalds, hot objects, or fire. Burn injuries are classified based on various causes, including burns from fire, burns from hot water, burns from chemicals, burns from electricity, lightning, and radiation, burns from sun exposure, burns from thermal contact, and burns from hot air/steam (Wolf et al., 2018).

Understanding the pathophysiology of burn injuries is crucial for effective management. Different causes lead to different injury patterns, necessitating varied treatments. Therefore, it is essential to comprehend the physiological responses triggered by these burn injuries.

The body's response to burn injuries can be classified into two categories: the response occurring in the local tissues at the site of the burn and the systemic response due to the release of inflammatory mediators. The local tissue response to burn injuries is classified based on the anatomical layers of the skin involved.

Jackson has divided tissue damage into three zones in burn injuries: the central coagulation zone with complete coagulation necrosis, the stasis zone with partial coagulation at the periphery that can undergo improvement after adequate resuscitation and proper wound care, and the outer hyperemic zone caused by vasodilation. Among these three zones, the hyperemic zone can undergo spontaneous healing.

Wound healing in burn injuries can occur secondarily in epidermal and superficial-dermal burns. Superficial-dermal burns heal completely within 5-7 days and result in scar formation without contraction or hypertrophic scarring. This is due to the presence of residual epithelial cells in the hair follicles located in the superficial dermal layer.

Glutathione is a tripeptide consisting of three amino acids: glutamate, cysteine, and glycine. Glutathione has various crucial functions within cells (Jefferies et al., 2003). It plays a role in catalysis, metabolism, signal transduction, gene expression, and apoptosis. Glutathione protects tissues by neutralizing free radicals. As a strong nucleophile, reduced Glutathione safeguards cells in the body from oxidative damage (Kopal et al., 2007).

An experimental study conducted by Buz et al. (2016) on the efficacy of Glutathione mesotherapy for burn injuries in rats showed that the group using Glutathione exhibited almost complete re-epithelialization, well-developed collagen bands in the dermis, dominance of mature fibroblasts, and well-developed neovascularization areas.

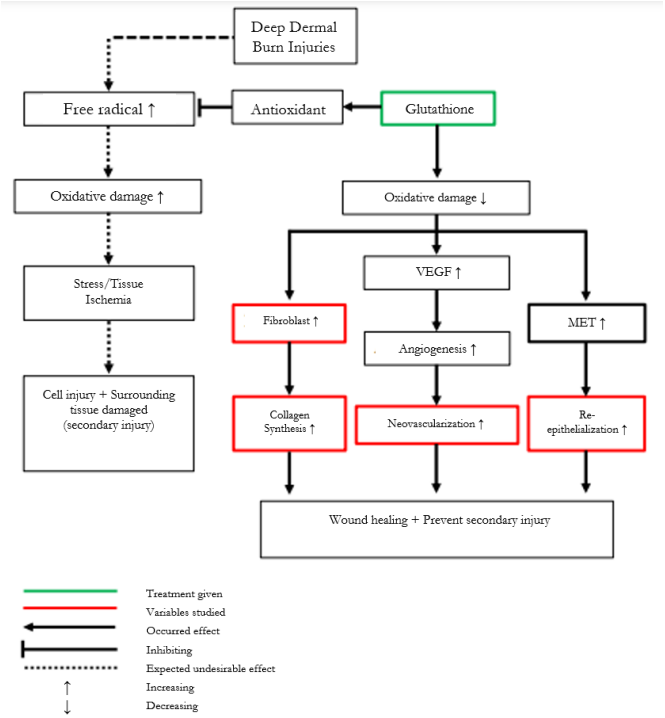

Figure 1. Conceptual Framework

The treatment provided, the variables studied, and the resulting effects are as follows: Deep dermal burn injuries lead to an increase in free radicals, causing oxidative damage that results in cell injury and damage to the tissues surrounding the burn (secondary injury). The administration of Glutathione is expected to function as an antioxidant, acting as a scavenger for free radicals, and preventing oxidative damage as well as protecting tissues from oxidative stress. Consequently, cellular capacity is anticipated to increase, inhibiting the undesirable effects.

The increased cell capacity with the administration of Glutathione is expected to enhance fibroblasts, promote neovascularization, improve re-epithelialization, and elevate collagen synthesis through heightened fibroblast activity. The ultimate desired outcome is the healing of the wound, and Glutathione is anticipated to prevent the occurrence of secondary injury.

Based on the theories and data mentioned, this study seeks to examine the effect of topical Glutathione on the healing process of deep dermal burns using male Wistar rats (*Rattus Novergicus*) as the experimental subjects. The objective is to validate the efficacy of Glutathione, with the expectation that it may offer a promising therapeutic approach for deep dermal burn management. This investigation aims to showcase the impact of topical Glutathione on the recovery of deep dermal burns in a male Wistar rat (*Rattus Novergicus*) model.

Research Hypotheses

The hypotheses proposed in this study include:

1. There is a difference in epithelial area in deep dermal burns caused by thermal contact in male Wistar rats (*Rattus Novergicus*) following administration of Glutathione.
2. There is a difference in the number of fibroblasts in deep dermal burns caused by thermal contact in male Wistar rats (*Rattus Novergicus*) following administration of Glutathione.
3. There is a difference in the amount of collagen in deep dermal burns caused by thermal contact in male Wistar rats (*Rattus Novergicus*) following administration of Glutathione.
4. There is a difference in the number of neovascularization in deep dermal burns caused by thermal contact in male Wistar rats (*Rattus Novergicus*) following administration of Glutathione.

2. Methodology

Research Design

This study employs a true experimental design using a post-test only control group design (data collection is done after the intervention), and subject groups are selected randomly. The grouping scheme is as follows (Figure 2):

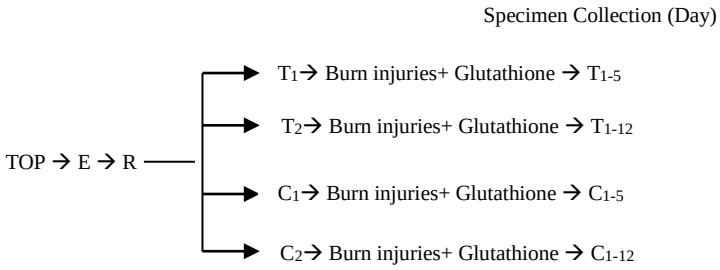

Figure 2. Research design

Explanation:
TOP: Treatment operational design
E: Experimental animals
R: Randomization
T: Treatment group
C: Control group

Experimental Animals

The experimental animals in this research are male Wistar strain rats (Rattus norvegicus) aged 3-4 months, weighing 250-300 grams, subjected to deep dermal burn injury. Each rat is housed individually, provided with the same amount and type of food and water in the Laboratory in Faculty of Pharmacy.

The inclusion criteria for this study involve male Wistar strain rats (Rattus norvegicus) aged 3 to 4 months, weighing between 250 and 300 grams. These rats should exhibit signs of good health, including active movement, thick fur, and clear eyes. The research subjects must be exclusively maintained and studied in the Pharmacy Laboratory. On the other hand, rats that have been previously used for other research or those with skin abnormalities or infections (such as redness, spots, or peeling) before treatment are excluded from the study.

Criteria for dropout from the research include a weight loss exceeding 10% during the adaptation period in the laboratory, the manifestation of illness symptoms (such as hair loss, eye discharge, or reduced activity) during the adaptation period, and instances of rats dying during the research.

The variables under investigation include fixed variables such as Glutathione and a Placebo group without Glutathione. Dependent variables encompass the epithelial area, fibroblasts, collagen, and neovascularization. Control variables involve assessing stress on the animals and monitoring the overall health conditions of the research subjects throughout the study.

Research Subjects

The research subjects are deep dermal burn wounds on rats, created by causing a 3 x 2 cm burn using a heated iron plate at a temperature of 100°C for 5 minutes, then applied to the dorsum (back) of Wistar strain rats (Rattus norvegicus) for 10 seconds, in the Faculty of Pharmacy Laboratory at Airlangga University.

Research Samples

Samples are groups of subjects under study that represent a population. In this research, there are four sample groups, namely:

1. Treatment group with deep dermal burns measuring 3 x 2 cm in rats (with topical administration of 0.2 grams (0.2 ml) of Glutathione once a day, applied daily), and specimen collection on the 5th day (D-5) post-burn (Group A1).
2. Treatment group with deep dermal burns measuring 3 x 2 cm in rats (with topical administration of 0.2 grams (0.2 ml) of Glutathione once a day, applied daily), and specimen collection on the 12th day (D-12) post-burn (Group A2).
3. Control group with deep dermal burns in rats (administered a placebo without the active ingredient Glutathione, at 0.2 grams (0.2 ml) once a day, applied daily), and specimen collection on the 5th day (D-5) post-burn (Group B1).
4. Control group with deep dermal burns in rats (administered a placebo, a cream without the active ingredient Glutathione, at 0.2 grams (0.2 ml) once a day, applied daily), and specimen collection on the 12th day (D-12) post-burn (Group B2).

Sample Size

The sample size of this study was determined based on the following formula (Charan & Kantharia, 2013):

$$n = \frac{a^2(Z_{1-\beta} + Z_{1-a})^2}{E^2}$$

Explanation:

n = Required sample size

σ = Standard deviation (Standard deviation from previous research)
E = Effect Size (Mean difference from previous research), namely $\mu 0 - \mu 1$
α = Significance level (0.05)
$Z1\text{-}\beta$ = Power (Standard > 0.8)

Based on the formula above, the calculation of the sample size is obtained as follows:

$$n = \frac{a^2(Z_{1-\beta} + Z_{1-a})^2}{E^2}$$

$$n = \frac{a^2(Z_{0,95} + Z_{0,8})^2}{(\mu_0 - \mu_1)^2}$$

$$n = \frac{13{,}914^2(1{,}96 + 1{,}84)^2}{(46{,}40 - 22{,}90)^2}$$

$$n = \frac{2.795{,}43}{552{,}25} = 5{,}06$$

From the calculation above resulting 5.06 if rounded, the sample size of each group is 6 (subjects) rats, multiplied by a correction factor of 1.3 (risk of dead rats); 6 x 1.3 = 7.8 subjects, if rounded to 8 subjects. The number of groups is 4, so the total number of subjects used in this study is 8 (subjects) x 4 (groups) = 32 rats.

Research Instruments

Assessment of the epithelial area

The assessment of the epithelial area involves measuring its extent using the imitoMeasure application on a smartphone. The imitoMeasure application is a non-contact digital planimetry application that offers advantages compared to other methods. The imitoMeasure application is a useful and practical method for area measurement with excellent accuracy (Biagioni et al., 2021). Assessment of wound dimensions with imitoMeasure is performed twice, on the D-5 and D-12. Each assessment is then documented using a smartphone camera, the imitoMeasure application, a meter for accuracy, and flash photography.

Histopathological Assessment

Histopathological assessment is used to calculate the number of fibroblasts, collagen, and neovascularization in the sample tissue, evaluated using an Olympus light microscope at 400x magnification.

Research Procedure

1. A total of 32 healthy male Wistar strain Rattus norvegicus rats aged around 3-4 months with a weight of approximately 250-300 grams were selected.
2. Each rat was assigned a number on the ear, and 32 rats were randomly selected to be divided based on specimen collection days (D-5 and D-12): 16 rats in the treatment group, 16 rats in the control group. Each group was further divided into two, resulting in a total of 4 groups. The treatment group included 8 rats (Group A1), 8 rats (Group A2), and the control group included 8 rats (Group B1) and 8 rats (Group B2).
3. Rats were anesthetized using a combination of 50 mg/kg ketamine and 5 mg/kg xylazine intramuscularly in a 3cc syringe (Zohdi et al., 2012).
4. Each rat was shaved on the dorsum (back), and a design for a burn wound measuring 3 x 2 cm was drawn on the dorsum.
5. Disinfection was performed with a 10% povidone iodine solution followed by Savlon: NaCl 0.9%, then rinsed with NaCl 0.9%.

6. A deep dermal burn wound was created using a heated brass bar measuring 3 x 2 cm, heated on a hot plate at 100° for 5 minutes, and then applied to the dorsum (back) of the rat for 10 seconds.
7. Wounds in the A1 group (treatment) were treated with Savlon and NaCl 0.9% 1:30, followed by the application of 2% Glutathione topical cream at 0.2 grams (0.2 ml) once a day for 5 days, and then covered with a transparent dressing.
8. Wounds in the A2 group (treatment) were treated with Savlon and NaCl 0.9% 1:30, followed by the application of 2% Glutathione topical cream at 0.2 grams (0.2 ml) once a day for 12 days, and then covered with a transparent dressing.
9. Wounds in the B1 group (control) were treated with a placebo, a topical cream without the active ingredient Glutathione, at 0.2 grams (0.2 ml) once a day for 5 days, and then covered with a transparent dressing.
10. Wounds in the B2 group (control) were treated with a placebo, a topical cream without the active ingredient Glutathione, at 0.2 grams (0.2 ml) once a day for 12 days, and then covered with a transparent dressing.
11. One rat was kept in each cage, provided with the same amount and type of food and drink in the Faculty of Pharmacy Laboratory.
12. Tissue specimens were collected from Group A1 and B1 on day 5, and Group A2 and B2 on day 12.
13. The skin was excised above the fascia, including 0.5 cm of healthy tissue around the wound. This aseptic procedure was performed, and epithelialization area on the skin was measured using the imitoMeasure application.
14. The excised skin tissue was stored in vials containing 10% Neutral Buffered Formalin (NBF) for histopathological examination in the Department of Anatomy Pathology.
15. Rats were euthanized by injecting 60-100 mg/kg of intraperitoneal Pentobarbital in the slightly mid-lateral area between the xyphoid process and pubic tubercle. Euthanasia was performed after skin tissue specimen collection.

Location and Research

This research was conducted at the Animal Testing Laboratory of the Faculty of Pharmacy, Universitas Airlangga, in December 2022 for the maintenance and treatment of experimental animals. The evaluation and collection of histopathological data on specimens were carried out at the Department of Anatomy Pathology, Faculty of Medicine, Universitas Airlangga, in January 2023.

Research Workflow

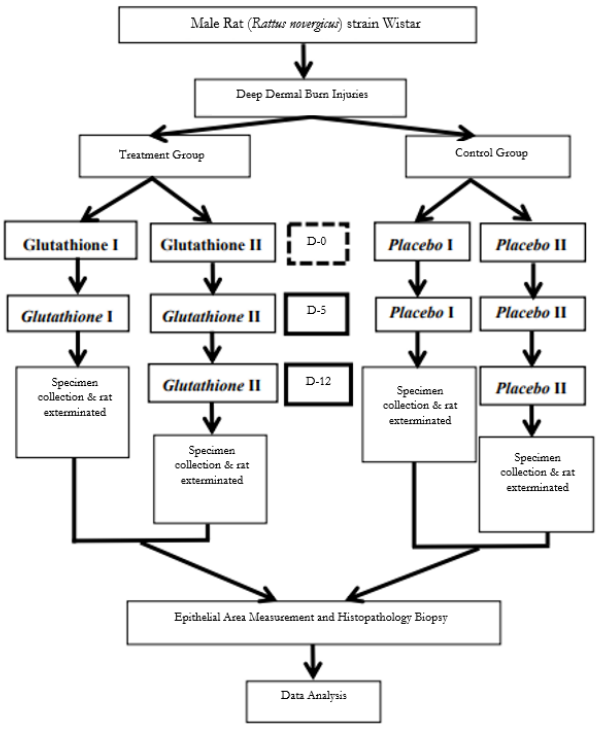

Figure 3. Research flowchart

Data Processing and Analysis

Data was collected in the form of a table, including sample numbers, treatments, and observation results containing epithelialization area data, the number of fibroblasts, collagen, and the amount of neovascularization on D-5 and D-12. The collected data was then processed with two measured groups. The first group compared the treatment group on D-5 with the control group on D-5, while the second group compared the treatment group on D-12 with the control group on D-12. Both groups were measured with Δ, using the Independent T-Test.

The Independent T-Test is employed to determine whether there is a difference in the mean of two unrelated samples. This test is a parametric statistical test where the data used are ratio-scale data.

Hypotheses and the decision-making basis for the Independent T-Test:

• *Hypotheses*

H0 = There is no difference between treatment and control groups

H1 = There is a difference between treatment and control groups

• *Decision-Making Basis*

1. If the Sig. value (2-tailed) < 0.05, then H0 is rejected, and H1 is accepted.
2. If the Sig. value (2-tailed) > 0.05, then H0 is accepted, and H1 is rejected.

3. ResultsandDiscussion

Laboratory experimental research with a post-testcontrolled group design was conducted to demonstrate the role of topical Glutathione in the healing process of deep dermal burns in male Wistar rats (Rattus norvegicus). The research population comprised 32 subjects grouped into 2 categories: 16 subjects in the treatment group observed on D-5 and D-12, and 16 subjects in the control group observed on D-5 and D-12.

The researcher intervened in the treatment group with topical Glutathione application on the skin subjected to measured deep dermal burns. Meanwhile, the control group received burn wound treatment on the skin of male Wistar rats (Rattus norvegicus) using a placebo (cream without the active ingredient Glutathione). The data results were documented in a

table and then measured using the Independent T-Test.

Epithelialization Comparison

The epithelial area variable was obtained through the comparison of wound areas on D-5 and D-12, measured using the wound measurement application, imitoMeasure. In Figure 4, for one sample from the treatment group, the wound area on D-0 was 5.32 cm². The sample was then treated with topical Glutathione application daily until D-5, and upon reevaluation on D-5, the wound area was 5.07 cm². The same treatment was continued daily until D-12, resulting in a wound area of 2.44 cm².

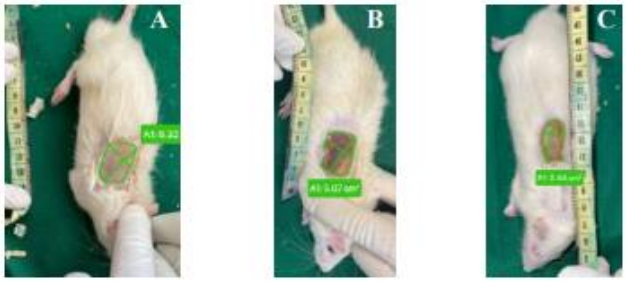

Figure 4. Wound Area of Treatment Group Samples (A. Wound Area at D-0 (5.32 cm²). B. Wound Area at D-5 (5.07 cm²). C. Wound Area at D-12 (2.44 cm²))

In Figure 5 below, for one sample from the control group, the wound area at D-0 was 4.92 cm². The sample was then treated with the application of placebo cream (without the active ingredient Glutathione) daily until D-5, and upon reevaluation on D-5, the wound area was 4.9 cm². The same treatment was continued daily until D-12, resulting in a wound area of 3.07 cm².

Figure 5. Wound Area of Control Group Samples A. Wound Area at D-0 (4.92 cm²). B. Wound Area

Table 1. Wound Area of the Treatment Group

Treatment (A)			
Subject Number (Rat)	**RS D-0 (cm²)**	**RS D-5 (cm²)**	**RS D-12 (cm²)**
1	5,32	5,07	2,44
2	5,75	5,2	2,25
3	4,88	4,78	3,1
4	3,37	2,94	2,5
5	2,99	2,19	0,7
6	4,36	2,73	1,85
7	4,59	4,44	2,19
8	4,17	3,55	2,99
9	4,59	3,21	-
10	4,79	4,33	-

Table 1. Wound Area of the Treatment Group

Treatment (A)			
Subject Number (Rat)	**RS D-0 (cm^2)**	**RS D-5 (cm^2)**	**RS D-12 (cm^2)**
11	4,57	2,99	-
12	4,6	4,16	-
13	4,94	4,81	-
14	3,63	2,51	-
15	4,86	4,56	-
16	4,44	2,99	-
Total	71,85	60,46	18,02
Average	4,490625	3,77875	2,2525

In Table 1, the wound area in the treatment group was determined for 16 research rat subjects subjected to burn wound treatment with a heated plate on the dorsum of the rat's back. Thus, at D-0, the average wound area was 4.49 cm^2. Subsequently, topical Glutathione application was administered daily, resulting in a reduced wound area on D-5, with an average of 3.77 cm^2 observed in 16 rat subjects. Eight of them were then sacrificed for microscopic observation. The remaining 8 subjects continued to receive topical Glutathione application daily until D-12, and their observed wound area further decreased to 2.25 cm^2.

Table 2. Wound area of the control group

Control (B)			
Subject Number (Rat)	**RS D-0 (cm^2)**	**RS D-5 (cm^2)**	**RS D-12 (cm^2)**
1	5,47	5,54	3,65
2	5,52	5,14	4,56
3	3,42	2,29	4,62
4	3,49	3,14	3,59
5	4,92	4,9	3,07
6	5,09	5,41	4,08
7	6,43	5,95	2,57
8	4,22	4,27	2,83
9	4,26	4,45	-
10	4,59	4,63	-
11	4,62	4,66	-
12	5,15	5,53	-
13	3,83	3,32	-
14	4,21	4,24	-
15	5,01	5,15	-
16	4,2	3,74	-
Total	74,43	72,36	28,97
Average	4,651875	4,5225	3,62125

In Table 2, the wound area in the control group was determined for 16 research rat subjects subjected to burn wound treatment with a heated plate on the dorsum of the rat's back. Thus, at D-0, the average wound area was 4.65 cm^2. Subsequently, placebo application (cream without the active ingredient Glutathione) was administered daily, resulting in a reduced wound area on D-5, with an average of 4.52 cm^2 observed in 16 rat subjects. Eight of them were then sacrificed for microscopic observation. The remaining 8 subjects continued to receive placebo application daily until D-12, and their observed wound area further decreased to 3.62 cm^2.

Comparison of Fibroblasts and Vascularization

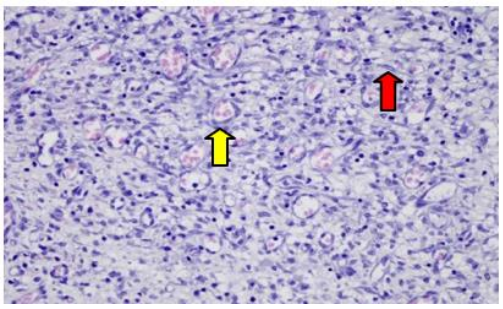

Figure 6. Microscopic observation of treatment group samples D-5 of the study at 400x magnification. (Yellow arrow blood vessels; Red arrows fibroblast cells)

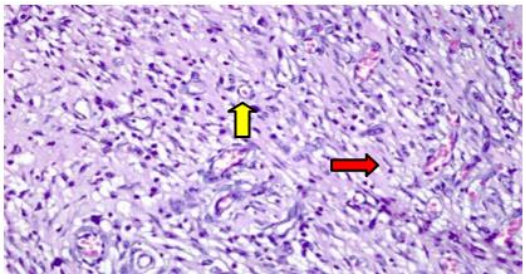

Figure 7. Microscopic observation of control group sample D-5 of the study at 400x magnification. (Yellow arrow blood vessels; Red arrows fibroblast cells)

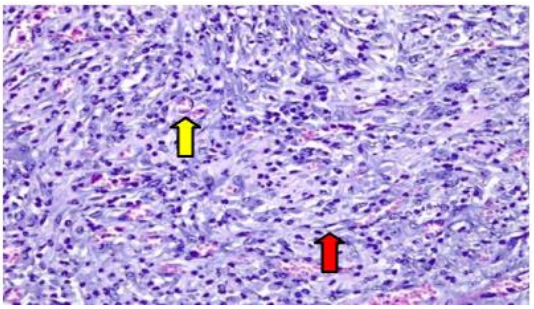

Figure 8. Microscopic observation of the sample of the treatment group D-12 of the study with 400x magnification. (Yellow arrow blood vessels; Red arrow fibroblast cells)

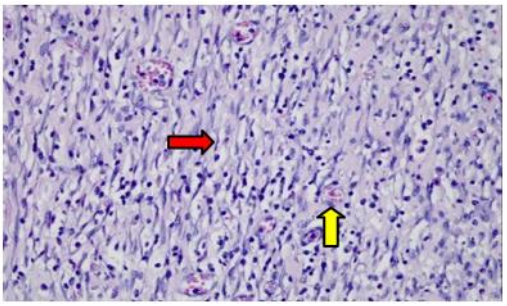

Figure 9. Microscopic observation of the treatment group sample D-12 of the study at 400x magnification. (Yellow arrows of blood vessels; Red arrow fibroblast cells)

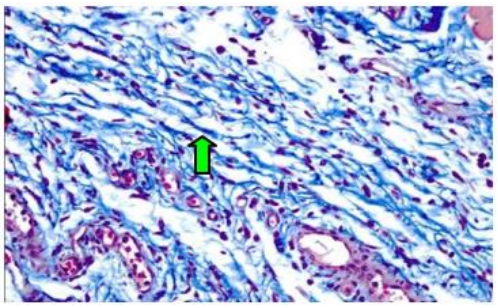

Figure 10. Microscopic observation of treatment group samples, 400x magnification. Scoring Collagen +1 (less than 10% per field of view). (Green arrows represent collagen)

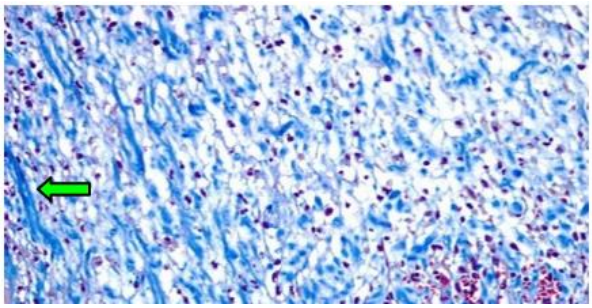

Figure 11. Microscopic observation of control group samples, 400x magnification. Collagen +2 scoring (10% to 50% per field of view). (Green arrows represent collagen)

Analysis

Table 3. Comparison of Epithelialization Area

Group	Description	Wound Area on D-0 (cm^2)	Epithelialization Area on D-5 (cm^2)	Epithelialization Area on D- 12 (cm^2)
Treatment (A)	Total (16 Rat)	71,85	11,39	53,83
	Average	4,490625	0,711875	2,238125
Control (B)	Total (16 Rat)	74,43	2,07	45,46
	Average	4,651875	0,129375	1,030625

The observed variables in this study are the speed of epithelialization area, the number of fibroblasts, and the number of neovascularization. For the epithelialization area speed variable, the wound area was measured using the imitoMeasure wound measurement application. In Table 3, at D-0, burn wounds were created on the dorsum (back) of the rats, with an average wound area per rat in the treatment group being 4.49 cm and in the control group being 4.65 cm^2. The development of the epithelialization area was measured by subtracting the burn wound area at D-0 from the wound area at D-5 and D-12. The epithelialization area at D-5 in the treatment group had an average per rat of 0.71 cm^2, while in the control group, the epithelialization area was 0.12 cm^2. At D-12, the epithelialization area in the treatment group per rat was 2.23 cm^2, and in the control group, it was 1.03 cm^2.

Based on the results of data analysis using the Independent T-Test with the assistance of SPSS 27 software, the following results were obtained.

Table 4. Wound area test results in D-0

Group	Mean ± SD	Mean difference	Sig.
Treatment	4.4906 ± 0.69581	-0.610	0.434
Control	4.6519 ± 0.79634		

Based on the table 4, it is obtained that the average value of the treatment group is 4.4906 with a standard deviation of 0.69581. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 4.6519 with a standard deviation of 0.79634. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is -0.610, and since this value is negative, it indicates that the treatment value is smaller than the control group value by 0.743. Additionally, a Sig. value of 0.434 > 0.05 is obtained, thus it can be concluded that there is no significant difference in the D-0 wound area values between the treatment and control groups.

Table 5. Wound Area Test Results in D-5

Group	Mean ± SD	Mean difference	Sig.
Treatment	3.778 ± 0.993	-0.743	0.043
Control	4.522 ± 0.997		

Based on the table 5, it is obtained that the average value of the treatment group is 3.778 with a standard deviation of 0.993. Since the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 4.522 with a standard deviation of 0.997. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is -0.743, and since this value is negative, it indicates that the treatment value is smaller than the control group value by 0.743. Additionally, a Sig. value of 0.043 < 0.05 is obtained, thus it can be concluded that there is a significant difference in values between the treatment and control groups.

Table 6. Wound area test results in D-12

Group	Mean ± SD	Mean difference	Sig.
Treatment	2.495 ± 1.011	-1.125	0.022
Control	3.621 ± 0.767		

Based on the table 6, it is obtained that the average value of the treatment is 2.495 with a standard deviation of 1.011. Since the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 3.621 with a standard deviation of 0.767. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is -1.125, and since this value is negative, it indicates that the treatment value is smaller than the control group value by 1.125. Additionally, a Sig. value of 0.022 < 0.05 is obtained, thus it can be concluded that there is a significant difference in values between the treatment and control groups.

Table 7. Fibroblast Test Results in D-5

Group	Mean ± SD	Mean difference	Sig.
Treatment	36.875 ± 2.941	21.775	0.000
Control	15.100 ± 2.937		

Based on the table 7, it is obtained that the average value of the treatment is 36.875 with a standard deviation of 2.941. Since the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 15.100 with a standard deviation of 2.937. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is 21.775, and since this value is positive, it indicates that the treatment value is greater than the control group value by 21.775. Additionally, a Sig. value of 0.000 < 0.05 is obtained, thus it can be concluded that there is a significant difference in values between the treatment and control groups.

Table 8. Fibroblast Test Results in D-12

Group	Mean ± SD	Mean difference	Sig.
Treatment	65.875 ± 4.319	20.975	0.000
Control	44.900 ± 2.092		

Based on the table 8, it can be seen that the average value for the treatment group is 65.875 with a standard deviation of 4.319. Since the average > standard deviation, it can be concluded that the average value effectively represents the data. In the control group, the average value is 44.900 with a standard deviation of 2.092, where the average > standard deviation, affirming that the average value adequately represents the data. The mean difference is 20.975, and since this value is positive, it indicates that the treatment group has a higher value compared to the control group by 20.975. Additionally, the obtained Sig. value is 0.000 < 0.05, leading to the conclusion that there is a significant difference in values between the treatment and control groups.

Table 9. Vascularization Test Results in D-5

Group	Mean ± SD	Mean difference	Sig.
Treatment	37.375 ± 1.148	22	0.000
Control	15.375 ± 2.682		

Based on the table 9, it is obtained that the average value of the treatment is 37.375 with a standard deviation of 1.148. Since the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 15.375 with a standard deviation of 2.682. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is 22, and since this value is positive, it indicates that the treatment value is greater than the control group value by 22. Additionally, a Sig. value of 0.000 < 0.05 is obtained, thus it can be concluded that there is a significant difference in values between the treatment and control groups.

Table 10. Vascularization Test Results in D-12

Group	Mean ± SD	Mean difference	Sig.
Treatment	46.650 ± 3.475	15.100	0.000
Control	31.550 ± 3.116		

Based on the table 10, it is obtained that the average value of the treatment is 46.650 with a standard deviation of 3.475. Since the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 31.550 with a standard deviation of 3.116. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is 15.100, and since this value is positive, it indicates that the treatment value is greater than the control group value by 15.100.

Additionally, a Sig. value of 0.000 < 0.05 is obtained, thus it can be concluded that there is a significant difference in values between the treatment and control groups.

Table 11. Collagen Test Results in D-5

Group	Mean ± SD	Mean difference	Sig.
Treatment	1.900 ± 0.213	0.800	0.000
Control	1.100 ± 0.151		

Based on the table 11, it is obtained that the average value of the treatment is 1.900 with a standard deviation of 0.213. Since the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 1.100 with a standard deviation of 0.151. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is 0.800, and since this value is positive, it indicates that the treatment value is greater than the control group value by 0.800. Additionally, a Sig. value of 0.00 < 0.05 is obtained, thus it can be concluded that there is a significant difference in values between the treatment and control groups.

Table 12. Collagen Test Results in D-12

Group	Mean ± SD	Mean difference	Sig.
Treatment	2.425 ± 0.291	0.925	0.001
Control	1.500 ± 0.534		

Based on the table 12, it is obtained that the average value of the treatment is 2.425 with a standard deviation of 0.292. Since the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. In the control group, the average value is 1.500 with a standard deviation of 0.534. As the average value is greater than the standard deviation, it can be concluded that the average value can be used to represent the data. The mean difference is 0.925, and since this value is positive, it indicates that the treatment value is greater than the control group value by 0.925. Additionally, a Sig. value of 0.00 < 0.05 is obtained, thus it can be concluded that there is a significant difference in values between the treatment and control groups.

Discussion

This study investigated four variables: epithelialization measured by wound area, the second variable being fibroblasts, the third variable being vascularization, and the fourth variable being collagen. All four variables were tested at D-5 and D-12 of the study, after the burned skin of the rats was treated with Glutathione in the treatment group and given a placebo in the control group.

For the epithelialization variable, the wound area was measured at D-0, and a Sig. value of 0.434 > 0.05 was obtained, indicating no difference in the wound area between the treatment and control groups at D-0 (D-0 being the first day the rat samples were subjected to burn injury with the rat's back burned with a hot plate). It was concluded that the wound area in the burn injury application in the treatment group and the control group was the same.

In the D-5 wound area test, a Sig. value of 0.043 < 0.05 was obtained, and a mean difference of -0.743 was found. In the D-12 wound area test, a Sig. value of 0.022 < 0.05 was obtained, and a mean difference of -1.125 was found. The test results indicate that there is a difference in values between the treatment group and the control group, with the wound area in the treatment group (in samples given Glutathione) being smaller than the wound area in the control group (samples given a placebo) at both D-5 and D-12 of the study. This is consistent with previous research conducted by Kopal et al., indicating that the treatment group with Glutathione application shows complete wound healing and epithelialization

(Kopal et al., 2007).

In the D-5 fibroblast observation test, a treatment value was obtained greater than the control value with a mean difference of 21.775 and a Sig. value of 0.000 < 0.05. In D-12, a treatment value was obtained greater than the control value with a mean difference of 0.975 and a Sig. value of 0.000 < 0.05. The test results indicate that there is a difference in values between the treatment group and the control group, with the observation of the number of fibroblasts in the treatment group being greater than in the control group, at both D-5 and D-12 of the study.

In the D-5 vascularization observation test, a mean difference of 22 was obtained, and a Sig. value of 0.000 < 0.05 was obtained, indicating a difference in values between the treatment group and the control group. In D-12, a mean difference of 15.100 was obtained, and a Sig. value of 0.000 < 0.05 was obtained. The test results indicate that there is a difference in values between the treatment group and the control group, with the observation of the number of vascularizations in the treatment group being greater than in the control group, at both D-5 and D-12 of the study.

In the D-5 collagen observation test, a value of 0.800 was obtained, and a Sig. value of 0.000 < 0.05 was obtained. In D-12, a mean difference of 0.925 was obtained, and this value is positive, indicating that the treatment value is greater than the control value by 0.925. Additionally, a Sig. value of 0.001 < 0.05 was obtained. The test results indicate that there is a difference in values between the treatment group and the control group, with the observation of the amount of collagen in the treatment group being greater than in the control group, at both D-5 and D-12 of the study.

The results of this study are consistent with the research conducted by Buz et al. in 2016, which found that the Glutathione group significantly outperformed other treatment groups in terms of increased maturation of fibroblasts, increased collagen deposition, re-epithelialization, and neovascularization.

4. Conclusion

In the initial phase of the study (D-0), there was no discernible difference in wound area between the treatment group and the control group. However, on both D-5 and D-12, it was evident that the treatment group exhibited a smaller wound area compared to the control group. This substantiates the conclusion that Glutathione has the potential to expedite the epithelialization process in burn wounds. Furthermore, the administration of Glutathione demonstrated a notable increase in the number of fibroblasts, vascularization, and collagen on both D-5 and D-12. The data revealed a higher quantity of these crucial elements in the treatment group, indicating the positive impact of Glutathione in enhancing the healing process.

Moving forward, it is advisable to conduct additional research to validate the efficacy of Glutathione in alternative formulations, such as injections or infiltrations, utilizing varying doses. This exploration aims to ascertain whether a concentrated form of Glutathione can exert its effects without encountering barriers presented by burn wounds. Moreover, future investigations should encompass a more extensive set of observation parameters and explore novel aspects, such as collagen thickness and the presence of growth factors in burn wounds. Extending the duration of the study could provide more comprehensive insights, substantiating the significant influence of Glutathione on wound healing.

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Conflicts of interest

There are no conflicts of interest.

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