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**EFFECT OF MORINGA OLEIFERA LEAF EXTRACT GEL ON FIBROBLAST IN
DIABETIC TRAUMATIC ULCER**

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a metabolic disease that impairs wound healing, including traumatic ulcers in the oral mucosa, by suppressing fibroblast proliferation. **Purpose:** This study aims to evaluate the effect of Moringa oleifera leaf extract gel on the number of fibroblasts during the healing process of traumatic ulcers in diabetic Wistar rats. **Methods:** This experimental study used a post-test only control group design. A total of 8 groups (n=4 rats/group) were observed across four time intervals (days 3, 5, 7, and 9). The rats received applications of either 15% Moringa oleifera leaf extract gel or 3% Na-CMC gel (control) three times daily. Labial mucosal tissues were harvested, and fibroblast counts were evaluated using Hematoxylin and Eosin staining. Data were analyzed using the Mann-Whitney U test. **Results:** The mean fibroblast ratios between the 15% Moringa gel and 3% Na-CMC gel groups were: day 3 (3.1 vs. 3.0), day 5 (4.3 vs. 3.85), day 7 (3.75 vs. 5.3), and day 9 (7.4 vs. 6.05). Statistical analysis revealed no significant differences on days 3, 5, and 9, whereas a statistically significant difference was observed on day 7. Antioxidant and anti-inflammatory compounds in Moringa leaves—such as flavonoids, phenolics, and vitamin C—reduce free radicals and inhibit pro-inflammatory cytokines, enhancing fibroblast survival. Additionally, Moringa oleifera promotes TGF- β 1 and VEGF expression, stimulating fibroblast proliferation and angiogenesis. **Conclusion:** Application of Moringa oleifera on days 3, 5 and 9 resulted in higher fibroblast counts than in the control group, except on day 7.

Keywords: Diabetes, Fibroblasts, Male wistar rats, Moringa leaf, Traumatic ulcers.

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INTRODUCTION

Oral health can be assessed by evaluating the health of both soft and hard tissues through the detection of pathological conditions in the tissues as indicators of health abnormalities. One such condition is the formation of traumatic ulcers in the oral cavity¹. Traumatic ulcers are oral cavity lesions associated with trauma that frequently occur on the buccal mucosa (42%), tongue (25%), and lower labial mucosa (9%). This occurs due to mechanical trauma such as biting and irritation from sharp objects, as well as from hot food and beverages. Traumatic ulcers appear as an erythematous area surrounding a yellow fibrinopurulent membrane that can be scraped off². Healing of traumatic ulcers takes approximately 10–14 days if the causative factor is eliminated; however, they can potentially interfere with normal oral function if left untreated. The body's natural healing mechanisms become active after a traumatic ulcer disrupts the continuity of the affected tissue. There are three distinct stages in the ulcer healing process: the inflammatory phase, the proliferative phase, and the maturation³.

The healing process for traumatic ulcers differs slightly from that of normal ulcers. The healing process can be significantly influenced by various medical conditions. For example, hyperglycemia is a hallmark of the metabolic disorder diabetes mellitus, which can lead to other complications, one of which is delayed ulcer healing⁴. Hyperglycemia causes cellular dysfunction, including reduced fibroblast activity and impaired angiogenesis. Ulcer healing in individuals with diabetes is disrupted by chronic hyperglycemia, which affects various aspects of tissue regeneration. According to Pratiwi⁽⁵⁾, hyperglycemia can increase levels of pro-inflammatory cytokines such as *tumor necrosis factor-alpha* (TNF- α) and interleukin-6 (IL-6), which cause prolonged inflammation and slow down fibroblast migration and proliferation. Additionally, research conducted by Wisesa⁶ shows that angiogenesis in diabetic patients is impaired due to reduced endothelial cell function, resulting in suboptimal oxygen and nutrient supply to the ulcer site. The combination of these factors makes patients more susceptible to infection, at risk of serious complications, and generally results in ulcers in diabetic patients being more difficult to heal. This also applies to the healing process of traumatic ulcers. Certain systemic conditions, such as diabetes, require consideration of several important factors, given that diabetes limits patients' ability to undergo optimal angiogenesis during ulcer healing.

Topical corticosteroids are one of the preferred treatments for traumatic ulcers.. Topical corticosteroids should be used in the form of ointments or gels because they act quickly and are easy to apply. The analgesic, anti-inflammatory, and anti-allergic effects of triamcinolone acetonide make it a popular topical corticosteroid for relieving pain and accelerating wound healing. However, this medication can cause a number of side effects if used regularly⁷. Corticosteroids also have side effects for people with diabetes. In patients with diabetes, corticosteroids can worsen glycemic control by increasing the risk of hyperglycemia and diabetic ketoacidosis. Patients with type 1 diabetes are more susceptible to diabetic ketoacidosis due to spikes in glucose levels triggered by corticosteroids. In patients with type 2 diabetes, corticosteroids worsen insulin resistance, thereby increasing the need for insulin therapy or other antidiabetic agents⁸.

These considerations, along with the lack of sufficient scientific evidence regarding specific treatments to support the healing process of ulcers in diabetic patients, have prompted the author to explore other alternatives for treating traumatic ulcers that can also have a positive impact on the condition of diabetic patients. Various other types of treatment have also emerged as alternatives to corticosteroids, namely herbal-based treatments. One plant chosen as an alternative treatment is the moringa tree (*Moringa oleifera*). The antioxidant properties of moringa leaves (*Moringa oleifera*) can help reduce the risk of hyperglycemia and hyperlipidemia associated with type 2 diabetes mellitus⁹. The flavonoids present in moringa leaves (*Moringa oleifera*) have the potential to lower blood glucose levels through the mechanism of s as antioxidants. These compounds function to inhibit beta cell apoptosis without affecting pancreatic beta cell proliferation. Additionally, flavonoids can scavenge free radicals, contributing to the reduction of insulin resistance. One of the main flavonoids, quercetin, plays a role in reducing the absorption of glucose and fructose in the intestines, thereby having a positive effect on lowering blood glucose levels.¹⁰

Moringa leaf extract has been shown to accelerate wound healing in several studies, including a study by Zakiyah et al., 2019 which found that ethanol extract from moringa leaves accelerates the healing of second-degree burns in mice.¹¹ Additionally, this is supported by research conducted by Herdiani⁽¹²⁾ who noted that *Moringa oleifera* leaf extract in liquid accelerates ulcer healing in rats, particularly when administered at a 15% concentration. However, no studies have yet examined the administration of moringa leaf extract in gel form to Wistar rats with traumatic ulcers and diabetes. Based on the problem statement and previous studies, research using diabetic rats to observe the ulcer healing process remains scarce.

The objective of this study is to determine the effect of moringa leaf gel extract (*Moringa oleifera*) at a 15% concentration on the increase in the number of fibroblasts during the healing of traumatic ulcers in male Wistar rats (*Rattus norvegicus*) with diabetes.

MATERIALS AND METHODS

This study began after obtaining *ethical clearance* from the Research Ethics Committee of Mahasaraswati University, Denpasar, No. 03.0007/KEP-Unmas/VII/2025. This was followed by the preparation of moringa leaf extract and gel formulations, which was carried out at the Laboratory of the Faculty of Agriculture, Warmadewa University, and experimental research at the Biocore Laboratory located at Jalan Raya Puputan No. 26 A, East Denpasar. The materials used in this study were *streptozotocin* (STZ), 15% moringa leaf

extract gel, 96% ethanol, *xylene*, sterile distilled water, alcohol, 3% NA-CMC, sterile cotton, ketamine anesthetic, 10% formaldehyde solution, male Wistar rats, standard rat feed, citrate buffer solution, and 10% sucrose/dextrose. In addition, this study also describes the equipment used, namely: a 5 mm diameter round *burnisher*, an electric light microscope, a measuring cylinder, a digital scale, a *rotary evaporator*, a Buchner funnel, a blender, an Optilab Pro camera, HPA slides, microscope slides, a 10 ml syringe, surgical forceps, surgical scissors, plastic filling material, Nierbeken, labels, mouse marking pens, glucometer, Bunsen burner, matches, spirit burner, cotton swabs, medical masks and gloves, aluminum foil, animal cages, and a rotary microtome. A total of 14 types of materials and 20 types of equipment will be used to support the research, ensuring that the research procedures can be carried out smoothly.

This study employs an in vivo laboratory experimental design using a Post-test Only Control Group Design. This study began with the preparation of a moringa leaf extract gel. The process of formulating the 15% moringa leaf extract gel began by weighing 10 grams of a 3% NA-CMC gel base using a digital scale. Next, 1.5 grams of moringa leaf extract solution were added to the gel base, which was then stirred thoroughly until a homogeneous gel formulation was formed. The study sample comprises normal rats induced with *Streptozotocin* (STZ) to induce a diabetic condition. The dose administered was 150 mg/kg of body weight. Wistar Rats were considered diabetic if their fasting glucose level was 126 mg/dL. In this study, the Wistar Rats were diabetic on the second day after induction. traumatic ulcers were induced in the mice by treating the labial mucosa with a 5-mm-diameter burnisher heated with a spirit burner for 60 seconds.¹⁶

The sample size was determined using *Federer's* formula, with assigned to 4 treatment groups and 4 control groups. This resulted in an ideal sample size of 4 or more experimental animals per group, yielding a total sample size of 32 rats. The sampling technique used in this study was *Purposive Sampling*, with inclusion and exclusion criteria. The independent variable in this study was a 15% moringa leaf extract in gel formulation, and the dependent variable was the number of fibroblasts in the healing process of traumatic ulcers in male Wistar rats with diabetes. The instrument used in this study was an Anatomical Histopathology (HPA) preparation, which was examined on days 3, 5, 7, and 9.

The research results were analyzed by counting the number of fibroblasts visible in the histological preparations using a ratio scale, yielding quantitative data. The collected data were processed using the *Statistical Product and Service Solutions* (SPSS) software. The tests used were the *Saphiro-wilk* normality test because of the sample size <50 and the *Levene's test* for homogeneity. After confirming that the data were normally distributed and homogeneous, parametric tests were performed using a two-way ANOVA to determine whether there were differences between the treatment groups. Subsequently, a *Tukey post hoc* test was conducted to identify differences among the treatments. If the test results are not normally distributed, then a nonparametric test—specifically, the Mann-Whitney U test—is used.



Figure 1. (A) Traumatic ulcers formed on the labial mucosa of male Wistar rats
(B) Application of moringa leaf extract gel on male Wistar rats

RESULT

The research results were obtained after conducting research at the Faculty of Agriculture Laboratory, Warmadewa University, and experimental research at the Biocore Laboratory, with the aim of determining the effect of *Moringa oleifera* leaf gel extract at a 15% concentration on the increase in the number of fibroblasts in

the healing of traumatic ulcers in male Wistar rats (*Rattus norvegicus*) with diabetes. The following data were obtained:

Table 1. Results of descriptive analysis

	N	Minimum	Maximum	Mean	Std. Deviation
P1	4	2.40	4.20	3.1000	.87178
P2	4	3.80	5.20	4.3000	.66332
P3	4	3.40	4.00	3.7500	.25166
P4	4	3.80	11.40	7.4000	3.76652
K1	4	2.20	4.40	3.0000	.96609
K2	4	2.40	5.60	3.8500	1.43643
K3	4	4.20	6.60	5.3000	1.03923
K4	4	4.60	8.20	6.0500	1.54380

Valid N

32

(listwise)

Note: N = Number of Treatments; Mean = Average; Std. Deviation = standard deviation.

Table 1 shows that the mean fibroblast counts in the treatment groups were 3.10 for P1 (15% concentration for 3 days), 3.75 for P2 (15% concentration for 7 days), and 7.40 for P4 (15% concentration for 9 days). These results indicate that the lowest mean value was found in group P1, while the highest mean value was found in group P4. Furthermore, in the control groups, the means were K1 (3.00), K2 (3.85), K3 (5.30), and K4 (6.05).

Table 2. Normality Test Results

		Tests of Normality					
		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
fibroblas	Group	.319	16	<.001	.679	16	<.001
	Group	.135	16	.200*	.953	16	.531

Based on the results of the normality test, the Sig value for Group P was <0.001, indicating that the data for Group P are not normally distributed; whereas for Group K, the Sig value was >0.05, indicating that the data for Group K are normally distributed.

Table 3. Homogeneity test results

	Levene Statistic	df1	df2	Sig.
Based on Mean	0.247	1	30	0.623
Based on Median	0.000	1	30	0.984
Based on Median				
FIBROBLAST	0.000	1	20,86	0,984
and with adjusted d				
Based on trimmed				
Mean	0,057	1	30	0,811

Based on Table 3 of the results of the Levene's Test for homogeneity of variances above, it is known that the value of Based on Mean in this study is $0.623 > 0.05$. Therefore, it can be concluded that the variances of the data for all groups in this study are homogeneous. Because one of the groups was not normally distributed, the conditions for a parametric test were not met, so a nonparametric test was conducted instead. In this study, the nonparametric test used was the Kruskal-Wallis test.

Table 4. Results of the Mann-Whitney U test

Day	Group P vs Group K		
	Mann-Whitney U	Z	Nilai Asymp. Sig. (2-tailed)
3	8,00	0,000	1,000
5	6,50	-0,438	0,661
7	0,00	-2,323	0,020
9	7,50	-0,145	0,885

Based on Table 4 of the Mann Whitney U results above, The results of the Mann-Whitney U test showed a U value of 8.000 with an Asymp. Sig. (2-tailed) value of 1.000 ($p > 0.05$). This indicates that on Day 3, there was no significant difference in the number of fibroblasts between Group P and Group K. On Day 5, the results of the Mann-Whitney U test showed a U value of 6.500 with an Asymp. Sig. (2-tailed) value of 0.661 ($p > 0.05$). This indicates that there was no significant difference in the number of fibroblasts between the two groups. On Day 7, the results of the Mann-Whitney U test showed a U value of 0.000 with an Asymp. Sig. (2-tailed) of 0.020 ($p < 0.05$), indicating a significant difference in the number of fibroblasts between Group P and Group K. On day 9, the results of the Mann-Whitney U test showed a U value of 7.500 with an Asymp. Sig. (2-tailed) value of 0.085 ($p > 0.05$), indicating that the fibroblast counts in both groups were again not significantly different.

DISCUSSION

In this study, diabetes was induced in the experimental animals using STZ. The mechanism of action of STZ involves the formation of free radicals that have the potential to damage pancreatic beta cells, thereby disrupting insulin production. STZ enters pancreatic beta cells via *glucose transporter 2* (GLUT 2) and causes alkylation of DNA¹⁴. In this study, fasting blood glucose measurements in all STZ-induced rats showed fasting blood glucose levels above 126 mg/dL, indicating blood glucose levels exceeding the normal range¹⁵. Subsequently, in the group of rats that had stabilized following STZ induction, the process of creating a traumatic ulcer was performed. The traumatic ulcer was created by applying a 5-mm burnisher, heated for 60 seconds over a Bunsen burner, to the lower labial mucosa of male Wistar rats; the ulcer on the rat's labial mucosa formed 24 hours after the traumatic ulcer was created¹⁶. Traumatic ulcers in the context of diabetes have a longer wound healing process, as diabetes or hyperglycemia leads to cellular dysfunction, including reduced fibroblast activity and impaired angiogenesis. As shown by Wisesa's research⁶, angiogenesis in diabetic patients is disrupted due to impaired endothelial cell function, resulting in suboptimal oxygen and nutrient supply to the ulcer site. This combination makes patients more susceptible to infection, at risk of serious complications, and ulcers in diabetic patients typically heal more slowly.

In this study, a 15% moringa leaf extract gel was used, based on a previous study by Herdiani¹² that compared the use of 5%, 10%, and 15% concentrations. The 15% concentration proved effective in healing when formulated as a topical gel and applied directly to the ulcer site three times daily—morning, afternoon, and night—for nine days. This study employed three daily applications of the moringa leaf extract gel to ensure sustained anti-inflammatory and wound-healing effects throughout the day. The gel was formulated by adding a gelling agent, which serves as the base or primary structural component to achieve the appropriate consistency of the preparation¹⁷.

The treatment groups (P1, P2, and P4) treated with 15% moringa leaf extract gel showed a faster increase in fibroblast numbers on days 3, 5, and 9 compared to the control group (NA-CMC 3%). This can be explained by the antioxidant and anti-inflammatory effects of moringa extract; compounds such as flavonoids, phenolics, and vitamin C can reduce free radicals and inhibit pro-inflammatory cytokines, allowing fibroblasts to survive better and promoting more active proliferation. Additionally, *Moringa oleifera* has been reported to increase the expression of wound-healing growth factors such as *Transforming Growth Factor-β* (TGF-β1) and *Vascular Endothelial Growth Factor* (VEGF) in diabetic wounds, which play a role in stimulating fibroblast proliferation and angiogenesis¹⁸. In contrast, the control group, which was administered only 3% NA-CMC (*Sodium Carboxymethyl Cellulose*) as the gel base, showed slower and more gradual fibroblast proliferation¹³. NA-CMC itself, in hydrogel form, is widely used as a wound dressing base due to its ability to maintain wound moisture; however, NA-CMC does not contain bioactive substances that can directly stimulate fibroblast

proliferation or regulate inflammation¹⁹. Therefore, although fibroblasts in the control group continued to increase from day 3 to day 9, the increase was not as efficient as in the treatment group.

The phenomenon observed on Day 7 (P3), where the number of fibroblasts in the moringa group was lower than in the control group. This decrease does not indicate treatment failure but rather suggests that the moringa group had entered the remodeling phase earlier. During this transitional phase, fibroblasts begin to differentiate into myofibroblasts, and some cells undergo apoptosis after completing their role in extracellular matrix formation. Conversely, in the control group, fibroblast levels remained high because proliferation persisted for a longer duration, and the transition to maturation was delayed due to metabolic and inflammatory disturbances associated with diabetes^{20,21}. Recent research suggests that *Moringa oleifera* can shorten the duration of the fibroblast proliferation phase by accelerating the transition to the remodeling phase in diabetic wounds²².

By P4 (day 9), fibroblasts in the Moringa gel treatment group increased again, exceeding the fibroblast levels in the control group. This indicates that fibroblasts in the Moringa group remained active during the remodeling phase and the deposition of more mature type I collagen. The combined effects of antioxidants, angiogenesis stimulation, and growth factors induced by moringa leaf extract appear to support fibroblasts in working more efficiently in scar formation and wound re-epithelialization. Conversely, in the control group, there were fewer fibroblasts and the remodeling process was slower, leading to suboptimal wound healing in the context of diabetes²³.

CONCLUSION

Based on the research findings and discussion, the administration of moringa leaf extract gel (*Moringa oleifera* L.) affected the number of fibroblasts in traumatic wounds in diabetic Wistar rats, with the treatment group showing a higher number than the control group on days 3, 5, and 9; however, these differences were not statistically significant, whereas on day 7, the fibroblast count in the treatment group was lower than that in the control group, with a statistically significant difference.

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