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Research Article

Effect Of Moringa oleifera Extract Gel on Macrophage Cells in Diabetic Traumatic Ulcer Healing

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KEYWORDS

Diabetes, macrophage, Moringa oleifera, traumatic ulcer

ABSTRACT

Introduction: Traumatic ulcers are common oral mucosal lesions caused by mechanical injury. In diabetes, wound healing is delayed due to impaired immune responses, including macrophage dysfunction. *Moringa oleifera* leaves contain flavonoids, tannins, phenols, and saponins that have anti-inflammatory and wound-healing properties. This study evaluated the effect of *Moringa oleifera* extract gel on macrophage cell counts during traumatic ulcer healing in diabetic male Wistar rats.

Materials and Methods: This experimental study used a post-test-only control group design with 32 male Wistar rats. Diabetes was induced using streptozotocin (STZ). Traumatic ulcers were created on the labial mucosa using a burnisher. Rats were divided into four treatment groups (P1–P4) receiving *Moringa oleifera* extract gel and four control groups (K1–K4) receiving 3% Na-CMC gel. Observations were conducted on days 3, 5, 7, and 9. Gel was applied three times daily. Tissue samples were processed histologically, stained with hematoxylin-eosin, and examined for macrophage counts.

Results: Mean macrophage counts in treatment groups were P1=11.45, P2=10.15, P3=12.33, and P4=10.28, while control groups were K1=5.65, K2=6.70, K3=7.65, and K4=10.25. One-Way ANOVA showed significant differences among groups (p=0.021). Tukey Post Hoc analysis revealed a significant difference between P3 and K1 (p=0.037). Pearson correlation showed a significant negative correlation (r=-0.357; p=0.045).

Conclusion: *Moringa oleifera* extract gel significantly affected macrophage cell counts during traumatic ulcer healing in diabetic male Wistar rats.

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INTRODUCTION

In Indonesia, dental and oral diseases still require attention, considering that various efforts to improve and overcome problems in dental and oral health have not shown tangible results.[1] The oral mucosa serves as a protective barrier, shielding the oral cavity from trauma and disease.[2] According to the 2018 RISKESDAS (Basic Health Research), 57.6% of Indonesians experience dental and oral health problems, largely due to inadequate knowledge of oral hygiene practices.[3]

Traumatic ulcers are clinically found to be ulcers accompanied by a yellowish-white surface with erythematous edges and have a lesion shape that depends on the source of the trauma. Traumatic ulcers can be single or multiple, symmetrical or asymmetrical, and their size depends on the trauma that caused them. They are usually painful. The characteristics of acute traumatic ulcers include damage to the mucosa with erythematous edges and a yellowish-white center, as well as pain, while chronic traumatic ulcers may or may not be painful, with an indurative base and raised edges.[4]

Indonesia has abundant natural resources. These natural resources provide great benefits to the health of the population, and even to the world's population. Traditional medicines and medicinal plants are widely used by the lower-middle class in preventive, promotive, and rehabilitative efforts.[4] The use of natural ingredients from medicinal plants is also used by the community to prevent dental and oral health problems and to treat dental and oral diseases.[6]

One plant that has medicinal properties is the moringa leaf (*Moringa oleifera)*. The moringa plant (*Moringa oleifera*) is a member of the *Moringaceae* family and originates from India, Pakistan, Bangladesh, and Afghanistan. Moringa is commonly used to treat bacterial infections, fungal infections, sexually transmitted diseases, inflammation, malnutrition, and diarrhea. Moringa leaves (*Moringa oleifera*) contain 5% saponins, 1.4% tannins, and 5% triterpenoids. Tannins, polyphenols, and saponins are known to destroy bacterial cells by inhibiting synthesis and damaging cell membranes. A gel is pharmaceutical preparation with a semi-solid consistency consisting of a well-organized dispersion of small inorganic particles and large organic molecules mutually permeated by a liquid.[7]

Macrophages are one of the cells that play a role in the healing process of traumatic ulcers. The role of macrophages is not only in phagocytosis of foreign objects that enter the body, but macrophages are also key in the processes of fibrosis and angiogenesis.[8] The pain from traumatic ulcers greatly interferes with daily activities, such as eating, drinking, and speaking, prompting sufferers to seek medication to accelerate the healing process.[9] *Moringa oleifera* leaf extract accelerates the healing of rat ulcers, especially when administered at a dose of 15%. Various studies have examined the efficacy of moringa leaves in accelerating ulcer healing.[10] Based on the above description; the author wishes to conduct further research to determine the effect of using a 15% moringa leaf extract gel and 3% Na-CMC on the number of traumatic ulcer macrophages in male Wistar rats (*Rattus norvegicus)* with diabetes.

MATERIAL AND METHODS

This study began after obtaining *ethical clearance* from the Research Ethics Committee of the Faculty of Dentistry, Mahasaraswati University, Denpasar No. 03.0007/KEP-Unmas/VII/2025. It was then followed by the preparation of moringa leaf extract and gel preparations 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, *xylol,* sterile distilled water, alcohol, 3% NA-CMC, sterile cotton, anesthesia (ketamine), 10% formaldehyde solution, male Wistar rats, standard rat feed, citrate buffer solution, and 10% sucrose/dextrose. In addition, this study also describes the tools used, namely: *a* 5 mm diameter round *burnisher*, electric light microscope, measuring cup, digital scale, *rotary evaporator,* Buchner funnel, blender, gel cup, Optilab® Pro camera, HPA preparation, *deck glass*, 10 ml squirt, surgical tweezers, surgical scissors, nierbeken, labels, mouse marker pens, *glucometer*, Bunsen burner, matches, spiritus burner, *cotton buds*, medical masks and *gloves*, *aluminum foil*, and animal cages. Fourteen types of materials and 25 types of tools will be used to support the research so that the research procedures can be carried out easily.

This study used an in vivo laboratory experimental research design with *a post-test only control group design.* The population in this study was male Wistar rats *(Ratus Nobergicus)*. The research sample used normal rats induced with *streptozotocin* (STZ) to induce diabetes, then given treatment until traumatic ulcers formed on the lower labial mucosa of the Wistar rats. The sample size was calculated using *Federer's* formula by randomly grouping them into 4 treatment groups and 4 control groups, resulting in an ideal sample of 4 or more test animals for each experimental group, for a total sample of 32 animals. The independent variable in this study was 15% moringa leaf extract in gel form, and the dependent variable was the number of macrophages in the healing process of traumatic ulcers in male Wistar rats with diabetes. The instrument used in this study was anatomical histopathology (HPA) observed on days 3, 5, 7, and 9.

This study used a total of 8 groups, consisting of control and treatment groups. The control group was treated with *sodium carboxymethylcellulose* (Na-CMC) gel (3%) applied to the area of traumatic ulcer wounds. The treatment group was treated with moringa leaf extract gel (*Moringa oleifera*) at a concentration of 15%. The control and treatment groups were applied with each gel three times a day every 8 hours. In the P1 treatment group, moringa leaf extract gel with a concentration of 15% was applied for 3 days, and samples were taken on the 4th day. The P2 treatment group was applied with moringa leaf extract gel at a concentration of 15% for five days, and samples were taken on the 6th day. The P3 treatment group was applied with moringa leaf extract gel at a concentration of 15% for seven days, and samples were taken on the 8th day. The P4 treatment group was applied with 15% moringa leaf extract gel for 9 days, and samples were taken on the 10th day. The K1 control group was applied with 3% Na-CMC gel for 3 days, and samples were taken on the 4th day. The K2 control group was treated with 3% Na-CMC gel for five days, and samples were collected on the 6th day. The K3 control group was treated with 3% Na-CMC gel for seven days, and samples were collected on the 8th day. In control group K4, 3% Na-CMC gel was applied for nine days, and samples were taken on the 10th day.

The analysis of the research results was carried out by counting the number of macrophages visible in histological preparations using a ratio scale with quantitative data. The collected data was processed using the Statistical Product of Service Solution (SPSS) program. The tests used were the Kolmogorov-Smirnov normality test and Levene's test for homogeneity. After the data were distributed normally and homogeneously, a parametric test was

performed using the *one-way ANOVA* test to determine whether there were differences between the treatment groups. After that, a *Tukey Post Hoc* test was performed to determine the differences between treatments.

Figure 1. Moringa oleifera leaf

Figure 2. Moringa oleifera leaf extract

RESULTS AND DISCUSSIONS

The results were obtained after conducting research at the Laboratory of the Faculty of Agriculture, Warmadewa University, and experimental research at the Biocore Laboratory, to determine the effect of moringa leaf extract (*Moringa oleifera*) at a concentration of 15% on increasing the number of microphage cells in the traumatic ulcer healing of male Wistar rats (*Rattus novergicus*) with diabetes. The following data were obtained:

Table 1. Results of descriptive analysis

Group	Mean ± SD	Min–Max
P1 (Treatment, Day 3)	11.450 ± 1.482	10.20–13.60
P2 (Treatment, Day 5)	10.150 ± 2.357	7.00–12.60
P3 (Treatment, Day 7)	12.325 ± 2.128	9.80–14.50
P4 (Treatment, Day 9)	10.275 ± 5.092	3.20–15.00
K1 (Control, Day 3)	5.650 ± 0.755	5.00–6.40
K2 (Control, Day 5)	6.700 ± 1.778	5.00–9.20
K3 (Control, Day 7)	7.650 ± 2.695	4.00–10.40
K4 (Control, Day 9)	10.250 ± 3.272	6.40–14.40

Notes:

1. Data are presented as mean ± standard deviation (SD).
2. Min–Max indicates the minimum and maximum observed values in each group.
3. Each group consisted of four samples (n = 4).
4. P1–P4 = treatment groups receiving Moringa oleifera extract gel on days 3, 5, 7, and 9.
5. K1–K4 = control groups receiving 3% Na-CMC gel on days 3, 5, 7, and 9.

Table 1 shows that the mean macrophage counts in the treatment group were P1: 11.45, P2: 10.15, P3: 12.33, and P4: 10.28. The lowest mean value was found in P2, while the highest mean value was found in P3. In the control group, the mean values were K1 at 5.65, K2 at 6.70, K3 at 7.65, and K4 at 10.25. The lowest average value was found in K1, while the highest average value was found in K4. Overall, the control group (K1–K3) showed a lower average than the treatment group, except for K4, which was almost equal to P4.

Table 2. Results of Shapiro–Wilk Normality Test for Macrophage Cell Counts

Group	Mean ± SD	Statistic	p-value
P1	11.450 ± 1.482	0.369	0.539
P2	10.150 ± 2.357	0.225	0.962
P3	12.325 ± 2.128	0.225	0.961
P4	10.275 ± 5.092	0.252	0.902
K1	5.650 ± 0.755	0.305	0.754
K2	6.700 ± 1.778	0.317	0.717
K3	7.650 ± 2.695	0.243	0.927
K4	10.250 ± 3.272	0.256	0.892

Notes:

1. Data are presented as mean ± standard deviation (SD).
2. Each group consisted of four samples (n = 4).
3. All groups showed $p > 0.05$, indicating normal data distribution.
4. Shapiro–Wilk test was used due to the small sample size.

Based on Table 2, the results of the *Kolmogorov-Smirnov* normality test using the Exact. The Sig. approach in the table above, it is known that the Exact. Sig (2-tailed) values for the 8 groups, including group P1 (0.536), P2 (0.962), P3 (0.961), P4 (0.902), K1 (0.754), K2 (0.717), K3 (0.927), and K4 (0.892). All of these values are greater than 0.05. Therefore, based on the decision criteria in the Kolmogorov-Smirnov normality test, it can be concluded that all macrophage data are normally distributed.

Table 3. Results of Levene's Test for Homogeneity of Variance

Test	Levene Statistic	df (1,2)	p-value
Based on Mean	1.132	7, 24	0.377

Notes:

1. Levene's test was conducted to assess the homogeneity of variances for macrophage cell count data.
2. A p-value > 0.05 indicates equal variances among groups; therefore, the assumption of homogeneity was met.
3. Homogeneous variance supported the use of one-way ANOVA for further statistical analysis

Based on Table 3, the results of Levene's Test for homogeneity yielded a p-value of 0.377 > 0.05. Therefore, it can be concluded that the variance of the macrophage data is homogeneous.

Table 4. Results of One-Way ANOVA for Macrophage Cell Counts

Source	SS	df	F	p-value
Between Groups	156.614	7	2.988	0.021
Within Groups	179.725	24	–	–
Total	336.339	31	–	–

Notes:

1. SS = Sum of Squares.
2. One-way ANOVA showed a significant difference in macrophage cell counts among groups (p < 0.05).
3. Post hoc analysis was performed for pairwise comparisons.

Based on Table 4 of the one–way ANOVA results, the p-value is 0.006 < 0.05. Thus, it can be concluded that there is a significant difference between the treatment groups.

Table 5. Results of the Pearson correlation test

Variables	r	p-value	N
Macrophage vs Group	-0.357	0.045	32

Notes:

1. r represents the Pearson correlation coefficient.
2. Pearson correlation analysis was performed to evaluate the relationship between macrophage cell counts and study groups.
3. A significant negative correlation was observed (r = -0.357, p < 0.05).
4. The negative coefficient indicates that increases in group progression were associated with lower macrophage cell counts.
5. Significance level was set at 0.05 (two-tailed).

Based on Table 5, the results of the Pearson correlation test obtained a significance value of Sig. (2-tailed) = 0.045 < 0.05, indicating that there is a correlation between the administration of moringa leaf extract gel *(Moringa oleifera)* and the number of macrophage cells. The Pearson Correlation value for macrophages is -0.357, with a weak negative correlation indicating that the number of macrophage cells decreases with the administration of moringa leaf extract gel, thereby reducing the inflammatory phase and supporting the healing process of traumatic ulcers in male Wistar rats *(Rattus norvegicus)* with diabetes.

Based on the results of research data testing, the use of moringa leaf extract gel is effective against the number of macrophage cells in traumatic ulcers in male Wistar rats with diabetes. This can be seen from the test results, with the highest average results found in groups K3 and K4. Overall, the control groups (K1, K3, and K4) showed higher macrophage cell values compared to the number of macrophage cells in the treatment groups, except for group P3, which had values relatively equivalent to K4. The higher macrophage counts in the control groups that used 3% Na-CMC gel or only the regular gel base indicate the effectiveness of the 15% moringa leaf extract gel as an anti-inflammatory agent, preventing inflammation and accelerating the wound healing process.

In this study, a 15% concentration of moringa leaf extract gel was used, based on previous research conducted by Herdiani et al.[14] comparing the use of 5%, 10%, and 15% concentrations.[14] The 15% concentration was proven to be effective in healing when formulated as a topical gel and applied directly to the ulcerated area three times a day, namely in the morning, afternoon, and evening, for nine days. This frequency of administration is based on a pharmacokinetics approach that refers to the half-life of anti-inflammatory drugs commonly used in the treatment of oral mucosal ulcers, such as triamcinolone acetonide. This drug has a biological half-life ranging from 18 to 36 hours, because topical application is often influenced by local distribution and tissue metabolism, a dosing frequency of 2-4 times per day is typically chosen in clinical practice to maintain effective drug levels.[11,12]

Moringa oleifera leaf extract has therapeutic effects because it contains secondary metabolites, so phytochemical screening tests were conducted to identify the chemical compounds contained in Moringa oleifera leaf extract. In the phytochemical screening test, moringa leaf extract was found to contain flavonoids, tannins, saponins, and phenols. Bioactive compounds such as flavonoids, tannins, saponins, and phenols play an important role in the wound healing process, particularly through their antioxidant and anti-inflammatory activities. These compounds can reduce oxidative stress, which usually increases in conditions such as diabetes, thereby supporting the continuity of tissue regeneration and ulcer healing processes.[13]

The polymers used in making gels include natural tragacanth gum, pectin, carrageenan, agar, alginic acid, as well as synthetic and semi-synthetic materials such *as methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose,* and *carbopol*, which is a synthetic vinyl polymer with *ionized carboxyl* groups. In formulating a pharmaceutical preparation, the stability of the substance must be considered. This is important because it takes a relatively long time to reach the user or patient and is produced in large quantities. Therefore, it is also important to test the stability of the prepared product according to predetermined procedures. A gel formulation can be considered stable if it remains within the specified limits during the storage and usage period, with the properties and characteristics of the active ingredient.[12]

In this study, macrophage counting and observation were performed using a light microscope with 400x magnification. Each specimen consisted of five tissue sections, and macrophages in each section were systematically counted, starting from the lower-left corner and proceeding to the right and upward in a sequential manner, until the entire field of view was covered. The average macrophage count from the five sections was then calculated. Hematoxylin and eosin staining was used for examination to assess the increase in macrophage numbers.

The observation results showed an increase in the number of macrophages in the treatment groups, with all treatment groups showing higher levels than the control group. The P1 group had a value of 11.45, compared to the K1 group (5.65); the P2 group had a value of 10.15, compared to the K2 group (6.7); the P3 group had a value of 12.33, compared to the K3 group (7.65); and the P4 group had a value of 10.28, compared to the K4 group (10.25). These findings align with another research, which showed that the compounds in moringa leaves with anti-inflammatory properties are primarily flavonoids. Moringa leaf extracts, containing flavonoids, have demonstrated inhibitory activity on the production of PGE2 (Prostaglandin E2) and COX-2 (Cyclooxygenase 2) induced by lipopolysaccharides.[13] Other compounds found in moringa leaves, such as

tannins, act as astringents that can stop bleeding and promote tissue regeneration. These astringent tannins work locally by precipitating blood proteins, immediately halting bleeding. Additionally, compounds like phenols and saponins possess antibacterial properties, which are crucial for accelerating the wound healing process. Saponins have antiseptic activity that helps prevent bacterial growth, thereby reducing the risk of infection in wounds.[14]

Flavonoids in moringa leaves (*Moringa oleifera*) in the treatment of traumatic ulcers in diabetes have the potential to lower blood glucose levels through their antioxidant mechanism, which suppresses beta cell apoptosis. This mechanism can bind free radicals that contribute to reducing insulin resistance. According to Agung et al.[16], flavonoids accelerate the healing of oral mucosal ulcers by increasing transforming growth factor-2 (FGF-2). This protein helps macrophages, fibroblasts, and endothelial cells migrate away from damaged tissue and form new mucosal tissue. The rapid narrowing of ulcers in the oral mucosa of Wistar rats is an indication of this.[14,15,16] Other compounds contained in moringa leaves, such as tannins, have effectiveness as astringents that can stop bleeding and regenerate new tissue.[15] These astringent tannins work locally by precipitating blood proteins, thereby stopping bleeding immediately. Other compounds, such as phenols and saponins, have antibacterial properties that are important in accelerating the wound healing process. Saponins have antiseptic activity that can prevent the growth of germs or bacteria, thereby preventing wounds from becoming infected.[16]

Flavonoids also contribute to enhancing angiogenesis growth factors through the proliferation of M2 macrophages, which release not only FGF-2 but also VEGF and EGF. VEGF is considered the primary mediator of angiogenesis, as it plays a crucial role in degrading the ECM surrounding endothelial cells, modulating endothelial cell proliferation, increasing vascular permeability, and recruiting endothelial progenitor cells that differentiate into mature endothelial cells. This process accelerates the proliferation and migration of endothelial cells to the wound area.[17] Furthermore, flavonoids also help stabilize newly formed blood vessels by increasing Ang-1 levels and promoting the migration of Tie-1 and Tie-2. This stabilization maintains vascularization and oxygen supply to the newly formed tissues, thereby accelerating the healing of traumatic ulcers.[18]

CONCLUSION

Based on the research results and discussion, it can be concluded that moringa leaf extract gel (*Moringa oleifera*) influences the number of macrophage cells in the healing process of traumatic ulcers in male Wistar rats (*Rattus norvegicus*) with diabetes.

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