Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2023 Jan 02; 11(A):23-30.
https://doi.org/10.3889/oamjms.2023.7551
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

Since 2002

OPEN ACCESS

Combined Treatment Effect of Topical CM-hWJMSCs and Oral *Moringa oleifera* extract on Wound Healing of Diabetic Rat

Dian Laksmitawati[1]*, Umi Marwati[1], Wahyu Widowati[2], Rachmawati Noverina[3], Tri Suciati[4], Ahmad Faried[5]

[1]Department of Pharmacology and Biochemistry, Pharmacy Faculty, Pancasila University, Jakarta, Indonesia; [2]Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung, West Java, Indonesia; [3]Laboratory Animal and Stem Cells, PT Bio Farma (Persero) Hospital, Bandung, West Java, Indonesia; [4]School of Pharmacy, Institute Technology Bandung, Bandung, West Java, Indonesia; [5]Department of Neurosurgery and Stem Cell Working Group, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin, Bandung, Indonesia

Edited by: Sinisa Stojanoski
Citation: Laksmitawati D, Marwati U, Widowati W, Noverina R, Suciati T, Faried A. Combined Treatment Effect of Topical CM-hWJMSCs and Oral *Moringa oleifera* extract on Wound Healing of Diabetic Rat. Open Access Maced J Med Sci. 2023 Jan 01; 11(A):23-30. https://doi.org/10.3889/oamjms.2023.7551
Keywords: Conditioned medium; Mesenchymal stemcell; *Moringa oleifera*; Wound healing; Streptozotocin; Diabetic rat
***Correspondence:** Dian Laksmitawati, Pharmacy Faculty, Pancasila University, Jakarta, Indonesia. E-mail: dian.ratih@univpancasila.ac.id
Received: 10-Oct-2021
Revised: 20-Dec-2021
Accepted: 26-Dec-2022

Funding: This study was supported by Incentive Program of National Innovation in Research system, Kemenristek-BRIN Republik Indonesia
Competing Interests: The authors have declared that no competing interests exist

Abstract

AIMS: The aim of the study was to determine the activity of topical conditioned medium (CM) mesenchymal stem cells as single or combined with oral therapy of *Moringa oleifera* extract (ME) in lowering blood glucose levels and healing diabetic wounds.

MATERIALS AND METHODS: Male Sprague-Dawley rats were induced hyperglycemia with Streptozotocin and injured by a biopsy punch on the dorsal side. Then, the rats were divided into five groups, namely, the CM group, which was given topically; the CM–ME group, which was given CM gel topically and ME orally; the CM–Met group, which was given Metformin (Met) as the positive control; commercial gel containing neomycin and placenta extract (BP); and placebo as the negative control. The treatments were given once daily for 16 days. On the 3rd, 8th, and 16th days of treatment, blood glucose level was measured, and anatomical observations of wounds and histology were performed using hematoxylin-eosin and Masson's Trichrome staining.

RESULTS: The wounds treated with CM–ME and CM–Met have a similar profile of lowering blood glucose levels; however, Met was faster to reach blood glucose levels below 250 mg/dL (day 8) compared with ME (day 16). As shown in data on anatomical and histological wound healing parameters, untreated mice exhibited moist wounds with poor healing. Meanwhile, the group that received CM gel alone or along with oral ME showed an improvement in wound parameters.

CONCLUSION: CM–ME has a similar profile to CM–Met in reducing blood glucose levels and healing diabetic wounds parameters in rats.

Introduction

The conditioned medium (CM) of human Wharton's jelly mesenchymal stem cells contains various types of growth factors that are needed in the wound healing process, including basic fibroblast growth, interleukin growth factor (IGF), vascular endothelial growth factor, and transforming growth factor-beta (TGF-β2 and TGF-β3), which can suppress inflammation, stimulate the growth of new capillaries, and accelerate epithelial formation in chronic wounds [1], [2], [3]. In addition, CM can accelerate wound closure by increasing epithelial and endothelial cell migration, cell infiltration, granulation formation, and angiogenesis in an existing wound model [2]. In this research, CM is expected to have the same effectiveness as placenta extract, which can increase collagen and tissue protein synthesis, as well as neoangiogenesis and epithelialization [4].

Moringa oleifera (family *Morigaceae*) is commonly known as drumstick tree and widely cultivated in several locations in the tropics area and is now distributed worldwide [4], [5]. The treatment of diabetic wounds not only gives therapy for a wound but also requires treatment to decrease blood glucose. Combined topical and oral treatment may give better results because normal blood glucose can speed up wound tissue healing. The previous study reported that *M. oleifera* extract (ME) has an antidiabetic effect and has antibacterial effects against gram-positive and gram-negative *bacteria*. Furthermore, ME can reduce blood glucose because it contains compounds that are believed to have antihyperglycemic activity [6], [7]. It is assumed that ME can be given as an antidiabetic therapy, such as Metformin (Met), which can reduce blood sugar levels [8].

The previous studies only discussed single topical treatment or single oral treatment. In this study, either single topical CM or along with oral ME was studied, and their effectiveness on healing in streptozotocin (STZ)-induced diabetic rat wounds was examined.

Materials and Methods

Materials

The chemicals used are STZ (Sigma-Aldrich); CM; a commercial gel containing neomycin sulfate; and placenta extract, ketamine, and xylazine.

The animals used were adult male Sprague-Dawley rats weighing 200–250 g. Animals were kept at room temperature with 12 h of light-dark lighting. Standard feeding and drinking were performed *ad libitum*. Animals are acclimatized for 1 week. The treatment of animals has been approved by the ethical committee of the Faculty of Medicine, University of Indonesia (Protocol number 19-01-0054).

Methods

Table 1 shows the gel formulations of CM and placebo gel. The gel formulation is the result of the stem cell consortium research with Biofarma in 2017. The pharmaceutical parameter test was conducted by another consortium member. The gelling process is performed in an aseptic procedure.

Table 1: Formulation of CM and placebo gel

Materials	CM gel (g)	Placebo gel (g)
Conditioned medium	2	–
Glycerin	20	20
Polyvinyl alcohol	5.6	5.6
Alginate	70	70
Phosphate buffer saline	2.4	2.4

CM: Conditioned medium.

Preparation of ME

The extract of *M. oleifera* leaf is powdered and sieved using a 4/18 sieve. The powder was macerated for 6 h by stirring (kinetic maceration) in 70% ethanol solvent (v/v) at a 1:10 ratio. The mixture was then precipitated for 24 h and filtered. The filtrate was separated, and the remnant of powder was macerated 3 times. The filtrate was collected and evaporated with a rotary evaporator until a thick extract was obtained [9].

Diabetes induction

Rats were injected with a SZT solution at a dose of 45 mg/kg BW by the intraperitoneal route [10]. The injection is carried out within 5 min [11]. Then, the rats were given a 10% ad libitum sucrose solution as a substitute for water until the next day to prevent hypoglycemia [8].

Fasting blood glucose measurement

The measurement of glucose levels was performed on the 0th day before diabetes induction using a glucometer (GlucoDr and glucose reagent strips); then, on the 4th day after diabetes induction, if diabetes has not been reached, the next measurement would be on the 7th day. The measurement of glucose levels was also performed after wound induction on days 3, 8, and 16 of therapy. Rats were considered diabetic when their glucose levels exceed 250 mg/dL. The rats were fasted for approximately 5–6 h before measuring glucose levels. Blood was drawn from the tail vein.

Excision wound model

Rats that reached blood glucose levels >250 mg/dL were divided into three groups. Wound induction using a 5 mm biopsy punch was performed on the dorsal part of the rats anesthetized with ketamine injection 50 mg/kg BW-xylazine 10 mg/kg BW by the intraperitoneal route [12]. Table 2 shows the grouping of experimental animals. Wound treatment was done once a day, for 16 days. On days 0, 3, 8, and 16 of treatment, two rats from each group were euthanized. Then, the skin on the back of the rats was cut and immersed in a 10% neutral buffered formalin solution for further histopathological examination.

Table 2: Group of diabetic wound therapy

Group	Treatment
1	The wound was given topical CM gel
2	The wound was given topical CM gel and oral ME
3	The wound was given topical CM gel and oral Met 45 mg/kg BW
4	The wound was given neomycin gel placenta extract
5	The wound was given placebo gel

CM: Conditioned medium.

Wound anatomy and macroscopic assessment

Diameter data were used to calculate the percentage of reduction in wound diameter by comparing the wound diameter on the 0th day of wound induction with that on the 3rd, 8th, and 16th days. Wounds were documented daily using a digital camera. In addition, the scabs and hair growth around the wound were observed every day.

Wound histopathological assessment

Microscopic observation of the wound was performed by histopathological examination, which included the hematoxylin-eosin (H&E) and Masson's Trichrome (MT) staining. The staining stages are as follows.

The skin tissue was washed with 0.9% physiological NaCl and then soaked in normal physiological formalin solution for 48 h. The skin tissue was sliced crosswise with a length of 0.5 cm and then transferred to 70%, 80%, 90%, 95%, absolute alcohol 1, and absolute alcohol 2. The tissue was transferred to liquid paraffin I and liquid paraffin II and then put into xylol I and xylol II. The tissue was cooled at −20℃ until the liquid paraffin freezes and then cut using a microtome. The cut is placed on a slide dripped and heated over an incubator with a temperature of 40℃–45℃ in an inclined position [13].

Hematoxylin-eosin staining was performed by soaking the tissue into a solution of xylol I and xylol II and then put into absolute alcohol, 95% alcohol, and 80% alcohol. The tissue was washed with Aquadest and then stained with Mayer's hematoxylin, washed again with Aquadest and stained with eosin, and then washed with Aquadest and dipped in 95% ethanol 10 times, absolute alcohol I 10 times, and absolute alcohol II 10 times. Then, the tissue was immersed in a solution of xylol I and xylol II. The histological preparation was covered with a coverslip and examined under a microscope [13].

MT staining was conducted by submerging the tissue with Weigert's Iron Hematoxylin for 10 min and then rinsed with distilled water and stained with a solution of Biebrich scarlet acid fuschin for 10–15 min. Then, the tissue was rinsed with distilled water and stained with a solution of phosphomolybdic-phosphotungstic acid for 10–15 min. The tissue was stained with aniline blue solution for 5–10 min, rinsed with Aquadest, and then soaked in 1% acetic acid solution. Before being immersed in 95% ethanol and absolute ethanol, the tissue was rinsed with distilled water. The preparation is covered with a coverslip and examined under a microscope. The parameters measured were the amount of neutrophil infiltration, the amount of mononuclear inflammatory cell infiltration, the number of neovascularization, the ratio of reepithelialization, and the area of collagen tissue [13].

Statistical analysis

To determine the significance of data on fasting blood glucose levels after STZ induction, a T-test analysis was conducted, comparing the mean blood glucose levels before induction and after induction. The profiles of these data at days 0, 3, 8, and 16 were compared descriptively between groups. Statistical analysis was performed on the 16th day of data, using Graphpad PRISM 5.

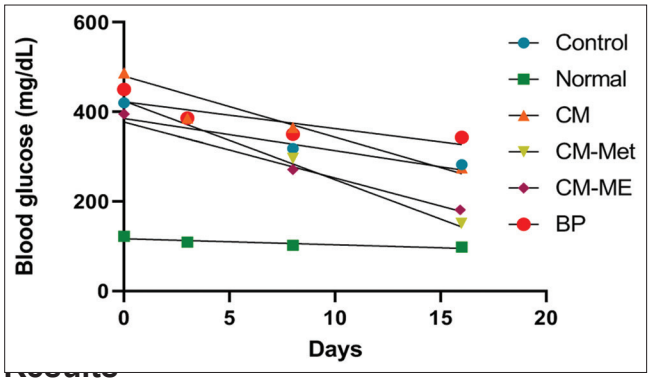

Figure 1: Graphic of blood glucose level in streptozotocin-induced diabetic rats

Level of blood glucose

According to data in Figure 1, the injection of STZ at a dose of 45 mg/kg BW can cause hyperglycemia conditions with an average range of blood glucose levels reaching 356–463 mg/dL. When calculated from the range of blood glucose levels under normal conditions, there was an increase in the range of 69.19–77.90%. Statistical analysis of the increase in blood glucose levels after STZ induction showed a significant value with a $p < 0.05$ using the Kruskal–Wallis test.

On day 16 of the CM–ME group, the decrease in blood glucose levels reached 31.30% with the final glucose level of 163.5 mg/dL. In the CM–Met group, the decrease in blood glucose was 23.06% and reached 163.5 mg/dL on day 16. Blood glucose levels in the CM group increased 16.87% on day 16 and were still classified as hyperglycemic with average blood glucose levels reaching 354.5 mg/dL.

Analysis of wound healing according to macroscopic anatomy

Diameter of the wound

In Figure 2, on the 16th day, the largest reduction in wound diameter occurred in the CM–ME group with

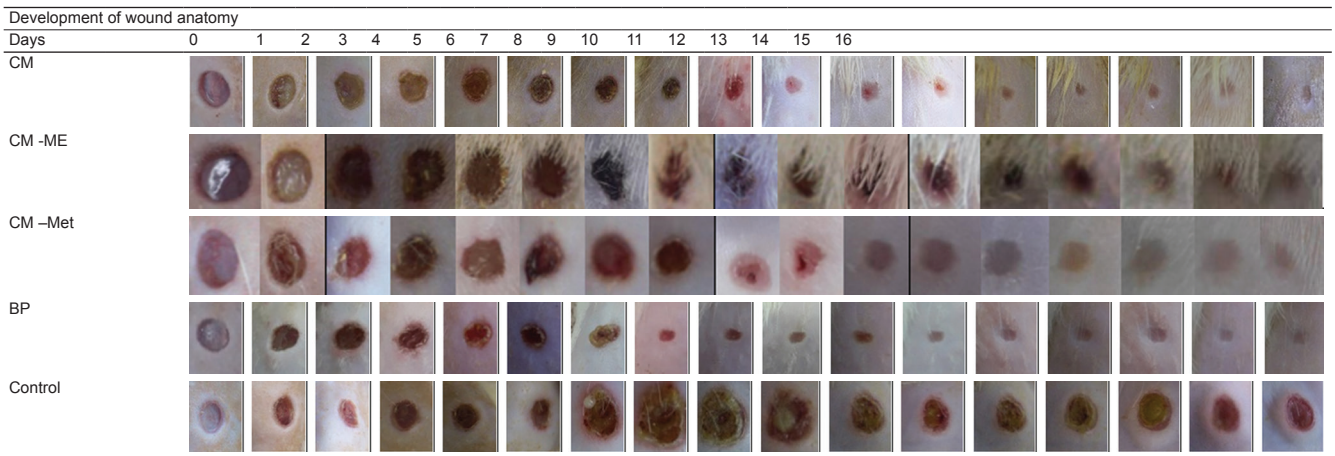

Figure 2: Diameter of the wound of streptozotocin-induced diabetic rats

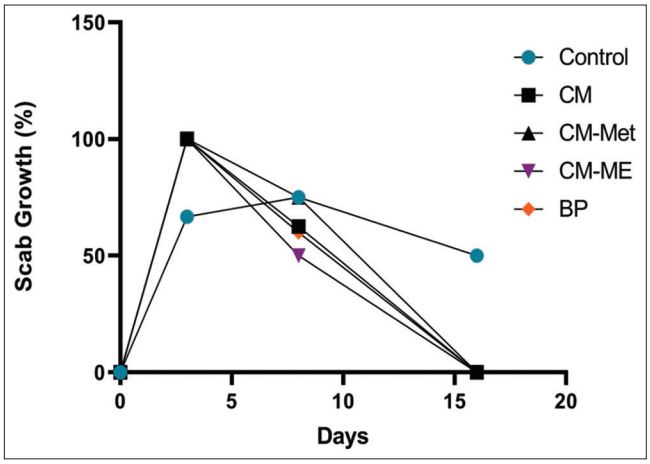

Figure 3: Graphic of scab growth in streptozotocin-induced diabetic rats

93.5% in comparison with the percentage on day 8, followed by the CM–Met group with 84.9%. The lowest percentage reduction (62.20%) was found in the group given topical CM therapy only, in comparison with the control group.

Scab growth on the wound

As shown in Figure 3, the highest percentage of wound scabs after the wound was made was in the CM–ME group, followed by the CM–Met group. The control group on day 3 was only 65% compared with the other group with 100% scab growth, which means that the scabs have formed all over the wound. Based on the result, the meantime of the scab release was in 6 days. After the scabs disappeared, the wound was reduced to the point where no red areas were visible.

Hair growth on the wound

The hair growth was seen more rapidly in wounds treated with the CM–Met and CM–ME groups on day 3 was 75%, followed by BP (43%) and CM gel (33%). The control group had 17% hair growth on day 3 and only 75% on day 16, whereas all treated groups had 100% hair growth (Figure 4).

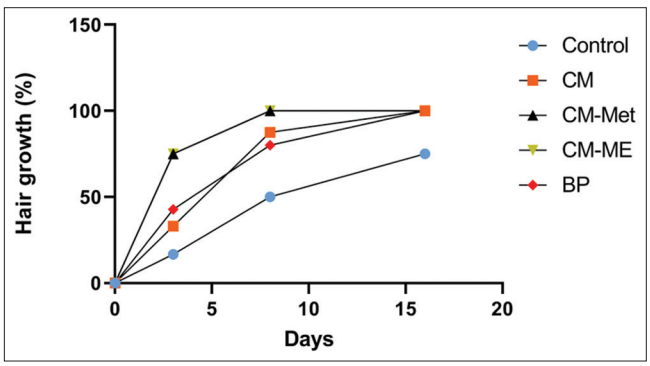

Figure 4: Graphic of hair growth on the wound in streptozotocin-induced diabetic rats

Analysis of wound healing according to microscopic anatomy

Neutrophil infiltration

Based on the data in Figure 5, the most neutrophil infiltration was found in the CM–Met group with the number of cells found as many as 228 cells formed on the 3rd day of therapy, followed by the CM group with 154.5 cells, and the CM–ME group with as many as 145.6 cells. The lowest number of neutrophil infiltration was found in the control group, that is, only 81.1, and on day 16, the number of neutrophil infiltration was high compared with all treated groups.

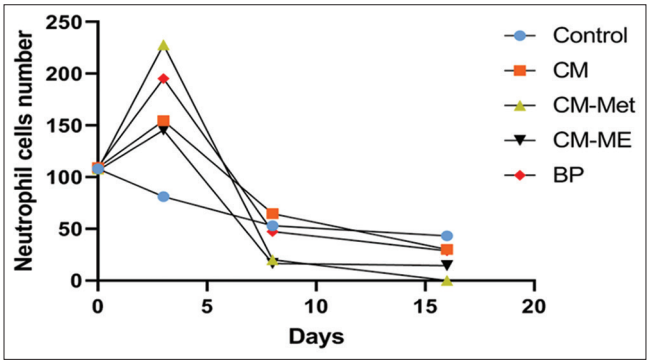

Figure 5: Graphic of neutrophil infiltration number in streptozotocin-induced diabetic rats

Mononuclear cells infiltration

Infiltration data of mononuclear cells also showed that the highest number of cells was in the CM–Met group, that is, 171.1 cells on day 3, followed by the CM group with cells. On day 16, the control group had the highest number of mononuclear cell infiltration compared with the treated group and the lowest number on day 16 was in the CM–Met group (Figure 6).

Neovascularization

The lowest number of neovascularization was in the CM–ME group on day 5, which was observed

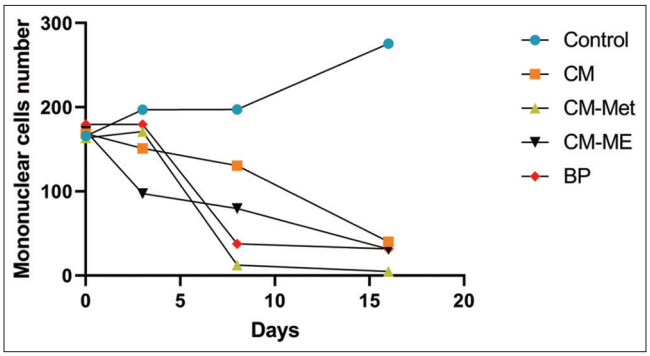

Figure 6: Graphic of mononuclear cell infiltration number in streptozotocin-induced diabetic rats

on day 16 of therapy, whereas the highest number was in the BP group, in comparison with the control group. Nevertheless, for the velocity profile, the CM group showed the highest number after 3 days of treatment compared with the other groups (Figure 7).

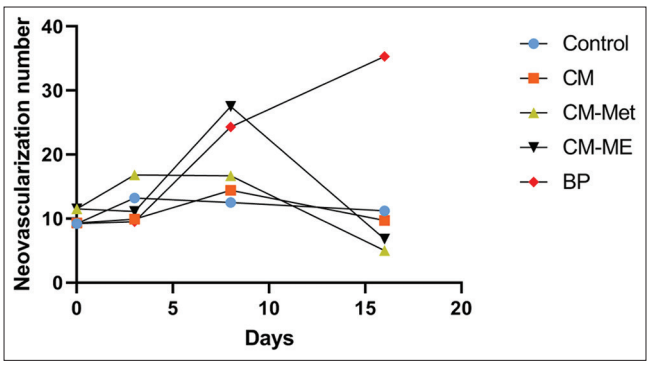

Figure 7: Graphic of neovascularization in streptozotocin-induced diabetic rats

Re-epithelization ratio

On day 8 of treatment, there was an increase in re-epithelialization ratio in the three groups with the greatest ratio in the CM–Met, CM–ME, and CM groups as shown in Figure 8. On days 8 and 16, the control group had a lower ratio compared with the treated group.

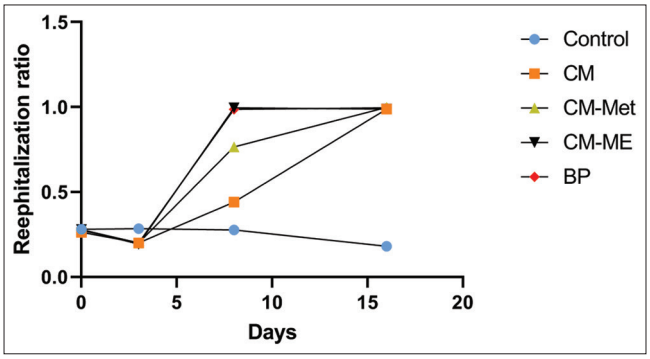

Figure 8: Graphic of re-epithelization ratio in streptozotocin-induced diabetic rats

Collagen tissue area

The largest area of collagen tissue formed was found in the control group on day 16 of therapy, whereas the smallest area of collagen tissue was seen in the BP group, in comparison with the other treated groups (Figure 9).

Discussion

In a recent study, it was shown that SZT can cause diabetes in rats with high blood glucose levels at a

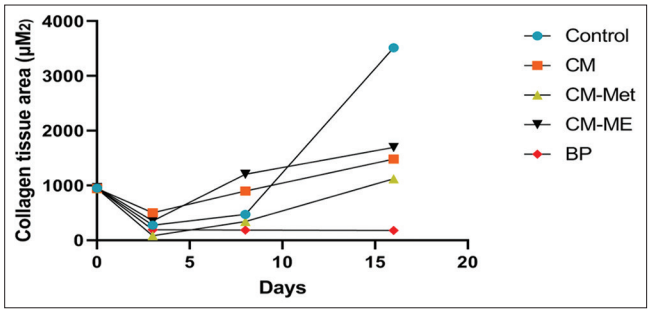

Figure 9: Graphic of collagen tissue area in streptozotocin-induced diabetic rats

range above 250–450 m/dL, which is quite stable until the 16th day. This was due to the activity of SZT in damaging pancreatic β cells through DNA alkylation, which causes necrosis of these cells [14]. After giving the therapy on the basis of the treatment group, the decrease in blood glucose levels in the CM–ME group showed that ME had the potential to reduce blood glucose because it contains compounds of antihyperglycemic activity, such as quercetin-3-glycoside, routine, kaempferol, glycosides, terpenoids, and saponins, which have antihyperglycemic activity although not as good as Met as a positive control [14], [15].

The decrease in blood glucose levels was also in line with the wound healing process, which was characterized by faster wound closure compared with the uncontrolled group with blood glucose levels. Diabetic wounds are difficult to heal because they are moist and are a preferred medium for *bacteria*, which can aggravate the wound or hinder the healing process. *M. oleifera* has been studied for its antidiabetic properties. The previous research regarding the effect of ethanol extract of *Moringa* leaves on STZ-induced diabetes has led to a significant reduction in blood glucose levels 1–7 h after administration of ethanol extract at doses of 250 and 500 mg/kg BW intraperitoneal [16]. Several studies regarding the antibacterial effect of *M. oleifera* leaves against gram-positive and gram-negative *bacteria Bacillus aureus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, and *Escherichia coli* showed activity against the inhibition zone of *bacterial* growth [17]. In addition, the antibacterial activity of *Moringa* leaves is identified from one of its compounds, pterygospermin, with the active substance glucosinolate 4 alpha-L-rhamnosyloxy benzyl isothiocyanate [18].

CM is a culture medium with secretions of stem cells and is not directly antibacterial but through the mechanism of the growth factors in CM and other proteins, such as cytokines, that contribute to the wound healing process. Some cytokines are pro-inflammatory agents that can stimulate tissues to respond to an immune system by eliminating foreign parts, such as *bacteria*, so that the CM gel can indirectly reduce infection rates [19]. Based on the previous research, MSC-CM involve in the wound healing process and specifically in reepithelialization process,

which is critical for optimal wound closure because of its role in wound contraction, which is mediated by keratinocyte proliferation and migration [20]. This reason can be the basis for explaining the phenomenon of why the CM–ME group can provide a good diabetic wound healing effect because it is supported by the antibacterial compounds in *Moringa* leaves that have been tested to inhibit *bacterial* growth. Wounds created on the backs of untreated mice exhibited moist wounds with poor healing, as evidenced by data on wound diameter closure slowing. Meanwhile, the group that received CM gel alone or along with oral showed an improvement in wound parameters.

The content of placenta extract in BP gel contributes to decreasing wound diameter because placenta extract can increase collagen, tissue protein synthesis, neoangiogenesis, and epithelialization. The BP gel-treated mice also did not experience swelling because the placenta extract reduced edema and inflammation in the surrounding tissue [4]. Meanwhile, the content of the neomycin sulfate is antimicrobial, so it can reduce the infection rate directly. Another factor that can affect the wound healing process is the stress factor. Stress increases glucocorticoid production and reduces levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 in the wound area. Stress also reduces the expression of IL-1α and IL-8 in wounds; these cytokines are chemoattractants that contribute to the inflammatory phase [21]. When stress occurs, reactive oxygen species (ROS) levels increase. ROS breaks down the extracellular matrix and reduces the function of fibroblasts and keratinocytes; low levels of ROS can improve wound repair, whereas high levels reduce wound healing rates [22].

Repair of wound tissue is characterized by a scab. A scab is a dry, crust-like layer that forms to protect the wound. A scab is a form of body defense in preventing microbes and other impurities from entering the wound. The formation of scabs can cause the epithelialization rate to decrease because the collagen tissue and fibrin layer interfere with the migration of epidermal cells from the oil glands and hair follicles. The scab will be released from the wound because the new epidermal layer under the wound has matured; thus, the new tissue will encourage collagen, fibrin stretching, or collagen dissolution due to enzymes produced by epidermal cells or leukocytes [23]. Based on the data in this study, on day 16, the wounds of the BP gel and CM gel group mice did not have a scab anymore, whereas 50% of the control gel group mice still had scabs.

Hair growth is a sign that the scar tissue around the hair follicle has improved. The CM–Met group gave the effect of scabbing on the wound in a fairly quick time because blood glucose levels were controlled by Met as an antihyperglycemic agent so that it could reduce high blood glucose levels in diabetic rats. Tissue repair can be caused by the secretion of growth factors in CM, which can stimulate hair growth in the wound area. The result of this study was in line with other studies. Research by Fukuoka *et al*. states that since a CM can cure alopecia in humans, it can lead to better hair growth than the saline-only group [24]. The BP group mice also experienced better hair growth than did the control group because the placenta extract contained growth factors.

Besides macroscopic anatomical data, wound healing can be done through histopathological observations with two types of staining, H&E staining, which was used to see neutrophil infiltration, mononuclear inflammatory cells, and neovascularization, and MT staining, which was used to determine the results of re-epithelialization and collagen identification. The high number of neutrophil infiltration indicates that the wound is in an inflammatory phase, which is a stage of wound healing. The low number of neutrophils in the control group indicated an inflammatory phase that was not as good as the BP and CM groups. It can also be caused by tissue stress that causes decreased expression of IL-1 α and IL-8 as a chemoattractant to initiate the inflammatory phase [21]. Neutrophil infiltration was reduced by days 8 and 16. This was because neutrophils contribute to the inflammatory phase that can last for up to 6 days afterward, which functions to phagocytose microbes around the wound [20], [24].

The rapid formation of neovascularization indicates a good wound healing process. This phenomenon occurs by the remodeling phase, that is, the angiogenesis process and blood flow will decrease. Shrestha *et al*. reported that the CM of mesenchymal stem cells can increase wound closure in diabetic mice, their injection can increase the neovascularization of injured tissue, and the compounds it secretes directly stimulate growth factors that may contribute to wound healing [6].

On day 8, the CM–ME group had the highest neovascularization when compared with the other treated and control groups. This could indicate that on day 8, the wounds in treated and control groups were in the proliferation phase, which was followed by the remodeling phase. In the remodeling phase, the angiogenesis process and blood flow decreased, and the newly formed blood vessels regressed; hence, the vascular density of the wound can return to normal [19], [20], [25] On day 16, the number of neovascularization in the BP group was greater than in the control group. It is because the content of growth factors in the placental extract can stimulate endothelial cell precursors, thereby increasing neovascularization [20]. On day 16, the control group had less neovascularization not because the wound had entered the remodeling phase because the neovascularization process was disrupted. The wounds in the control group had not yet entered the remodeling phase. It could be seen from the result that the percentage reduction in diameter was lower than the decrease in diameter in the BP and CM groups, and the re-epithelialization ratio was very small (0.1804).

Disruption of the neovascularization process is also a cause of chronic diabetic wounds [21].

Reepithelialization comprises various stages including migration, mitosis, and differentiation of epithelial cells [25]. This process is influenced by various growth factor molecules, such as KGF, IGF-1, NGF, and EGF [19]. CM gel and BP gel contain a lot of growth factors; hence, they help the re-epithelialization process better than control gels. One of the stages in the proliferation phase is collagen synthesis, which is stimulated by growth factors, for example, FGF-2, IGF-1, and TGF-β. Macrophages can also synthesize growth factors, one of which is TGF-β, which can affect collagen synthesis [26]. The extent of the collagen tissue in the wound that was given CM on day 8 was due to the content of the growth factor in CM. On the 8th day, the wound treated with BP gel had the smallest collagen area; this could be because the wound had entered the remodeling phase. The remodeling phase can overlap with the proliferation phase on days 8–21. In the remodeling phase, collagen begins to degrade in reducing scars or to prevent keloid formation [27]. Wounds in the BP group had entered the remodeling phase on day 8, which could be seen from their large reapplication ratio and a large percentage of reduction in diameter. Macroscopically, it was also seen that the wound of the BP group on day 8 was almost completely closed.

The 16th day is the last phase of wound healing, namely, remodeling. This phase is characterized by a balance between deposition and degradation of collagen [27]. In the CM group, the area of collagen was not as large as the control group because the wound had entered the remodeling phase; thus, collagen was degraded not to form hypertrophic scars or keloids. Collagen in the control group on day 16 was very extensive, which indicated the inhibition of collagen degradation.

Collagen synthesis is also influenced by macrophages. Platelet-derived growth factors and EGF from macrophages are the main signals for fibroblasts. Then, fibroblasts will migrate to the wound area in the tissue and synthesize collagen and proliferate [23]. On day 16, the area of the wound collagen tissue in the control group was large, based on the amount of mononuclear inflammatory cell infiltration in the wound.

CM can repair skin wounds according to controlled natural healing mechanisms. The wound healing process is a complex process that may be divided into stages, such as homeostasis, inflammation, proliferation, and remodeling. CM participates in all of these processes in a controlled manner, and when combined with oral medications that reduce blood glucose levels, it can enhance wound healing compared with CM alone. Untreated wounds may close and heal, but they are not controlled, and there is a risk of hypertrophic scars due to excessive collagen synthesis [28].

Conclusion

CM–ME has a profile similar to CM–Met in reducing blood glucose levels and healing diabetic wounds in rats. CM had diabetic wound healing activity equivalent to BP. Oral and topical combinations are required to produce effective outcomes in the diabetic wound model. Oral treatment is systemic (comprehensive), such as Met, which can enhance insulin sensitivity and reduce blood glucose levels. Blood glucose levels influence the wound healing process. The effectiveness of *Moringa* extract with topical treatments was caused by several properties of extract, including antibacterial, antioxidant, and immunomodulatory properties. The limitation in this study is that the active compound and concentrations of CM and ME are not yet known so that further research is needed to measure the type and amount of active compound from both CM and ME.

Acknowledgment

This study was supported by Incentive Program of National Innovation in Research system, Kemenristek-BRIN Republik Indonesia. We thank our students, Virandita Diandra and Dita Primandari Putri from Pancasila University who provided technical assistance.

References

1. Yuan X, Li X, Hao H, Cui Y, Chen M, Liu L, *et al*. Secretome of mesenchymal stem cells. In: Essentials of Mesenchymal Stem Cell Biology and its Clinical Translation. Netherlands: Springer; 2013. p. 33-46.
2. Lopes L, Setia O, Aurshina A, Liu S, Hu H, Isaji T, *et al*. Stem cell therapy for diabetic foot ulcers: A review of preclinical and clinical research. Stem Cell Res Ther. 2018;9(1):188. https://doi.org/10.1186/s13287-018-0938-6
   PMid:29996912
3. Navadiya SK, Vaghani YL, Patel MP. Study of topical placental extract versus povidone iodine and saline dressing in various diabetic wounds. Natl J Med Res. 2012;2(4):411-3.
4. Lambole V, Kumar U. Effect of *Moringa oleifera* Lam. on normal and dexamethasone suppressed wound healing. Asian Pac J Trop Biomed. 2012;2(1):S219-23. https://doi.org/10.1016/S2221-1691(12)60163-4
5. Panchal MA, Murti K, Lambole V, Gajera V. Pharmacological properties of *Moringa oliefera* lam.-a review. Pharmacologyonline. 2010;1(2):768-75.
6. Shrestha C, Zhao L, Chen K, He H, Mo Z. Enhanced healing of diabetic wounds by subcutaneous administration of human umbilical cord derived stem cells and their conditioned media. Int J Endocrinol. 2013;2013:592454. https://doi.org/10.1155/2013/592454
   PMid:24089612

7. Villarruel-López A, la Mora DA, Vázquez-Paulino OD, Puebla-Mora AG, Torres-Vitela MR, Guerrero-Quiroz LA, *et al*. Effect of *Moringa oleifera* consumption on diabetic rats. BMC Complement Altern Med. 2018;18(1):127. https://doi.org/10.1186/s12906-018-2180-2
   PMid:29636032
8. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2015;70(1):5.47.1-5.47.20. https://doi.org/10.1002/0471141755.ph0547s70
   PMid:26331889
9. Laksmitawati DR, Prasanti AP, Larasinta N, Syauta GA, Hilda R, Ramadaniati HU, *et al*. Anti-inflammatory potential of gandarusa (*Gendarussa vulgaris* nees) and soursoup (*Annona muricata* L) extracts in LPS stimulated-macrophage cell (RAW264.7). J Nat Remedies. 2016;16(2):73-81. https://doi.org/10.18311/jnr/2016/5367
10. Azevedo IM, Araújo-Filho I, Teixeira MM, de Carvalho Moreira MD, Moreira AC. Wound healing of diabetic rats treated with *Moringa oleifera* extract. Acta Cir Bras. 2018;33(9):799-805. https://doi.org/10.1590/s0102-865020180090000008
    PMid:30328912
11. Nagarchi K, Ahmed S, Sabus A, Saheb SH. Effect of streptozotocin on glucose levels in Albino Wister rats. J Pharm Sci Res. 2015;7(2):67-9.
12. Galehdari H, Negahdari S, Kesmati M, Rezaie A, Shariati G. Effect of the herbal mixture composed of aloe vera, henna, *Adiantum capillus-veneris*, and myrrha on wound healing in streptozotocin-induced diabetic rats. BMC Complement Altern Med. 2016;16(1):386. https://doi.org/10.1186/s12906-016-1359-7
    PMid:27716154
13. Suvik A, Effendy AW. The use of modified Masson's trichrome staining in collagen evaluation in wound healing study. Malays J Vet Res. 2012;3(1):39-47.
14. Goud BJ, Dwarakanath V, Swamy BK Streptozotocin-a diabetogenic agent in animal Models. Int J Pharm Pharm Res. 2015;3(1):253-69.
15. Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J Diabetes Metab Disord. 2013;12(1):60. https://doi.org/10.1186/2251-6581-12-60
    PMid:24364898
16. Chinedu AA, Alani SO, Olaide AO. Effect of the ethanolic leaf extract of *Moringa oleifera* on insulin resistance in streptozotocin induced diabetic rats. J Plant Sci. 2014;2(6-1):5-12. https://doi.org/10.11648/j.jps.s.2014020601.12
17. Salkovic-Petrisic M, Knezovic A, Hoyer S, Riederer P. What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research. J Neural Transm (Vienna). 2013;120(1):233-52. https://doi.org/10.1007/s00702-012-0877-9
    PMid:22886150
18. Utary N, Murti K, Septadina IS. Effects of Moringa (*Moringa oleifera*) leaf extract on alveolar diameter of breastfeeding and weight of infant Wistar rats. J Phys Conf Ser. 2019;12067:012067. https://doi.org/10.1088/1742-6596/1246/1/012067
19. Reinke JM, Sorg H. Wound repair and regeneration. Eur Surg Res. 2012;49(1):35-43. https://doi.org/10.1159/000339613
    PMid:22797712
20. Li M, Zhao Y, Hao H, Dai H, Han Q, Tong C, *et al*. Mesenchymal stem cell-conditioned medium improves the proliferation and migration of keratinocytes in a diabetes-like microenvironment. Int J Low Extrem Wounds. 2015;14(1):73-86. https://doi.org/10.1177/1534734615569053
    PMid:25759411
21. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010;89(3):219-29. https://doi.org/10.1177/0022034509359125
    PMid:20139336
22. Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, *et al*. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. Int Wound J. 2017;14(1):89-96. https://doi.org/10.1111/iwj.12557
    PMid:26688157
23. Wu AS, Kalghatgi S, Dobrynin D, Sensenig R, Cerchar E, Podolsky E, *et al*. Porcine intact and wounded skin responses to atmospheric nonthermal plasma. J Surg Res. 2013;179(1):e1-12. https://doi.org/10.1016/j.jss.2012.02.039
    PMid:22480830
24. Fukuoka H, Narita K, Suga H. Hair regeneration therapy: Application of adipose-derived stem cells. Curr Stem Cell Res Ther. 2017;12(7):531-4. https://doi.org/10.2174/1574888X12666170522114307
    PMid:28530535
25. Winarsih W, Wientarsih I, Sutardi LN The activity of turmeric extract ointment in the wound healing process of induced diabetic mice. J Vet. 2012;13(3):242-50.
26. Widowati W, Wijaya L, Murti H, Widyastuti H, Agustina D, Laksmitawati DR, *et al*. Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation. Biomarkers Genomic Med. 2015;7(1):8-17. https://doi.org/10.1016/j.bgm.2014.08.008
27. Prasetyono TO. General concept of wound healing, revisited. Med J Indones. 2009;18:208-16.
28. Xue M, Jackson CJ. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv Wound Care (New Rochelle). 2015;4(3):119-36. https://doi.org/10.1089/wound.2013.0485
    PMid:25785236