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Gel formulations of *Merremia mammosa (Lour.)* accelerated wound healing of the wound in diabetic rats

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A B S T R A C T

Background and aim: The treatment of diabetic ulcers is difficult because of defective blood vessels and frequent co-occurrence of bacterial infections. In a previous study, we found a water fraction of *Merremia mammosa (Lour.) (Mm(Lour.))* had beneficial effects on wound healing in diabetic rats. This study aimed to evaluate the influence of different gelling agents added to *Mm(Lour.)* water fraction gel on wound healing treatment in diabetic rats.

Experimental procedure: Diabetic Wistar rats were divided into the following five groups: 1. positive control (Neomycin Sulfate 0.5% and Placenta Extract 10%), 2. negative control (distilled water), and 10% water fraction of *Mm(Lour.)* extract in 3. HPMC, 4. Carbopol, and 5. CMC Na gelling agents. The wound was made by the Morton method and treatment applied every other day for 25 days, then the wound healing process was observed. Data were observed and analysed using appropriate statistic tools.

Results: Histopathology observation, VEGF expression and hydroxyproline levels showed a significant acceleration of wound healing in all treatment groups compared to the negative control group. This study showed all of *Mm(Lour.)* gel formulations could restore the delayed healing process on wound in diabetic rats and were equally effective in accelerating wound healing. CMC Na was the most preferable because it did not irritate.

Conclusion: The results suggest that *Mm(Lour.)* water fraction in CMC Na gelling agent provided an option to be developed as a topical drug on diabetic wound healing treatment, showed by enhancement of collagen synthesis and angiogenesis.

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Taxonomy

Diabetic foot ulcer, Animal model for drug effect, Antioxidant studies, Anti-inflammatory activity, Antibacterial activity, Collagen

1. Introduction

Wound healing involves complex processes including the inflammatory phase, granulation, and tissue remodeling.[1] This process is triggered by growth factors and cytokines, and complications may be occur, influenced by many factors like Diabetes mellitus (DM).[2] The high costs incurred treating wounds in DM patients, the risk of amputation,[3] the fact that topical drugs of choice do not contain comprehensive activities[4,5] and the difficulty of handling diabetic wounds[6,7] requires the development of effective drugs that come from local Indonesian resources.

Indonesia has about 40,000 endemic plants (typical of the

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List of abbreviations

BW	body weight
DM	Diabetes Mellitus
CMC	Na carboxymethylcellulose sodium
FGF	Fibroblast Growth Factor
HCl	Hydrochloric acid
HE	Hematoxylin-Eosin
HPMC	hydroxypropylmethylcellulose
LSD	Least Significant Difference
Mm (Lour.)	*Merremia mammosa (Lour.)*
MT	Masson's Trichrome
PDGF	Platelet-Derived Growth Factor
ROS	Reactive Oxygen Species
SOD	Super Oxide Dismutase
STZ	Streptozotocin
TEA	triethanolamine
TGF-β	Transforming Growth Factor β
VEGF	Vascular Endothelial Growth Factor

region) and 7,000 of them are considered medicinal in some way by local cultures. However, there are only about 200 medicinal plants that have been studied extensively. *Merremia mammosa (Lour.) (Mm (Lour.))* is locally used as antidiabetic, antibacterial and anti-inflammation therapy.[8–10] Our previous study examined extract and extract fractions of *Mm (Lour.)* showed that each could accelerate wound closure in diabetic wound healing and increase the density of collagen in diabetic wounds.[11–13] The effective dose of a water extract fraction of Mm (*Lour.*) was 50 mg/625 mm^2 wounded area.[12]

Modern treatment protocols for wound advise maintaining moist environmental conditions on the wound itself,[14] which could not be achieved if simply applying a water fraction. Therefore, this study aimed to develop the Indonesian original plant, *Mm (Lour.)*, preparation in a gel form for the treatment of diabetic wounds to maintain a moist environment for a longer period of time. Gel formulations are also more easily spread, greaseless, and are also water-soluble.[15] However, in a topical preparation, drug release from the matrix is an important parameter for bioavailability and efficacy. Thus, the choice of gelling agent can influence drug release in topical gel preparations.[16,17]

In this study, we used a gel containing neomycin and placenta extract as a positive control. Topical gels with neomycin and placenta extract have been shown to have a regenerating effect with antibacterial, anti-inflammatory, antioxidant, and pro-angiogenesis activities. For comparison *Mm (Lour.)* fraction were prepared using cellulose derivates of gelling agents i.e., hydroxypropylmethylcellulose (HPMC), and carboxymethylcellulose sodium (CMC Na), as well as a synthetic gelling agent (Carbopol). These gelling agents have high stability and compatibility, low toxicity, and increase skin contact time. Thereby, they can increase the effectiveness of gel usage.

2. Materials and methods

2.1. Chemicals and reagents

Ethanol, n-hexane, ethyl acetate, chloroform, ether, gallic acid, HPMC, CMC Na, Carbopol, triethanolamine (TEA), propylene glycol, and xylol were purchased from Merck [Indonesia]. Other chemicals such as ketamine hydrochloric acid (HCl), xylazine and vascular endothelial growth factor (VEGF) polyclonal antibody were purchased from Guardian Pharmatama [Indonesia], Interchemie Werken [imported from Holland], Bioss [imported from USA] and the *General Hydroxyproline Assay Kit®* [imported from, China]. Trichrome stain, hematoxylin-eosin (HE) stain, citrate buffer solution, and Streptozotocin (STZ) were purchased from Sigma-Aldrich [Indonesia]. All other chemicals and reagents used for the analysis were analytical grade.

2.2. Preparation of plant extract and fraction

Mm (Lour.), was collected from Klaten, Central Java Province, Indonesia. Plant identification was carried out by referring to the Tropical Plants Database, Ken Fern.[18] Plants were labelled and deposited in the Herbarium Jemberiense, Biology Department, Mathematics and Natural Science Faculty, University of Jember (84/HB/7/2017). Extraction and fractionation of the plant tissues were carried out based on protocols from previous research.[11,12] Briefly, a total of 1 kg of simplicia powder was extracted by ultrasound using 70% ethanol solvent for 1 h. Then, it was filtered with a Buchner funnel to obtain the filtrate. The residue was re-extracted once. The resulted filtrate was concentrated with a rotary evaporator until a thick ethanol extract was obtained.

The ethanol extract was added to water in a ratio 1:2 and stirred until homogeneous. This water fraction was subsequently placed in a successive partition using n-hexane and ethyl acetate with a ratio of 2:3. This process was repeated three times and then the resulting solution freeze-dried until viscous water fraction was obtained. The water fraction was standardized before being used for formulation. The parameters of standardization were organoleptic, drying shrinkage, thin layer chromatography profile and total flavonoids content. The total flavonoids content of Mm (*Lour.*) water fraction using the $AlCl_3$ method was $0.17 \pm 0.009\%$ w/w[11].

2.3. Formulation and physical properties testing of gels

Mm (Lour.) gels were prepared by incorporating different gelling agents (HPMC, Carbopol or CMC Na) to the most potent dose and extract fraction of *Mm (Lour.)* according to our previous study (a 10% water fraction).[11,12] The formulation was as follows: 10% water fraction of *Mm (Lour.)* ethanol extract, 1.5% gelling agent, 0.5% triethanolamine, 20% propylene glycol, and 68% distilled water. The process of making a gelling agent began by developing the gelling agent in hot water, stirring until homogeneous, and then adding TEA slowly until a gel mass was formed. The water fraction was mixed with propylene glycol until it was homogeneous. Then it was mixed into the gelling agent and stirred again until homogeneous. The remaining distilled water was added slowly until homogeneous. The physical properties of gel formulation to be tested, including organoleptic test, pH, viscosity and spread ability.

2.4. Diabetic induction and wound excision

This study used a post-test only control group design. Early adulthood male Wistar rats weighing between 200 and 250 g kept in individual cages with a standard feed of ad libitum food and water. Fifty rats were divided into five groups ($n = 10$ per group), which consisted of positive control (Neomycin Sulfate 0.5% and Placenta Extract 10%), negative control (distilled water) and 10% water fraction of *Mm (Lour.)* extract in each 1.5% gelling agent (i.e., HPMC, Carbopol, CMC Na). Diabetes induction was carried out using STZ solution in 0.05 M (pH 4.5) citrate buffer at a dose of 40 mg/kg body weight (BW), given to rats intraperitoneally. Random blood glucose level examinations were carried out 24 h before and after induction each week during the experiment to monitor the diabetic condition of the rats.[19]

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Wound excision was carried out in rats that had GDA levels ≥200 mg/dL. Ketamine of 80 mg/kg BW and xylazine of 10 mL/kg BW were injected intramuscularly as anesthesia. Excision was done on the rat backs 1 cm from the left side of the vertebral column by the Morton method.[20] A 2 × 2 cm area of skin was excised from the epidermal layer to the subcutaneous layer as well as the connective tissue below it. Wound healing rate was calculated according to Heidari et al..[21] This study followed the standard of ethics of Health Law research number 23/1992 and obtained ethical approval number 1175/H25.1.11/KE/2017 from the Faculty of Medicine, University of Jember.

2.5. Wound healing parameters

Wound healing processes were observed at day 3, 10, and 25 after excision ($n = 3$; $n = 4$; $n = 3$, respectively, for each treatment group of $n = 10$), following wound healing phases. At days 3, 10, and 25, the observed rats were then sacrificed for further test via cervical dislocation. Excisions were performed to obtain tissue for histopathological examination with HE staining and, at day 10 only, for VEGF immunohistochemical examination. Histopathological observation with HE staining was performed according to methods listed in a previous study.[12]

VEGF examination on day 10 groups was performed using an immunohistochemical kit (BIOSSUSA) with paraffin blocks cut 4 μm thick and then deparaffinized and rehydrated. Then, 0.5% endogenous peroxidase was added for 30 min for blocking, and placed in decloaking chamber at 110 C with Diva solution added and blocked with 5% normal horse serum for 30 min. Immunostaining used the indirect method with polyclonal rabbit primary antibody at a 1: 100 standard dilution incubation for 60 min, and incubated with Universal Link secondary antibody for 30 min. Trekavidin HRP labels were added and incubated for 30 min before counterstaining with HE. VEGF expression was assessed using a 400x magnification Olympus CX21LED microscope on five visual fields with the help of ImageJ software. Assessment of VEGF expression was carried out quantitatively. A histology score was used in calculating VEGF expression by multiplying the percentage of the browned areas with brown intensity and averaging over each field of view.[22,23]

Connective tissues were observed at day 10 after excision using histopathological examination with modified Masson's Trichrome (MT) staining specific to collagen, which stained collagen fibers a bright blue color,[13,24] and measuring hydroxyproline levels. Observation of collagen density was carried out with 400x magnification at 6 fields of view, and then, pictures were taken using Olympus DP21 series microscope. The percentage of collagen density was measured using imageJ software according to a previous study.[13] Hydroxyproline level measurement followed the *General Hydroxyproline Assay Kit*® protocol. A standard curve to measure concentration of hydroxyproline was made by measuring standard concentration 0; 7,5; 15; 30; 60; 120; and 240 ng/mL at 450 nm wavelength absorbance.

2.6. Safety test

A skin irritation test based on OECD guideline 404 using rabbits (three animals) was carried out for the safety test. A 2 × 2 cm area of hair on the back of each rabbit was carefully shaved off 24 h before treatment. A total of 0.5 g of each gel was applied to the 2 × 2 cm area, then it was covered with gauze and tape. After 4 h, the gels were washed off with water and the appearance of erythema and oedema observed at 1, 24, 48, and 72 h after the gels were removed. Erythema and oedema were assessed via a scoring method ranking severity on a 0–4 scale.

2.7. Statistical analysis

Statistical tests were performed with a one-way ANOVA test or Kruskal-Wallis test, depending on the normality of the data distribution and were followed by post hoc Least Significant Difference (LSD) or Mann-Whitney test as appropriate. SPSS v15 software was used for analysis. $P < 0.05$ showed statistical significance.

3. Results

3.1. Wound healing parameters

The percentage of reduction in wound size comparison showed a difference for every gelling agent when compared with negative control, although it was not statistically significant (Fig. 1A). The study showed a tendency that among the three gelling agents, HPMC and CMC Na had a similar healing rate, relevant to the high bioavailability and Carbopol was the less. This result showed that although not significant, different gelling agents provide a tendency for different release rate that may affect the topical drug potency.

Photomicrograph observation of HE staining in each healing phase showed that there were no observable differences yet on day 3 after wound excision. On day 10 and 25, Carbopol had a slower wound healing rate compared to other gelling agents, as seen by evaluating the expression of angiogenesis, macrophage, fibroblast and collagenas well as macroscopic appearance. Other gelling agents were similar to the positive control healing phase, as described by an optimum level of VEGF expression, and no macrophage (Figs. 2 and 3). VEGF expression data were normally distributed, and with same variants, analysis used a one way ANOVA test, showing a significant treatment effect with $p = 0.017$. The data, then, were tested with post hoc LSD test, which showed the negative control (C(−)) significantly difference than the positive control (C(+)), T1, T2, and T3 ($p = 0.023$; $p = 0.005$; $p = 0.017$; $p = 0.002$, respectively). C(+) did not differ significantly from T1, T2, and T3. There was also no significant difference among T1, T2 and T3 ($p > 0.05$).

Collagen density results by modified MT staining in diabetic wound of rats can be seen in Figs. 1C and 4. The one way ANOVA test result showed $p = 0.001$, which means that there were significant differences in collagen density among the groups. Then, LSD test was conducted to find out the differences in each group and significant results are shown in Fig. 1C. Hydroxyproline measurement in skin tissue represented the collagen index. Kruskal-Wallis test results showed $p = 0.033$, which means that there were significant differences in hydroxyproline level among the groups. The results of the Mann-Whitney test are shown in Fig. 1D.

3.2. Gel physical properties

The organoleptic examination was done to assess the properties of each gel. Several physical properties, such as, odor, taste, and others, which are shown in Table 1.

3.3. Safety test

The wound irritation test to see the safety of the gel showed that only the rabbit with the CMC Na gelling agent applied did not experience any erythema or oedema during the test (Table 2).

4. Discussion

The diabetic animal model was STZ injected Wistar rats; the injection created a hyperglycemic condition characterized by

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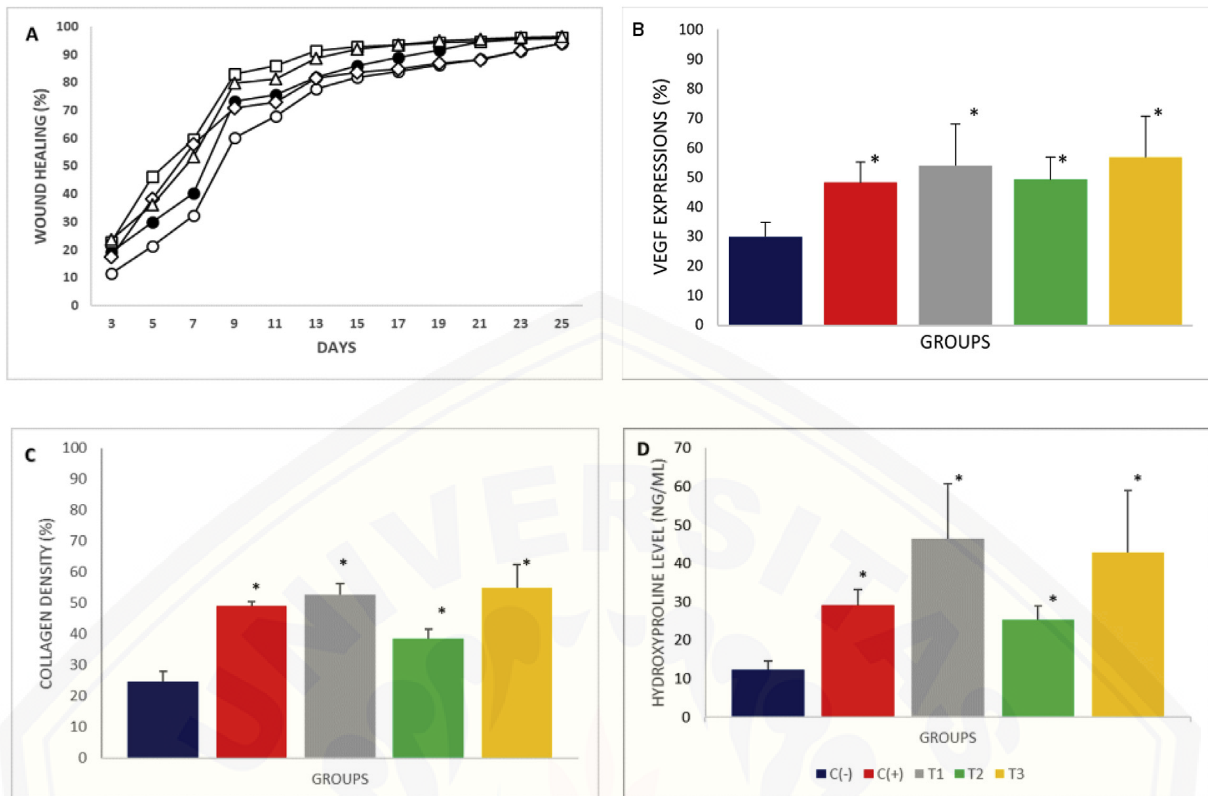

Fig. 1. The evaluation of diabetic wound healing in rats treated with *Merremia mammosa (Lour.)* gel formulation by parameters of, (A): Wound healing percentage, (B): VEGF expressions, (C): Collagen density by modified Masson's Trichrome staining, (D): Hydroxyproline level, all data represented in percentage as mean ± SEM (n = 3 or 4) except hydroxyproline level in ng/ml, *significant value at $P < 0.05$ compared negative control (groups in sequence C(−): negative control group with aquadest administration (open circle symbol), C(+): positive control group with Neomycin Sulfate 0.5% + Placenta Extract 10%administration(closed circle symbol) and *Merremia mammosa (Lour.)* gel in; T1: HPMC, T2: Carbopol, T3: CMC Na (square, diamond, triangle symbols) gelling agents administration).

random blood glucose levels of more than 200 mg/dL during the experiment. The wound healing in diabetic rats as an experimental model was expected to resemble wound conditions in diabetes patients. Diabetic wounds have significant fibroblast dysfunction and a disruptive maturation of epithelium and granulation tissue, resulting in decreased collagen synthesis acting as an extracellular matrix, as well as hydroxyproline and VEGF levels also low.[25] An ANOVA test of blood glucose levels was carried out in all groups of animals, and the result was $p > 0.05$ (data not shown), showing there were no significant differences in blood glucose levels among the groups. This result indicated that the differences in blood glucose, which served as the confounding variable that affected wound healing in each Wistar rats could be ignored.

Group C(−) with distilled water treatment as the negative control, showed lower wound healing parameters, with significant differences ($p < 0.05$) when compared to C(+), T1, T2, and T3 (Fig. 1B, C, 1D). Distilled water would not be expected to improve diabetic wound healing and would only function as wound cleanser.[26] The transition from the inflammatory phase to the proliferative phase was hampered. This was relevant to the our previous studies[11–13] of diabetic wound, which showed that wound healing in the negative control took longer than in the positive control and treatment groups, as assessed via several wound parameters. This was also similar with the result of Ackermann et al. study on diabetic wounds.[27]

Improved wound healing was observed in the treatment groups compared to the negative control group. The time needed to achieve 50% closure of the wound decreased from 8.3 ± 0.6 days (n = 6) in control negative group to 5.7 ± 0.4 days (n = 18) in the *Mm (Lour)* gels (Fig. 1A). Although not statistically significant, probably due to high variation in control negative data, as shown by the higher standard error. The reason for that were perhaps because of individual non-treatment responses in diabetic condition related to various factors.[28]

When we examined photomicrographs of HE staining, the negative control group at day 25 still showed a low density of extracellular matrix, a small amount of fibroblasts and prominent macrophages, which indicated incomplete healing, in contrast to the other groups (Fig. 2). This result was consistent with our previous study on *Mm(Lour)* extract fractions.[11-13] Therefore, the findings in this study allowed the presumption that the gel formulation of *Mm (Lour.)* water fraction possibly restored the delayed process of diabetic wound healing. This result might be attributed by the activities of merremosida and mammoside, a group of resin glycosides compound mainly found in *Mm (Lour.)*[10] and also because of flavonoid activity. Based on the standardization result, the water fraction of Mm (Lour.) contained flavonoids (0.17 ± 0.009% w/w).

The treatment of wounds requires the combined effects of antibiotics, anti-inflammatory agents, astringents, and antipyretics.[29] Resin glycosides as antibacterial agents have the activity of inhibiting pump efflux on bacterial membranes so that resistant bacteria become more sensitive.[30] The anti-inflammatory activity of resin glycosides can inhibit COX-1 and COX-2 enzymes that are overproduced in DM[31] while flavonoids that are also contained in *Mm (Lour.)*[11] inhibit bacterial cell wall synthesis and damage cell walls directly.[32] The anti-inflammatory activity of flavonoids are in the form of activation of M2 macrophages secreting various growth

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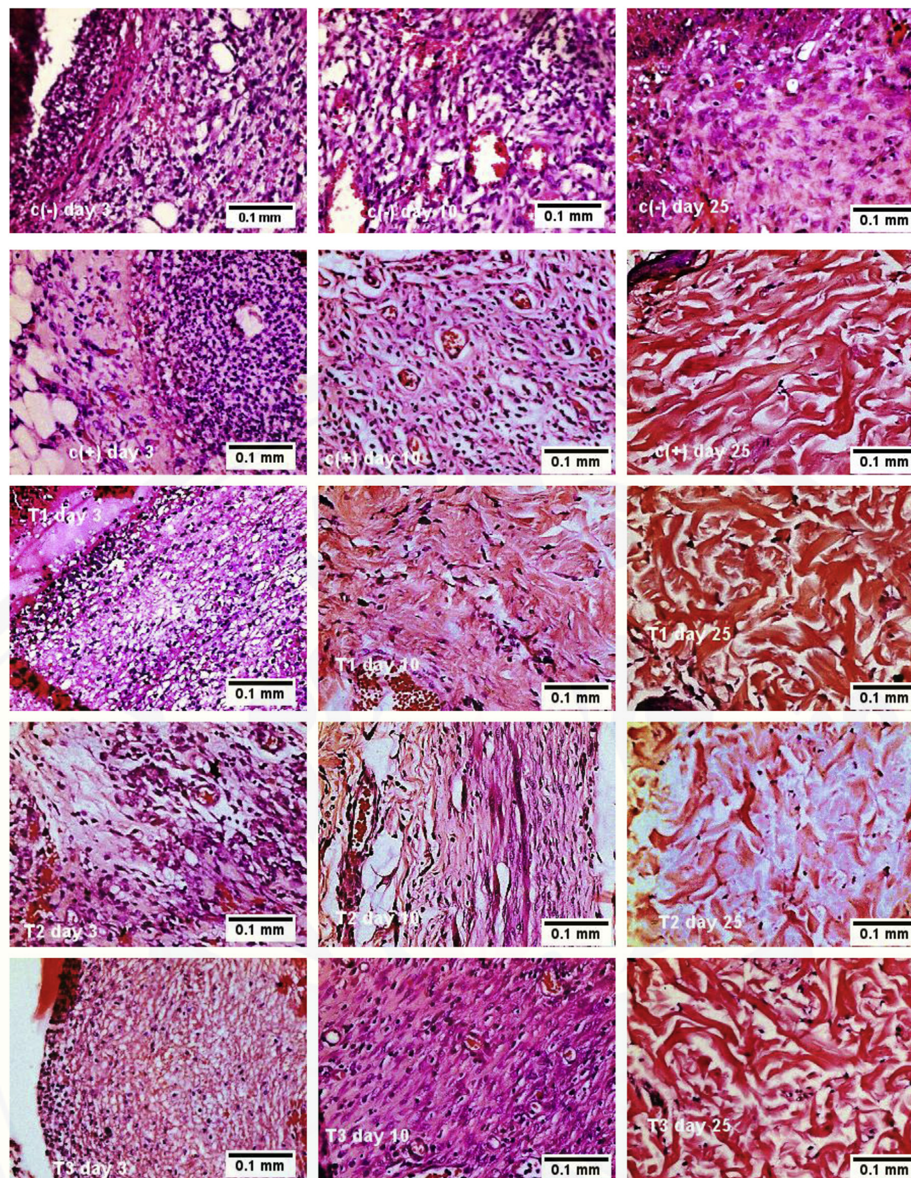

Fig. 2. Photomicrographs of rat skin sample on hematoxylin-eosin stain with at 3rd, 10th and 25th day post excision showing density of angiogenesis, fibroblast, macrophage and collagen in each group at high power view (magnification 400×) (C(−): negative control group with aquadest administration, C(+): positive control group with Neomycin Sulfate 0.5% + Placenta Extract 10% administration and *Merremia mammosa (Lour.)* gel in; T1: HPMC, T2: Carbopol, T3: CMC Na gelling agents).

factors such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), and fibroblast growth factor (FGF).[33] These mechanisms can reduce the risk of infection in diabetic wounds that experience decreased immunity.[34]

The growth factors induced by flavonoids of *Mm (Lour.)* accelerate the transition from the inflammatory phase to the proliferation phase by stimulating migration, proliferation, and activity of fibroblasts in collagen synthesis. When there is an increase in collagen synthesis activity done by fibroblasts, the synthesis of hydroxyproline by cells, which acts as a base material for collagen increases as well, which results in increasing hydroxyproline levels.[35,36] This statement is relevant to the results of this study, where collagen density, hydroxyproline and VEGF in all groups of *Mm (Lour.)* gel were significantly higher than the negative control group (Fig. 1B, C, 1D).

Flavonoids have an antioxidant activity by binding to free radicals and preventing oxidative reactions by increasing the activity of the super oxide dismutase (SOD) enzyme.[12] This enzyme will reduce reactive oxygen species (ROS) in the form of superoxide anion (O_2-) to H_2O_2, which will then undergo catalysis by catalase enzyme to neutral H_2O. If the level of ROS exceeds the amounts of antioxidants as in DM conditions, oxidative stress will occur and increase the risk of lipid peroxidation (the release of electrons in the lipid layer of cell membranes that can cause damage to membrane stability).[37] The decrease in ROS caused by flavonoids will prevent tissue damage during the inflammatory phase so that the transition to the proliferative phase will be faster and the tissue hydroxyproline level and VEGF expression higher. Juneja et al.[38] reported that the flavonoids from *Boerhavia diffusa leaf* extracts improved wound healing by enhancement in fibroblast growth and collagen fibrils, similar to our result.

Making gel formulations from *Mm (Lour.)* aims to maintain skin moisture, increase the penetration of active substances into wounds, and protect skin from the external environment. A humid

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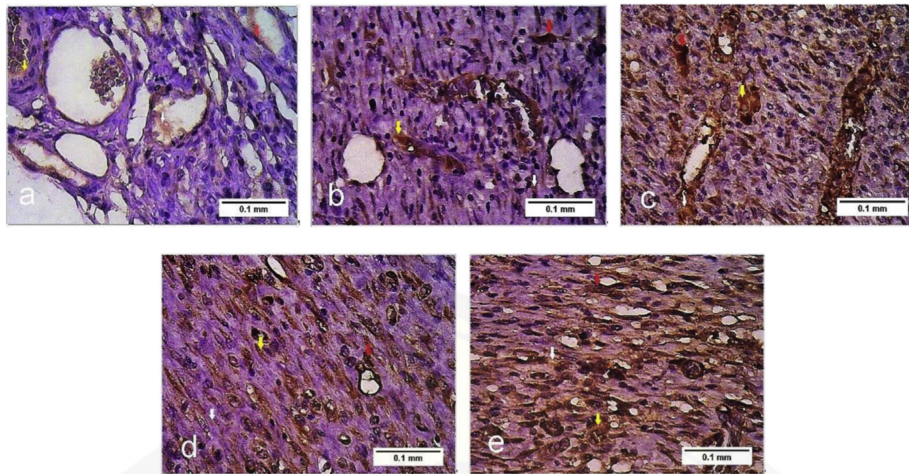


Fig. 3. VEGF expression of *Merremia mamosa (Lour.)* gel formulation treated rats at day 10 after excision with magnification of x 400, A: negative control group with aquadest administration, B: positive control group with Neomycin Sulfate 0.5% + Placenta Extract 10% administration and *Merremia mammosa (Lour.)* gel in; C: HPMC, D: Carbopol, E: CMC Na gelling agents, brown color in the image shows VEGF expression (white arrow: low intensity; yellow arrow: medium intensity; red arrow: high intensity).

environment can improve retention of growth factors such as TGF-β, PDGF, and FGF so that the proliferation response of fibroblasts and extracellular matrix synthesis increases. Moist conditions will also increase autolytic debridement.[39] Furthermore, the gel also plays a role in helping the drug penetrate the skin by changing the nature of the stratum corneum to become more tenuous, so that the drug can reach the dermis layer and affect fibroblast cells, macrophages and other immune cells located there.[40]

The C(+) group containing neomycin and placenta extract showed higher levels of hydroxyproline and differed significantly

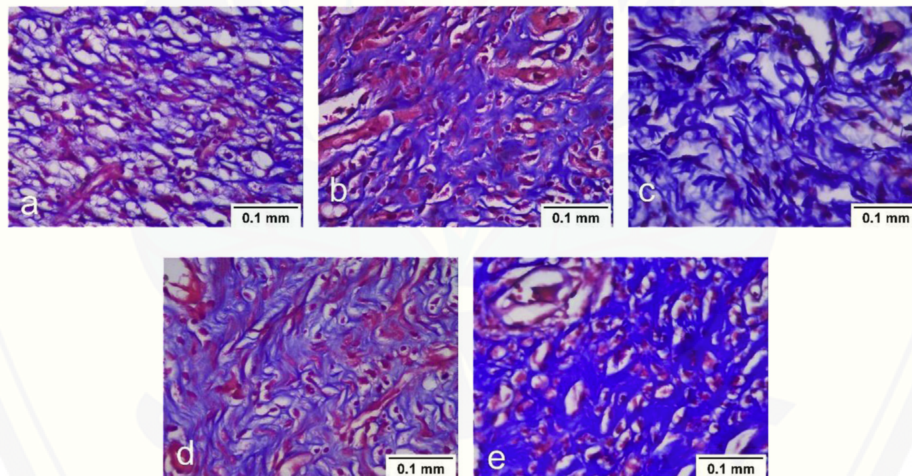


Fig. 4. Observation of *Merremia mamosa (Lour.)* gel formulation treated rats at day 10 after excision stained with modified Masson's Trichrome, A: negative control group with aquadest administration, B: positive control group with Neomycin Sulfate 0.5% + Placenta Extract 10% administration and *Merremia mammosa (Lour.)* gel in, C: HPMC, D: Carbopol, E: CMC Na gelling agents, blue color in the image shows VEGF expression.

Table 1
Gel physical properties examination.

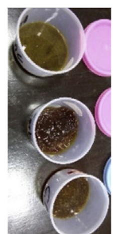
Gelling agent	Spread Ability	pH	Viscosity	Form	Colour	Separation	Picture
HPMC	5,8 cm	6,89	120 dpas	thick	brown	none	
Carbopol	4,7 cm	6,62	>100 dpas	thick solid	brown	none	
CMC Na	7,5 cm	6,48	200 dpas	thick	brown	none	

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Table 2
Observation of the safety test of gel preparations.

Gelling agent	1st hour		24th hour		48th hour		72nd hour	
	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema
HPMC	–	–	–	1st degree	–	1st degree	–	–
Carbopol	–	1st degree	–	1st degree	–	1st degree	–	–
CMC Na	–	–	–	–	–	–	–	–

when compared to the C(−) group (p = 0.034), but there was no significant difference when compared to the T1 group (p = 0.513), T2 (p = 0.480), and T3 (p = 0.480). This result likely happened because all these groups have a comprehensive activity on diabetic wound healing, which includes anti-inflammatory, antioxidant, antibacterial, and stimulation of collagen synthesis. Neomycin is a broad-spectrum antibiotic in the aminoglycoside group that is commonly used topically for infections of the skin and mucous membranes.[41] The activity of placenta extract in wound healing is as an anti-inflammatory, antioxidant, and stimulant of collagen synthesis.[42] Aside from their activities, the positive control and treatment groups were in the form of gels suitable for wound therapy.

The statistical analysis of the results showed a significant difference in collagen density between the HPMC gelling agent group compared to the Carbopol gelling agent group, where the average percentage of collagen density in the HPMC gelling agent group (52.71%) was higher than the Carbopol gelling agent group (38.59%). Meanwhile, the comparison of collagen density between the HPMC gelling agent group and the CMC Na group did not show any significant difference. On the other hand, there was a significant difference in collagen density between the gelling agent CMC Na group compared to the Carbopol gelling agent group where the average percentage of collagen density in the gelling agent CMC Na group (54.88%) was higher than the Carbopol gelling agent group (38.59%). As other parameters showed no significant difference among treatment groups, it can be concluded that there were no differences in the effect of gelling agents (HPMC, Carbopol, and CMC Na) on the activity of the water fraction gel of *Mm (Lour.)* in diabetic wound healing.

The effect of *Mm (Lour.)* gel on T1 (HPMC) and T3 (CMC-Na) groups were mostly similar, likely because HPMC and CMC-Na were both cellulose derivative polymer gelling agents that had good viscosity and swelling properties. Tas et al.[43] compared HPMC, CMC-Na, and methylcellulose as gelling agents to the active ingredient chlorpheniramine maleate and proved that HPMC showed a higher drug release rate compared to CMC-Na. This was probably the caused at day 10; HPMC group showed a tendency to be the fastest healed, but at day 25 the HPMC and CMC-Na groups healed similarly.

The analysis of differences in almost all wound healing parameters in groups T1, T2, and T3 showed non-significantly different results. These data demonstrated that the gelling agent played no significant role in the wound healing enhancing effect of Mm (*Lour.*) gels. Based on the physical properties and safety test results, it appeared that CMC Na gel with pH 6.48, the highest viscosity (200 dpas), the widest scattering power (7.5) and having no erythema or oedema on the skin was the better option to be chosen as a gelling agent. Our study showed promising results for further development for clinical use since it is safer (as a natural product) and the activity can still be enhanced by purification as well as combination treatment. Further experiments are needed to continue the development of gel *Mm (Lour.)* water fraction as a topical drug, such as clinical trial studies.

5. Conclusion

The gel formulation of *Mm (Lour.)* water fraction possibly restored the delayed process of wound healing in the diabetic rat model. It can be concluded that there were significant differences in diabetic wound healing between the negative control group and every other group. There was no significant difference between the positive control group and *Mm (Lour.)* water fraction gel in HPMC, Carbopol, or Na-CMC gelling agents. There was also no significant difference of wound healing between the different gelling agent, groups: HPMC and Carbopol; Carbopol and Na-CMC; Na-CMC and HPMC. However, *Mm (Lour.)* water fraction gel with CMC Na base showed a tendency to be the best in accelerating healing rate, meets the gel property requirements and was the only base that showed no erythema or oedema during the safety test. Therefore, the safest and suggested gel formulation to be developed as a topical drug is *Mm (Lour.)* water fraction gel with CMC Na gelling agent.

Declaration of competing interest

None

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2019.12.002.

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