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Potential of Jatropha Multifida L.- Placenta - Carboxymehyl Chitosan (CMC) Formulation as Regenerative Therapy for Burn Wounds

Mellyna Mellyna[1], Juniarti Juniarti[2], Nunung Ainur Rahmah[3]
Universitas YARSI, Indonesia
Email: dr.mellyna@gmail.com[1]*, juniarti@yarsi.ac.id[2], nunung.ainur@yarsi.ac.id[3]

Keywords

Burns; CMC; Fibroblasts; Jatropha multifida L; Placenta extract

Abstract

Second-degree burns are tissue injuries that often cause complications and require a long healing time. Previous studies have reported that natural ingredients such as Jatropha multifida L., placenta extract, and Carboxymethyl Chitosan (JM-P-CMC) have the potential to accelerate tissue regeneration, but no combined studies have been conducted on the three. The study aimed to evaluate the effect of the Jatropha multifida L.-Plasenta-CMC combination formulation on the number of fibroblasts as an indicator of tissue regeneration in second-degree burns. This experimental study used a post-test only control group design with 24 male Sprague dawley rats (200–250 g) induced burns on the back using a hot metal plate, then divided into four groups: control (Bioplacenton ®), JM5%-P-CMC, JM10%-P-CMC, and JM15%-P-CMC. Topical treatment was carried out twice a day for 14 days. Data collection was carried out on days 5, 9, and 14, followed by histological analysis of skin tissue with Hematoxylin Eosin staining to count the number of fibroblasts. The results of the Kruskal-Wallis and Mann-Whitney tests showed a significant increase in the number of fibroblasts in all treatment groups compared to the control, with the highest results in the JM15%-P-CMC group. This study concluded that the combination was effective in enhancing tissue regeneration and has the potential to be developed as an innovative topical therapy based on natural ingredients.

INTRODUCTION

Burns are a common form of tissue injury and cause serious health problems, both in developing and developed countries (Agnieszka Surowiecka et al., 2022; Lars-Peter Kamolz and Andrzej Hecker, 2023). Second-degree burns, which affect the epidermis and part of the dermis, often require a long healing time and are susceptible to infection and complications (Agnieszka Surowiecka et al., 2022; Lars-Peter Kamolz and Andrzej Hecker, 2023). In the wound healing process, there are three main phases, namely the inflammatory, proliferative, and remodeling phases (Halil İlhan Aydoğdu et al., 2020; Soheila S. Kordestani and Soheila S. Kordestani, n.d.).

One of the important parameters in the proliferative phase is the activity of fibroblasts, namely mesenchymal cells that play a role in collagen synthesis, extracellular matrix formation, and tissue remodeling (Abazari et al., 2022; Lars-Peter Kamolz and Andrzej Hecker, 2023). As the need for more effective, safe, and naturally based wound therapies increases, the combination approach of phytopharmaceuticals and biological materials such as placenta is gaining more attention. Jatropha multifida L., commonly known as the “jatropha cina” or “jatropha tintir” plant, is a tropical plant known to have pharmacological potential, including

anti-inflammatory, antimicrobial, and wound-healing properties (Juniarti et al., 2013). This plant contains active compounds such as flavonoids, tannins, and saponins, which are known to increase fibroblast activity and angiogenesis (Aryantini et al., 2021; Vieira et al., 2021). On the other hand, animal placenta has long been used in various medical products due to its content of growth factors such as VEGF, FGF, and EGF, which support tissue regeneration (Covarrubias et al., 2023; Naumenko et al., 2021). To ensure optimal penetration of active ingredients into the wound surface, carboxymethyl chitosan (CMC) is used as a hydrophilic topical base material that can maintain wound moisture (Sarymsakov et al., 2022; Mansur et al., 2023). The main problem in burn therapy is the lengthy healing process and the low effectiveness of single topical agents. Therefore, innovative formulations are needed that combine active ingredients with mutually supporting mechanisms of action. This study aims to evaluate the effect of a combination formulation of Jatropha multifida L., placenta, and CMC (JM–Placenta–CMC) on the number of fibroblasts as an indicator of tissue regeneration in second-degree burns using an animal model of Sprague Dawley rats.

Several previous studies have proven the potential of various natural and biological materials in accelerating the wound healing process. The study by Juniarti et al. (2013) examined the effect of Jatropha multifida L. on the number of fibroblasts in wounds in animal models. According to Covarrubias et al. (2023), mammalian placental extract contains growth factors that can stimulate fibroblast migration and proliferation effectively, as well as reduce local inflammation. Carboxymethyl chitosan has been used in various topical preparations due to its ability to maintain wound moisture and accelerate epithelial cell migration (Geng et al., 2023). Such combination formulations, when properly prepared, have the potential for stronger synergistic effects than single agents.

Although numerous studies have examined each component individually, very limited research has evaluated the combined effects of Jatropha multifida L., placenta, and CMC as a single formula in burn wound therapy. Previous research has generally focused on single active ingredients without examining the synergistic effects of combining natural and biological materials. Furthermore, most studies have only evaluated macroscopic parameters of wound healing, such as epithelialization time, without observing histological parameters such as fibroblast count, which are more representative in assessing dermal tissue regeneration. Scientific data quantitatively measuring changes in fibroblast activity over time—particularly on days 5, 9, and 14 post-injury—is also still limited. Understanding these cellular dynamics is crucial for determining effective timing and dosage of therapy. Another limitation is the lack of reports on the dose effects of graded concentrations of Jatropha multifida L. in combination with placenta and CMC. Therefore, this study is crucial to fill this gap and provide a scientific basis for developing more efficient, natural-based topical burn therapies.

This study aims to evaluate the effectiveness of Jatropha multifida L., placenta, and CMC formulations on increasing the number of fibroblasts as an indicator of second-degree burn tissue regeneration in Sprague Dawley rats. The main focus is to analyze the cellular response (fibroblast count) histologically on days 5, 9, and 14 after wound induction. The formulations were prepared in three different concentrations (5%, 10%, and 15% Jatropha multifida L.) to observe the effect of dose on therapeutic effectiveness. The novelty of this study lies in the multi-component approach based on phytopharmaceuticals and biological materials, which has not been widely explored systematically. This formulation combines the

anti-inflammatory and proliferative actions of bioactive compounds in Jatropha multifida L., the regenerative effects of placenta, and the CMC base that maintains wound moisture and prolongs exposure time of active ingredients. With observations carried out in stages, this study provides detailed information on fibroblast dynamics during the healing process. The benefits of this research are to contribute scientifically to developing more effective, safe, and local natural resource-based topical burn therapies. The results can serve as a foundation for developing environmentally friendly and economical pharmaceutical preparations while expanding knowledge on the use of medicinal plants and biological materials in regenerative medicine. Furthermore, if proven effective, this formulation has the potential to be further developed into clinical trials as an alternative burn treatment for the community.

RESEARCH METHOD

Research Design

This study is a laboratory experimental study using a post-test-only control group design. The study aims to evaluate the effectiveness of topical gel formulations made from Jatropha multifida L., placenta, and CMC on the number of fibroblasts in a second-degree burn model in Sprague Dawley rats. The research was conducted at the Herbal Laboratory and Animal Research Facility of YARSI University, Jakarta, in 2025. All procedures obtained ethical approval from the YARSI University Health Research Ethics Committee with number: 076/KEP-UY/EA.10/11/2025.

Sprague Dawley rats weighing 200–250 grams and aged ±2–3 months were used in this study. The sample size was determined based on the Federer formula, which suggests a minimum of six rats per group. A total of 24 rats were randomly divided into four treatment groups, namely:

a. Positive control group: given Bioplasenton® ointment.
b. JM 5% + Placenta + CMC group: given 5% Jatropha multifida L. gel formulation.
c. JM 10% + Placenta + CMC group: given 10% Jatropha multifida L. gel formulation.
d. JM 15% + Placenta + CMC group: given 15% Jatropha multifida L. gel formulation.
e. All rats were given second-degree burn induction on the back, then topical treatment was administered daily for 14 days.

Data Collection Techniques

Second-degree burns were induced on the backs of the rats after being anesthetized with 50 mg/kgBW ketamine and 5 mg/kgBW xylazine intraperitoneally, by attaching a hot metal rod (1 × 1 cm diameter) at a temperature of 100°C for 15 seconds (Cai et al., 2014). After wound induction, each group received topical treatment according to their group allocation. The gel formulation was applied evenly twice a day for 14 days

Tissue sampling was carried out on days 5, 9, and 14 (Krissanti et al., 2023). Rats at each period were decapitated, and skin tissue was collected from the wound area for histological examination. The tissue was fixed in formalin, stained with Hematoxylin–Eosin (HE), and observed under a light microscope.

Research Instruments

The instruments used in this study included a water bath, mortar and pestle, shaving machine, microtome, embedding set, light microscope (Olympus CX33, 400× magnification), sterile containers (for collecting sap and tissue), hot metal rod (for wound induction), SPSS version 27 (for statistical analysis), and gel base (as a carrier medium for topical active ingredients).

The number of fibroblasts was counted manually in three random fields of view of each histological preparation by identifying spindle-shaped cells in the dermis layer.

Data Analysis Techniques

Fibroblast count data obtained on days 5, 9, and 14 were analyzed using the Kruskal–Wallis test to determine significant differences between treatment groups. If a significant difference was found ($p < 0.05$), the Mann–Whitney post hoc test was used to determine which groups differed significantly. The analysis was performed using SPSS version 27. The results are presented in tables and graphs to illustrate the trend of changes in fibroblast count in each group and across the observation periods.

RESULT AND DISCUSSION

This study aims to evaluate the effect of *Jatropha multifida* L. formulation with a combination of placenta and CMC on the number of fibroblasts as an indicator of tissue regeneration after second-degree burns in *Sprague Dawley rats.*

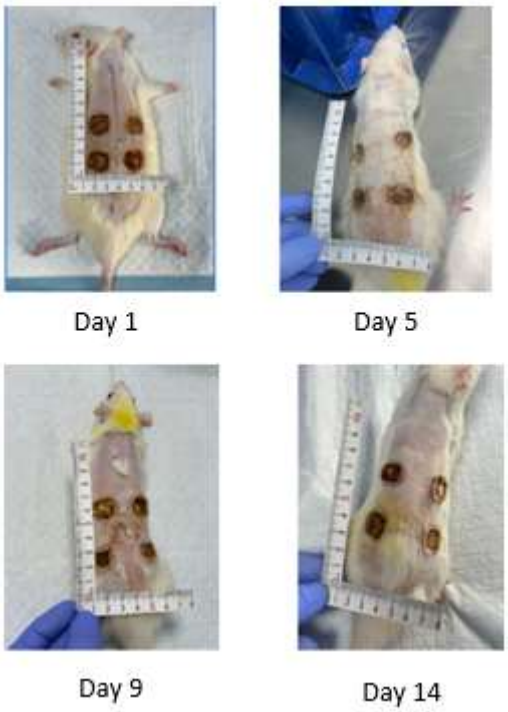

Figure 1. Progression of burn wound healing in male white rats of *the Spraque dawley strain* given 4 treatments. Visual comparison between groups, with emphasis that JM 15%-P -CMC

had better visual performance at the end of observation (day 14). (upper left wound: JM 5%-P-CMC; upper right wound JM 10%-P-CMC; lower left wound JM 15%-P-CMC; lower right wound Bioplacenton).

The histological picture of the number of fibroblasts on days 5, 9 and 14 after the burn was induced showed that:

Table 1. Average Number of Fibroblasts on Days 5, 9 and 14

TREATMENT	DAY 5	DAY 9	DAY 14
Bioplacenton ®	89.33	104	234.33
JM5%-P-CMC	101	132.33	276.00
JM10%-P-CMC	108.33	151.33	291.33
JM15%-P-CMC	121.33	143.33	401.00

The results of histological examination showed a significant increase in the average number of fibroblasts in the treatment group compared to the bioplacenton control. On day 5, the highest number of fibroblasts was found in the JM15%-P-CMC group (101 cells), followed by JM10%-P-CMC (108.33), and JM5%-P-CMC (121.33), compared to the control (89.33). On day 9, the increase continued with the highest number in JM10%-P-CMC (151.33), followed by JM15%-P-CMC (143.33), and JM5%-P-CMC (132.33). Day 14 showed a large spike, especially in JM15%-P-CMC which reached 401, much higher than the control group (234.33).

Microscopic image of skin tissue with Hematoxylin Eosin staining is shown in Figure 2.

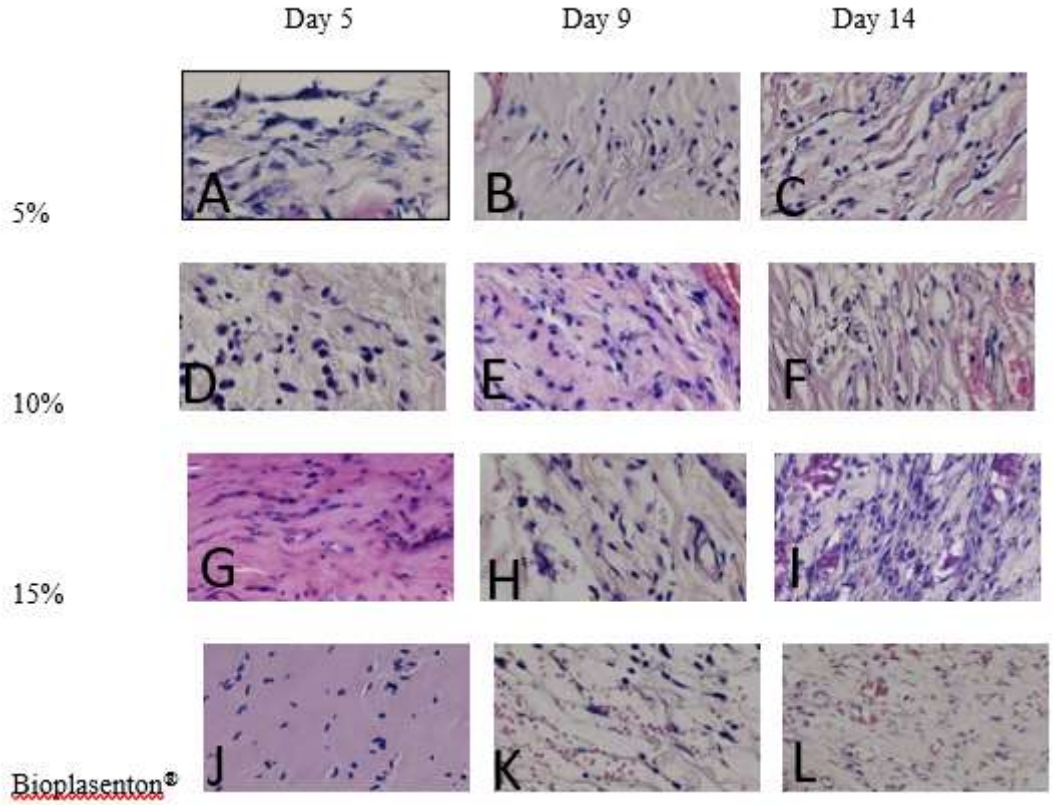

Figure 2. Histopathological picture of fibroblast cells in burn skin tissue of male *Sprauge dawley rats* treated (HE, 400x). A. JM (Jatropha multifida L.) 5%+Placenta (P)+CMC day 5; B. JM 5%+P+CMC day 9; C. JM 5%+P+CMC day 14; D. JM 10%+P+CMC day 5; E. JM 10%+P+CMC day 9; F. JM 10%+P+CMC day 14; G. JM 15%+P+CMC day 5; H. JM 15%+CMC+P day 9; I. JM 15%+CMC+P day 14; J. Bioplacenton day 5; K. Bioplacenton day 9; L. Bioplacenton day 14.

Figure 2 shows that the number of fibroblasts in each treatment increased as the dose of Jatropha multifida resin used in the formulation increased. Comparative fibroblast data are presented in the graph in Figure 3

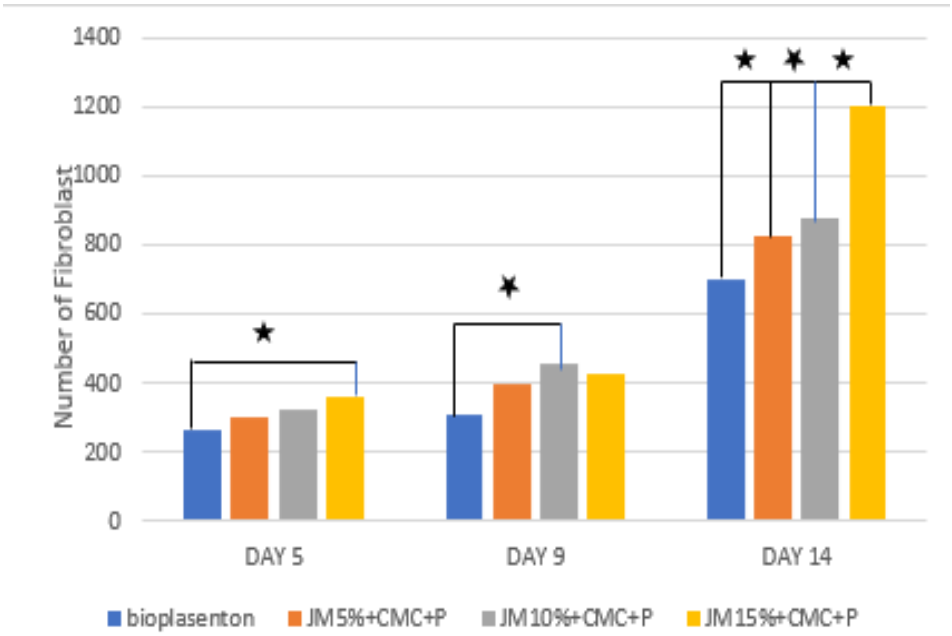

Figure 3. Graph of the number of fibroblasts based on treatment groups (*meaning **p<0.05).**

The JM15%+CMC+P formulation consistently showed an effect of increasing the number of fibroblasts. fibroblasts highest**,** especially significant for Bioplacenton on days 5, 9, and 14. Day 14 was the peak point of the healing effect, marked by a significant increase compared to the control group.

This graph indicates that increasing concentrations of *Jatropha multifida* L. are directly proportional to increased fibroblastic activity in the wound healing process. The combination of active ingredients with anti-inflammatory, regenerative, and growth factor-rich properties provides a synergistic effect in accelerating fibroblast proliferation. These results support the potential of *the Jatropha multifida* L.-Placenta-CMC combination as an innovative topical therapy for second-degree burns.

Burns are a form of tissue trauma that requires appropriate treatment to prevent complications and speed up the healing process. (Abazari *et al* ., 2022; Kamolz and Hecker,

2023) . One of the main indicators in the wound healing process is the presence and activity of fibroblasts, cells that play a crucial role in collagen and extracellular matrix synthesis, as well as tissue re-epithelialization (Ardizzone *et al* ., 2023; Tottoli *et al* ., 2020) . In this study, a formulation based on *Jatropha multifida* L., placenta, and CMC showed the potential to accelerate tissue regeneration by increasing the number of fibroblasts in a rat model of second-degree burns.

Fibroblasts play an important role in the proliferative phase of wound healing and are an indicator of wound healing. (Abazari *et al* ., 2022; Hye-na Ahn *et al* ., 2020) . These cells migrate to the wound area and produce collagen and other extracellular matrix components that are essential in rebuilding dermal tissue (Jaurila *et al* ., 2021; Winkler *et al* ., 2020) . The high number of fibroblasts, as shown in the JM15%-P-CMC group, reflects an active regenerative response. The significant increase in the number of fibroblasts, especially on day 14, indicates that this formulation not only stimulates the initial migration of fibroblasts but also maintains their activity over a longer period of healing. This is an indicator of the therapeutic effectiveness of the formulation against the proliferative phase of the wound.

Jatropha multifida L. is known to contain phytochemicals such as flavonoids, tannins, and saponins that have anti-inflammatory, antioxidant, and antimicrobial activities (Aryantini *et al* ., 2021; Nouvlessounon *et al* ., 2023) . Flavonoids, in particular, have been known to stimulate angiogenesis and fibroblast proliferation, as well as inhibit free radical activity that can slow the wound healing process (Chaker *et al* ., 2023; Taheri *et al* ., 2022) . The results of this study showed that increasing the dose of *Jatropha multifida* L. from 5% to 15% was directly proportional to the increase in the number of fibroblasts. This supports the hypothesis that the bioactive compounds in this plant work dose-dependently in accelerating tissue regeneration.

The mammalian placenta is rich in growth factors such as VEGF (*Vascular Endothelial Growth Factor*), FGF *(Fibroblast Growth Factor*), and EGF (*Epidermal Growth Factor*), which support fibroblast migration and proliferation as well as angiogenesis (Covarrubias *et al* ., 2023; Naumenko *et al* ., 2021) . Several studies have shown that placenta extract can accelerate wound healing time through cellular stimulation effects and modulation of the local immune response (Covarrubias *et al* ., 2023; Bobrova *et al* ., 2022) . The results of this study indicate that the combination of *Jatropha multifida* L. with the placenta provides a synergistic effect, where growth factors from the placenta strengthen the regenerative stimulation provided by the active compounds *of Jatropha multifida* L. This combination has been shown to be more effective than the use of Bioplacenton alone.

Carboxymethyl Chitosan is used as a vehicle in the formulation (Khan *et al* ., 2021) . *Carboxymethyl Chitosan* is hydrophilic, maintains a *moist wound healing environment* , and prolongs the contact time of the active ingredient with the tissue (Sarymsakov *et al* ., 2022; Geng *et al* ., 2023) . Moist environmental conditions have been shown to accelerate cell migration and collagen synthesis by fibroblasts, compared to dry wounds which tend to experience crusting and delayed healing (Sarymsakov *et al* ., 2022; Budiman *et al* ., 2021; Tottoli *et al* ., 2020) . The role of CMC in the formulation of this study allows optimal penetration of the active ingredient, while maintaining the integrity of the formulation during topical application.

The advantage of the formulation in this study is the multi-component combination approach: medicinal plants (*Jatropha multifida* L), natural biological materials (placenta), and

moisturizing base (CMC), which have not been comprehensively studied. This formulation shows higher efficiency compared to single therapies such as bioplacenton or plant extracts alone. The synergistic effect of *Jatropha multifida* L, placenta, and CMC likely works through several simultaneous mechanisms; (1) as an anti-inflammatory: flavonoids reduce pro-inflammatory cytokines such as TNF-α and IL-1β; (2) as a fibroblast stimulant: growth factors from the placenta and flavonoids promote the proliferative cycle of fibroblasts; (3) as angiogenesis: VEGF from the placenta accelerates the formation of new capillaries, supporting tissue oxygenation; (4) as *a moist wound healing* : CMC maintains a moist wound environment and supports epithelial cell migration.

While the results of this study are promising, there are limitations that need to be considered. These include: the study was limited to a single parameter (fibroblast count). Other parameters such as collagen gene expression, neovascularization, and epidermal thickness were not analyzed. The study was conducted only on male mice. Hormonal responses in female mice may differ.

For further research, it is recommended to evaluate the toxicity and skin irritation tests of the formulation, evaluation of the molecular mechanism through immunohistochemistry or RT-PCR, and initial clinical trials in humans to evaluate the clinical effectiveness of the formulation.

CONCLUSION

This study successfully achieved its objectives by demonstrating that the formulation of Jatropha multifida L., placenta, and CMC was able to increase the number of fibroblasts as an indicator of tissue regeneration in second-degree burns. These findings provide an important contribution in the development of topical therapies based on natural ingredients that are safer, more effective, and have the potential to be widely developed in the community as an alternative treatment for wounds that are economical and environmentally friendly. In addition, the results of this study enrich the scientific literature in the field of regenerative medicine and herbal pharmacy, and open up opportunities for innovation in phytopharmaceutical products that support the principle of sustainability in the use of local resources. Therefore, it is recommended that further research be conducted by expanding biological parameters such as wound healing gene expression, toxicity testing, and testing the stability and effectiveness of the formulation in other test models and human clinical trials, to strengthen the validity and applicability of these findings in broader clinical practice.

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