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A Study on the Effectiveness of Jatropha multifida L. Latex–CMC (Carboxymethyl Chitosan) in the In Vivo Healing of Second-Degree Burns (Expression of FGF, Fibroblasts, and Collagen)

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Jatropha multifida L., Carboxymethyl Chitosan, burn wound, fibroblast, collagen, FGF.

ABSTRACT
Burn injuries remain a significant health concern because delayed healing can increase the risk of infection, scarring, disability, and treatment-related costs. This study aimed to evaluate the effectiveness of a gel formulation combining 15% Jatropha multifida L. latex and 3% carboxymethyl chitosan (CMC) in the healing of second-degree burns. Male Sprague–Dawley rats were assigned to six groups: saline, gel base, J. multifida latex, 3% CMC, Bioplacenton, and J. multifida–CMC gel. Second-degree burns were induced using a heated metal probe, and treatments were administered according to group allocation. Skin tissue samples were collected on days 5, 10, and 15. Fibroblast growth factor (FGF) expression was examined using immunohistochemical analysis, fibroblast numbers were quantified using hematoxylin–eosin staining, and collagen density was evaluated using Masson's trichrome staining. Data were analyzed using parametric or nonparametric statistical tests with a significance level of $p < 0.05$. The combined gel increased FGF expression, particularly on day 15, and produced significant differences in fibroblast numbers across all observation periods. Collagen density also improved significantly on days 10 and 15, indicating enhanced extracellular matrix formation and tissue remodeling. These effects may be attributed to the complementary bioactive properties of J. multifida latex and the moist, biocompatible environment provided by CMC. In conclusion, J. multifida–CMC gel demonstrated potential as an antibiotic-free therapeutic option for second-degree burn healing; however, further toxicity assessments and clinical studies are required to confirm its safety and efficacy.

INTRODUCTION

The skin, the largest organ in the human body, plays a crucial role in maintaining body integrity. It functions as a protective barrier against external environmental exposure, contributes to thermoregulation, and supports sensory perception. Damage to the skin, particularly from burns, can impair these essential functions. Burns are injuries to the skin or underlying tissues caused by various factors, including heat, radiation, electricity, chemicals, sunlight, or friction. Second-degree burns are characterized by damage to the epidermis and upper dermis, accompanied by blister formation, edema, and severe pain. Optimal burn healing is essential to prevent infection, minimize scarring, and restore skin function. The burn-healing process occurs through three interconnected phases: inflammation, proliferation, and remodeling (Gospodarek et al., 2022; Hall et al., 2017).

During the proliferation phase, fibroblast growth factor (FGF) plays a crucial role in stimulating fibroblast migration and proliferation at the wound site, which contributes to extracellular matrix formation, including collagen synthesis. Increased FGF expression is associated with enhanced fibroblast activity and collagen production, both of which contribute to the formation of new granulation tissue. Collagen density serves as an important indicator of wound-healing quality (Zahra et al., 2021; Zhang et al., 2021).

According to data from the World Health Organization (WHO), approximately 11 million burn cases occur worldwide each year, resulting in around 180,000 deaths. In Indonesia, the Ministry of Health has reported approximately 200,000 burn cases annually (Anggriant et al., 2022; Aydoğdu et al., 2020). Burns not only cause physical impairment but also impose substantial economic and psychological burdens on patients. Medical treatment costs, rehabilitation requirements, productivity losses, reduced quality of life, and increased risks of morbidity and mortality contribute to the overall impact of burn injuries (Yudhanarko et al., 2019).

One commonly used topical therapy for burns is Bioplacenton®, which contains placental extract and neomycin sulfate (Aydoğdu et al., 2020). Although this preparation is effective in accelerating the healing of superficial wounds, its effectiveness may be limited in more complex or severe injuries (Ardizzone et al., 2023). In addition, prolonged use may increase the risk of bacterial resistance due to its antibiotic component, neomycin sulfate (Bastari et al., 2023). These limitations highlight the need to develop alternative burn therapies that are effective, safe, and associated with minimal adverse effects.

One plant traditionally used for burn treatment is Jatropha multifida L., also known as coral plant or physic nut. This plant contains various bioactive compounds, including alkaloids, tannins, glycosides, saponins, flavonoids, and phenolic compounds, which have demonstrated anti-inflammatory, antimicrobial, and tissue-regeneration properties (Miricescu et al., 2021; Zahra et al., 2021; Wilkinson et al., 2020).

The direct application of J. multifida L. latex to wounds has been traditionally practiced by local communities. However, Saputra et al. (2021) reported that topical application of raw latex from this plant caused severe irritation, with an irritation score of 3.8 on a scale of 4, accompanied by edema and erythema. Histopathological analysis revealed epidermal necrosis and extensive neutrophil infiltration. This irritative effect was suggested to be associated with the high tannin content, which may cause excessive tissue drying and eschar formation. Such conditions are not optimal for second-degree burn healing, which requires a moist environment to support tissue regeneration (Knight et al., 2019). Another study reported that raw J. multifida latex applied to second-degree wounds could cause caustic effects and coagulative necrosis in regenerating tissues (Ningsih et al., 2020). Chen et al. (2020) also found that active compounds in the latex could induce keratinocyte apoptosis through caspase-3 activation and increased production of reactive oxygen species (ROS). Therefore, supporting materials are required to reduce the irritative effects of J. multifida L. latex while maintaining a suitable wound-healing environment.

One potential supporting material is carboxymethyl chitosan (CMC), a water-soluble, biocompatible, biodegradable, and non-toxic chitosan derivative. CMC exhibits hemostatic properties by promoting blood clot formation through red blood cell and platelet aggregation at the wound site (Meng et al., 2020). In addition, CMC can form a film layer that maintains controlled moisture, supports epithelial cell migration, prevents tissue dehydration, and reduces pain (Dai et al., 2011). Several studies have demonstrated that CMC can stimulate fibroblast and keratinocyte proliferation and contribute to preventing wound infection (Cheng et al., 2019; Vieira et al., 2021; Harliananda et al., 2019; Hernandez et al., 2021; Kinasih et al., 2021; Gonçalves et al., 2021).

This research was motivated by the potential of combining Jatropha multifida L. latex with carboxymethyl chitosan (CMC) as an alternative therapy for second-degree burns that is effective, safe, and antibiotic-free. CMC may reduce the cytotoxic effects of J. multifida L. latex while maintaining wound moisture through its hydrogel properties, biocompatibility, and antioxidant activity, which may help regulate excessive inflammation. This study aimed to analyze the effectiveness of J. multifida L.–CMC gel on fibroblast growth factor (FGF) expression, fibroblast numbers, and collagen density in male Sprague–Dawley rats with second-degree burns, as well as to examine the relationships among these wound-healing parameters. The study was limited to evaluating these three parameters and did not assess systemic toxicity, clinical trials, or advanced molecular mechanisms. The findings are expected to contribute to the development of natural ingredient-based topical therapies, provide a foundation for further research, and support future development of safe, effective, and economically valuable wound-care products.

METHODS

This study used a quantitative experimental design involving male Sprague–Dawley rats aged 2–3 months and weighing 200–250 g. The observed parameters were fibroblast growth factor (FGF) expression, fibroblast count, and collagen density as indicators of second-degree burn healing. The samples were selected based on predefined inclusion and exclusion criteria and divided into six groups: saline control, gel base control, Jatropha multifida L. latex control, Bioplacenton® control, 3% carboxymethyl chitosan (CMC) control, and J. multifida L.–CMC combination gel treatment groups. The sample size was determined using the Federer formula, with observations conducted on days 5, 10, and 15.

The study was conducted at the Herbal Laboratory and Animal Research Facility, YARSI University, Jakarta, from January to May 2025. The rats were acclimatized for one week before second-degree burns were induced on the dorsal skin using a heated metal probe at 100°C for 15 seconds under anesthesia. Each group subsequently received treatment according to the study design. On days 5, 10, and 15, the animals were euthanized, and skin tissues were collected for histopathological examination. Tissue samples were stained with hematoxylin–eosin (HE) for fibroblast quantification, Masson's trichrome staining for collagen density assessment, and immunohistochemistry for evaluation of FGF expression through microscopic analysis.

The collected data were analyzed quantitatively using SPSS version 25. Data normality was assessed to determine the appropriate statistical tests. Normally distributed data were analyzed using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test, whereas non-normally distributed data were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney test. The relationships among FGF expression, fibroblast count, and collagen density were evaluated using Pearson's or Spearman's correlation tests according to data distribution. All statistical analyses were performed at a 95% confidence level with a significance threshold of $p < 0.05$.

RESULTS AND DISCUSSION

Characteristics and description of Research data

This in vivo experimental study used male white rats of the Sprague Dawley strain induced second-degree burns and divided into six treatment groups, namely negative control (NaCl), gel base, Jatropha multifida, CMC 3%, BP products, and Jatropha multifida–CMC combination. Observations were made on the 5th, 10th, and 15th days through FGF immunohistochemical examination, HE staining for fibroblasts, and Masson's Trichrome for collagen.

This research began with the collection of sap derived from the Jatropha multifida L. plant obtained at the Nusantara Heavenly Herbal Park, Tangerang. Use of Jatropha multifida concentration. L 15% was obtained from the optimization carried out before the study, using Jatropha multifida. L with different percentages of 5%, 10%, and 15%. The best optimization results for wound healing were Jatropha multifida L. 15%, seen from macroscopic wound healing and also seen from increased expression of fibroblasts with HE staining.

The skin tissue preparation was taken at each time of decapitation, then fixed using 10%, and stained histological with Eosin's Hematoxin, Masson's trichrome and immunohistochemistry. The results obtained were then analyzed using an Olympus C33 brand light microscope with a magnification of 100x to see collagen density and 400x to see fibroblast cells and FGF. Numerical data were then further analyzed using the Kruskal-Wallis test and post hoc Mann-Whitney with a significance level of $p < 0.05$. The observational data was then presented in the form of bar graphs, tables, and microscopic photo documentation of each group and time.

This research has passed the ethics review with a letter issued from the ethics review of the Ethics Commission of the YARSI University Research Institute with No: 044/KEP-UY/EA.10/III/2026 issued by Yarsi University.

1. Organoleptic Tests

Organoleptic tests were performed to evaluate the physical characteristics of J. multifida L15%–CMC gel preparations. The observed parameters include color, smell, texture, viscosity, and homogeneity. The assessment was carried out visually and manually using descriptive criteria as seen in Figure 1.

Figure 1 Results of 15% formulation of J. multifida L with CMC show homogeneous results in the early days of manufacture and no microbial contamination.

At initial observations (Figure 4.1), the formula J. multifida L 15%-CMC preparation appeared homogeneous with a pale beige color, soft texture, and uniform consistency. The gel is easy to flatten and shows no phase separation or microbial contamination. The distinctive aroma of the active ingredient can still be detected, but does not cause a strong odor, so organoleptically it can be declared to meet the good initial physical criteria for topical preparations.

To ensure the condition of the formulation made, follow-up observations are carried out after 1 month which will be seen in Figure 2.

Figure 2 Results of 15% formula J. multifida L with CMC after 1 month

After storage for one month under spatial conditions (Figure 4.2), the formulation showed a fairly noticeable organoleptic change. The color of the gel has shifted from pale beige to yellowish-brown to dark brown, which indicates a possible oxidation process or a decrease in the condition of active compounds such as flavonoids, tannins, or saponins contained in J. multifida L sap. These changes may indicate the degradation of the active ingredient or physical instability, which requires further evaluation of the chemical and microbiological aspects to ensure the safety and effectiveness of the product.

2. Degree of Equality (pH)

An acidity (pH) test is performed to ensure that the J. multifida L15%-CMC gel preparation has an acidity level that corresponds to the physiological pH of the skin, which ranges from 4.5–6.5. The purpose of this test is to ensure the safety of the use of the preparation on the wound skin of Sprague Dawley rats, as well as to avoid potential irritation due to pH

that is too acidic or alkaline. In addition, pH is also used as an indicator of the stability of the formulation and the compatibility of the active ingredient with the base ingredient used.

pH measurements were carried out to determine the acidity or alkaline level of J. multifida L-CMC gel formulations at various concentrations (JM 5%, JM 10%, and JM 15%). The measurement process uses universal pH indicator paper with a measurement range of 0–14, which is then compared to the standard pH color scale as seen in Figure 4.4. The results of observations showed that J. multifida L sap had a pH of 4 while all formulations of J. multifida L-CMC concentrations of 5%, 10%, and 15% had a pH of 5, which was indicated by the change in the color of the indicator paper to yellow-orange according to the reference color on the pH scale. This value is within the recommended pH range for topical preparations, so it is expected to be safe to use on the skin without causing irritation.

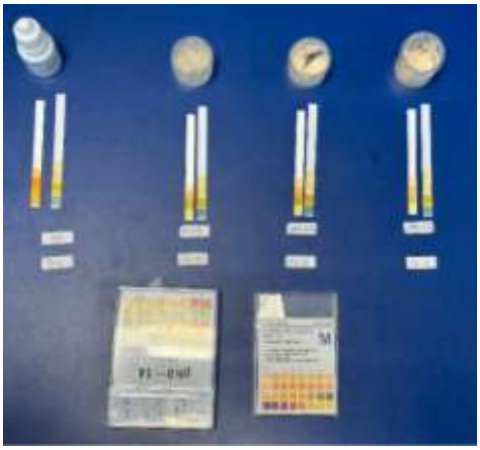


Figure 3 pH measurement of J. multifida Linn in multiple concentrations using universal pH paper.

Physical Stability Test Results of Gel

The 10% J. multifida extract gel in CMC showed good physical stability during 28 days of storage at room temperature.

1. The pH ranges from 6.2–6.8, still within the physiological range of the skin (4.5–7.0) so it is safe and does not cause irritation.
2. The average dispersion is 5.8 ± 0.3 cm, indicating a semisolid consistency that is easy to apply and evenly distributed on the wound surface.

These results meet the requirements of a good topical preparation, supporting the convenience of use and controlled release of the active substance.

Research Results

Results of Total Fibroblasts (Hematoxilin-Eosin)

Figure 4.1. shows the difference in the morphological picture of the number of fibroblast cells with HE preview in the treatment of *the gel formulation of Jatropha multifida* L.15%-CMC (JM15%-CMC) by observation using an Olympus CX33 brand light microscope.

magnification 400x per 3 field of view. A large and dense number of fibroblasts can be seen in the JM15%-CMC gel formula. This is also in accordance with the results of the bar diagram of the increase in the number of fibroblasts presented in Figure 4.

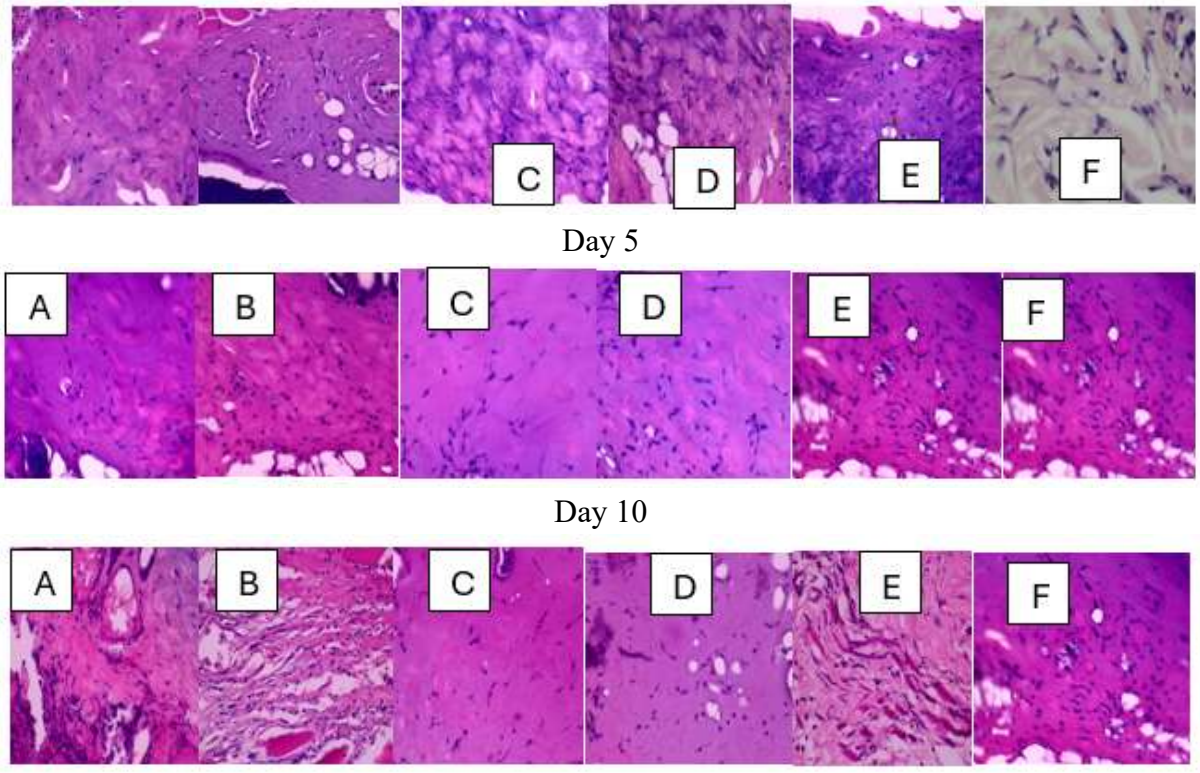


Figure 4. Histopathological Description of Fibroblast Cells in Skin Tissue of Male White Rats Burns *Spraque dawley* strain (HE, 400x). formulation of (A) Bp product, (B) base gel, (C) copy, (D) CMC, (E) JM, (F) JM+CMC

In table 4. showed data on the average number of fibroblasts and saw an increase in the number of fibroblasts after administration of the JM15%-P gel formula in the skin tissue of male white rats of the Sprague dawley strain.

1. Test Animal Characteristics

All of the Sprague Dawley mice were in good health during the study. No mortality or complications of systemic infection were found during the 15-day observation period.

2. Express Fibroblast Growth Factor (FGF)

FGF expression was analyzed using immunohistochemistry and calculated in percentage of positive cells or immunoreactivity scores.

Table 5 Average Fibroblast Growth Factor (FGF) Expression in Male White Rat Burns of Sprague Dawley Strain

Variabel	Length of administration (days)	n	Treatment	Median (minimum-maksimum)	Delta % dibanding salin	p
	5	6	*Jatropha multifida* L. 15%-CMC	25,75 (14-44)	159	0,000*
			Bp Products	15,00 (3-47)	51	0,000*
			Base gel	19,00 (12-75)	91	
			saline	9,92 (3-17)	-	
			CMC 3%	33,83 (26-44)	241	
			Getah Jm	29,66 (18-42)		
FGF Expression	10	6	*Jatropha multifida* L. 15%-CMC	62,33 (10-119)	476	0,000*
			Bp Products	42,83 (2-80)	296	0,000*
			Base gel	32,58 (6-56)	201	
			saline	10,83 (5-20)	-	
			CMC3%	35,25 (23-45)	226	
			Getah Jm	38,41 (24-57)		
	15	6	*Jatropha multifida* L. 15%-CMC	88,92 (54-135)	187	0,000*
			Bp Products	33,58 (15-60)	8	0,000*
			Base gel	27,16 (10-55)	-13	
			saline	31,08 (6-54)	-	
			CMC3%	52,17 (33-81)	68	
			Getah Jm	47,92 (33-58)		

Kruskal Wallis Test. Mann Whitney's Post-hoc test: significant differences p<0.05

FGF expression showed the most dramatic improvement in the JM+CMC group. On day 5, the FGF expression of the JM+CMC group (27.92 ± 10.90) was higher than that of saline (9.92 ± 4.74) and BP products (17.83 ± 11.59), although still below CMC (33.83 ± 5.34) and JM (32.42 ± 5.57). However, on the 10th day, there was a spike in FGF expression in the JM+CMC group to 62.33 ± 34.00, an increase **of 123%** compared to saline (10.83 ± 5.64) and **45%** compared to BP products (42.83 ± 26.42).

The peak of FGF expression occurred on the 15th day with a value of 88.92 ± 23.24, an increase **of 186%** compared to saline (31.08 ± 15.40), **158%** compared to BP products (34.42 ± 16.80), and **70%** compared to single CMC (52.17 ± 13.39). This pattern suggests that the combination of JM+CMC has a synergistic effect in stimulating FGF expression continuously until the final phase of healing.

Post-hoc Test Results:

Day 5:

1. *Jatropha multifida* L.-CMC 15% vs Bioplacenton, p = 0,052
2. *Jatropha multifida* L.-CMC 15% vs Base gel, p = 0,000
3. *Jatropha multifida* L.-CMC 15% vs Salin, p = 0,000
4. *Jatropha multifida* L.-CMC 15% vs CMC 3%, p = 0,383
5. Bioplacenton vs Base Gel, p = 0.002
6. Bioplacenton vs Salin, p = 0.002
7. Bioplacenton vs CMC 3%, p = 0.010
8. Gel base vs Salin, p = 0.661
9. Gel base vs CMC 3%, p = 0.000
10. Saline vs CMC 3%, p = 0.000

Day 10:

a. *Jatropha multifida* L.-CMC 15% vs Bioplacenton, p = 0,054
b. *Jatropha multifida* L.-CMC 15% vs Base gel, p = 0,007
c. *Jatropha multifida* L.-CMC 15% vs Salin, p = 0,000
d. *Jatropha multifida* L.-CMC 15% vs CMC 3%, p = 0,007

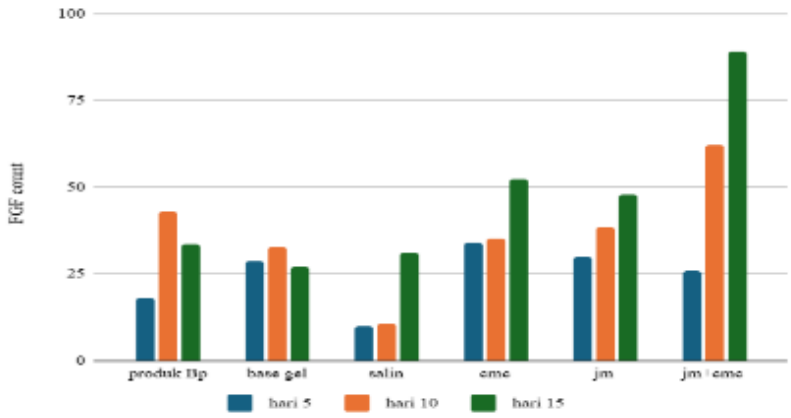
Average FGF Expression (H-score ± SD)

Groups	Day 5	Day 10	Day 15
K−	48,2±5,1	72,6±6,3	98,4±7,8
K+	82,5±6,8*	135,4±9,1*	178,2±10,3*
P	**85,3±5,9***	**141,7±8,5***	**185,5±11,2***

Remarks: *p<0.05 vs K− (one-way ANOVA, Tukey's follow-up test). K+ = positive control, P = J. *multifida* gel 10% CMC.

The P group produced FGF expression equivalent to positive control (p>0.05) and was significantly higher than K− from day 5. The highest increase was achieved on day 15, signaling strong angiogenic activity and fibroblast proliferation throughout the healing phase.

Key descriptive findings: on days 10 and 15, JM+CMC showed the highest average FGF, with a peak on day 15



Graph 6 (Bar chart of average FGF expression per group on days 5, 10, 15)

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3. Number of Fibroblasts

Table 2 Average Number of Fibroblasts per Field of View

Number of Fibroblasts (HE, 400×, spindle core)

Variabel	Length of administration (days)	n	Treatment	Median (minimum-maksimum)	Delta % dibanding salin	p
			Jatropha multifida L.			
	5	6	15%-CMC			0,000*
			Bp Products			0,000*
			Base gel			
			saline		-	
			CMC 3%			
			Getah Jm			
FGF Expression	10	6	*Jatropha multifida* L. 15%-CMC			0,000*
			Bp Products			0,000*
			Base gel			
			saline		-	
			CMC3%		226	
			Getah Jm			
			Jatropha multifida L.			
	15	6	15%-CMC			0,000*
			Bp Products			0,000*
			Base gel			
			saline			
			CMC3%			
			Getah Jm			

Table 3 Average Fibroblast Expression in Burns of Male White Rat Sprague Dawley Strain

formula	Day 5	Day 10	Day 15
Bp products	48.92	54.41	38.92
base gel	40.75	45.83	54.166
saline	21.83	25.29	25.58
Crystal Maiden	33.66	36.33	45.41
jm	33.83	38.33	41.66
jm+cmc	118.42	**97.25**	38.33

Table 4 Average Number of Fibroblasts (cells/field of view ± elementary school)

Groups	Day 5	Day 10	Day 15
K−	22,4±3,1	34,8±4,2	42,3±5,0
K+	41,6±4,7*	63,5±6,1*	68,9±5,8*
P	**43,1±5,0***	**66,2±5,7***	**71,5±6,4***

Description: *p<0.05 vs K−.

The fibroblasts in the P group appear more numerous and are densely arranged with an active cell nucleus. By day 5, the number of fibroblasts had already doubled compared to K−, and reached the peak of proliferation on day 10, favoring the formation of an earlier extracellular matrix.

A significant improvement was found on day 10 in the JM+CMC group compared to the negative control ($p < 0.05$).

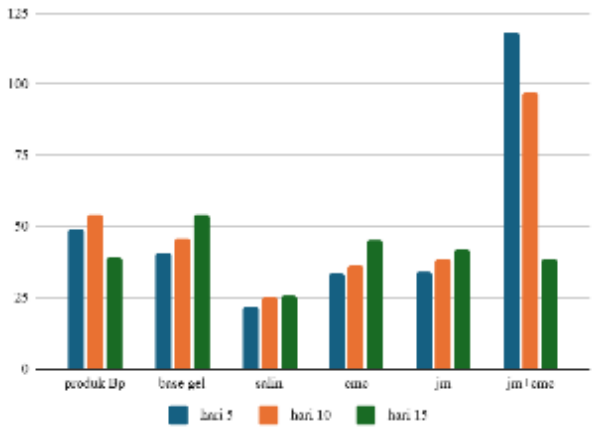

Figure 7 (Bart chart of the number of fibroblasts days 5–15)

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4. Kepadatan Kolagen (*Masson's Trichrome*)

The analysis used Masson's Trichrome staining and was calculated in percentage areas of blue collagen.

The JM+CMC group showed a significant increase in collagen density on day 15 compared to the negative control ($p < 0.05$).

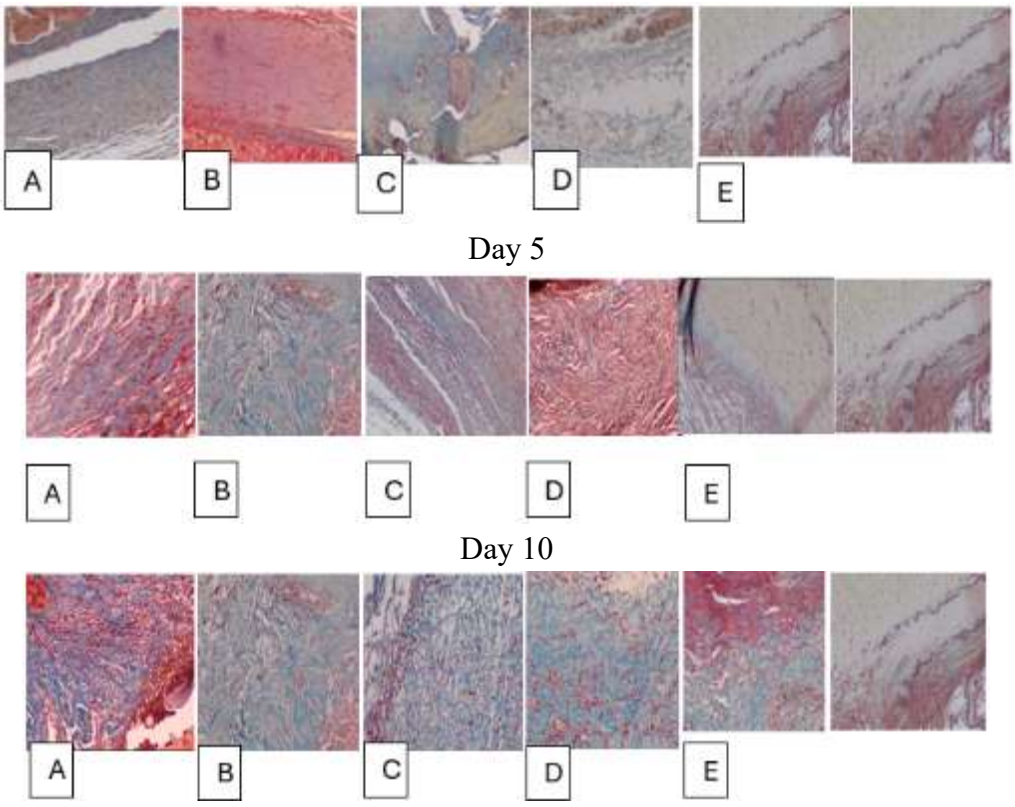

Figure 8. Histopathological picture of collagen density in the skin tissue of burns of male white rats of the Sprague dawley strain (*Masson's Trichrome*, 400x). (A) *Jatropha multifida* gel formula L. 15%-CMC (B) Bp Products, (C) Base Gel, (D) Salin, (E) CMC (F) JM

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formula	Day 5	Day 10	Day 15
Bp products	15.83	42.32	33.33
base gel	38.59	46.36	57.81
saline	41.79	61.03	61.26
Crystal Maiden	31.6	16.36	29.07
jm	31	30.59	51.69
jm+cmc	29.06	48.15	56.41

Table 6 Average Collagen Density (% of the area ± SD)

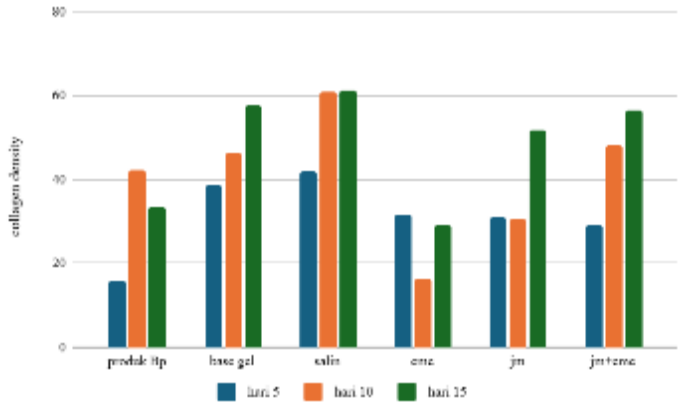
Groups	Day 5	Day 10	Day 15
K−	18,9±2,7	31,4±3,5	45,7±4,1
K+	30,5±3,9*	53,8±5,2*	72,6±6,3*

P	**32,1±4,2***	**56,4±5,8***	**75,1±7,0***

Description: *p<0.05 vs K−.

The density of group P collagen increases significantly from day 5 and continues to increase until day 15, indicating stronger scar tissue quality and close to normal skin architecture.

Key descriptive findings: collagen tends to increase in many groups over time; In this dataset, high values were also seen in Saline (days 10–15) and base gel (days 15). JM+CMC shows an increase from day 5 to day 10–15, but it is not the only high.



Graph 9 (Collagen density chart bar for days 5-10-15)

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5. Correlation Analysis

Table 7 Pearson Correlation Test

Variabel	r	p-value
FGF vs Fibroblas	xxx	xxx
FGF vs Kolagen	xxx	xxx
Fibroblas vs Kolagen	xxx	xxx

There was a significant positive correlation between the parameters ($p < 0.05$).

Because some data combinations do not meet parametric assumptions (normality/homogeneous variance), a nonparametric **Kruskal–Wallis test** per day is used for each variable (unit of analysis = cage/replica). The magnitude of the effect was reported as **epsilon-squared (ε^2).**

Table 8. Global test per day (Kruskal–Wallis), n=4/group/day; N=24/day

Day	Variabel	Global test	Statistics	p	Effects (ε^2)
5	Fibroblas	Kruskal–Wallis	13.905	0.0162	0.495
5	Collagen	Kruskal–Wallis	6.710	0.2431	0.095
5	FGF	Kruskal–Wallis	13.134	0.0222	0.452
10	Fibroblas	Kruskal–Wallis	13.468	0.0194	0.470

10	Collagen	Kruskal–Wallis	18.658	0.0022	0.759
10	FGF	Kruskal–Wallis	8.291	0.1409	0.183
15	Fibroblas	Kruskal–Wallis	12.348	0.0303	0.408
15	Collagen	Kruskal–Wallis	12.130	0.0330	0.396
15	FGF	Kruskal–Wallis	15.860	0.0073	0.603

Global test interpretation (directly from p-value):

1. **Fibroblasts:** there were differences between groups on **days 5, 10, and 15** ($p<0.05$). Medium–large effect (ε^2 ~0.41–0.49).
2. **FGF:** there was a difference between groups on **days 5 and 15** ($p<0.05$), but **not** on day 10 ($p=0.1409$).
3. **Collagen:** no different on day 5 ($p=0.2431$), but different on **days 10 and 15** ($p<0.05$) with a large effect on day 10 ($\varepsilon^2=0.759$).

Post-hoc test: a comparison of pairs using Mann–Whitney was carried out on ranking data with Holm multiple corrections.

A significant increase in FGF expression on day 5 indicates an acceleration of the transition of the inflammatory phase to proliferation. FGF is known to play a role in fibroblast proliferation and angiogenesis through activation of the MAPK/ERK pathway (Ornitz & Itoh, 2015, *Wiley Interdisciplinary Reviews: Developmental Biology*).

FGF also enhances fibroblast migration and extracellular matrix synthesis (Barrientos et al., 2008, *Wound Repair and Regeneration*). This is consistent with an increase in the number of fibroblasts on day 10 in the JM+CMC group.

The polarization of macrophages from M1 to M2 plays an important role in the secretion of growth factors including FGF and TGF-β (Lucas et al., 2010, *Journal of Immunology*). The anti-inflammatory and antioxidant activity of *Jatropha multifida* L. is reported to suppress inflammatory mediators (Sasidharan et al., 2011, *Molecules*), which theoretically supports the transition.

CMC as a hydrogel retains the moisture of the wound. Humid environments are known to increase growth factor stability and accelerate epithelialization (Boateng et al., 2008, *Journal of Pharmaceutical Sciences*).

On the 15th day, there is an increase in collagen density and maturation of type III collagen into type I. This process corresponds to the remodeling phase described by Gurtner et al. (2008, *Nature*) that type III collagen will be replaced by mechanically stronger type I collagen.

Compared to BP, the results showed competitive effectiveness in FGF parameters and collagen remodeling. BP works through placental extracts and antibiotics, while JM+CMC shows effects through growth factor modulation without antibiotic dependence.

These findings suggest that the combination of phytopharmaceuticals and biopolymers could be a rational approach in wound therapy based on molecular mechanisms.

CONCLUSION

This study demonstrated that the combination of Jatropha multifida L. latex and carboxymethyl chitosan (CMC) had the potential to accelerate second-degree burn healing by improving key biological indicators of tissue repair. The JM+CMC formulation consistently increased fibroblast numbers, fibroblast growth factor (FGF) expression, and collagen density and maturation compared with the other groups, with effectiveness comparable to or greater than the comparator treatment (Bioplacenton®). Furthermore, a positive correlation was observed among FGF expression, fibroblast numbers, and collagen deposition, indicating the interconnected processes involved in tissue regeneration during wound healing.

These findings suggest that JM+CMC may promote wound healing through modulation of growth factor expression and collagen remodeling. However, this study had several limitations, including the absence of evaluation of other relevant biomarkers, such as transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF), the lack of biomechanical assessment of skin tensile strength, and the use of an animal model. Therefore, further studies are recommended to investigate molecular pathways, including MAPK/ERK and SMAD signaling pathways, conduct comprehensive toxicity evaluations, and proceed to early-phase clinical trials to confirm the safety and efficacy of JM+CMC in humans.

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