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Effect of jatropha-placenta-cmc formulation on second-degree burn wound healing in rats: Fibroblast growth factor expression

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ABSTRACT

Introduction: Second-degree burns require effective topical therapy to accelerate tissue regeneration and reduce complications. Current treatments remain limited by side effects and suboptimal regenerative outcomes. This study aimed to evaluate the effectiveness of a combination gel of Jatropha multifida L. latex, placenta extract, and carboxymethyl chitosan (JM–P–CMC) on burn wound healing by assessing fibroblast growth factor (FGF) expression, fibroblast count, and collagen density.

Research Methodology: A post-test-only controlled experimental design was conducted using 18 male Sprague-Dawley rats, allocated into 6 groups. Standardised second-degree burns (1×1 cm) were induced and treated for 15 days. Histological and immunohistochemical analyses were performed at days 5, 10, and 15. Data were analysed using the Kruskal–Wallis and Mann–Whitney tests (α=0.05), with effect sizes expressed as median differences (%) and 95% confidence intervals (CI).

Results: The JM–P–CMC group demonstrated significantly higher FGF expression compared to saline (Day 15 median: 75 vs 33; =42%; p=0.0015; 95% CI: 18–60). Fibroblast counts were also significantly increased (median: 55 vs 17; =38%; p=0.0001; 95% CI: 20–64). Collagen density was markedly higher in the intervention group (58.83% vs 21.70%; p<0.01). A strong positive correlation was observed between fibroblast count and collagen density on day 10, whereas FGF showed an inverse regulatory relationship with collagen during the remodelling phase.

Conclusion: The JM–P–CMC gel significantly enhances burn wound healing through modulation of FGF expression, fibroblast proliferation, and collagen deposition. This formulation represents a promising, safe, and cost-effective alternative for topical burn management and supports the integration of bioactive-based therapies into clinical and nursing practice.

Keywords: burns; carboxymethyl chitosan; fibroblast growth factor; placenta extract; wound healing.

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INTRODUCTION

Burns are one of the most common skin injuries and have a significant impact on public health, both locally and globally. Burns not only cause extensive tissue damage but also lead to serious complications such as infection, organ dysfunction, and long-term disability, requiring prompt and appropriate medical intervention. Globally, the WHO reports that more than 11 million people experience burns each year, with approximately 180,000 deaths, making them a leading cause of injury-related morbidity and mortality (Bastari et al., 2023). In Indonesia, the Ministry of Health records approximately 200,000 burn cases annually, demonstrating that this issue is not only international but also highly relevant in the national context and health care delivery (Keshri et al., 2023).

Burn wound healing is a complex biological process involving inflammation, proliferation, and remodelling, during which fibroblasts and collagen play crucial roles (Ren, 2025). Fibroblast Growth Factor (FGF) is a key mediator of fibroblast migration and proliferation, and influences collagen synthesis, which determines the quality of tissue healing (Chandra et al., 2026). Although various conventional therapies, such as bioplastics, are available, their use still has limitations, including the risk of allergies, bacterial resistance to neomycin sulfate, and suboptimal effectiveness in more severe burns (Mai et al., 2020; Yang et al., 2022). Several herbal studies have evaluated the ability of Jatropha multifida L., placenta extract, and Carboxymethyl Chitosan (CMC) to accelerate wound healing; however, the available evidence remains partial and fragmented and has not been fully tested in the context of applied nursing and clinical practice (Syaharuddin & Fardi, 2026).

A number of previous studies provide preliminary evidence regarding the potential of each of these ingredients (Zhao et al., 2026). Jatropha multifida L. extract can increase fibroblast proliferation and granulation tissue formation in incision wounds, although this has not been tested in burns. (Ardizzone et al., 2023) found that placenta extract is rich in growth factors, such as EGF and TGF-β, which can accelerate re-epithelialization; however, this study did not evaluate histological or molecular parameters simultaneously. Ardizzone et al. (2023) reported that CMC effectively increases fibroblast activity and possesses antimicrobial properties that support wound healing. Additional research by Budiman et al. (2021) confirmed that herbal bioactive ingredients can improve collagen quality by modulating cytokines. However, this finding was general and not specific to the combination of ingredients studied. Furthermore, this study reported that natural-based therapy for burns can reduce inflammation and accelerate regeneration, but it did not simultaneously evaluate fibroblasts, FGF, and collagen (Wang et al., 2022).

From this series of previous studies, it is clear that existing studies still assess the effects of each ingredient separately, have not tested the synergy of the three in a single formulation, and have not comprehensively utilized biomolecular and histological parameters. Furthermore, most studies focus solely on pharmacological perspectives and have not examined the therapeutic implications in a nursing context, despite the crucial role nurses play in wound management, dressing selection, and monitoring patient healing progress (Miri et al., 2024; Shore et al., 2022). Therefore, this study aims to fill a scientific gap by providing more comprehensive data on the effectiveness of the Jatropha-placenta-CMC combination as a burn wound therapy.

This research gap arises because most previous studies have evaluated only one therapeutic component, have not tested all three in combination, and have rarely integrated molecular parameters, such as FGF expression, with histological indicators, such as fibroblast count and collagen density, simultaneously. Furthermore, previous research has not examined the effectiveness of this therapy from a nursing perspective, even though nurses play a direct role in

wound management, dressing selection, monitoring clinical signs of healing, and reducing the risk of infection. Therefore, research combining these bioactive ingredients into a single gel formulation and testing their effects on wound-healing parameters is urgently needed to address this unmet clinical need.

The novelty of this research lies in the development of a gel formulation that combines Jatropha multifida L. latex, bovine placenta extract, and CMC as a single therapeutic unit, along with a comprehensive evaluation using three key indicators: FGF expression as a molecular parameter, fibroblast count as an indicator of proliferation, and collagen density as a reflection of new tissue quality. Such an integrated approach has never been studied before, particularly in a second-degree burn model and from a nursing practice perspective.

This research also brings novelty to the context of clinical applications, as the resulting product has the potential to be a safer, more affordable, and more suitable therapeutic alternative for nursing care.

This research is based on the Roy Adaptation Model, which views individuals as adaptive systems that respond to internal and external stimuli to achieve a state of healing. In burns, tissue damage is a negative stimulus that disrupts physiological integrity. Interventions such as Jatropha-placenta-CMC gel serve as positive stimuli that help enhance the body's adaptive response, including fibroblast activity, FGF expression, and collagen formation. Within this framework, the relationship between variables becomes clear: increased FGF expression promotes fibroblast proliferation, which in turn increases collagen deposition, thereby accelerating the proliferative phase and improving the quality of wound healing.

The contribution of this research is significant for nursing and healthcare practice. The results have the potential to enrich nurses' knowledge in selecting topical therapies that are safe, effective, and minimize side effects. Furthermore, these findings can strengthen burn wound management strategies in primary care and hospitals, open up opportunities for the development of standardized herbal products, and inform health policy considerations to provide more economical and accessible therapeutic alternatives. Nurses' understanding of the molecular mechanisms of wound healing can also improve the accuracy of nursing care, clinical monitoring, and counseling for patients and families regarding safer therapeutic options.

Given this urgency and gap, this study aims to analyse the effectiveness of a Jatropha multifida L.-placenta-CMC gel formulation on second-degree burn wound healing in mice by assessing FGF expression, fibroblast count, and collagen density, and to evaluate the relationship among these parameters in supporting tissue regeneration.

RESEARCH METHODOLOGY

Study Design

This study employed a laboratory experimental design with a post-test approach at three observation time points: days 5, 10, and 15. This design was chosen to directly assess the effect of the Jatropha multifida L.-placenta-CMC gel formulation on the healing process of second-degree burns in mice. This design allows for a controlled causal relationship, particularly in changes in FGF expression, fibroblast count, and collagen density, as key indicators of tissue healing.

Participants

The study participants were male Sprague-Dawley rats selected using purposive sampling based on their suitability for physiological characteristics in wound-healing research. Inclusion criteria included rats aged 2–3 months, weighing 200–250 grams, active, and showing no signs

of disease. Exclusion criteria included animals that became ill during the adaptation period, developed wound infections, or died during treatment. The sample size was determined using the Federer formula, yielding a minimum of 4 rats per group across 6 treatment groups. Considering three terminations, a total of 18 animals were required.

Instrument

The research instruments included a set of histological tools and materials: an Olympus CX33 light microscope with a trinocular camera; immunohistochemical staining to detect FGF expression; Hematoxylin-Eosin staining for fibroblast counting; and Masson's Trichrome staining to measure collagen density. Tissue image analysis was performed using Image Raster and ImageJ software, which boast high accuracy in cell counting and tissue density. All instruments are standardized and commonly used in biomedical research.

Intervention

The intervention consisted of a Jatropha multifida L.-placenta-CMC combination gel, formulated based on optimized compositions of 15% Jatropha multifida L. latex, 10% placenta, and 3% CMC. This gel was applied to the burn wounds twice daily for 15 days. Six study groups were selected: a negative control (0.9% NaCl or gel base), a positive control (bioplacenton), and four treatment groups given the combination gel. Burn wounds were created using standardized metal heating devices at a specific temperature for 15 seconds, and the gel was applied using sterile techniques to ensure the validity of the research results.

Data Collection

Data were collected at the Herbal Laboratory and Animal Research Facility of YARSI University from January to May 2025. Skin tissue samples were taken on days 5, 10, and 15 after treatment using standard euthanasia procedures. The tissue was then processed into histological slides and stained using techniques relevant to each research variable. Observations were made in three representative fields for each preparation to obtain accurate and consistent data.

Data Analysis

Data analysis was performed quantitatively by calculating the mean FGF expression, fibroblast count, and collagen density. Data were analysed using statistical software such as SPSS, including a one-way ANOVA or other tests appropriate to the data distribution. Results are expressed as the mean ± standard deviation with a significance level of 0.05. This analysis allowed researchers to assess differences between treatment groups and the relationships between healing parameters.

Ethical Considerations

This research was conducted in accordance with the ethical principles of animal research, including the 3Rs (Replacement, Reduction, and Refinement), to minimise animal discomfort. All procedures, from adaptation and burn induction to intervention and euthanasia, were conducted in accordance with internationally accepted ethical protocols. The research has received official approval from the YARSI University Research Ethics Committee, ensuring that all stages of the study meet animal welfare standards.

Ethical Approval

Ethical clearance for this experimental animal study was obtained from the Research Ethics Committee of YARSI University, Jakarta, Indonesia (Approval No.: 076/KEP-UY/EA.10/II/2025). All procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals and adhered to the principles of the 3Rs (Replacement, Reduction, and Refinement) to minimize animal suffering. Sprague Dawley rats were handled under controlled laboratory conditions, including appropriate

housing, acclimatisation, and monitoring throughout the study period. Burn induction, treatment application, and tissue sampling were performed according to standardised protocols to ensure animal welfare. Euthanasia procedures were conducted humanely following approved ethical standards. No human participants were involved in this study; therefore, informed consent was not required.

RESULT

This study evaluated the effectiveness of the Jatropha multifida L.–placenta–carboxymethyl chitosan (JM–P–CMC) gel formulation on second-degree burn wound healing in Sprague Dawley rats through three primary indicators: fibroblast growth factor (FGF) expression, fibroblast count, and collagen density. Observations were conducted at three time points (days 5, 10, and 15) to capture the dynamic progression of wound healing phases, including inflammation, proliferation, and remodelling. Overall, the JM–P–CMC formulation demonstrated a consistent trend toward improved healing outcomes compared with control groups. Increases in FGF expression and fibroblast proliferation were observed predominantly during the proliferative phase, followed by enhanced collagen deposition during the remodelling phase. Statistical analysis revealed significant differences between treatment and control groups across several parameters, indicating that the combination gel exerts a measurable biological effect on tissue regeneration. These findings provide a basis for further analysis of each variable and its interrelationships in supporting accelerated wound healing.

The FGF expression data obtained through observations using an Olympus Trynocular CX33 microscope at 400× magnification across three fields of view are presented in Table 1. Administration of the JM-P-CMC gel formulation showed an increase in the number of FGF-expressing cells in the skin tissue of male Sprague Dawley rats (Rattus norvegicus).

Table 1. Number of Fibroblast Growth Factor (FGF)–Expressing Cells in Second-Degree Burn Wounds of Male Sprague Dawley Rats

Formulation	Day 5	Day 10	Day 15
Saline	9	9.5	33
Base gel	20	36	21.5
Bioplasenton	14	44.5	30
Placenta	22	10	14
CMC	33.5	33.3	50
JM-P-CMC	38	35.5	75

Table 1 displays the immunohistochemical staining results, in which FGF expression was assessed by the number of cells showing brown staining. On day 5, the JM-P-CMC gel formulation already demonstrated increased FGF expression. By day 10, the JM-P-CMC group continued to show higher expression compared with the saline group. On day 15, the JM-P-CMC formulation exhibited the greatest increase in FGF expression, exceeding all control groups. Therefore, day 15 can be considered the optimal time point for stimulating FGF expression. Figure 1 presents the immunohistochemical images illustrating differences in FGF expression following administration of the Jatropha multifida L.–Placenta–CMC gel formulation (JM-P-CMC), specifically on day 10 during the proliferative phase. FGF expression appeared most optimal at this stage, with nearly all fibroblasts showing positive immunostaining. FGF expression patterns for all treatment groups on days 5, 10, and 15 are presented in Figure 1.

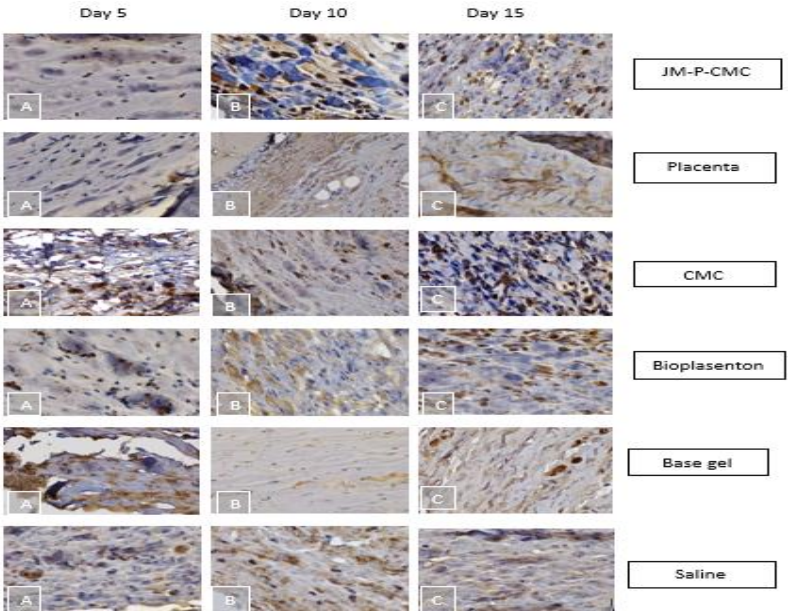

Figure 1. Histopathological depiction of Fibroblast Growth Factor (FGF) expression (brown-stained cells) in burn-injured skin tissue of male Sprague Dawley rats (Rattus norvegicus) (IHC, 400×). (A) Day 5, (B) Day 10, (C) Day 15,

The normality test results showed that the treatment groups were not normally distributed. Therefore, the significance test for FGF expression in this study was conducted using the nonparametric Kruskal–Wallis test, followed by Mann–Whitney post hoc analysis.

Table 2. Fibroblast Growth Factor (FGF) Expression in Second-Degree Burn Wounds of Male Sprague Dawley Rats

Variable	N	Day	Formulation	Median (min–max)	Delta (%) Compared to Saline	p-value
FGF Expression	12	**5**	JM-P-CMC	38 (19–58)	29	
			Placenta	22 (11–83)	13	
			CMC	33.5 (26–44)	24.5	
			Bioplasenton	14 (7–47)	5	**0.016***
			Base gel	20 (12–75)	11	
			Saline	9 (3–17)	0	
FGF Expression	12	**10**	JM-P-CMC	35.5 (19–54)	26	
			Placenta	10 (6–18)	0.5	
			CMC	33.5 (23–45)	24	**0.002***
			Bioplasenton	44.5 (2–80)	35	
			Base gel	36 (6–56)	26.5	
			Saline	9.5 (5–20)	0	
FGF Expression	12	**15**	JM-P-CMC	75 (45–104)	42	
			Placenta	14 (6–26)	–19	
			CMC	50 (33–81)	17	**0.001***
			Bioplasenton	30 (10–55)	–3	
			Base gel	21.5 (10–55)	–11.5	
			Saline	33 (6–54)	0	

The Kruskal–Wallis test with Mann–Whitney post-hoc comparisons produced the following results:

Day 5:

JM-P-CMC vs Bioplasenton ($p = 0.0018$); Bioplasenton vs Base gel ($p = 0.0431$); Bioplasenton vs CMC ($p = 0.0011$); Base gel vs Saline ($p = 0.0003$); Base gel vs CMC ($p = 0.322$)

Day 10:

JM-P-CMC vs Saline ($p = 0.0001$); JM-P-CMC vs Placenta ($p = 0.0179$); Base gel vs Saline ($p = 0.0084$); Bioplasenton vs CMC ($p = 0.0402$); Bioplasenton vs Saline ($p = 0.0001$)

Day 15:

JM-P-CMC vs Bioplasenton ($p = 0.0324$); JM-P-CMC vs Placenta ($p = 0.0001$); JM-P-CMC vs Saline ($p = 0.0015$)

The Mann–Whitney test was used to evaluate differences between treatment groups on days 5, 10, and 15. On day 5, FGF expression showed statistically significant differences among the formulation groups compared with saline. The FGF expression data are presented in Figure 2.

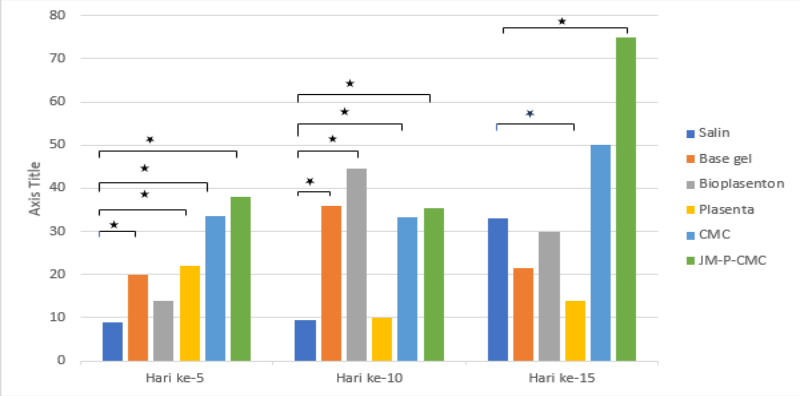

Figure 2. FGF expression graph for all treatments on days 5, 10, and 15 (Asterisk indicates significant difference compared with the saline group, $p < 0.05$).

On day 5, the JM-P-CMC formulation demonstrated the highest median FGF expression, followed by CMC and placenta. On day 10, JM-P-CMC continued to outperform other groups, showing a significant increase in FGF expression. Placenta and CMC formulations exhibited moderate expression levels, whereas only JM-P-CMC reached statistical significance. Bioplasenton and base gel did not differ significantly from saline. By day 15, the JM-P-CMC formulation consistently exhibited the highest FGF expression and remained significantly different from saline. Other formulations showed reduced effectiveness relative to JM-P-CMC. These findings reinforce the potential of JM-P-CMC in supporting long-term tissue regeneration during the remodelling phase.

Fibroblast Count Results (Hematoxylin-Eosin)

The mean number of fibroblast cells obtained through observations using an Olympus Trynocular CX33 microscope at 400× magnification across three fields of view is presented in Table 3. Administration of the JM-P-CMC gel formulation showed an increase in fibroblast counts in the skin tissue of male Sprague Dawley white rats (Rattus norvegicus).

Table 3. Mean Number of Fibroblasts per Three Fields of View in Burned Skin Tissue of Male Sprague Dawley Rats

Formulation	Day 5	Day 10	Day 15
Saline	22.75	19.67	22.71
Base gel	40.96	39.79	46.83
Bioplasenton	37.88	47.71	35.25
Placenta	37.88	31.29	18.83
CMC	31.00	26.46	41.21
JM-P-CMC	37.79	54.08	54.17

On Day 5, fibroblast counts had not yet increased significantly across all groups; however, by Day 10, the JM-P-CMC gel formulation showed a notable increase in fibroblast numbers, which continued through Day 15. Figure 3 illustrates variations in fibroblast counts stained with Hematoxylin–Eosin (HE) in the JM-P-CMC treatment group compared with other control groups. The JM-P-CMC gel formulation exhibited higher fibroblast density and quantity. These findings are consistent with the data in Figure 4, which displays fibroblast counts in bar chart format.

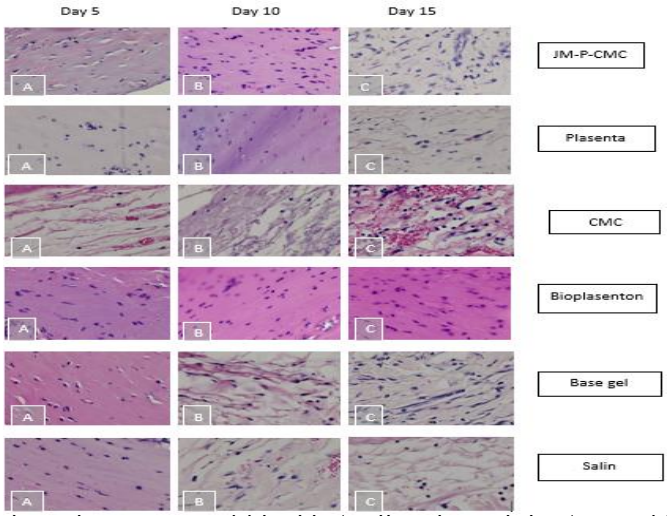

Figure 3. Histopathological appearance of fibroblast cells in burned skin tissue of Sprague Dawley rats (HE 400×). (A) Fibroblast count on Day 5, (B) Day 10, (C) Day 15**.**

Normality testing indicated that the data were not normally distributed. Therefore, the significance of fibroblast counts was analysed using the nonparametric Kruskal–Wallis test, followed by the Mann–Whitney post hoc test. Table 4 presents the results of the Kruskal–Wallis test, followed by Mann–Whitney post hoc testing to identify meaningful differences among treatment groups.

Table 4. Significance Test of Fibroblast Counts in Burn Wounds of Male Sprague Dawley Rats

Variable	N	Day	Formulation	Median (min–max)	Delta (%) vs Saline	p-value
Fibroblast Count	–	**5**	JM-P-CMC	41.0 (11–53)	20	
			Placenta	38.5 (17–58)	17.5	
			CMC	28 (7–77)	7	**0.0574**
			Bioplasenton	50.5 (19–80)	29.5	
			Base gel	39.5 (28–71)	18.5	
			Saline	21 (2–59)	0	
Fibroblast Count	12	**10**	JM-P-CMC	57 (37–107)	35.5	**0.0006***

			Placenta	25.5 (8–106)	4	
			CMC	19 (11–99)	–2.5	
			Bioplasenton	55.5 (37–65)	34	
			Base gel	44 (13–91)	22.5	
			Saline	21.5 (9–38)	0	
Fibroblast Count	–	**15**	JM-P-CMC	55 (37–101)	38	**0.0001***
			Placenta	21 (5–39)	4	
			CMC	37.5 (22–89)	20.5	
			Bioplasenton	32 (9–73)	15	
			Base gel	48 (19–92)	31	
			Saline	17 (6–66)	0	

Post-hoc Mann–Whitney Test

Day 5:

Bioplacenton vs Saline (p = 0.0061); Base gel vs Saline (p = 0.0152); JM-P-CMC vs Saline (p = 0.099); CMC vs Saline (p = 0.470); Placenta vs Saline (p = 0.064)

Day 10:

Bioplacenton vs Saline (p = 0.0001); Bioplacenton vs CMC (p = 0.0402); Base gel vs Saline (p = 0.0084); Saline vs JM-P-CMC (p = 0.0001); Placenta vs JM-P-CMC (p = 0.0179); CMC vs JM-P-CMC (p = 0.0086)

Day 15:

Bioplacenton vs Placenta (p = 0.0492); Bioplacenton vs JM-P-CMC (p = 0.0324); Base gel vs Saline (p = 0.0072); Base gel vs Placenta (p = 0.0032); Saline vs CMC (p = 0.0129); Saline vs JM-P-CMC (p = 0.0015); Placenta vs CMC (p = 0.0055); Placenta vs JM-P-CMC (p = 0.0001)

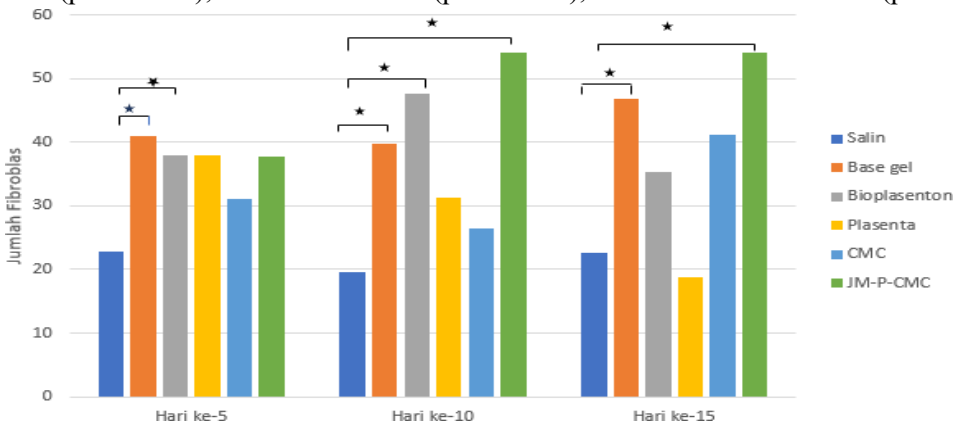

Figure 4. Graph of fibroblast counts in all treatment groups on days 5, 10, and 15 (connecting lines and asterisks (*) indicate significant differences compared to the saline group, $p < 0.05$).

Collagen Density Results (Masson's Trichome)

Collagen density data were obtained through microscopic observation using an Olympus Trinocular CX33 microscope at 400× magnification across three fields of view. The data are presented in Table 5. Administration of the JM-P-CMC gel formulation demonstrated an increase in collagen density in the skin tissue of male Sprague Dawley rats (Rattus norvegicus).

Table 5. Percentage of Collagen Density in Male Sprague Dawley Rats

Formulation	Day 5	Day 10	Day 15
Saline	20.68	34.26	21.70
Base gel	38.59	26.50	33.46
Bioplasenton	21.10	18.98	22.14
Placenta	11.38	12.12	35.67
CMC	31.62	16.52	29.07
JM-P-CMC	30.77	40.03	58.83

The percentage of collagen density on day 5 did not show a substantial increase across all groups. However, on day 10, collagen density increased notably in the JM-P-CMC group, and this further increased on day 15. Figure 5 illustrates variations in fibroblast distribution stained with H&E in the JM-P-CMC group compared with other control groups. The JM-P-CMC gel formulation showed higher collagen density, as shown in Figure 5, which presents the increase in fibroblast count in bar chart form. Figure 5 presents Masson's Trichrome staining results, highlighting collagen density in burn-injured skin. The JM-P-CMC gel formulation exhibits denser collagen compared to Bioplacenton and other control formulations. These findings indicate that the JM-P-CMC gel formulation positively influences the wound healing process.

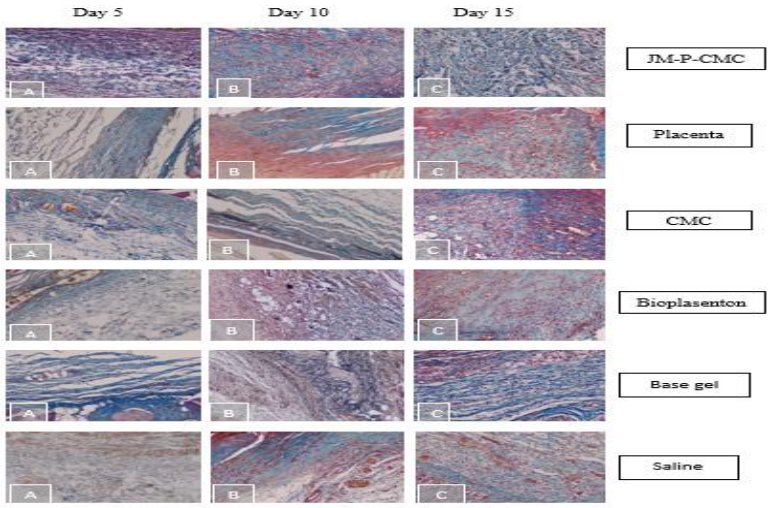

Figure 5. Histopathological Features of Collagen Density in Burn-Injured Skin of Sprague Dawley Rats (Masson's Trichrome, 400×): (A) Day 5; (B) Day 10; (C) Day 15.

An increase in collagen density is an important indicator of wound healing, particularly during the proliferative and remodelling phases. Quantitative analysis of collagen density at the three observation points (days 5, 10, and 15) showed that the JM-P-CMC formulation consistently produced higher median collagen levels compared with the saline control and most other treatment groups. The significance test for collagen density in this study used the Kruskal–Wallis test, followed by the Mann–Whitney post hoc test. Table 6 presents the statistical analysis results.

Table 6. Significance Test of Collagen Density in Burn Wounds of Male Sprague Dawley Rats

Variabel	N	Hari	formulasi	Median (min-max)	Delta vs Salin	*p*
Persentase kepadatan Kolagen	12	5	JM-P-CMC	32,17 (15,20-46,18)	15,77	0,0021*
			Plasenta	8,48 (2,26-28,63)	-7,92	
			CMC	24,9 (2,29-66,30)	8,5	
			Bioplasenton	15,24 (3,84-31,35)	-1,16	
			Base gel	42,83 (6,99-57,26)	26,43	
			Salin	16,40 (2,07-45,89)	0	
		10	JM-P-CMC	40,48 (20,74-49,84)	6,19	0,00014*
			Plasenta	10,96 (2,41-31,11)	-23,61	
			CMC	17,94 (3,79-37,29)	-16,36	
			Bioplasenton	20,78 (3,80-35,88)	-13,52	
			Base gel	26,46 (2,82-52,79)	-7,84	
			Salin	34,30 (16,76-53,69)	0	
		15	JM-P-CMC	55,09 (44,56-82,57)	32,79	0,0000044*
			Plasenta	35,44 (23,85-46,64)	13,13	
			CMC	25,31 (7,39-64,56)	3,01	
			Bioplasenton	24,49 (4,44-43,78)	2,19	
			Base gel	36 (15,54-66,38)	13,7	
			Salin	22,3 (16,76-28,63)	0	

JM-P-CMC vs placenta ($p = 0.0043$); base gel vs bioplacenton ($p = 0.0017$)

Day 10:

JM-P-CMC vs base gel ($p = 0.0007$); JM-P-CMC vs bioplacenton ($p = 0.0035$); JM-P-CMC vs placenta ($p = 0.0166$)

Day 15:

JM-P-CMC vs CMC ($p = 0.0011$); JM-P-CMC vs placenta ($p = 0.0001$); base gel vs placenta ($p = 0.0001$)

The analysis of collagen density in all treatment groups on days 5, 10, and 15 is presented in Figure 6.

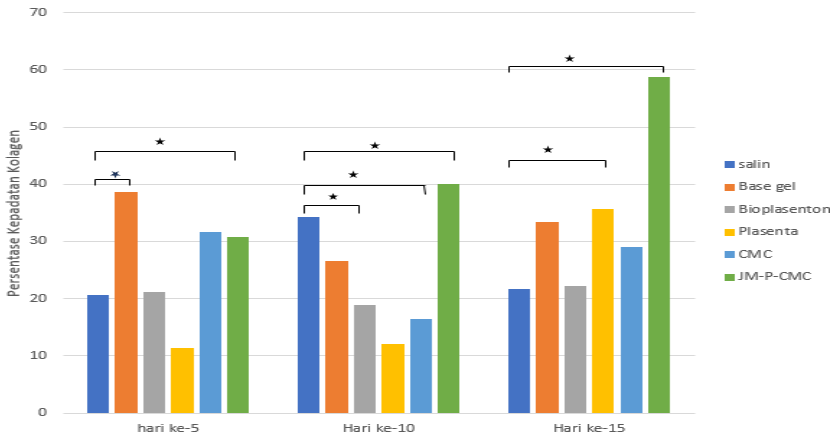

Figure 6. Analysis of collagen density in all treatment groups on days 5, 10, and 15 (asterisks (*) indicate significant differences compared with the saline group, $p < 0.05$).

Correlation of Increased Fibroblast Number and Fibroblast Growth Factor (FGF) Expression

The correlation between fibroblast count and Fibroblast Growth Factor (FGF) expression was analyzed using Spearman's rank test because the dataset did not meet normality assumptions. On day 5 of observation, the correlation showed a weak, non-significant negative relationship.

This result suggests that during the early inflammatory phase, fibroblast activity was not yet directly regulated by FGF expression. At this stage, FGF levels are typically still low, and inflammatory signals rather than proliferative cues dominate cellular responses. On day 10, the direction of the correlation shifted to positive, although it remained statistically non-significant. This change reflects biological progression toward the proliferative phase, in which fibroblasts begin to respond more strongly to growth factors, including FGF. However, variability in tissue responses and the influence of multiple inflammatory mediators may weaken this correlation. The transitional nature of day 10 indicates that fibroblast activation and FGF signalling are increasing but are not yet fully synchronised.

By day 15, the correlation was strongly positive, although it remained statistically insignificant. This pattern suggests that during the remodelling phase, fibroblasts increasingly rely on FGF for regulating proliferation, migration, and extracellular matrix synthesis. The strong correlation supports the established biological role of FGF in coordinating fibroblast function as the wound matures and reorganises. Overall, although none of the correlations reached statistical significance, the temporal trend demonstrates increasing alignment between fibroblast activity and FGF expression over the wound-healing timeline. The early phase shows weak coupling, but the late phase reveals a strong directional relationship. This progression highlights the increasing influence of FGF on fibroblast behavior as the tissue shifts from inflammation to proliferation and ultimately to remodelling.

Correlation of Increased Fibroblast Growth Factor (FGF) Expression and Collagen Density

The correlation between FGF expression and collagen density on day 5 was very weak and statistically nonsignificant. This result is expected, as the early inflammatory phase is not yet characterized by substantial collagen deposition. FGF expression at this stage tends to initiate fibroblast activation rather than directly promote measurable collagen synthesis. Therefore, the minimal relationship reflects the biological reality that collagen matrix formation has not significantly begun.

On day 10, the correlation remained weak and non-significant, despite fibroblasts actively producing collagen during the proliferative phase. One explanation for this weak correlation is the temporal delay between FGF-induced fibroblast activation and the subsequent deposition of collagen fibres. Another possibility is that multiple other growth factors—such as TGF-β and PDGF—play more dominant roles in early collagen production, thereby reducing the measurable influence of FGF. A striking change occurred on day 15, when a perfect and statistically significant negative correlation emerged between FGF expression and collagen density. This finding suggests that these two variables move in opposite directions during the remodelling phase. As collagen becomes denser and matures, the need for FGF-driven proliferation declines. Biologically, FGF may even modulate or limit excessive collagen deposition to optimise tissue structure.

Taken together, the results indicate that the relationship between FGF and collagen is highly phase-dependent. FGF does not have a strong measurable influence on collagen deposition during early or middle phases of healing. However, during the remodelling phase, their relationship becomes negatively associated, suggesting an important regulatory mechanism. Thus, FGF may shift from being a stimulator of early fibroblast activity to a modulator of matrix maturation in later stages.

Correlation of Increased Fibroblast Number and Collagen Density

On day 5, the correlation between fibroblast counts and collagen density was essentially absent. This aligns with the biological understanding that fibroblasts have only begun to

proliferate, and collagen deposition is still minimal in the early inflammatory phase. The cellular environment is dominated by immune cell infiltration, so fibroblast-driven collagen synthesis has not yet occurred. Therefore, the lack of relationship reflects expected physiological conditions.

By day 10, a perfect and statistically significant positive correlation was observed between fibroblast numbers and collagen density. This result strongly supports the role of fibroblasts as the primary producers of collagen during the proliferative phase. As fibroblast proliferation increases, collagen production naturally rises, explaining the perfect correlation. This finding confirms that day 10 represents the peak period of extracellular matrix synthesis.

On day 15, however, the correlation direction shifted to negative, although it remained statistically non-significant. This indicates that collagen accumulation no longer increases in proportion to fibroblast numbers during the remodelling phase. Instead, collagen undergoes reorganization, cross-linking, and partial degradation, meaning its quantity may not change in parallel with fibroblast levels. The dynamic turnover of collagen at this stage contributes to this inverse trend. Overall, the relationship between fibroblast count and collagen density evolves in accordance with the stages of wound healing. The proliferative phase shows strong alignment between the two variables, while early and late phases show weaker or negative relationships. These findings reinforce the concept that fibroblasts are central to collagen synthesis but that collagen dynamics become more complex during tissue maturation. Thus, the correlation patterns provide insight into the shifting biological priorities throughout wound repair.

DISCUSSION

The results of this study indicate that the JM–P–CMC gel formulation significantly increased FGF expression compared to the control group, particularly on day 15. This increase aligns with FGF's role as a key mediator in fibroblast proliferation, angiogenesis, and accelerated tissue regeneration. Recent studies have reported that FGF plays a crucial role in increasing cell migration and accelerating the transition from the inflammatory phase to the proliferative phase in second-degree burns (Ali Khan et al., 2021). Therefore, these findings strengthen evidence that FGF modulation is a crucial pathway that can be targeted to accelerate wound healing.

The increased number of fibroblasts in the JM–P–CMC group also supports efficient healing mechanisms. Fibroblasts play a central role in extracellular matrix formation by producing collagen and glycosaminoglycans. Previous research has reported that fibroblasts reach maximum activity in the proliferative phase, between days 10 and 15, particularly in wounds treated with bioactive therapeutics (Alven & Aderibigbe, 2020; Cheng et al., 2021). In this study, a significant increase in fibroblast count on day 15 supports the hypothesis that the combination of Jatropha multifida, placenta, and CMC creates a microenvironment conducive to cell proliferation.

Furthermore, the results showed that collagen density increased most significantly in the JM–P–CMC group on day 15. This increase in collagen density is a key indicator of successful tissue remodelling (Fahrurroji et al., 2026). Recent research suggests that good collagen quality in burn wounds is associated with a reduced risk of hypertrophic scar formation and increased tissue strength (Čoma et al., 2021; Hernandez-Hernandez et al., 2021). The synergistic effect of the three active ingredients in the JM–P–CMC formulation appears to support collagen synthesis via FGF-mediated fibroblast activation, resulting in more organised, stable tissue (Fahrurroji et al., 2026).

The positive correlation between FGF expression and fibroblast count in this study aligns with recent research showing that increased FGF levels directly enhance fibroblast proliferation

and accelerate matrix deposition (Dah-Nouvlessounon et al., 2023). However, the negative correlation between FGF and collagen density on day 15 indicates that, in the final phase of healing, the role of FGF begins to decline as collagen maturation progresses (Elbialy et al., 2020; Farooq et al., 2021). This pattern aligns with the theory that as fibroblast activity declines, the focus of healing shifts from proliferation to tissue remodelling (Sánchez-Hernández & Martínez-Flor, 2022). Thus, the dynamic relationship between these three parameters found in this study illustrates a normal physiological transition in wound healing (Berry et al., 2024).

Overall, the findings of this study strengthen scientific evidence that a combination of natural bioactive ingredients can be an effective alternative for burn wound therapy (Asl et al., 2026). These results are also consistent with studies reporting that herbal formulations or polysaccharide-based biomaterials such as CMC can accelerate re-epithelialization and improve granulation tissue quality (Bobrova et al., 2022). The advantage of the JM–P–CMC formulation lies in the synergistic effect of the three components, which not only enhances the proliferative response but also enhances tissue maturation. Therefore, this study makes an important contribution to the development of topical natural-based therapies that are safe, affordable, and potentially applicable in nursing and clinical practice.

Recommendations for Future Research

Future studies should expand sample size and include diverse animal models or clinical populations to improve generalizability. Randomized controlled clinical trials are needed to validate the safety and effectiveness of the JM–P–CMC formulation in human burn patients. Further investigation of molecular mechanisms, including additional growth factors and inflammatory markers, is recommended to clarify the pathways involved in tissue regeneration. Standardization of formulation dosage and long-term outcome evaluation, including scar quality and functional recovery, should also be prioritized.

CONCLUSION

This study demonstrates that the combined gel formulation of Jatropha multifida L. latex, placenta extract, and carboxymethyl chitosan (JM–P–CMC) effectively accelerates the healing of second-degree burn wounds, as evidenced by significant increases in fibroblast growth factor (FGF) expression, fibroblast proliferation, and collagen density. The intervention showed the most pronounced effects during the proliferative and remodelling phases, particularly on day 15, indicating its role in sustaining tissue regeneration rather than only modulating early inflammation. The observed relationships among FGF, fibroblasts, and collagen confirm an integrated biological mechanism in which growth factor signalling promotes cellular activity and extracellular matrix formation. These findings directly address the study objective by confirming the therapeutic effectiveness of the JM–P–CMC formulation through both molecular and histological indicators. From a practical perspective, this formulation offers a promising alternative to conventional topical agents, with potential advantages in safety, cost, and biological efficacy. In terms of policy and clinical implications, the results support the development of standardized bioactive-based topical therapies for burn management, particularly in resource-limited settings. Integration of such formulations into clinical guidelines and nursing practice may enhance treatment outcomes while reducing reliance on synthetic agents that can cause adverse effects and promote antimicrobial resistance.

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Conflict of Interest

The authors declare no conflict of interest related to this study.

Authors' Contributions

Mellyna Mellyna: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft, Supervision.
Juniarti: Methodology, Validation, Formal Analysis, Writing – Review & Editing.
Nunung Ainur Rahmah: Investigation, Data Curation, Resources, Visualization.
Restu Syamsul Hadi: Formal Analysis, Software, Validation, Writing – Review & Editing.
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Declaration of Generative AI Use

The authors declare that generative artificial intelligence tools were used solely to assist in language editing and manuscript refinement. All scientific content, data analysis, interpretation, and conclusions remain the responsibility of the authors.

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