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Guava leaf extract and polyvinylpyrrolidone hydrogel for rabbit oral wounds

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Summary

Introduction: A wound is defined as damage or loss of continuity to the skin or body tissue which can cause disruption to the body's anatomical structure. Wounds can be caused by several things, such as post-operative wounds, trauma, contact with heat sources, chemicals, and accidents. One of the potential complications from wounds and maxillofacial surgery is dehiscence. The combination of guava leaf ethanol extract (Psidium guajava Linn) and polyvinylpyrrolidone (PVP) hydrogel is expected to help the healing process of traumatic wounds on the oral mucosa by increasing the number of fibroblasts, epithelialization, vascular endothelial growth factor (VEGF) levels, and decreasing interleukin-16 (IL-16) levels. **Methods:** This research was an experimental test, using 28 rabbits as experimental animals. A traumatic wound is an incision made 2 cm laterally from the left central incisor and along the curve of the tooth. The wound reaches the gum line from the incision on the facial aspect to the midline of the papilla. The animals were divided into four treatment groups.: group 1 – the intervention group with wounds healed and given a combination gel; group 2 – the intervention group with wounds not healed and given a combination gel; group 3 – the observed group with wound healed and given placebo gel; group 4 – the observed group with wounds not healed and given placebo gel. There was an increase in the number of fibroblasts, epithelialization, VEGF levels, and a decrease in IL-16 levels between the treatment and control groups. **Results:** This study showed an increase in fibroblast levels in the treatment group (64.50 ± 4.43) which was higher than the control group (55.67 ± 4.04), with P = 0.041 as well as an increase in epithelialization in the treatment group. The results of the analysis in this study support the superiority of this gel combination in accelerating wound healing through a positive influence on VEGF levels and angiogenesis. In this study, IL-6 levels on day 3 showed a decrease of 25% in the treatment group. **Conclusion:** The combination of guava leaf ethanol gel and PVP hydrogel is an innovative therapy with high potential to accelerate wound healing, especially in chronic or difficult-to-heal wounds, with minimal risk of excessive inflammation and scar tissue formation.

Key words
wound – guava leaf ethanol extract – oral mucosa – fibroblasts – VEGF levels – IL-16 levels

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Introduction

Wound healing restores tissue integrity through inflammatory, proliferative, and remodeling phases, with fibroblast proliferation playing a key role [1]. Maxillofacial trauma, including mandibular fractures, requires advanced trauma life support-based (ATLS) management due to its impact on airway function [2–4]. Surgical treatment often involves open reduction and internal fixation (ORIF) via intraoral or extraoral approaches, each with benefits and risks. Intraoral techniques minimize scarring but increase the risk of infection, while extraoral approaches improve visualization but may cause nerve injury and scarring [5]. A major complication is intraoral wound dehiscence, prolonging recovery and increasing costs [6].

Wound dehiscence management requires meticulous care, but persistent cases impair healing and quality of life [7,8]. Herbal medicine, including guava (Psidium guajava Linn) leaves, shows potential due to antimicrobial, anti-inflammatory, and astringent properties [9,10]. polyvinylpyrrolidone sodium hyaluronate (PVP-NaHA) hydrogel accelerates oral wound healing, but studies on its combination with guava leaf extract are limited [11]. This study explores their synergistic effects in promoting oral wound healing.

Material and methods

This experimental study used a randomized post-test only control group design with 28 rabbits divided into four groups: P1 group (guava leaf extract + PVP hydrogel + suturing), K1 (extract without suturing), P2 group (placebo + suturing), and K2 group (placebo without suturing). Rabbits were housed under controlled conditions, receiving standard feed and care. Anesthesia was administered intramuscularly using a ketamine-xylazine-acepromazine (Ket-A-Xyl®; Ethica, Indonesia) mixture. Standardized incisional wounds were created on the gingival mucosa, with suturing using 5-0 Vicryl where applicable. The guava leaf extract gel was prepared through ethanol maceration, followed by formulation with carboxymethylcellulose – natrium (Na-CMC), propylene glycol, nipagin, glycerin, and triethanolamine. The gel was applied twice daily. Euthanasia was performed on day 14 using high-dose ketamine (Ketalar®; PT Pfizer Indonesia, Indonesia) and xylazine (Xyla®; Interchemie Werken De Adelaar Nederland B.V., The Netherlands), with tissue samples collected for histopathological analysis of IL-6, VEGF, and fibroblast count. Statistical analysis included Shapiro-Wilk test for normality, Levene's test for homogeneity, and one-way analysis of variance (ANOVA) with *post-hoc* comparisons using Tukey or Games-Howell tests. The data were analyzed in the Statistical Package for the Social Sciences (SPSS) 26.0, with significance set at $P < 0.05$. The study was conducted at Udayana University, Indonesia, from November to December 2024.This study was approved by the Committee of Research and Experimental, Udayana University, Bali, Indonesia, under the number of 2608/UN14.2.2.VII.14/LT/2024. There are no human subjects mentioned in this article, therefore no informed consent is applicable.

Results

The 28 male rabbits were divided into four groups. On day 3, fibroblast count was highest in P1 (64.50 ± 4.43) and lowest in K1/P2 (55.67 ± 4.04). By day 14, P1 increased to 76.75 ± 7.68, while K1/P2 remained at 66.67 ± 1.53. Epithelialization in P1 was 131.34 ± 3.88 on day 3, rising to 176.95 ± 11.99 by day 14, whereas K2 remained the lowest (83.21 ± 16.87 to 94.65 ± 14.37).

VEGF levels in P1 were highest (day 3: 68.99 ± 4.77; day 14: 81.95 ± 5.23), while K2 had the lowest increase (53.17 ± 8.26 to 58.87 ± 6.02). IL-6 levels in P1 were the lowest (day 3: 38.28 ± 0.95; day 14: 23.87 ± 1.88), while K1/P2 remained elevated (48.56 ± 1.10 to 47.40 ± 1.21). The results indicate significant improvements in fibroblast proliferation, epithelialization, and VEGF levels while reducing IL-6.

Data characteristics are summarized in Tab. 1.

The ANOVA analysis in Tab. 2 showed significant differences in fibroblast counts among groups on days 3 ($P = 0.009$) and 14 ($P = 0.035$). On day 3, P1 had a significantly higher fibroblast count than K1 (mean difference 8.833; $P = 0.041$; 95% CI 0.33–17.34). K1 had significantly lower counts than P2 (−12.083; $P = 0.007$; 95% CI −20.59 to −3.58) and K2 (−9.333; $P = 0.044$; 95% CI −18.42 to −0.24). By day 14, P1 maintained the highest fibroblast count, though differences with K1 (10.083; $P = 0.33$) and other groups were not significant. K1 remained lower than P2 (−17.833; $P = 0.042$; 95% CI −35.01 to −0.66), but no significant differences were found between K1 and K2 ($P = 0.979$) or P2 and K2 ($P = 0.08$).

The analysis in Tab. 3 showed significant differences in epithelial length on days 3 ($P = 0.001$) and 14 ($P = 0.000$), confirming the efficacy of P1 in accelerating epithelialization. On day 3, P1 had significantly longer epithelium than P2 (−40.57; $P = 0.003$;

Tab. 1. Data characteristics, normality test, and homogeneity test.

Variable	Time	P1 Mean ± SD	K1 Mean ± SD	P2 Mean ± SD	K2 Mean ± SD	Shapiro-Wilk and Levene tests (P)
fibroblasts	day 3	64.50 ± 4.43	55.67 ± 4.04	55.67 ± 4.04	65.00 ± 3.61	> 0.05
	day 14	76.75 ± 7.68	66.67 ± 1.53	66.67 ± 1.53	69.00 ± 4.36	> 0.05
epithelization	day 3	131.34 ± 3.88	109.49 ± 5.03	109.49 ± 5.03	83.21 ± 16.87	> 0.05
	day 14	176.95 ± 11.99	129.48 ± 7.29	129.48 ± 7.29	94.65 ± 14.37	> 0.05
VEGF	day 3	68.99 ± 4.77	57.34 ± 1.54	57.34 ± 1.54	53.17 ± 8.26	> 0.05
	day 14	81.95 ± 5.23	46.48 ± 1.31	46.48 ± 1.31	58.87 ± 6.02	> 0.05
IL-16	day 3	38.28 ± 0.95	48.56 ± 1.10	48.56 ± 1.10	49.37 ± 2.01	> 0.05
	day 14	23.87 ± 1.88	47.40 ± 1.21	47.40 ± 1.21	28.92 ± 2.98	> 0.05

The data are presented as mean ± SD unless otherwise indicated. $P < 0.05$ was considered statistically significant.
IL-16 – interleukin-16, SD – standard deviation, VEGF – vascular endothelial growth factor

Tab. 2. One-way ANOVA and Tukey HSD tests for fibroblasts.

Group I	Group II	Mean difference	P-value	95% CI lower bound	95% CI upper bound	ANOVA P-value
Fibroblasts – day 3						
P1	K1	8.833	0.041	0.33	17.34	0.009
P1	P2	–3.25	0.604	–11.12	4.62	
P1	K2	–0.5	0.998	–9.01	8.1	
K1	P2	–12.083	0.007	–20.59	–3.58	
K1	K2	–9.333	0.044	–18.42	–0.24	
P2	K2	2.75	0.759	–5.75	11.25	
Fibroblasts – day 14						
P1	K1	10.083	0.33	–7.09	27.26	0.035
P1	P2	–7.75	0.477	–23.65	8.15	
P1	K2	7.75	0.538	–9.47	24.97	
K1	P2	–17.833	0.042	–35.01	–0.66	
K1	K2	–2.333	0.979	–20.7	16.3	
P2	K2	15.5	0.08	–1.68	32.68	

P < 0.05 was considered statistically significant.
CI – confidence interval, HSD – honestly significant difference

Tab. 3. One-way ANOVA and Tukey HSD tests for epithelization.

Group I	Group II	Mean difference	P-value	95% CI lower bound	95% CI upper bound	ANOVA P-value
Epithelization – day 3						
P1	K1	21.85	0.129	–5.4	49.1	0.001
P1	P2	40.57	0.003	15.34	65.79	
P1	K2	48.13	0.001	20.88	75.38	
K1	P2	18.72	0.217	–8.53	45.97	
K1	K2	26.28	0.08	–2.85	55.41	
P2	K2	7.56	0.83	–19.69	34.81	
Epithelization – day 14						
P1	K1	47.47	0.001	23.35	71.59	0.000
P1	P2	56.8	0.000	34.47	79.13	
P1	K2	82.3	0.000	58.18	106.42	
K1	P2	9.33	0.65	–14.79	33.45	
K1	K2	34.82	0.009	9.04	60.61	
P2	K2	25.49	0.038	1.37	49.62	

P < 0.05 was considered statistically significant.
CI – confidence interval, HSD – honestly significant difference

Tab. 4. One-way ANOVA and Tukey HSD tests for VEGF.

Group I	Group II	Mean difference	P-value	95% CI lower bound	95% CI upper bound	ANOVA P-value
VEGF – day 3						
P1	K1	11.65	0.13	–2.92	26.21	0.009
P1	P2	–2.1	0.962	–15.59	11.38	
P1	K2	15.82	0.033	1.26	30.38	
K1	P2	–13.75	0.066	–28.31	0.81	
K1	K2	4.17	0.844	–11.4	19.74	
P2	K2	17.92	0.016	3.36	32.49	
VEGF – day 14						
P1	K1	35.47	0.000	23.61	47.33	0.000
P1	P2	–5.41	0.469	–16.39	5.57	
P1	K2	23.08	0.001	11.22	34.94	
K1	P2	–40.88	0.000	–52.74	–29.02	
K1	K2	–12.39	0.056	–25.07	0.28	
P2	K2	28.49	0.000	16.63	40.34	

P < 0.05 was considered statistically significant.
CI – confidence interval, HSD – honestly significant difference, VEGF – vascular endothelial growth factor

95% CI 15.34–65.79) and K2 (–48.13; P = 0.001; 95% CI 20.88–75.38), while differences with K1 were not significant (P = 0.129). On day 14, P1 remained the highest, significantly differing from K1 (–47.47; P = 0.001; 95% CI 23.35–71.59), P2 (–56.8; P = 0.000; 95% CI 34.47–79.13), and K2 (–82.3; P = 0.000; 95% CI 58.18–106.42). K1 vs. P2 was not significant (P = 0.65), but K1 vs. K2 (–34.82; P = 0.009) and P2 vs. K2 (–25.49; P = 0.038) were.

P1 significantly enhanced epithelialization at both time points. Sutures in K1 did not consistently improve healing, as differences with K2 were only significant on day 14. P2 showed better healing than K2 but was less effective than P1.

The analysis in Tab. 4 showed a significant increase in VEGF levels in the treatment groups compared to controls on days 3 (P = 0.009) and 14 (P = 0.000), indicating enhanced angiogenesis and tissue regeneration. On day 3, P1 had significantly higher VEGF than K2 (+15.82; P = 0.033; 95% CI 1.26–30.38), while differences in K1 (P = 0.13) and P2 (P = 0.962) were not significant. P2 also had higher VEGF than K2 (+17.92; P = 0.016). On day 14, P1 had significantly higher VEGF than K1 (+35.47; P = 0.000; 95% CI 23.61–47.33) and K2 (+23.08; P = 0.001; 95% CI 11.22–34.94), while the difference in P2 was not significant (P = 0.469). P2 also had significantly higher VEGF than K1 (–40.88; P = 0.000) and K2 (+28.49; P = 0.000), but K1 vs. K2 was not significant (P = 0.056). These results confirm that P1 consistently enhances VEGF expression, particularly on day 14, supporting its role in promoting angiogenesis and tissue repair. The effect was more pronounced in sutured wounds, suggesting an optimal environment for VEGF activation.

The analysis in Tab. 5 indicates that the combination gel significantly reduces IL-16 levels, a pro-inflammatory cytokine, during wound healing (P = 0.000 for both day 3 and day 14). On day 3, P1 had significantly lower IL-16 than K1 (–10.28; P = 0.000; 95% CI –14.17 to –6.40) and K2 (–11.09; P = 0.000; 95% CI –14.98 to –7.21), indicating reduced inflammation. However, P1 had higher IL-16 than P2 (+8.09; P = 0.000). Among controls, K1 and K2 showed no significant difference (P = 0.931), but P2 had significantly higher IL-16 than K2 (+19.19; P = 0.000). On day 14, P1 maintained lower IL-16 than K1 (–23.54; P = 0.000; 95% CI –28.01 to –19.06) and K2 (–5.06; P = 0.027; 95% CI –9.53 to –0.58). No significant difference was observed between P1 and P2 (P = 0.325). K1 had significantly higher IL-16 than P2 (+25.98; P = 0.000), and K1 vs. K2 also showed a significant difference (+18.48; P = 0.000).These findings confirm that P1 consistently reduces IL-16 levels, with a more pronounced effect in sutured wounds.

The analysis in Tab. 6 reveals significant changes in fibroblast count, epithelial length, VEGF, and IL-16 levels in group P1 between days 3 and

Tab. 5. One-way ANOVA and Tukey HSD tests for IL-16.

Group I	Group II	Mean difference	P-value	95% CI lower bound	95% CI upper bound	ANOVA P-value
IL-16 – day 3						
P1	K1	–10.28	0.000	–14.17	–6.40	0.000
P1	P2	8.09	0.000	4.50	11.69	
P1	K2	–11.09	0.000	–14.98	–7.21	
K1	P2	18.38	0.000	14.50	22.26	
K1	K2	–8.09	0.931	–4.96	3.34	
P2	K2	19.19	0.000	15.30	23.07	
IL-16 – day 14						
P1	K1	–23.54	0.000	–28.01	–19.06	0.000
P1	P2	2.45	0.325	–1.70	6.59	
P1	K2	–5.06	0.027	–9.53	–0.58	
K1	P2	25.98	0.000	21.51	30.46	
K1	K2	18.48	0.000	13.7	23.26	
P2	K2	–7.5	0.002	–11.98	–3.03	

P < 0.05 was considered statistically significant.
CI – confidence interval, HSD – honestly significant difference, IL-16 – interleukin 16

Tab. 6. Paired t-test for P1 group at days 3 and 14.

Variable pair	Mean ± SD	95% CI lower bound	95% CI upper bound	P-value
fibroblasts	–12.25 ± 4.79	–19.87	–4.63	0.014
epithelization	–45.62 ± 8.82	–59.66	–31.57	0.002
VEGF	–12.96 ± 5.16	–21.16	–4.76	0.015
IL-16	14.41 ± 1.31	12.32	16.5	0.000

The data are presented as mean ± SD unless otherwise indicated. P < 0.05 was considered statistically significant.
CI – confidence interval, IL-16 – interleukin-16, SD – standard deviation, VEGF – vascular endothelial growth factor

14 (P < 0.05, paired t-test), reflecting gingival wound healing dynamics. Fibroblast count significantly decreased by −12.25 ± 4.79 (P = 0.014; 95% CI −19.87 to −4.63), indicating a transition from the proliferative to the maturation/remodeling phase as collagen synthesis demand declines. Epithelial length decreased by −45.62 ± 8.82 (P = 0.002; 95% CI −59.66 to −31.57), suggesting rapid epithelialization peaking at day 3 and stabilizing by day 14, marking near-complete tissue regeneration. VEGF dropped by −12.96 ± 5.16 (P = 0.015; 95% CI −21.16 to −4.76), indicating reduced angiogenesis demand as vascularization stabilizes, highlighting the gel's role in early wound healing. IL-16 increased by +14.41 ± 1.31 (P = 0.000; 95% CI 12.32 to 16.5), possibly reflecting residual inflammation during ongoing healing, warranting further investigation.

The analysis in Tab. 7 reveals significant changes in fibroblast count, epithelial length, VEGF, and IL-16 levels in the control group (K1) between days 3 and 14 (P < 0.05, paired t-test), reflecting natural wound healing without active intervention. Fibroblast count decreased by −11.00 ± 2.65 (P = 0.019; 95% CI −17.57 to −4.43), indicating a transition from proliferation to maturation. Compared to the treatment group (P1), fibroblast regeneration was slower, highlighting the gel's role in accelerating this phase. Epithelial length decreased by

Tab. 7. Paired t-test for K1 group at days 3 and 14.

Variable pair	Mean ± SD	95% CI lower bound	95% CI upper bound	P-value
fibroblasts	−11.00 ± 2.65	−17.57	−4.43	0.019
epithelization	−19.99 ± 2.27	−25.62	−14.36	0.004
VEGF	10.87 ± 2.54	4.55	17.18	0.018
IL-16	−1.16 ± 0.14	−1.50	−0.81	0.005

The data are presented as mean ± SD unless otherwise indicated. P < 0.05 was considered statistically significant.
CI – confidence interval, IL-16 – interleukin-16, SD – standard deviation, VEGF – vascular endothelial growth factor

Tab. 8. Paired t-test for P2 group at days 3 and 14.

Variable pair	Mean ± SD	95% CI lower bound	95% CI upper bound	P-value
fibroblasts	−16.7 5± 9.57	−31.98	−1.52	0.039
epithelization	−29.38 ± 13.53	−50.90	−7.85	0.023
VEGF	−16.26 ± 4.09	−22.77	−9.76	0.004
IL-16	8.68 ± 1.96	5.57	11.80	0.003

The data are presented as mean ± SD unless otherwise indicated. P < 0.05 was considered statistically significant.
CI – confidence interval, IL-16 – interleukin-16, SD – standard deviation, VEGF – vascular endothelial growth factor

Tab. 9. Paired t-test for K2 group at days 3 and 14.

Variable pair	Mean ± SD	95% CI lower bound	95% CI upper bound	P-value
fibroblasts	−4.00 ± 1.00	−6.48	−1.52	0.02
epithelization	−11.45 ± 3.23	−19.47	−3.43	0.026
VEGF	−5.37 ± 1.82	−9.9	−0.84	0.036
IL-16	20.45 ± 4.94	8.17	32.73	0.019

The data are presented as mean ± SD unless otherwise indicated. P < 0.05 was considered statistically significant.
CI – confidence interval, IL-16 – interleukin-16, SD – standard deviation, VEGF – vascular endothelial growth factor

−19.99 ± 2.27 (P = 0.004; 95% CI −25.62 to −14.36), suggesting slower epithelialization than the treatment group, indicating that the placebo had minimal effect in enhancing tissue regeneration. VEGF increased by 10.87 ± 2.54 (P = 0.018; 95% CI 4.55 to 17.18), reflecting ongoing angiogenesis in the control group.

The analysis in Tab. 8 demonstrates the gel's effectiveness in wound healing without sutures, though healing was slower than in P1. Fibroblast count decreased by −16.75 ± 9.57 (P = 0.039), indicating transition to maturation, with a greater decline than in P1, suggesting mechanical stabilization enhances fibroblast activity. Epithelial length decreased by −29.38 ± 13.53 (P = 0.023), reflecting slowed epithelialization after day 3, confirming sutures create a more favorable healing environment. VEGF declined by −16.26 ± 4.09 (P = 0.004), suggesting reduced angiogenesis as vascular stabilization occurs, with a sharper decline than P1, implying sutures aid gel distribution. IL-16 increased by 8.68 ± 1.96 (P = 0.003), indicating persistent inflammation at day 14, suggesting the gel is less effective in controlling inflammation without sutures. Overall, P2 showed significant wound healing, but at a slower rate than P1, highlighting the role of mechanical stabilization in optimizing regeneration and reducing inflammation.

The analysis in Tab. 9 illustrates natural wound healing without treatment or mechanical stabilization. Fibroblast count decreased by −4.00 ± 1.00 (P = 0.02), indicating a transition to maturation, but with minimal support for fibroblast ac-

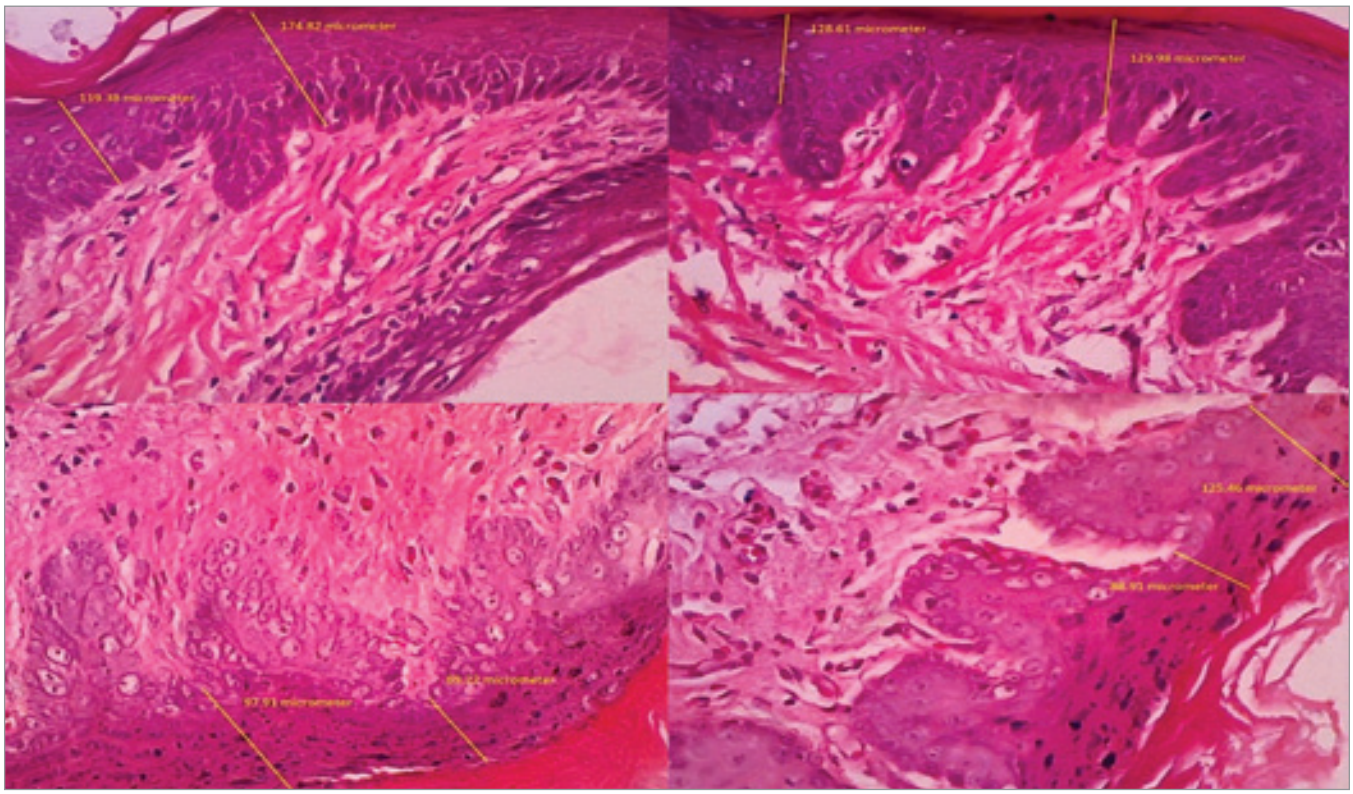

Fig. 1. Histology results from gingiva mucous epithelization of rabbit at day 3 after combination of guava leaf ethanol gel (Psidium guajava Linn) and polyvinylpyrrolidone hydrogel.

tivity. Epithelial length decreased by −11.45 ± 3.23 (P = 0.026), reflecting slower epithelialization compared to treated and sutured groups, confirming that both mechanical stabilization and active treatment accelerate healing. VEGF declined by −5.37 ± 1.82 (P = 0.036), showing reduced angiogenesis, with a smaller decline than active treatment groups, suggesting prolonged vascular maturation. IL-16 increased by 20.45 ± 4.94 (P = 0.019), indicating prolonged inflammation at day 14, delaying the transition to proliferation.

Histological analysis of rabbit gingival mucosa on day 3 post-treatment with guava leaf ethanol gel and polyvinylpyrrolidone hydrogel is shown in Fig. 1. The treatment group (P1) exhibited greater epithelial thickness (128.61–129.98 µm) than controls (69–97 µm), indicating enhanced epithelialization. Flavonoids, tannins, and saponins in guava extract stimulated keratinocyte migration and collagen synthesis, while hydrogel maintained wound moisture, promoting regeneration. Reduced IL-16 levels accelerated the transition to the proliferative phase, with improved tissue organization and angiogenesis marked by increased VEGF expression

Histological analysis on day 14 post-treatment is shown in Fig. 2, indicating near-complete healing in the treatment group (P1), with thicker, well-organized epithelium (161.74–173.22 µm) compared to controls (128.53–136.82 µm). Fibroblast activity declined, marking the transition to the maturation phase with stabilized collagen synthesis and the extracellular matrix (ECM) organization. VEGF levels decreased, indicating sufficient vascularization, while IL-16 levels were significantly lower, suggesting reduced inflammation. Unlike the untreated control (K2), which still showed inflammation, P1 exhibited optimal regeneration, confirming the gel's effectiveness in accelerating wound healing.

Discussion

Wound healing involves inflammation, proliferation, and remodeling, with fibroblasts playing a crucial role in ECM production, collagen synthesis, and immune regulation [12]. This study demonstrated that a combination of ethanol extract of guava leaves (Psidium guajava Linn) and PVP hydrogel significantly enhances fibroblast proliferation, VEGF expression, and epithelialization while reducing inflammation. On day 3, fibroblast counts in the treatment group (P1) were 64.50 ± 4.43, significantly higher than controls (P = 0.041), with further increases by day 14 (76.75 ± 7.68 vs. 66.67 ± 1.53) [13]. The hydrogel sustained fibroblast activity into the remodeling phase, minimizing fibrosis [14]. VEGF, a key angiogenic factor, was also significantly elevated in P1 (68.99 ± 4.77 vs. 57.34 ± 1.54 on day 3; 81.95 ± 5.23 vs. 58.87 ± 6.02 on day 14), suggesting enhanced vascularization and oxygen supply [15,16]. The gel also modulated inflammation by re-

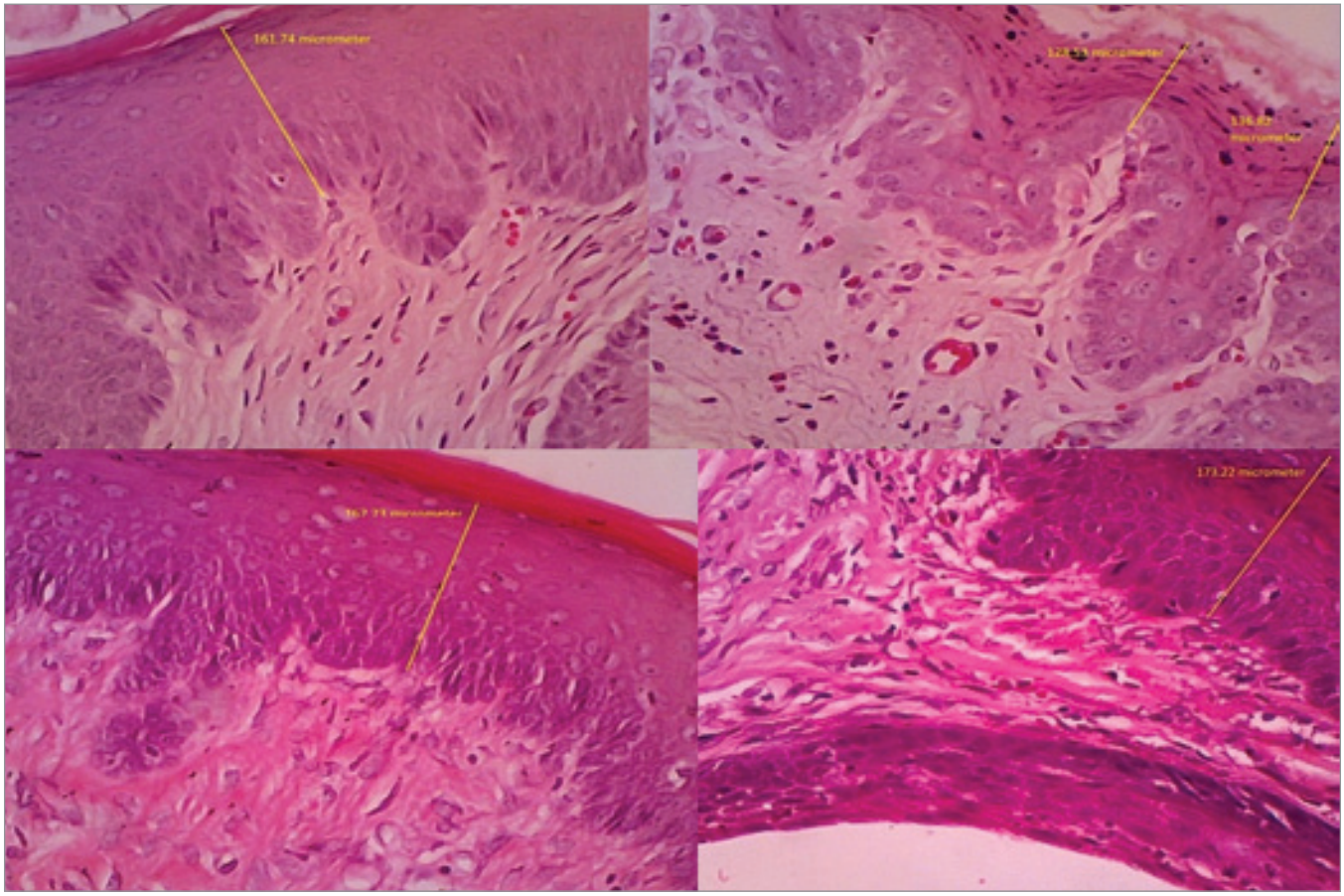

Fig. 2. Histological evaluation of mandibular gingival mucosal wound healing in rabbits on day 14 post-treatment with a combination of ethanol extract of guava leaves and polyvinylpyrrolidone hydrogel.

ducing IL-6 (38.28 ± 0.95 on day 3 to 23.87 ± 1.88 on day 14; P < 0.000), expediting the transition to proliferation [17]. Additionally, IL-16, an inflammatory marker, decreased by 45% in P1 by day 3, reaching 27.34 ± 1.21 pg/mL on day 14 compared to 41.78 ± 2.87 pg/mL in controls, fostering a favorable environment for regeneration [18]. The hydrogel's moisture-retaining properties supported keratinocyte migration and gradual bioactive release, further enhancing healing efficiency [19]. Histological analysis confirmed superior epithelialization in P1, with epithelial thickness reaching 176.95 ± 11.99 µm by day 14 compared to 129.48 ± 7.29 µm in controls [20]. Guava leaf extract's flavonoids and tannins played essential roles in reducing oxidative stress, forming a protective barrier, and enhancing collagen deposition [21,22]. The antibacterial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa* further reduced infection risks, crucial for chronic wound management [18]. These findings suggest that integrating herbal bioactive compounds with biomaterials like PVP hydrogel provides a promising therapeutic strategy for enhancing wound healing, optimizing fibroblast activity, angiogenesis, and inflammation control [15,23,24].

Conclusion

The combination of guava leaf ethanol gel and PVP hydrogel significantly enhances gingival wound healing in rabbits by increasing fibroblast count, epithelialization, VEGF levels, and reducing IL-16 compared to controls. Faster epithelialization was observed in the treatment group, particularly on day 14, indicating accelerated tissue regeneration. IL-16 levels decreased significantly at both time points, demonstrating effective inflammation control and a favorable wound microenvironment. The synergy between bioactive compounds (flavonoids, tannins, terpenoids) and PVP hydrogel improves bioavailability, maintains moisture, promotes angiogenesis, and prevents excessive inflammation. Sutures combined with the gel enhance results by ensuring uniform distribution and better wound stabilization. This innovative therapy shows high potential for accelerating wound healing, particularly in chronic or non-healing wounds, with minimal inflammation and scarring risks.

Conflicts of interest

None declared. No financial support.

Roles of the authors

All authors contribute to the creation of this original article. IGAABJ designed the study, collected the data, drafting the manuscript and

funding the research. ARRHH and IGPHS also designed the study and contribute in drafting the manuscript. GWS, IWN, IMD, SDS, and ALS supervised the whole process and gave revision on the writing on this journal. All authors reviewed and approved the final manuscript.

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