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The Effectiveness of Manuka Honey on Fibroblast and Collagen Growth in Gastric Rupture Repair

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ABSTRACT

Background: Gastric rupture is a rare but life-threatening surgical emergency characterized by leakage of gastric contents into the peritoneal cavity, leading to severe inflammation and infection. Optimal wound healing depends on fibroblast proliferation and collagen deposition. Manuka honey has shown anti-inflammatory and regenerative properties, yet its efficacy in gastric tissue repair remains unclear.

Objective: To evaluate the effect of topical and oral Manuka honey on fibroblast proliferation and collagen deposition in gastric wound healing after gastric rupture in rabbits.

Methods: Eighteen male New Zealand White rabbits were induced with a standardized gastric rupture, surgically repaired, and randomly allocated into three groups: Group A received topical Manuka honey (2 mL, UMF 16+, MGO 573+) applied to the sutured area; Group B received oral Manuka honey (2 g/kg/day for 7 days); Group C served as control with standard feeding only. On day 7, gastric tissues were collected for histopathological examination. Fibroblast and collagen levels were evaluated using hematoxylin–eosin and Masson's Trichrome staining, scored semi-quantitatively (0–4). Statistical analysis used the Kruskal–Wallis and post-hoc Mann–Whitney tests ($p < 0.0167$).

Results: The oral group showed the highest median fibroblast and collagen scores (3+), significantly greater than topical and control groups ($p = 0.014$ and $p = 0.011$, respectively). Post-hoc analysis confirmed significant differences between the oral group and both topical ($p = 0.013$) and control groups ($p = 0.007$) for collagen deposition, while fibroblast proliferation showed a similar trend.

Conclusion: Oral administration of Manuka honey significantly enhances fibroblast proliferation and collagen deposition in early gastric wound healing following gastric rupture in rabbits. Compared with topical application, oral therapy provides superior regenerative outcomes, suggesting its potential as an adjunct treatment in gastrointestinal surgical repair.

KEYWORDS: Manuka honey, gastric rupture, fibroblasts, collagen, wound healing

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INTRODUCTION

Gastric rupture is a rare yet life-threatening condition that requires immediate surgical intervention. It occurs when the gastric wall tears, allowing acidic gastric contents to spill into the peritoneal cavity and trigger severe peritonitis and systemic infection [1]. Although its incidence is low—less than 0.1% of all acute abdominal emergencies—gastric rupture is more frequently associated with blunt abdominal trauma, where it may represent up to 5% of documented stomach injuries [2]. Despite its rarity, the condition carries a high mortality rate, especially when complicated by septic shock, massive internal bleeding, or widespread peritonitis [3,4].

The pathophysiology of gastric rupture involves the leakage of digestive enzymes and acidic contents into the peritoneal cavity, resulting in intense inflammation, infection, and systemic disruption. This process may impair blood flow, cause additional damage to nearby organs, and heighten the risk of severe complications [5]. Successful healing of gastric injuries relies heavily on fibroblast proliferation and collagen deposition, which are essential for forming strong connective tissue and stabilizing the repair [6,7].

Manuka honey has gained attention as a natural therapeutic agent due to its anti-inflammatory, antioxidant, and antimicrobial properties. Its bioactive components—including methylglyoxal and flavonoids—help modulate inflammatory cytokines such as TNF-α and IL-1β, while nitric oxide pathways contribute to enhanced immune regulation and tissue repair [8,9]. Recent

experimental evidence also suggests that Manuka honey promotes cellular regeneration, as demonstrated in a rabbit mandibular fracture model by Herjuno *et al.* (2024) [10]. However, its potential role in accelerating healing after gastric rupture remains insufficiently explored. This study therefore aims to evaluate the effectiveness of Manuka honey in increasing fibroblast proliferation and collagen formation during gastric wound healing in rabbits.

MATERIALS AND METHODS

This experimental study used 18 clinically healthy male New Zealand White rabbits (3–4 months old, 2,500–3,000 g), maintained at the Experimental Animal Unit of the Faculty of Veterinary Medicine, Universitas Airlangga. Sample size was determined using the resource equation method, providing 6 animals per group. Inclusion criteria controlled for species, age, sex, and weight, while animals that failed fasting, exhibited aggressive behavior, or developed postoperative infection were excluded. Death during the experiment was classified as a drop-out.

Under ketamine anesthesia (50 mg/kg IM), all rabbits underwent a standardized midline laparotomy, induced gastric rupture, and surgical repair using uniform suturing techniques. Animals were subsequently randomized into three groups : Topical Manuka honey group received 2 mL of pure Manuka honey (UMF 16+, MGO 573+) applied directly to the sutured gastric area, oral Manuka honey group received 2 g/kg/day orally for 7 days, control group received standard postoperative feeding without honey.

On postoperative day 7, rabbits were euthanized using carbon monoxide, and gastric samples were collected. Tissues were fixed in 10% formalin and stained with hematoxylin–eosin to quantify fibroblasts and Masson's Trichrome to evaluate collagen deposition. Scoring followed the Ehrlich and Hunt semi-quantitative scale (0–4), based on 10 random high-power fields per sample, examined by a blinded pathologist. Data were analyzed using SPSS with non-parametric tests: Kruskal–Wallis for group comparison followed by Mann–Whitney with Bonferroni correction ($p < 0.0167$ as significant).

RESULTS

This study involved 18 male New Zealand White rabbits, which were allocated into three treatment groups. Group A (n = 6) received gastric repair followed by topical application of Manuka honey at the suture site; Group B (n = 7) received gastric repair followed by oral administration of Manuka honey; and Group C (n = 5) served as the control group, receiving gastric repair and standard feeding without honey. The baseline characteristics of the animals, including age and body weight, are presented as mean ± SD and summarized in Tabel 1.

Tabel 1. Characteristics of Study Subjects.

Variable	Group A (n = 6)	Group B (n = 7)	Group C (n = 5)	*p-value*
Age (month)	3,67 ± 0,52	3,57 ± 0,54	3,60 ± 0,55	0,942
Body weight (kg)	2,64 ± 0,30	2,73 ± 0,29	2,42 ± 0,41	0,286

Note: * = Significant at p-value < 0.05.

The body weight of the subjects was also within the predetermined range of 2.5–3 kg, with a p-value of 0.286 ($p > 0.05$), indicating no significant difference among the treatment groups. Thus, it can be concluded that the subjects in all intervention groups had comparable baseline ages and body weights in this study.

This study assessed the number of fibroblasts and collagen deposition following the interventions through histopathological examination. Fibroblast and collagen levels were quantified and evaluated histologically using the Ehrlich and Hunt numerical scale ranging from 0 to 4. Additionally, because the variables were measured on an ordinal scale, fibroblast and collagen data are summarized using median and minimum–maximum values. These results are presented in Tabel 2.

Tabel 2. Fibroblast and Collagen Counts Across Intervention Groups

Variable	Outcome Categories	Group A (n = 6)	Group B (n = 7)	Group C (n = 5)
Fibroblast Count	0	0 (0%)	0 (0%)	0 (0%)
	1+	0 (0%)	0 (0%)	2 (40%)
	2+	6 (100%)	3 (42,86%)	3 (60%)
	3+	0 (0%)	4 (57,14%)	0 (0%)

	4+ Median	0 (0%) 2+	0 (0%) 3+	0 (0%) 2+
Collagen Count	0 1+ 2+ 3+ 4+ Median	0 (0%) 0 (0%) 4 (66,67%) 2 (33,33%) 0 (0%) 2+	0 (0%) 0 (0%) 0 (0%) 7 (100%) 0 (0%) 3+	0 (0%) 2 (40%) 2 (40%) 1 (20%) 0 (0%) 2+

Explanation:
0 = None;
1+ = Occasional (Minimum);
2+ = Light scattering (Mild);
3+ = Numerous findings (Moderate);
4+ = Confluent cells/fibers (Intense/High).

Based on Table 5.2, fibroblast and collagen counts varied among the intervention groups. Group A predominantly showed scores of 2+ (mild), while Group B demonstrated higher scores of 3+, and Group C mostly remained at 2+. These histopathological differences are illustrated in Figure 5.1. Median values also differed, with Group A at 2+, Group B at 3+, and Group C at 2+. No degenerative changes were observed. Differences in median scores were further analyzed using the Kruskal–Wallis non-parametric test to assess statistical significance among the three groups.

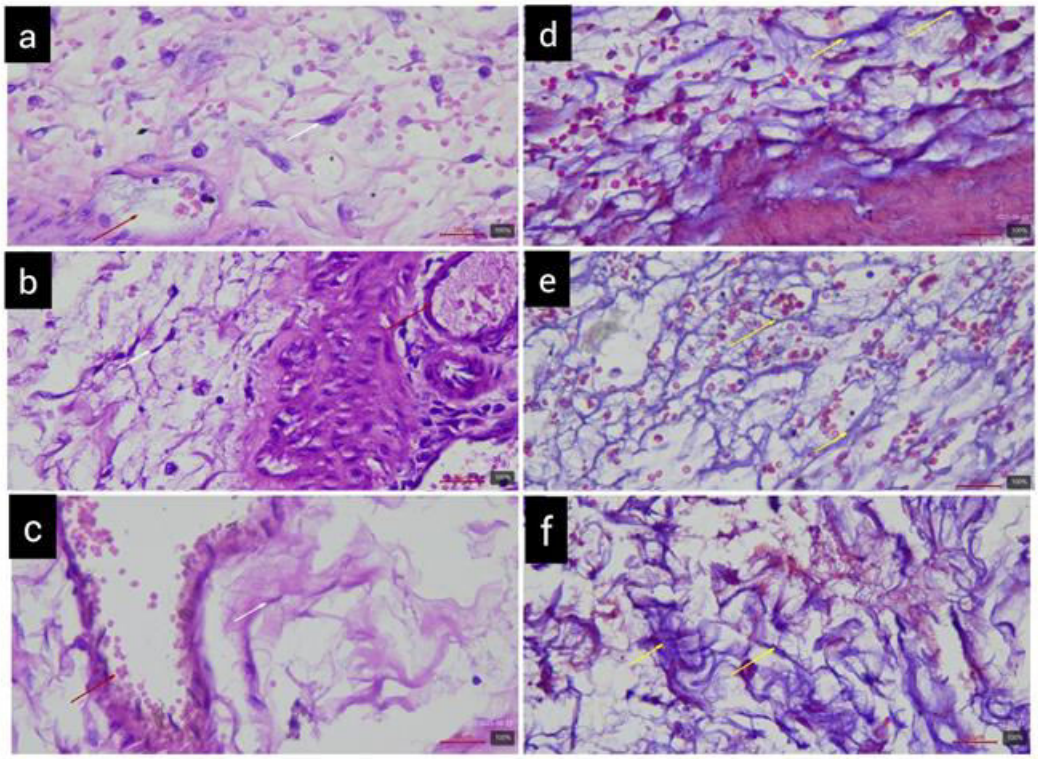

Fig 1. Histopathological Findings of Fibroblasts and Collagen in Gastric Tissue Across Intervention Groups (HE stain for fibroblasts and MT stain for collagen, 400× magnification). (a) Fibroblast 2+ (Group A); (b) Fibroblast 3+ (Group B); (c) Fibroblast 2+ (Group C); (d) Collagen 2+ (Group A); (e) Collagen 3+ (Group B); (f) Collagen 2+ (Group C).

Tabel 3. Results of the Comparative Analysis of Fibroblast Counts Across Intervention Groups.

Group	Median (Min - Max)	p-value (Kruskall Wallis test)
Group A (Topical)	2+ (2+ - 2+)	0,014*

Group B (Oral)	3+ (2+ - 3+)	
Group C (Control)	2+ (1+ - 2+)	

Note: * = Significant at p-value < 0.05.

A comparative analysis of fibroblast counts among Groups A, B, and C was conducted using the non-parametric Kruskal–Wallis test, as the data were ordinal and involved comparison of more than two independent groups. The results of the comparative analysis are presented in Table 3.

Based on the Kruskal–Wallis test (Tabel 3), a p-value of 0.014 ($p < 0.05$) indicated a significant difference in fibroblast counts among the intervention groups. Therefore, post-hoc analysis using the Mann–Whitney test with Bonferroni correction was performed to compare each pair of groups (A vs B, A vs C, and B vs C). A result was considered statistically significant when the adjusted p-value was below 0.0167 (0.05/3). The detailed post-hoc comparisons are presented in Tabel 4.

Tabel 4. Results of the Post-Hoc Comparative Analysis of Fibroblast Counts Between Groups.

Group	Group A	Group B	Group C
Group A		0,033[A]	0,102
Group B			0,021[A]
Group C			

Note: A = Significant at p-value < 0.05 using the Mann–Whitney test; B = Significant at p-value < 0.0167 with Bonferroni correction.

Based on the post-hoc Mann–Whitney test with Bonferroni correction, none of the group comparisons reached statistical significance at the adjusted $\alpha^* = 0.0167$ (A vs B, $p = 0.033$; A vs C, $p = 0.102$; B vs C, $p = 0.021$). However, when evaluated without Bonferroni correction, the oral group (B) showed significantly higher fibroblast counts compared with the control group (C) ($p = 0.021$) and the topical group (A) ($p = 0.033$), while no significant difference was observed between Groups A and C ($p = 0.102$). The comparison of collagen counts among Groups A, B, and C was conducted using the non-parametric Kruskal–Wallis test, as shown in Tabel 5.

Tabel 5. Results of the Comparative Analysis of Collagen Counts Across Intervention Groups

Group	Median (Min - Max)	*p-value* *(Kruskall Wallis test)*
Group A (Topical)	2+ (2+ - 3+)	0,011*
Group B (Oral)	3+ (3+ - 3+)	
Group C (Control)	2+ (1+ - 3+)	

Note: * = Significant at p-value < 0.05.

Based on the Kruskal–Wallis test results in Table 5.5, a p-value of 0.011 ($p < 0.05$) indicates a significant difference in collagen counts among the intervention groups. Therefore, a post-hoc analysis using the Mann–Whitney test with Bonferroni correction (significance threshold $p < 0.0167$) was performed to identify differences between individual group pairs. The results of the post-hoc comparisons are presented in Table 6.

Table 6. Results of the Post-Hoc Comparative Analysis of Collagen Counts Between Groups

Group	Group A	Group B	Group C
Group A		0,013^{AB}	0,226
Group B			0,007^{AB}
Group C			

Note: A = Significant at p-value < 0.05 using the Mann–Whitney test; B = Significant at p-value < 0.0167 with Bonferroni correction.

Based on the post-hoc Mann–Whitney test with Bonferroni correction (α* = 0.0167), significant differences were found between Groups A and B (p = 0.013) and between Groups B and C (p = 0.007), while no significant difference was observed between Groups A and C (p = 0.226) (Table 6). Group B demonstrated the highest median collagen score (3+), whereas Groups A and C both showed median scores of 2+. These findings indicate that oral administration of Manuka honey is more effective in enhancing collagen deposition than topical application or no treatment.

This study demonstrated that Manuka honey, particularly when administered orally, enhances fibroblast proliferation and collagen deposition in the early phase of gastric wound healing in a rabbit model of gastric rupture. Although both topical and oral administration showed better outcomes than the control group, the oral route consistently produced the highest median scores for fibroblasts and collagen (3+), indicating a more robust proliferative response. These findings suggest that the systemic bioavailability of Manuka honey's bioactive compounds plays a more significant role in promoting deep tissue regeneration than topical application alone. Given the severity and complexity of gastric rupture, which involves both mucosal and serosal injury, the ability of orally administered honey to exert systemic anti-inflammatory, antioxidant, and immunomodulatory effects appears critical in supporting optimal wound healing.

The enhanced fibroblast proliferation observed in the oral group aligns with several previous studies demonstrating the ability of Manuka honey to stimulate cell migration, proliferation, and extracellular matrix formation. In vitro research by Ranzato et al. (2012) and Minden-Birkenmaier et al. (2015) showed that Manuka honey accelerates fibroblast migration and proliferation, improving closure of scratch wounds and enhancing cellular infiltration into biomaterials [6,11]. These findings are supported by in vivo data from Oryan and Zaker (1998), who demonstrated accelerated fibroplasia and neovascularization in rabbit skin wounds treated with honey [12]. In the present study, the oral route delivered consistent exposure of gastric tissues to honey's bioactive molecules—including methylglyoxal (MGO), hydrogen peroxide, and flavonoids—which may have facilitated fibroblast recruitment and proliferation across tissue layers. The observed pattern (B > A > C) supports this mechanism, indicating that systemic absorption and distribution likely enhance the regenerative potential of honey at wounded gastrointestinal sites [13].

Collagen deposition followed a similar trend, with the oral group showing significantly higher levels than both the topical and control groups. Collagen synthesis is critically dependent on fibroblast activity; therefore, the parallel increase in fibroblasts and collagen supports the mechanistic relationship between the two parameters [14]. Previous work by Suguna et al. (1992) demonstrated that oral honey increased the synthesis and cross-linking of collagen more effectively than topical application [15]. This is consistent with the present findings, suggesting that systemic effects—such as improved nutritional status, enhanced antioxidant capacity, and modulated inflammatory cytokines—contribute to more efficient collagen production. Additionally, studies on gastric ulcer models have shown that oral Manuka honey reduces oxidative stress, increases anti-inflammatory cytokines (e.g., IL-10), and suppresses pro-inflammatory mediators (TNF-α, IL-1β, IL-6), contributing to mucosal regeneration and structural repair [16-18]. These systemic benefits likely explain the superior collagen deposition observed in the oral treatment group.

Topical application of Manuka honey showed moderate improvement compared with the control group but did not reach statistical significance for fibroblast counts after Bonferroni correction. This may be attributed to the limitation that honey was applied only once at the time of gastric repair, unlike skin wound models where repeated daily application produced stronger [12,19]. Moreover, the gastric serosal environment is moist and dynamic, and the honey applied topically may have been diluted or displaced shortly after surgery. Topical honey's known benefits—such as lowering wound pH, inhibiting proteases, providing antibacterial protection, and supporting autolytic debridement—may be less impactful in internal surgical wounds that lack prolonged surface contact [20,21]. Despite this limitation, the topical group still showed slightly better collagen and fibroblast scores than the control group, suggesting at least partial benefit when Manuka honey is placed directly on the wound site.

Mechanistically, Manuka honey contains several bioactive components that could account for the observed improvements. MGO exerts broad-spectrum antibacterial activity and promotes mucosal immune activation by influencing MAIT cell responses. Flavonoids and phenolic acids act as potent antioxidants that modulate NF-κB and MAPK signaling pathways, reducing excessive inflammation and preventing secondary tissue injury [13]. Hydrogen peroxide produced from glucose oxidase supports fibroblast migration, angiogenesis, and collagen maturation through VEGF upregulation and redox signaling. Additionally, the acidic pH

(3.5–4.5) of Manuka honey stabilizes the extracellular matrix by inhibiting protease activity, enhancing oxygen release, and supporting fibroblast metabolism [11]. These synergistic properties contribute not only to fibroblast proliferation but also to improved collagen synthesis, cross-linking, and tissue stability.

Although the findings of this study are promising, several limitations must be acknowledged. First, the observation period of seven days captures primarily the inflammatory and early proliferative phases of wound healing but does not fully represent the remodeling phase. Future studies with longer follow-up periods (14–21 days) may provide a clearer picture of long-term collagen maturation and tissue integrity. Second, the topical intervention was applied only once, which may underestimate its potential efficacy. Repeated dosing protocols could yield more comparable effects to the oral route. Third, the severity and dynamics of gastric rupture differ from external wounds, and factors such as digestive enzymes, gastric acidity, and peritoneal fluid turnover could influence therapeutic effectiveness. Additional parameters—such as tensile strength testing, hydroxyproline assays, or immunohistochemical markers for angiogenesis and inflammation—may also enhance future investigations.

Overall, this study provides new evidence that oral administration of Manuka honey significantly improves gastric wound healing by enhancing fibroblast proliferation and collagen deposition, outperforming topical application. These findings support the potential use of Manuka honey as a natural adjunct therapy in managing gastrointestinal injuries and postoperative gastric repair. Further studies are warranted to clarify dosing strategies, long-term healing outcomes, and translational relevance to clinical settings.

CONCLUSION

This study demonstrates that oral administration of Manuka honey is effective in enhancing fibroblast proliferation and collagen deposition during the healing process of gastric rupture in rabbits. Compared with topical application and the control group, the oral route consistently produced superior histopathological outcomes, indicating better tissue regeneration. Overall, Manuka honey—particularly when administered orally—shows promising potential as a natural adjunct therapy to support wound healing in cases of gastric injury.

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