JTL 14 (1) June 2025
Page: 31-39

Contents list available at Jurnal Teknologi Laboratorium

JURNAL TEKNOLOGI LABORATORIUM

Journal Homepage: www.teknolabjournal.com
ISSN 2580-0191(Online) I ISSN 2338 – 5634(Print)

Original Research

OPEN ACCESS

Evaluating royal jelly as a therapeutic agent for enhancing epithelial thickness in bacterial-induced wound healing

Fitri Nuroini[1*], Aprilia Permatasari[1], Tulus Ariyadi[1], Maya Damayanti[1,2]

[1] Medical Laboratory Technology, Faculty of Nursing and Health Science, Universitas Muhammadiyah Semarang, Indonesia
[2] Department of Pathology Anatomy, RSUD Dr. Adhyatma – Tugurejo, Semarang, Indonesia

Abstract: Open wounds provide an entry point for pathogenic microorganisms into the body, making proper wound management essential. A wound is considered healed when re-epithelialization occurs, with epithelial thickness serving as a key indicator of successful closure. Royal jelly, a natural supplement commonly used to boost energy and immunity, has shown potential as a therapeutic agent in wound healing due to its bioactive components, particularly royalactin and 10-hydroxy-2-decenoic acid (10-HDA). This study aimed to evaluate the effect of royal jelly on epithelial thickness during the proliferative phase of bacterial-induced wound healing through microscopic observation on day 14 post-treatment. Male white rats (200–250 grams, 2–3 months old) were used as subjects. The research involved bacterial culturing and suspension preparation, followed by royal jelly administration and histological assessment of epithelial regeneration. Data were analyzed using SPSS with the Shapiro-Wilk test, One-Way ANOVA, and LSD Post Hoc test. The results showed that 45 mg of royal jelly restored epithelial thickness (43.21 µm) to normal levels in wounds induced by *Pseudomonas aeruginosa*. Meanwhile, a 30 mg dose produced epithelial thickness (38.05 µm) comparable to that observed in the control group treated with amoxicillin. These findings indicate that higher doses of royal jelly promote greater epithelial regeneration. In conclusion, royal jelly can enhance epithelial tissue formation in infected wounds, as evidenced by increased epithelial thickness in treated groups.

Keywords: Royal Jelly; Epithelial Thickness; Proliferation; Wound Healing; *P. Aeruginosa* infection

INTRODUCTION

The skin is the largest organ in the human body and plays a vital role in maintaining homeostasis by regulating body fluids, electrolytes, and essential nutrients[1]. As the outermost protective barrier, the skin is highly susceptible to injuries, including both closed and open wounds. One common type of open wound is a linear laceration caused by sharp objects[2]. Open wounds can act as entry points for pathogenic microorganisms, and if not properly treated, may lead to infections that disrupt physiological balance and wound sterility[3,4].

Pathogenic organisms such as viruses, bacteria, fungi, and parasites may penetrate the body through breaches in the skin, potentially causing local or systemic infections[4,5]. Among these, *Pseudomonas aeruginosa* is a notable opportunistic bacterium known to cause skin infections, often characterized by bluish-green pus resulting from the pigment pyocyanin[6]. This pathogen invades host tissues through extracellular enzymes, toxins, and pilus-associated protein

Corresponding author.
E-mail address: fitrinuroini@unimua.ac.id (Fitri Nuroini)
DOI: 10.29238/teknolabjournal.v14i1.361
Received 07 June 2022; Received in revised form 24 December 2024; Accepted 16 January 2025

receptors (e.g., PilY1), which facilitate adhesion to wound surfaces. Additionally, *P. aeruginosa* can alter redox activity in host cells via NADPH pathways, triggering excessive production of reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2), which leads to epithelial membrane necrosis and infection exacerbation[7].

The standard treatment for such wounds often involves systemic broad-spectrum antibiotics to inhibit bacterial proliferation[8]. However, the overuse or inappropriate administration of antibiotics can result in bacterial resistance[9]. This concern highlights the need for alternative therapies that can effectively support wound healing without contributing to antimicrobial resistance.

Royal jelly, a natural secretion produced by worker bees, is widely known for its ability to boost energy and modulate the immune system[10]. It has been studied for its beneficial effects on reproductive health, neurodegenerative disorders, aging, and wound healing[11]. Its broad pharmacological properties include anti-lipidemic, antioxidant, antiproliferative, antimicrobial, anti-inflammatory, immunomodulatory, vasodilatory, hypotensive, hypocholesterolemic, anticancer, and estrogenic activities[12,13].

The composition of royal jelly includes water (50–60%), proteins (18%), carbohydrates (15%), sugars (7%), lipids (3–6%), minerals (1.5%), and vitamins[14–17]. Spectrometric analyses have identified over 185 organic compounds in royal jelly[11,18–20]. Key bioactive components include peptides such as royalisin, jelleines, aspimin, and royalactin; as well as polyphenols, phenolic acids, flavonoids, and unique fatty acids like 10-hydroxy-2-decenoic acid (10-HDA). Other notable compounds include AMP N1 oxide, adenosine, acetylcholine, and various hormones (e.g., testosterone, progesterone, prolactin, estradiol)[12,13,19–22]. Among these, royalactin and 10-HDA are particularly noted for their role in tissue regeneration and antimicrobial activity.

Wound healing is a complex physiological process involving numerous cell types, signaling molecules, and vascular responses. Normally, wounds heal within 4–6 weeks. However, chronic wounds, particularly those complicated by bacterial infections, may fail to heal within this period[23]. The healing process consists of three overlapping phases: inflammation, proliferation, and remodeling[24]. The proliferative phase, occurring between days 3 and 14, is essential for granulation tissue formation and tissue regeneration[25]. It is marked by the presence of fibroblasts, macrophages, endothelial cells, granulocytes, collagen fibers, and newly formed blood vessels within the extracellular matrix[26].

Epithelial regeneration and scar tissue formation are crucial aspects of wound healing[27]. Re-epithelialization involves epidermal cell proliferation and differentiation, mediated by growth factors such as VEGF, EGF, FGF, TGF-β, PDGF, and IGF-1[28–33]. This process is critical to wound closure and recovery. In chronic wounds, epithelialization is often impaired, delaying healing[34].

Given the regenerative and antimicrobial properties of royal jelly, further investigation into its effects on epithelialization is warranted. Although royal jelly has demonstrated potential in supporting wound healing, its comparative efficacy against standard antimicrobial treatments such as amoxicillin, especially in bacterial-infected wound models, remains underexplored. This study aims to address this gap by evaluating the role of royal jelly as a therapeutic agent to enhance epithelial thickness during the proliferative phase of wound healing. The evaluation was conducted via histological examination of hematoxylin-eosin (HE)-stained tissue sections, focusing on the measurement of neo-epithelium length on day 14 of treatment in a rat model.

MATERIAL AND METHOD

This study employed an experimental post-test only control group design to evaluate the effect of royal jelly on epithelial thickness in bacterial-induced wound healing. A total of 15 male white rats (*Rattus norvegicus*), aged 2–3 months and

weighing 200–250 grams, were used as experimental subjects. Prior to treatment, all animals were acclimated for 7 days under controlled conditions at the Experimental Animal Laboratory of Universitas Muhammadiyah Semarang.

Tools and materials
The tools used in this study included a micropipette (Onelab), incubator (Binder BD 115), animal hair shaver (Kemei), scalpel blade no. 18, camera, animal cages, microtome (Leica), water bath, microscope (Olympus CX25), hotplate, tissue processor (Leica), and staining jars. The reagents and materials comprised raw royal jelly (RAW Royal Jelly), amoxicillin (IndoFarma), BHI and BHIA media (Merck), MacConkey (MC) media (Oxoid), *Pseudomonas aeruginosa* isolate (Microbiology Lab, Unimus), 10% neutral-buffered formalin (NBF), ether, hematoxylin-eosin staining kits (Thermo), and Entellan® mounting medium.

Experimental design
Table 1. Research Group Design

Group	Description
Normal Control Group (KN)	Normal rats without any treatment
Negative Control Group (K-)	Rats treated incision and suspension of *P. aeruginosa*
Positive Control Group (K+)	Rats treated incision, suspension of *P. aeruginosa, amoxicillin*
Treatment group 1 (P1)	Rats treated incision, suspension of *P. aeruginosa* and Royal Jelly 30mg
Treatment group 2 (P2)	Rats treated incision, suspension of *P. aeruginosa* and Royal Jelly 45mg

The selected dosages of 30 mg and 45 mg of royal jelly were based on previous findings indicating their effectiveness in modulating inflammation and promoting epithelial cell proliferation, making them suitable for assessing therapeutic effects.

Bacterial suspension and sulture
A pure culture of *P. aeruginosa* was first inoculated in BHI liquid media and incubated at 37°C for 3–6 hours. The culture was then streaked onto MacConkey (MC) agar and incubated for 24 hours. Upon colony growth, it was further inoculated into BHIA and incubated for another 24 hours at 37°C. The bacterial suspension was prepared by diluting colonies in 0.9% NaCl solution, adjusted to a 0.5 McFarland standard for consistency.

Wound induction and treatment administration
All procedures were approved by the Ethics Committee of the Faculty of Public Health, Universitas Muhammadiyah Semarang (Approval No. 478/KEKP-FKM/UNIMUS/2021). Rats were anesthetized using ether, and their dorsal fur was shaved and sterilized with 70% alcohol. A 2 cm-long and 2 mm-deep incision was made using scalpel blade no. 18. Subsequently, 20 µL of *P. aeruginosa* suspension was topically administered to the wound site in groups K−, K+, P1, and P2. The treatments were administered orally using a 3 mL syringe fitted with gastric sonde no. 6. Group K+ received 10 mg of amoxicillin; P1 and P2 received 30 mg and 45 mg of royal jelly, respectively. Oral treatments were given once daily at the same time for 14 consecutive days.

Tissue collection and histological processing
On day 14, the rats were euthanized, and a 2 × 1 cm section of skin tissue (3 mm depth) from the wound area was excised. The tissue samples were fixed in 10% neutral-buffered formalin and underwent standard histological processing, including dehydration, clearing, paraffin embedding, and microtome sectioning at

5 µm thickness. The tissue sections were stained with hematoxylin-eosin (HE) for microscopic analysis.

Microscopic observation and measurement
Wound healing was evaluated through measurement of epithelial thickness using an Olympus CX25 light microscope at 400x magnification, connected to a DinoLite digital camera. Observations were captured and analyzed using Dino Capture 2.0® software, with calibration performed prior to measurement. Epithelial thickness was measured in micrometers from the outermost stratum corneum to the basal stratum basale layer on three locations of each sample (left, center, right). The final value was the average of these three measurements[35].

Statistical analysis
Data were analyzed using SPSS software. After confirming normality using the Shapiro–Wilk test, one-way ANOVA was performed, followed by LSD post-hoc testing to assess significant differences between groups. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

This study investigated the effect of royal jelly on epithelial thickness during the proliferative phase (day 14) of wound healing in white rats with incisions induced by *Pseudomonas aeruginosa*. Table 2 and Figure 1 present the mean epithelial thickness for each group: normal control (KN), negative control (K−), positive control (K+), and treatment groups P1 (30 mg royal jelly) and P2 (45 mg royal jelly).

Table 2. Average Epithelial Thickness in Skin Wound White Rats on Day 14

Group	Average Epithelial Thickness ± SD (µm)
KN	43,42 ± 2,65
K-	32,42 ± 6,16[a]
K+	38,81 ± 9,13
P1	38,05 ± 3,44
P2	43,21 ± 5,91[a]

Information: KN= normal control, K- = negative control; K+ = positive control; P1 = *royal jelly* 30mg; P2 = *royal jelly* 45mg. Note a indicate significant differences in the LSD test (<0.05)

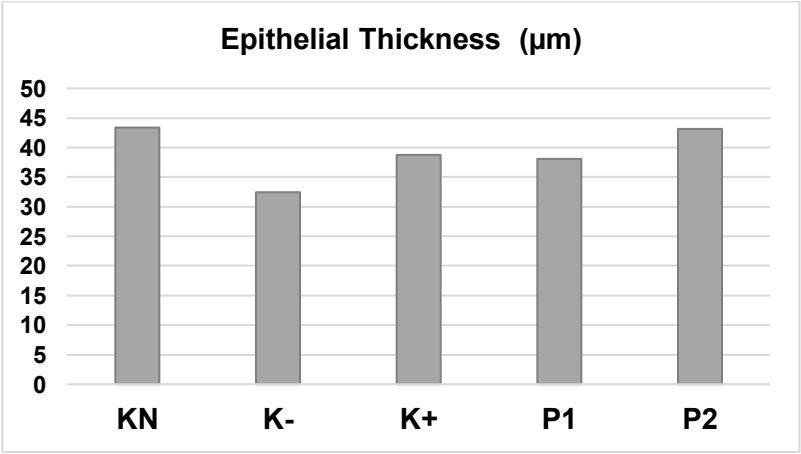

Figure 1. Graph of Average Epithelial Thickness in Skin Wound White Rats on Day 14

Based on the data, the KN and P2 groups exhibited the highest epithelial thickness, followed by the K+ and P1 groups, while the lowest thickness was observed in the K− group. Notably, the mean epithelial thickness in the P2 group was comparable to that of the normal control group, indicating complete epithelial restoration. Meanwhile, the P1 group showed results similar to the K+ group, which received amoxicillin.

These findings demonstrate that oral administration of 45 mg royal jelly effectively restored epithelial thickness to normal levels in rats with *P. aeruginosa*-induced wounds. This suggests that royal jelly has promising potential as a therapeutic agent not only for managing infections but also for enhancing tissue regeneration. The dual action of royal jelly—antimicrobial activity and promotion of epithelial repair—offers advantages over conventional antibiotics like amoxicillin, which primarily target pathogens without directly supporting tissue repair.

Although the data show positive trends, statistical analysis using one-way ANOVA followed by LSD post-hoc tests revealed no significant differences among groups ($p > 0.05$). This lack of statistical significance is likely due to the small sample size and individual variability in response. Nevertheless, the biological relevance of the findings remains noteworthy and warrants further investigation with larger sample sizes to strengthen statistical power and confirm efficacy.

Microscopic observations on day 14, as shown in Figure 2, illustrated the extent of epithelial regeneration. The re-epithelialization process, which involves covering the wound surface with new epithelial cells, is essential for wound closure[34]. This stage is universally critical across all species during the wound healing process[33]. The average neo-epithelial length in the treatment groups (P1 and P2) further supports the role of royal jelly in promoting epithelial growth, with a dose-dependent trend observed—the higher the dose, the thicker the epithelial layer formed.

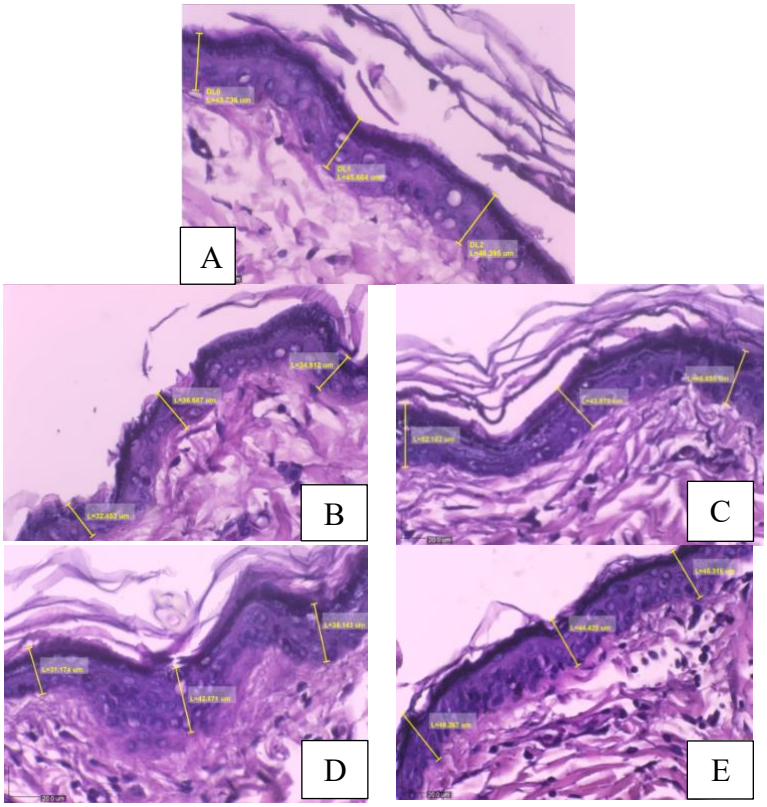

Figure 2. Microscopic Image of White Rat Skin Epithelium on Cut Wounds of White Rat Day 14 (A) Normal Control (B), Negative Control (C), Positive Control (D), Royal Jelly 30mg (E), and Royal Jelly 45mg (HE, 400X)

The therapeutic effect of royal jelly in wound healing may be attributed to its rich bioactive content, including antimicrobial peptides (e.g., jelleines), growth-stimulating proteins like royalactin, and fatty acids such as 10-hydroxy-2-decenoic acid (10-HDA)[16],[22]. These compounds are known to facilitate keratinocyte proliferation and migration, key processes in re-epithelialization[33]. Royal jelly also stimulates matrix metalloproteinases (MMPs), particularly MMP-1, which aid in extracellular matrix remodeling, cell migration, and reorganization of the actin cytoskeleton, further promoting tissue regeneration[16],[36].

Keratinocytes play a central role in epidermal repair. They originate from the basal layer, characterized by keratin intermediate filaments, and migrate upward through the spinous, granular, and stratum corneum layers. During migration, they undergo differentiation and require disassembly of desmosomes for effective movement[34]. Royal jelly supports this process by enhancing keratinocyte activity and extracellular matrix interactions, facilitating the formation of a cohesive epithelial barrier.

Although no statistically significant differences were found, the visible trends and histological observations suggest that royal jelly particularly at 45 mg has a biologically meaningful impact on epithelial regeneration. The minimal difference in dosage between the 30 mg and 45 mg groups may also explain the limited contrast in outcomes. According to Alfiyaturrohmah (2020), variations in individual responses to dosages must be considered within the framework of the dose-response relationship[37].

Over the 14-day observation period, all rats treated with royal jelly exhibited improved epithelial regeneration compared to untreated controls. This supports previous findings by Pasupuleti et al.[22], who reported that royal jelly enhances wound healing by promoting fibroblast migration, increasing sphingolipid levels, and modulating collagen synthesis in both in vivo and in vitro models. Furthermore, jelleines I–III, peptides rich in lysine, arginine, and histidine, contribute to antimicrobial activity by disrupting bacterial cell membranes[16].

The proliferative phase of wound healing is marked by granulation tissue formation, re-epithelialization, and neovascularization[23]. The epithelial restoration observed in the P2 group underscores the potential of royal jelly to support these critical processes. The action of myofibroblasts in contracting granulation tissue towards the end of epithelialization also plays a crucial role in wound closure[38].

In conclusion, while statistical significance was not achieved, the overall biological trends and histological evidence suggest that royal jelly, particularly at 45 mg, is a promising agent for enhancing epithelial regeneration in infected wounds**.** Further research with larger sample sizes and mechanistic studies is recommended to validate these findings and explore their application in clinical settings.

CONCLUSION

In summary, the administration of royal jelly was found to stimulate epithelial tissue formation, as indicated by increased epithelial thickness in the treatment groups 38.05 µm in the P1 group (30 mg) and 43.21 µm in the P2 group (45 mg). Notably, royal jelly at a dose of 45 mg restored epithelial thickness to levels comparable to the normal (uninjured) control group, highlighting its potential effectiveness in promoting epithelial regeneration in bacterial-induced wounds.

Although statistical analysis did not reveal significant differences between groups ($p > 0.05$), the biological trends observed suggest that royal jelly particularly at higher doses may serve as a promising alternative or adjunctive therapy for enhancing wound healing. Its dual action in combating infection and supporting tissue regeneration sets it apart from conventional antibiotic treatments. Further studies with larger sample sizes and focused mechanistic investigations are

recommended to validate these findings and explore the molecular pathways underlying royal jelly's regenerative effects.

AUTHORS' CONTRIBUTIONS
All authors contributed equally to this work.

ACKNOWLEDGEMENT
The authors would like to thank the Medical Laboratory Technology, Faculty of Nursing and Health Science, Universitas Muhammadiyah Semarang, and LPPM Universitas Muhammadiyah Semarang.

FUNDING INFORMATION
The founding of this research was from Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM), Universitas Muhammadiyah Semarang.

DATA AVAILABILITY STATEMENT
The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT
The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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