EXPERIMENTAL STUDY

Comparison of the efficacy of royal jelly and melatonin combinations in experimentally induced wounds in geriatric and young mice

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ABSTRACT

BACKGROUND: Wound healing involves the repair of skin and other soft tissues after an injury. Royal jelly, a product of bees, possesses antioxidant, anti-inflammatory, antibacterial, and antiviral properties. Melatonin, a circadian indoleamine, is produced in the pineal gland and other organs. This study explores the effects of melatonin and royal jelly, both individually and combined, on wound healing in geriatric and young mice.

METHODS: The study includes 90 Balb/C mice divided into ten groups to assess the effects of royal jelly and melatonin on wound healing. Royal jelly was applied topically at a concentration of 300 mg/kg. Melatonin was formulated in a vaseline-based pomade at a concentration of 5 mg/kg. The substances were applied either separately or in combination to wounds created on the mice.

RESULTS: Both substances significantly enhanced wound healing at a macroscopic level in both age groups. Melatonin was found to be more effective during the initial wound formation process, whereas royal jelly was more beneficial during the granulation phase. However, significant results at a histopathological level were observed only in geriatric animals.

CONCLUSION: The findings suggest a potential new therapeutic approach to enhance wound healing, particularly in elderly individuals. However, these findings need to be supported through further research and clinical trials.

Keywords: Apitherapy; epithelization; granulation; healing.

INTRODUCTION

The loss of integrity of skin and the underlying tissue is defined as a wound. Wound healing comprises a series of events, including the accumulation of a fibrin-platelet clot at the wound site, recruitment of white blood cells to protect the area from infection, neovascularization, cellular proliferation, and tissue remodeling.[1] Organic molecules and various cell types are integral to the healing process. A deficiency in one of the essential elements of the healing process can impede recovery.[2] Following an injury, there is an immediate inflammatory response, succeeded by new tissue formation and the maturation stage. Numerous neutrophils and macrophages from the circulation are attracted to the wound area by chemotactic factors secreted during the early inflammation stages. Alongside existing fibroblasts, these newly arrived cells contribute to collagen synthesis, contraction, and ultimately, wound closure. In the absence of circulatory or metabolic complications, wound healing proceeds swiftly through its own active mechanisms.[3] Several local and systemic factors influence wound

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healing. Local factors include the wound's location, depth, moisture, and oxygenation, while systemic factors encompass existing medical conditions, nutrition, mobility, obesity, infection, medication use, and age.[4] As individuals age, the onset of the wound healing process is delayed, and its progression slows. Research on geriatric rats and mice indicates more adverse effects on wound healing phases compared to younger animals. In geriatric individuals, various factors such as reduced cellular turnover, diminished collagen production, impaired immune response, and decreased blood flow to the wound site may compromise healing.[5]

N-acetyl-5-methoxytryptamine, commonly known as melatonin, is a circadian indoleamine primarily produced in the pineal glands following a 24-hour day-night rhythm.[6] Reports also indicate that various organs and glands secrete melatonin, with mitochondria considered the predominant sites of melatonin production in peripheral tissues.[7] As an anti-inflammatory agent, melatonin reduces several inflammation hallmarks. It inhibits the binding of nuclear factor kappa B to DNA and the translocation of numerous pro-inflammatory cytokines, including interleukin (IL)-1b, IL-6, and tumor necrosis factor-alpha.[8] Melatonin also mitigates oxidative stress and curbs the production of adhesion molecules that promote leukocyte adhesion to endothelial cells.[9] All living things, from single-celled organisms to mammals, contain melatonin. Its most significant characteristic is that it and its metabolites are potent antioxidants. Melatonin regulates circadian rhythms and has a profound impact on several systems, including the immune system, which it notably supports.[10] This molecule is regularly released following a nocturnal rhythm and possesses hormonal properties.[11] In animal experiments, activity of N-acetyltransferase (NAT), the rate-limiting enzyme in melatonin synthesis, peaks during darkness. During these periods, sympathetic nerve fibers innervate the pineal gland, and the increase in cyclic adenosine monophosphate (cAMP) mediated by norepinephrine boosts NAT enzyme activity, converting serotonin to melatonin. This results in decreased serotonin levels at night. Exposure to light, however, suppresses this sympathetic activity, leading to a rapid decrease in NAT enzyme activity and subsequently lower melatonin levels in the blood.[12] Melatonin is known to influence a variety of biological processes, including biological rhythms, sexual development, reproduction, sleep, mood, immunity, and aging.[13] Melatonin levels in humans and many animals decline with age. In humans, melatonin levels begin to rise between three to six months of age, establishing a clear distinction between daytime and nighttime levels. Nocturnal melatonin levels vary with age as follows: approximately 250 pg/mL between one and five years of age; around 65 pg/mL between 5 and 15 years; and about 20 pg/mL between 50 and 70 years of age. During the daytime, melatonin levels are approximately 20 pg/mL.[14] Previous studies on melatonin's role as a therapeutic mediator for wound healing have shown inconsistent results.[15,16]

Royal jelly is a bee product secreted by the hypopharyngeal glands of worker bees aged 5-15 days. This gel-like fluid is creamy-white, sour in taste, with a sharp phenolic odor, and has a density of 1.1 g/cm³, being partially soluble in water.[17] Royal jelly serves as the exclusive food for the queen bee and is noted for its high nutritional value. The significant lifespan of a queen bee, who is fed royal jelly throughout her life, is the most important indication of its benefits.[18] Its composition includes approximately 66% water, 12.34% protein, 5.46% fat, 12.49% sugars, 0.82% minerals, and 2.84% other components, along with vitamins A, D, E, K, and C. Royal jelly not only provides nutritional value but also offers functional and biological benefits.[19] It displays important activities, such as antioxidant, anti-inflammatory, antibacterial, and antiviral properties. The proteins in royal jelly, primarily albumin, have been scientifically proven to exhibit various beneficial effects, including antioxidant, immunomodulatory, antibacterial, anti-inflammatory, fatigue-reducing, antihypertensive, antidiabetic, and collagen-enhancing properties.[20-26] According to another study, the pineal gland regulates collagen synthesis in healthy skin, and melatonin significantly reduces skin collagen synthesis.[27] Given its wide range of promising properties, melatonin appears to be an excellent candidate for accelerating wound healing.

It is important to consider these factors when managing wounds in older individuals to optimize the healing process and prevent complications. Currently, no study specifically evaluates the effects of local melatonin and royal jelly on wound healing in geriatric animals. The proposed study aims to investigate the effects of melatonin and royal jelly, both individually and in combination, on wound healing in geriatric mice and to compare these effects with those observed in young animals.

MATERIALS AND METHODS

Animals

The study was conducted with the approval of Muğla Sıtkı Koçman University, under permit number 30.05.2022-22/22. Forty-five 14-month old male and 45 3-6 month-old male Balb/C mice, weighing between 20-35 g, were acquired from the MUDEM-HADYEK Experimental Animals Application and Research Center. The mice were maintained in a 12/12-hour light/dark environment at a temperature of 22±2 °C, with ad libitum access to food and water.

The Preparation of Royal Belly

The royal jelly used in the study was produced at the Mugla Sıtkı Koçman University, Faculty of Milas Veterinary Medicine apiary. In the apiary, the queen bee from a 10-frame hive was transferred to another queenless beehive, rendering the original hive queenless. One to two-day-old larvae were grafted into royal jelly production cells and placed in the queenless hive. The royal jelly accumulated in the queen cups was harvested five days later and stored at -20 °C. Before use, the royal jelly was thawed at +4 °C one day in advance and topically administered at a concentration of 300 mg/kg.[28]

The Preparation of Melatonin Pomade

Melatonin (CAS 73-31-4) used in the study was obtained from Sigma, Germany. A cream containing melatonin at a concentration of 5 mg/kg in vaseline was prepared for application.[29]

The Preparation of Royal Jelly and Melatonin Combination

In the combined application, melatonin and royal jelly were applied at the same doses consecutively. Due to its water-based nature, the royal jelly was applied first, followed by the melatonin cream one hour later.

Wound Model and Measurements

Mice were anesthetized by administering 10 mg/kg Xylazine hydrochloride (Rompun, Bayer 23.32 mg/mL, Germany) followed by 70 mg/kg Ketamine hydrochloride (Ketalar, Parke-Davis, 50 mg/mL, USA) injection intramuscularly to create a skin wound model. The dorsal area was shaved along a 3 cm line surrounding the interscapular region. After disinfecting the area with an antiseptic solution, a full-thickness skin wound model was created using a 5 mm punch trephine. Following the operation, the wound site was measured in terms of length and width in millimeters until the day of sacrifice and documented by taking photographs (Figures 1 and 2). The photographs were transferred to the "Image J" program in a computer to calculate the wound surface areas.

Experimental Design

Ten groups were formed, each comprising nine animals, using a total of 90 Balb/C mice. The groups and their respective treatments were as follows:

1. Group GC (Geriatric Control): No medication or treatment was applied to the geriatric mice (14 months old) from the time of wound formation.
2. Group GCV (Geriatric Control - Vaseline): In this group of geriatric mice (14 months old), Vaseline was topically applied (0.1 ml) twice daily for 15 days following wound formation.
3. Group GRjM (Geriatric Royal Jelly + Melatonin): In this group of geriatric mice (14 months old), a combination of royal jelly (0.05 ml) and melatonin (0.05 ml) was topically applied twice daily for 15 days following wound formation.
4. Group GRj (Geriatric Royal Jelly): In this group of geriatric mice (14 months old), royal jelly was topically applied (0.1 ml) twice daily for 15 days following wound formation.
5. Group GM (Geriatric Melatonin): In this group of geriatric mice (14 months old), melatonin pomade was topically applied (0.1 ml) twice daily for 15 days following wound formation.
6. Group YC (Young Control): No medication or treatment was applied to the young mice (3-6 months old) from the time of wound formation.
7. Group YCV (Young Control - Vaseline): In this group of young mice (3-6 months old), Vaseline was topically applied (0.1 ml) twice daily for 15 days following wound formation.
8. Group YRjM (Young Royal Jelly + Melatonin): In this group of young mice (3-6 months old), a combination of royal jelly (0.05 ml) and melatonin (0.05 ml) was topically applied twice daily for 15 days following wound formation.
9. Group YRj (Young Royal Jelly): In this group of young mice (3-6 months old), royal jelly was topically applied (0.1 ml) twice daily for 15 days following wound formation.
10. Group YM (Young Melatonin): In this group of young mice (3-6 months old), melatonin pomade (0.1 ml) was topically applied twice daily for 15 days following wound formation.

Histopathological Examinations

A wound tissue sample was taken from each euthanized mouse. For this purpose, the wound area and surrounding full-thickness skin, including the panniculus layer, were excised from the body. The skin samples were fixed in a 10% formaldehyde solution for histopathological examination. Following fixation, the tissues underwent a tissue processing procedure that included a series of alcohol and xylene treatment before being embedded in paraffin blocks. Sections with a thickness of 3 µm were cut from the paraffin blocks, transferred to slides, and stained with hematoxylin-eosin. Microscopic examination was then performed on these sections.

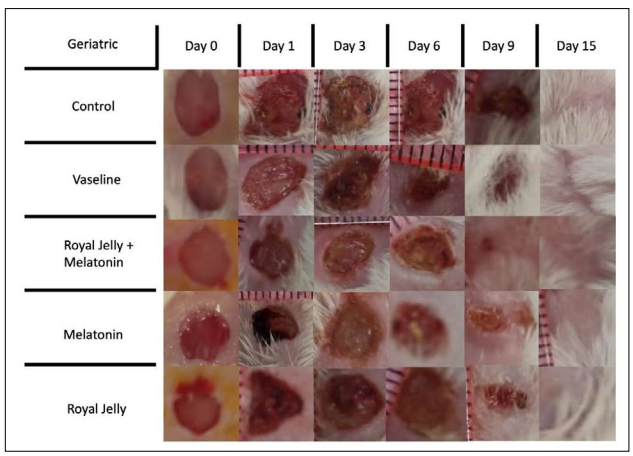

Figure 1. Wound healing by groups and days in geriatric mice.

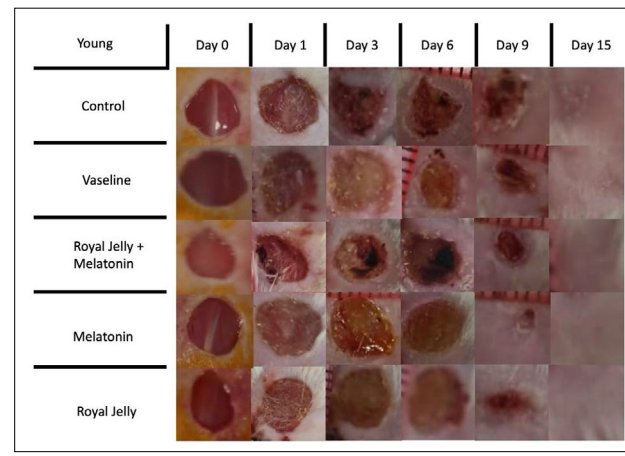

Figure 2. Wound healing by groups and days in young mice.

Statistical Analysis

The data was evaluated using the SPSS (Statistical Package for the Social Sciences) 26 statistical package program (Inc., Chicago, IL, USA). The normality of the variables was assessed using analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). The one-way Analysis of Variance (ANOVA) test was applied for group comparisons of normally distributed variables related to wound area, and the Kruskal-Wallis test was utilized for non-normally distributed variables. In instances of significant differences between groups for normally distributed variables, pairwise post-hoc comparisons were conducted using the Tukey test. The Independent T-test (Student T-test) was employed to compare geriatric and young animals in terms of wound area for normally distributed variables, while the Mann-Whitney U test was used for non-normally distributed variables. The Kruskal-Wallis test was used for histopathological examinations. For non-normally distributed variables, pairwise comparisons were conducted using the Mann-Whitney U test and evaluated with Bonferroni correction. Cases where the p-value was less than 0.05 were considered statistically significant.

RESULTS

Macroscopic Results

The results obtained using the Image J program to measure daily photographs from the onset of the wound until the day of euthanasia are presented in Tables 1 and 2.

In all trial groups, complete recovery was observed by the ninth day in both geriatric and young mice. Recovery rates in young groups were found to be higher than in geriatric animals (Figures 3 and 4). Within each age category, a significant difference was noted between the groups on the first and ninth days ($p<0.0001$). A statistical difference was observed between the Rj, M, and RjM groups and the control and vaseline groups on all days ($p<0.05$), indicating that the Rj, M, and RjM treatments significantly enhanced wound healing ($p<0.05$). The effectiveness of these treatments was statistically evident from the first day. Overall, the M group was identified as the most effective in promoting wound healing. No synergistic or antagonistic effects were detected, as the differences in the RjM combination did not appear significant ($p>0.005$). A similar process was observed between days 2 and 6 in both groups, and wound healing occurred rapidly. In both geriatric and young mice, the RjM and M groups demonstrated more than a 50% improvement within the first four days.

Geriatric Groups

In the analysis of geriatric groups, the RjM, Rj, and M groups showed the most improvement without significant differences among them from the first day to the seventh to ninth days ($p<0.0001$). From the second to the sixth days, the Rj, M, and RjM groups significantly enhanced the rate of wound healing and reduced the wound area compared to control groups ($p<0.0001$). Statistically, the M and RjM groups were found to be more effective than the Rj group during this period ($p<0.0001$). From the seventh to the ninth day, although there was a statistical difference between the Rj, M, and RjM groups and the control groups ($p<0.0001$), no significant differences were observed among these groups ($p>0.005$) (Table 1).

Young Groups

For young mice, statistically significant improvements in wound healing speed and the percentage of wound area re-

Table 1. Wound areas in geriatric mice (mm^2)

Days	Control		Vaseline		Royal Jelly + Melatonin		Royal Jelly		Melatonin		
	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	p-value
Day 0	9	30.29	0.06	30.24	0.16	30.27	0.09	30.49	0.09	30.55	0.05	0.093
Day 1	9	31.32^{a}	0.62	29.26^{b}	0.27	25.48^{c}	0.66	26.87^{c}	0.49	25.60^{c}	0.20	<0.0001
Day 2	9	30.49^{a}	0.55	28.45^{b}	0.60	20.31^{d}	0.38	25.52^{c}	0.49	21.10^{d}	0.29	<0.0001
Day 3	9	28.65^{a}	0.49	26.51^{b}	0.50	16.87^{d}	0.27	22.10^{c}	0.42	18.08^{d}	0.26	<0.0001
Day 4	9	26.58^{a}	0.41	23.54^{b}	0.50	14.28^{d}	0.21	17.14^{c}	0.42	14.64^{d}	0.26	<0.0001
Day 5	9	23.86^{a}	0.36	20.64^{b}	0.56	10.77^{d}	0.42	13.84^{c}	0.26	10.85^{d}	0.24	<0.0001
Day 6	9	19.96^{a}	0.59	16.93^{b}	0.42	8.54^{d}	0.49	10.41^{c}	0.20	8.51^{d}	0.26	<0.0001
Day 7	9	16.87^{a}	0.63	12.00^{b}	0.39	6.18^{c}	0.24	7.34^{c}	0.25	6.03^{c}	0.18	<0.0001
Day 8	9	13.45^{a}	0.56	7.95^{b}	0.34	3.66^{c}	0.46	4.04^{c}	0.19	3.54^{c}	0.15	<0.0001
Day 9	9	10.59^{a}	0.42	4.71^{b}	0.31	1.09^{c}	0.36	1.88^{c}	0.26	1.07^{c}	0.29	<0.0001
Day 15	9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	–

$^{a-d}$: Different letters in the same row indicate statistically significant differences ($p<0.05$).

Table 2. Wound areas in young mice (mm^2)

Days	Control		Vaseline		Royal Jelly + Melatonin		Royal Jelly		Melatonin		
	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	p-value
Day 0	9	30.54	0.09	30.55	0.08	30.38	0.07	30.48	0.06	30.41	0.07	0.421
Day 1	9	29.96^{a}	0.18	28.78^{b}	0.23	25.14^{cd}	0.18	26.03^{c}	0.25	24.86^{d}	0.29	<0.0001
Day 2	9	29.21^{a}	0.19	27.62^{b}	0.28	20.21^{d}	0.26	22.00^{c}	0.28	20.52^{d}	0.18	<0.0001
Day 3	9	27.98^{a}	0.20	25.84^{b}	0.41	16.72^{d}	0.26	18.85^{c}	0.35	17.32^{d}	0.11	<0.0001
Day 4	9	25.95^{a}	0.23	22.75^{b}	0.35	14.05^{d}	0.31	16.44^{c}	0.22	13.38^{d}	0.19	<0.0001
Day 5	9	22.32^{a}	0.23	19.49^{b}	0.45	10.14^{d}	0.29	13.15^{c}	0.21	9.79^{d}	0.13	<0.0001
Day 6	9	17.98^{a}	0.24	16.50^{b}	0.35	7.74^{d}	0.33	9.94^{c}	0.18	6.72^{d}	0.15	<0.0001
Day 7	9	13.21^{a}	0.38	13.21^{a}	0.26	6.18^{c}	0.25	7.57^{b}	0.22	3.53^{d}	0.13	<0.0001
Day 8	9	9.00^{a}	0.37	9.24^{a}	0.29	3.47^{b}	0.11	3.68^{b}	0.16	2.17^{c}	0.20	<0.0001
Day 9	9	6.16^{a}	0.37	4.82^{b}	0.30	0.54^{d}	0.21	1.79c	0.31	0.45^{d}	0.23	<0.0001
Day 15	9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-

$^{a-d}$: Different letters in the same row indicate statistically significant differences ($p<0.05$).

duction were observed in the trial groups compared to the control groups on all days ($p<0.0001$). From the first to the sixth days, the RjM and M groups demonstrated the most healing, with no significant differences between them. On the seventh day, a statistical difference emerged among the Rj, M, and RjM groups, with the M group showing the most healing and the Rj group the least. By the eighth day, the M group was statistically better than both the RjM and Rj groups. On the ninth day, the RjM and M groups again showed the most healing without significant differences between them ($p<0.0001$) (Table 2).

Histopathological Results

In the histopathological examination, the groups were semi-quantitatively evaluated for epithelialization, mononuclear cell infiltration, and granulation tissue formation, rated as follows: 0: Absent; 1: Mild; 2: Moderate; 3: Severe. This evaluation assessed the presence or severity of these histological features in the wound tissues across different groups (Fig. 5).

Geriatric Groups

Upon examining all groups, significant differences were observed between the Rj, M, and RjM groups and the control groups, with the former contributing to wound healing at histopathological levels ($p<0.005$). In terms of epithelization, the RjM and M groups were identified as the most contributive to healing, with no significant differences between them ($p>0.05$). These groups also exhibited less cellular infiltration. During this process, the Rj group did not show significant differences from the other test groups ($p>0.05$). Regarding granulation tissue formation, it was noted that Rj was more prevalent than in the other groups (Table 3).

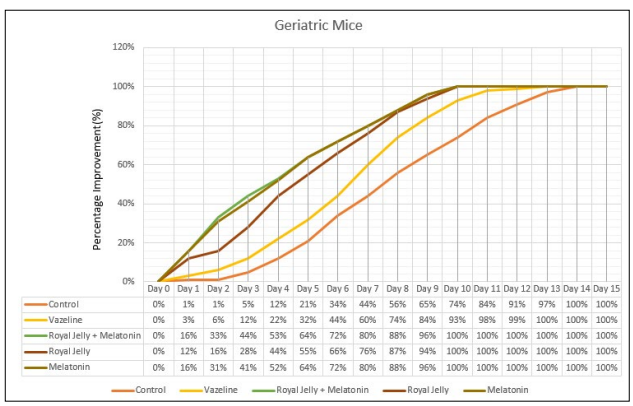

Figure 3. Percentage of wound healing improvement over days in geriatric mice.

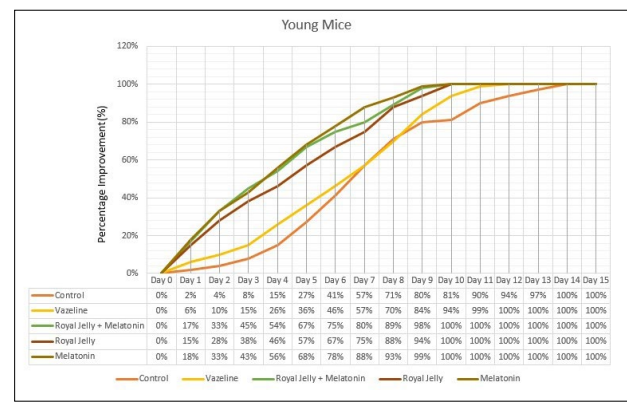

Figure 4. Percentage of wound healing improvement over days in young mice.

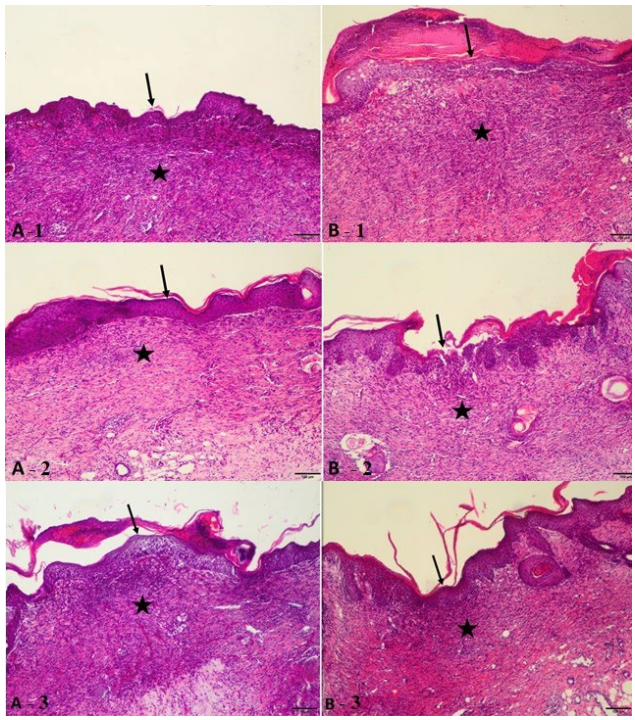

Figure 5. A-1: YRj; B-1: GRj; Arrow: More pronounced epithelization in YRj; Star: Inflammatory cell infiltration and granulation tissue formation. A-2: YM; B-2: GM; Arrow: Epithelization more pronounced in YM; Star: Decreased inflammatory cell infiltration and significant granulation tissue formation in both groups. A-3: YRjM; B-3: GRjM; Arrow: Significant epithelization in both groups; Star: Inflammatory cell infiltration and granulation tissue formation observed in both groups, with more severe granulation tissue formation in YRjM.

Young Groups

At the histopathological level in young animals, no significant differences were observed in terms of epithelization, inflammatory cell infiltration, and granulation tissue formation ($p>0.05$). Considering only epithelization, the RjM and M groups showed a tendency to contribute to healing, similar to their effect in geriatric animals, compared to other groups (Table 4).

DISCUSSION

Previous studies have documented the effects of royal jelly and melatonin on wound healing through various animal experiments.[30-32] However, the impact of these substances and their combination on wound healing in geriatric and young animals has not been previously studied. This study determined that royal jelly, melatonin, and their combination significantly contributed to wound healing at the macroscopic level in both geriatric and young animals. However, at the histopathological level, significant findings were noted in geriatric animals regarding epithelization, inflammatory cell infiltration, and granulation tissue formation, but no notable results were found in young animals except for epithelization. This situation indicates that the histopathological methods used in this study are insufficient to fully explain the cause of healing observed at the macroscopic level. Therefore, employing immunohistochemical, biochemical, and molecular methods is important to elucidate the underlying mechanisms of wound healing. In one study, molecular methods confirmed that royal jelly accelerates wound healing by regulating the inflammatory phase. Our current study shows that during the inflammatory process, wound healing did not differ from the control group, but granulation tissue formation was more effective.[33,34] Similarly, another study conducted like the current one showed that royal jelly contributed to the formation of granulation tissue and accelerated wound healing by influencing the proliferation phase. It was found that royal jelly increased inflammatory cell migration to the wound tissue and enhanced the proliferation phase.[34,35] In contrast, our study determined that royal jelly did not aid in the migration of inflammatory cells but was effective during the granulation tissue stage of the proliferation phase. This effect is akin to findings from another study, which demonstrated that oral use of royal jelly stimulated platelet-derived growth factor, thus contributing to wound healing. It is possible that wound healing in our study was facilitated by a similar mechanism, albeit locally.[34,36]

Table 3. Histopathological evaluation in geriatric mice

Factors		Epithelization		Inflammatory Cell Infiltration		Granulation Tissue Formation	
	n	Mean	SE	Mean	SE	Mean	SE
Control	9	0.44^{b}	0.18	2.56^{a}	0.18	0.44^{c}	0.18
Vaseline	9	0.67^{b}	0.17	2.56^{a}	0.18	0.89^{bc}	0.26
Royal Jelly + Melatonin	9	2.00^{a}	0.33	1.11^{b}	0.26	1.56^{ab}	0.34
Royal Jelly	9	1.22^{ab}	0.28	1.56^{ab}	0.29	2.11^{a}	0.35
Melatonin	9	2.33^{a}	0.24	0.89^{b}	0.26	1.78^{ab}	0.32
p-value		<0.0001	<0.0001	0.004			

$^{a-c}$: Different letters in the same column indicate statistically significant differences ($p<0.05$).

Table 4. Histopathological evaluation in geriatric mice

Factors		Epithelization		Inflammatory Cell Infiltration		Granulation Tissue Formation	
	n	Mean	SE	Mean	SE	Mean	SE
Control	9	1.00	0.29	2.00	0.29	1.33	0.37
Vaseline	9	1.33	0.37	1.78	0.32	1.56	0.34
Royal Jelly + Melatonin	9	2.11	0.26	1.78	0.32	2.22	0.43
Royal Jelly	9	1.33	0.37	2.00	0.24	2.11	0.26
Melatonin	9	2.11	0.35	1.33	0.24	1.67	0.24
p-value		0.080	0.360	0.253			

[a-c]: Different letters in the same column indicate statistically significant differences ($p<0.05$).

In a study on the effects of royal jelly on wound healing, it was observed that royal jelly sourced from the chestnut region was particularly effective. The study reported that it enhanced cutaneous wound healing by increasing the growth and mobility of keratinocytes, modulating the expression of aquaporin 3 (AQP3), regulating pathways involving mitogen-activated protein kinase and calcium, and mediating inflammatory responses.[37] The royal jelly used in our study was harvested in April, during the presence of spring flowers. It is thought that the reasons for the wound healing, which cannot be fully explained by histopathological results, may be attributed to these mechanisms.

Recent studies have established that melatonin accelerates wound healing.[38,39] Its effects on wound healing are provided through various mechanisms.[16] Since aging has been shown to significantly reduce melatonin plasma levels, researchers hypothesize a correlation between melatonin levels and aging.[40] Considerable problems in wound healing occur due to the decreased metabolism, reduced melatonin levels, diabetes, liver insufficiency, and diminished protein synthesis in the body, particularly in old age or with various chronic diseases. Similar challenges with delayed wound healing are observed in young individuals due to factors like prioritized tissue needs during rapid growth of the organism or an undeveloped immune system.[41] The study detected positive effects of melatonin and a melatonin-royal jelly combination on wound healing at a macroscopic level in both young and old groups. As the observed positive effect in groups treated only with royal jelly was not statistically significant, it is believed that melatonin contributed to the healing. At the histopathological level, although similar findings were obtained in geriatric animals, the results did not reach statistical significance among the groups in young animals. It was determined that different histopathological methods are necessary to identify the source of this effect and further elucidate the mechanism involved.

Pugazhenthi et al. (2008) reported that melatonin contributes to wound healing by increasing nitric oxide release during granulation tissue formation.[39] In the current study, while melatonin contributes to wound healing in the inflammatory phase, no difference was observed in terms of granulation tissue formation in both geriatric and young animals compared to the control group. It is assumed that royal jelly significantly influences the formation of granulation tissue. One potential reason for this effect could be the dilation of regional vessels and enhanced nourishment of the tissue, facilitated by the release of nitric oxide during this process. Similarly, Soybir et al. (2003) noted that melatonin enhances vascularization and epithelization in their studies.[42]

In the study conducted by Zarei et al. (2023), it was indicated that the combination of royal jelly and melittin, a component of bee venom, exhibited a positive synergistic effect in wound healing.[43] Although the combination of royal jelly and melatonin contributed to wound healing at the macroscopic level, it did not exhibit a significant synergistic effect at the histopathological level in young animals. Additionally, the application of melatonin was found to significantly impact both epithelialization and inflammatory cell migration. Evaluating these results, it can be concluded that each treatment has its strengths during specific phases. It is suggested that applications be tailored to these phases, using melatonin during the initial inflammatory cell migration and epithelialization phase, and royal jelly during the granulation process, which may yield better results.

The presence of similarities between aging processes in humans and mice suggests that the findings of this study may also be applicable to human wound healing. Both species exhibit signs of cellular aging such as DNA damage, mitochondrial dysfunction, and oxidative stress [44]. With aging, declines in immune function and metabolic rate, as well as decreased insulin sensitivity, are observed in both humans and mice.[45] Thus, it is important to emphasize that the use of mice as a model organism for humans has a molecular basis in this study. Despite these similarities, there are differences between humans and mice, such as lifespan, biological characteristics, predisposition to different diseases, and exposure to environmental factors.[46]

CONCLUSION

Wound healing is a highly complex process influenced by various factors, necessitating multiple assessment criteria. The conducted study determined the effectiveness of royal jelly, melatonin, and their combinations at the macroscopic level in both geriatric and young rats in terms of healing rate and reduction in wound area. However, melatonin and the combination of melatonin with royal jelly were found to be relatively more effective in terms of healing rate and reduction in wound area. Melatonin was found to be more effective during the initial wound formation process, while the application of royal jelly yielded better results during the granulation process. At the histopathological level, the effectiveness of royal jelly, melatonin, and their combinations was statistically established in geriatric animals, particularly affecting healing in terms of epithelialization. In young animals, the effects of royal jelly, melatonin, and their combinations were relatively positive, with the combination of melatonin and royal jelly specifically influencing epithelialization. By studying the effects of these substances on wound healing in geriatric animals and comparing them to young animals, researchers may uncover novel therapeutic approaches to enhance wound healing and promote better outcomes in the elderly population. However, it is important to emphasize that further research and clinical studies are necessary to draw definitive conclusions and translate these findings into clinical practice for both human and animal use.

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DENEYSEL ÇALIŞMA - *ÖZ*

Geriatrik ve genç farelerde deneysel olarak oluşturulmuş yaralarda arı sütü ve melatonin kombinasyonlarının etkinliğinin karşılaştırılması

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AMAÇ: Yara iyileşmesi, travma sonucunda deri ve diğer yumuşak dokuların onarılma sürecini ifade etmektedir. Bir arı ürünü olan arı sütü, antioksidan, anti-inflamatuar, antibakteriyel ve antiviral özelliklere sahiptir. Pineal bezde ve diğer organlarda üretilen melatonin ise bir sirkadiyen indol amindir. Bu çalışma yaşlı ve genç farelerde yara iyileşmesi üzerin melatonin ve arı sütünün tek başlarına ve kombine bir şekilde kullanılmasının etkilerinin araştırılmasını hedeflemektedir.

GEREÇ VE YÖNTEM: Çalışmada arı sütü ve melatoninin yara iyileşmesi üzerindeki etkilerinin değerlendirilmesi için, 10 gruba ayrılmış toplam 90 Balb/C fare kullanılmıştır. Arı sütü topikal olarak 300 mg/kg konsantrasyonunda, melatonin ise 5 mg/kg dozunda vazelin pomad ile karıştırılarak uygulanmıştır. Arı sütü ve melatoninin yara üzerindeki etkilerinin değerlendirilmesi için bu maddeler ayrı ayrı ve kombinasyon halinde yara bölgesinde kullanılmıştır.

BULGULAR: Her iki uygulamada her iki yaş grubunda makroskobik seviyede yara iyileşmesinde istatistiksel açıdan önem arz edecek düzeyde katkıda bulunmuştur. Melatonin yara oluşumunu takiben başlangıç dönemde daha etkili bulunurken, arı sütü granülasyon sürecinde daha etkili bulunmuştur. Ancak histopatolojik düzede önemli sonuçlar sadece geriatrik hayvanlarda gözlenmiştir.

SONUÇ: Çalışma bulguları özellikle yaşlı bireylerde yara iyileşmesine destek olmak için potansiyel yeni bir terapotik yaklaşım sunarken, daha fazla araştırma ve klinik çalışmalar ile bulguların desteklenmesi gerektiği sonucuna varılmıştır.

Anahtar sözcükler: Apiterapi; epitelizasyon; granülasyon; iyileşme.

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