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Synergistic effects of royal jelly and glycine on the healing of skin wounds in mice

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ABSTRACT

Aims: This study aims to assess how the synergistic effects of royal jelly (RJ) and glycine, which are easily accessible and economically viable substances, can enhance the restoration of skin affected by wounds. This study provides valuable insights into pioneering strategies for addressing the critical concern of effectively treating skin wounds.

Methods: The experimental design of this study involved the formulation of different concentrations of RJ and glycine. A total of 80 male NMARI mice were categorized into two groups of 10, each following specific oral and topical treatment protocols. Additionally, a 5-mm diameter wound was created on the back of the neck. These wounds were then treated orally and topically with varying doses of RJ and glycine, and their combination, over 9 days. Wound measurements were taken and recorded daily throughout the study period. On the 10th day, mice were anesthetized under ethical conditions, and skin tissue samples were collected for subsequent histological examinations and hydroxyproline measurements.

Results: The synergistic effects of combined oral treatment with RJ (50 mg/kg) and glycine (3 and 12 mg/kg) and simultaneous topical treatment of RJ (2.5%) and glycine (0.2 and 1%) caused a significant reduction in skin wound diameter ($p<0.001$) as well as an increase in new blood vessels, fibroblast accumulation, epithelial tissue formation, and collagen synthesis in histopathological sections compared with their single doses.

Conclusions: The results showed that RJ and glycine significantly increased collagen synthesis, epithelial formation, and hydroxyproline levels in wound tissue.

Introduction

The skin acts as a protective barrier for living organisms, covers the entire body surface, and has a high regenerative capacity. However, deep injuries, such as deep burns or extensive cuts, are associated with scar tissue and require quick and effective repair. Achieving healthier skin may require complementary treatments (1).

Wound healing therapies can generally be divided into two categories: traditional and modern, with varying degrees of efficacy, clinical acceptance, and side effects. Contemporary treatments for skin ulcers primarily involve chemical agents and invasive procedures, which can be expensive, time-consuming, and detrimental in the long term (2,3). Bee products are not only utilized in therapy and skincare as cosmetic ingredients. Royal jelly (RJ) serves as a traditional remedy for wound repair; nevertheless, the underlying mechanisms and ingredient profiles remain largely unknown. RJ is a yellow-white viscous substance with a sweet and sour taste and a faint phenol odor and is

secreted by the hypopharyngeal glands of young worker bees (4). RJ has many useful properties, including anti-inflammatory, antioxidant, anti-microbial, anti-tumor, and wound healing properties (5). In recent years, the use of RJ has increased because of its many benefits as a natural honeybee product and its great potential for use in medical and pharmaceutical products (6-8). In particular, in the field of healing skin wounds due to the antioxidant and anti-inflammatory properties of RJ, this substance can be an alternative treatment for many chemical compounds in the future (9). Glycine, the simplest amino acid characterized by its single carbon atom and side chain, accounts for approximately 11.5% of total amino acids and 20% of nitrogen content in body proteins (10). Moreover, it serves as a widely used analyte in clinical applications because of its prominence (11). Glycine functions include cell protection, anti-inflammatory responses, and body growth (12,13). Its involvement in the synthesis of glutathione, a natural antioxidant crucial for diminishing free radicals and thus mitigating risks of diseases and aging, further underscores its significance (14). Proline, glycine, and hydroxyproline are involved in 57% of all amino acids in collagen, and glycine is used as a cost-effective additive in animal diets more than other amino acids (15). Glycine is one of the amino acids found in collagen, and the abundance of skin collagen can be a sensitive indicator of the importance of glycine in various metabolic processes (16). The collagen index can be measured by measuring hydroxyproline because in creature tissues hydroxyproline and hydroxylysine are found as in collagen (17). Hydroxyproline-containing di- and tripeptides in human blood plasma increment in a dose-dependent manner after the expending hydrolyzed collagen (18). Prolyl hydroxyproline and hydroxypropyl glycine are the major food-derived collagen peptides found in human blood plasma (19).

The objective of this study was to investigate the potential of readily available substances such as glycine and RJ in enhancing collagen production in skin injuries, a topic explored comprehensively within this research.

Methods

Study design

The experimental design involved the preparation of different material concentrations for oral and topical treatments, the formation of experimental groups of mice, wound induction, histological and biochemical studies, and subsequent statistical analysis.

Animals

Eighty male NMARI mice (20-30 g) were obtained from Pasteur Institute (Iran). Forty mice were divided into 10 groups for oral and topical treatments (Table 1).

Procedures

RJ was obtained (Roodin Company, Iran) and concentrations of 50, 100, and 200 mg/kg of body weight were prepared for oral treatment with gavage (20) and concentrations of 2.5%, 5%, and 10% were arranged by physiological serum for topical treatment (21).

Glycine was obtained (Pajuhesh Chemistry Company, Iran) in concentrations (3, 12, and 50 mg/kg) for oral treatment with gavage, and concentrations of 0.2%, 1%, and 2% were arranged for topical treatment. The combined concentrations for oral treatment (RJ 50 mg/kg + glycine 3 mg/kg and RJ 50 mg/kg + glycine 12 mg/kg) and the combined concentration for topical treatment (RJ 2.5% + glycine 0.2% and RJ 2.5% + glycine 1%) were arranged.

Table 1. Experimental groups

Experimental groups for oral treatment			Experimental groups for topical treatment		
Group number	**Group name**	**Treatment**	**Group number**	**Group name**	**Treatment**
1	Intact group	No wound, no treatment	1	Intact group	No wound, no treatment
2	Control group	Wounded, no treatment	2	Control group	Wounded and received physiological serum
3	Experimental group	RJ 50 mg/kg	3	Experimental group	RJ 2.5%
4	Experimental group	RJ 100 mg/kg	4	Experimental group	RJ 5%
5	Experimental group	RJ 200 mg/kg	5	Experimental group	RJ 10%
6	Experimental group	Glycine 3 mg/kg	6	Experimental group	Glycine 0.2%
7	Experimental group	Glycine 12 mg/kg	7	Experimental group	Glycine 1%
8	Experimental group	Glycine 50 mg/kg	8	Experimental group	Glycine 2%
9	Experimental group	RJ 50 mg/kg + glycine 3 mg/kg	9	Experimental group	RJ 2.5% + glycine 0.2%
10	Experimental group	RJ 50 mg/kg + glycine 12 mg/kg	10	Experimental group	RJ 2.5% + glycine 1%

Note: Each group consisted of 4 mice for both oral and topical treatments. RJ: Royal jelly

5-mm diameter wound on the back of the neck of mice using a skin punch (14). The size of the wound was measured and recorded daily for 9 days. Approval was obtained from the Ethical Committee on the Use and Care of Laboratory Animals of Islamic Azad University of Varamin Pishva Branch, Iran.

Outcomes

The thin tissue pieces, measuring 5 m thick (22), were sliced with a microtome and stained with hematoxylin and eosin. The changes considered in tissue samples included the arrangement of epithelium, collagen synthesis, formation of new blood vessels and presence of fibroblasts. All the above items were scored (23) and measured using ImageJ software (INH, USA) and compared with the control group.

Tissue samples were analyzed using a hydroxyproline measurement kit (Kiazist Company, Iran). The sample was mixed in the presence of strong acid, and after oxidation, it reacted with chromogen, and its absorbance was measured at a wavelength of 540-560 nm.

Statistical Analysis

The Statistical Package for the Social Sciences Software (version 21, IBM Crop., Armonk, NY, USA) was used in the data analyses. The data are expressed as mean±standard error of the mean (SEM). Before conducting parametric tests, the normality of the data was evaluated using the Shapiro-Wilk test. Variances among the groups were examined using the Student's t-test and one-way analysis of variance (ANOVA), followed by Tukey's test. P<0.05 was considered statistically significant.

Results

Effects of oral and topical treatments on skin wound diameter

Measurements of the progression of skin wounds and the effects of oral and topical treatments were performed over 9 days. Wound size significantly decreased compared with the control group in the group of animals receiving oral glycine at 50 mg/kg on day 7 and RJ at a concentration of 200 mg/kg on day 8 ($p<0.05$). The combination of oral RJ (50 mg/kg) and glycine (3 and 12 mg/kg) resulted in a significant reduction in skin wound diameter compared with isolated doses of each substance ($p<0.001$). Topical wound treatment using 10% RJ solution or glycine solution (1 and 2%) also significantly decreased skin wound diameter on day 7 compared with the control group ($p<0.05$). Additionally, combining RJ (2.5%) with glycine (0.2 and 1%) led to a substantial reduction in skin wound diameter on day 7 compared with the use of each substance alone ($p<0.001$) (Figure 1).

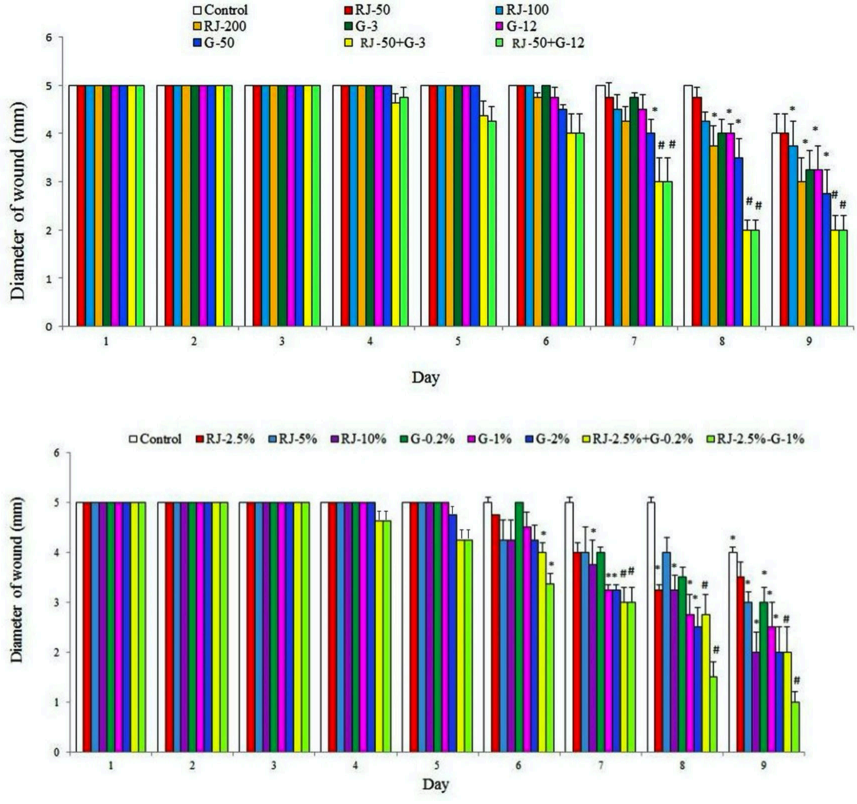

Figure 1. The effects of oral RJ and glycine (marked with G) on skin wound diameter in small laboratory mice for 9 days (top chart). The effects of topical RJ solutions and glycine on skin wound diameter in mice small laboratory for 9 days (down chart). *p<0.05 vs control group. #p<0.001 synergistic treatment vs single dose of RJ and glycine

RJ: Royal jelly

Results of the histological studies

Histological studies revealed that compared with the control group, oral RJ or glycine significantly increased epithelial tissue repair, collagen synthesis, fibroblast accumulation, and new blood vessel formation in a dose-dependent manner for 9 days ($p<0.001$). The combination of these substances also resulted in a significant increase in epithelial tissue repair compared with the individual doses of each ($p<0.01$) (Figure 2). Similarly, topical treatment with RJ or glycine solution in a dose-dependent manner significantly increased epithelial tissue repair, collagen synthesis, fibroblast accumulation, and new blood vessel formation compared with the control group ($p<0.001$). Combining RJ and glycine caused a notable increase in epithelial tissue repair compared with individual doses of each ($p<0.01$) (Figure 3).

Measurement results for hydroxyproline

After a 9-day treatment period, oral administration of RJ or glycine significantly increased hydroxyproline levels in the injured tissue in a dose-dependent manner compared with the control group ($p<0.001$). Moreover, the oral combination of these substances resulted in a significant increase in hydroxyproline concentration compared with individual doses of each ($p<0.05$) (Figure 4a). Similarly, topical treatment with RJ (5 and 10%) or glycine solution (1 and 2%) led to a significant increase in hydroxyproline levels in the wounded tissue compared with the control group ($p<0.001$). Combining RJ and glycine led to a distinctly higher concentration of hydroxyproline than when either substance was individually applied in topical treatment ($p<0.05$) (Figure 4b).

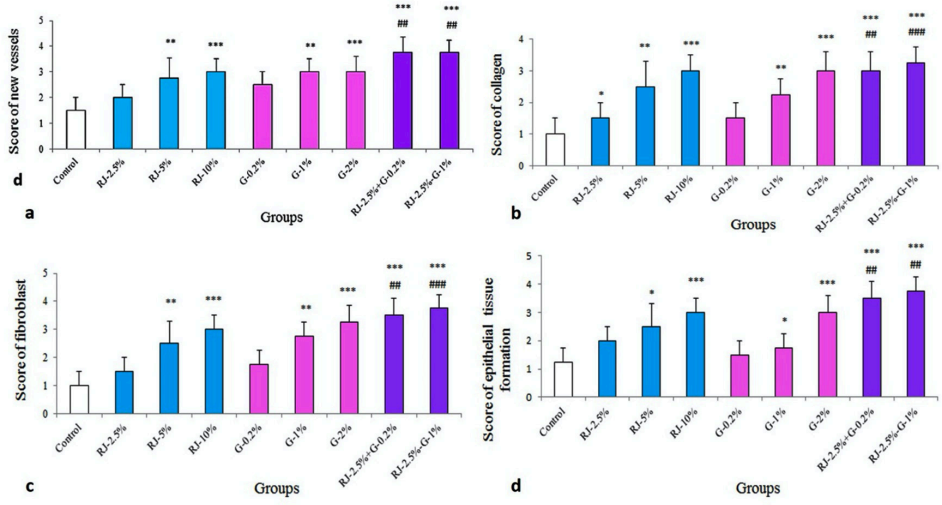

Figure 2. The effects of oral treatment on the number of new blood vessels (a), collagen synthesis (b), presence of fibroblasts (c), and formation of epithelial tissue (d). *p<0.05, ***p<0.001 vs control group. ##p<0.01 synergistic treatment vs single dose of RJ and glycine
RJ: Royal jelly

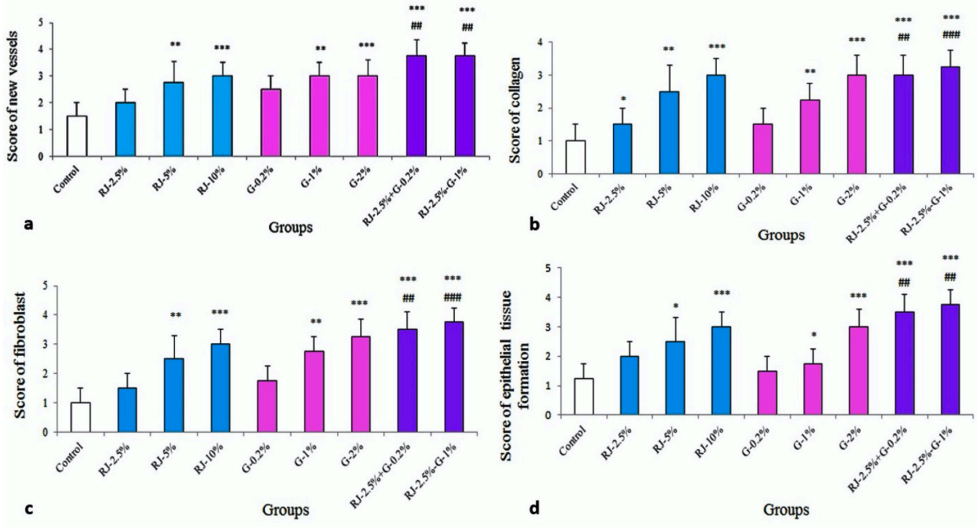

Figure 3. The effects of topical wound treatment on the number of new blood vessels (a), collagen synthesis (b), presence of fibroblasts (c), and formation of epithelial tissue (d). *p<0.05, ***p<0.001 vs control group. ##p<0.01 synergistic treatment vs single dose of RJ and glycine
RJ: Royal jelly

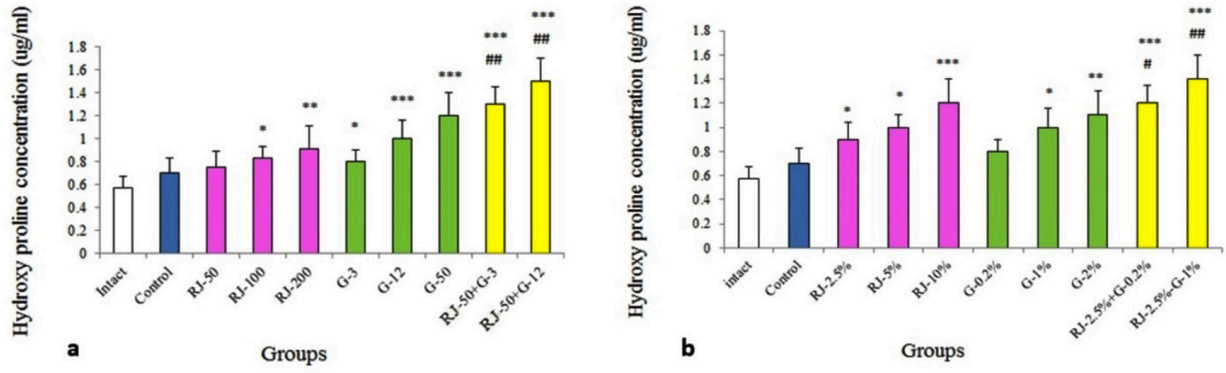

Figure 4. The effects of oral treatments on the amount of hydroxyproline (a). The effects of topical treatments on the amount of hydroxyproline (b), *p<0.05, ***p<0.001 vs control group. #p<0.05, ##p<0.01 synergistic treatment vs single dose of RJ and glycine
RJ: Royal jelly

Discussion

The present study demonstrated that administering higher doses of oral and topical treatments with RJ or glycine yielded more favorable effects than lower doses. Particularly, RJ at a concentration of 200 mg/kg and glycine at a concentration of 50 mg/kg led to significant reductions in the diameter of the skin wound on the 8th and 6th days, relative to the control group in oral treatment. In addition, the topical treatment of RJ with a concentration of 10% and glycine solution of 2% caused a significant decrease in the diameter of the skin wound on the 7th and 6th days, respectively, compared with the control group. Furthermore, oral administration of RJ and glycine yielded greater improvements when compared with topical treatment. This disparity was evident in the daily photographic documentation, where the wounds exhibited a smaller and more closed diameter following the oral regimen of RJ and glycine compared with their topical application. The results obtained from histological examinations and hydroxyproline measurements emphasize the enhanced effectiveness of the synergistic interaction between RJ and glycine in wound healing compared with their isolated applications. Similar results from other studies showed the validity of our results; for example, in past studies, it has been proven that RJ induces the proliferation of fibroblasts that produce collagen, thereby healing the wound (24). RJ is also effective in healing the skin through other mechanisms; RJ promotes wound healing by increasing the activity of keratinocytes (25), increasing nitric acid (26), modulating inflammation (27), increasing transforming growth factor-β secretion and decreasing tumor necrosis factor-alpha (28). RJ increases the migration of human skin fibroblasts and prevents skin aging in mice in an in vivo model by determining the expression levels of procollagen type I protein and matrix metalloproteinase (29).

In this study, glycine increased the number of skin repair cells both orally and topically, and similar to the results of scientists who had already discovered the various functions of glycine (30,31), this study showed the repair properties of this amino acid. The main function of glycine is the synthesis of proteins. Most proteins contain small amounts of glycine; an exception in this regard is collagen, which contains approximately 33% glycine due to the formation of a spiral structure (32). Glycine and proline can increase collagen synthesis in pig, chicken, and fish skin (12). Overall, our study proved that using RJ and glycine to treat wounds helps in effective skin healing. In addition, when RJ and glycine are used in combination, they show more effectiveness in wound healing and collagen synthesis than either treatment alone.

Study Limitations

The study was conducted on male NMARI mice, which may limit the generalizability of the findings to other species. The relatively short study duration (9 days) may not have captured the long-term effects of the combined treatment of RJ and glycine on wound healing. The study was conducted on mice, and the direct applicability of the findings to human subjects remains to be established.

Conclusion

This study showed the effectiveness of RJ and glycine alone and in combination on skin wound healing in mice. The observed synergistic effect of RJ and glycine in wound healing offers a promising direction for potential applications in both traditional and modern skin treatments. Further studies and testing in human populations are necessary to explore their clinical utility in disease conditions.

Ethics

Ethics Committee Approval: Approval was obtained from the Ethical Committee on the Use and Care of Laboratory Animals of Islamic Azad University of Varamin Pishva Branch, Iran.

Informed Consent: Animal experiment.

Authorship Contributions

Surgical and Medical Practices: Z.P., S.Z., N.P., Concept: Z.P., M.E., Design: M.E., S.Z., Data Collection or Processing: Z.P., M.E., N.P., Analysis or Interpretation: Z.P., Y.E., N.P., Literature Search: Y.E., S.Z., Writing: Y.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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