Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Effects of royal jelly on sterile skin cut repair

Maryam Shirzad[1], Mahboubeh Yousofi[2], Behnam Zamanzad[1], Akram Sedaghat[2], Masih Hosseini[1], Najmeh Shahinfard[1], Hedayatollah Shirzad[2*]

[1]Medical Plant Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 25 July 2014
Accepted: 7 October 2014
ePublished: 1 December 2014

Keywords:
Royal jelly
Skin cut repair
Nitrofurazon
Balb/C mice

ABSTRACT

Introduction: Following injury, inflammatory response occurs and the cells below the dermis begin to increase collagen production, then, the epithelial tissue is regenerated. Royal jelly (RJ) has anti-inflammatory activity hence, the aim of this study was to examine the effect of RJ on the induction of sterile skin incision in Balb/C mice.

Methods: In an experimental study 60 female Balb/C mice (8 weeks old) were anaesthetized with ether and a longitudinal para vertebral full thickness incision of 10 mm long was made. The animals were divided into six equal groups. Group 1 was considered as negative control. Group 2 (positive control) was treated topically with Nitrofurazon ointment, group 3 with RJ (200 mg/kg) every day, group 4 with RJ (200 mg/kg) every two days, group 5 with RJ (300 mg/kg) every day and group 6 with RJ (300 mg/kg) every two days. The wound length was measured with vernier capilar every two days up to full healing occurred and compared in different groups.

Results: There was significant difference between groups 1 or 2 and other groups ($p<0.05$). RJ promoted wound healing activity significantly in group 3, 5 compared to negative and positive control groups. There was no significant difference between the uses of 200 mg/kg and 300 mg/kg RJ ($p>0.05$).

Conclusion: The results of this study indicate that daily application of RJ possesses betters wound healing effects than Nitrofurazon and every two days usage of RJ.

Implication for health policy/practice/research/medical education:
Royal jelley has beneficial effect on burn healing with better wound healing effects when is used daily compared to every two days usage.

Please cite this paper as: Shirzad M, Yousofi M, Zamanzad B, Sedaghat A, Hosseini M, Shahinfard N, *et al.* Effects of royal jelly on sterile skin cut repair. J HerbMed Pharmacol. 2014; 3(2): 97-100.

Introduction

Skin is the body's most extensive organ and is considered as a main physical barrier between a creature and its surrounding. Human's skin protects his body against numerous harmful agents (1).

The wounds of skin are categorized into two groups, acute and chronic. Surgical wounds could be healed through different ways (2). The primary healing occurs when the tissue is incised with no infection and clean, non-infectious edges of scalpel only destroy the center of basement membrane integrity and kill a small number of epithelial and connective tissue cells. In these wounds, cut edges are close to each other and healing is complication-free. The wounds with delayed healing, has a lower tensile strength initially, but the consistency and strength become similar to normally healed wounds' ultimately (1,2).

The stages of wound healing comprise of hemostasis and inflammation, proliferation, differentiation and reformation. Hemostasis occurs before initiation of wound inflammation. Macrophages cause regulation of cell proliferation, matrix formation, and angiogenesis through releasing of mediators such as transforming growth factor beta, vascular endothelial growth factor, insulin-like growth factor, epithelial growth factor, and lactate. In addition, macrophages contribute greatly to regulating angiogenesis as well as matrix deposition and

***Corresponding author**: Hedayatollah Shirzad,
Email: shirzadeh@yahoo.com

reformation (1).

Proliferation is the second step of wound healing and lasts about 12-14 days. Throughout this step, tissue integrity is restored and established. Fibroblasts and endothelial cells are the last cell population which is infiltrated into the site of healing wound. The growth factor derived from platelet is the strongest chemotactic growth factor that attracts fibroblasts into the wound site. In this stage, collagens and proteoglycans are formed. Collagen type I and III are the main collagens involved in the wound regeneration (1,3).

The final step of wound healing is scar formation which occurs in fibroblastic step of wound healing and is characterized by reorganization of previously formed collagens. Factors such as patient's age, hypoxia, anemia, steroids and chemotherapy, metabolic disorders, nutrition, and infections affects wound recovery (1,2).

Royal jelly is one of bee products, which is produced in worker bees' stomach from honey partial digestion. This substance is released by hypopharyngeal and mandibular glands to develop the queen bee (4,5). Royal jelly is a milky white gelatinous substance with a pungent smell, fruit taste, and high nutritional value (6). Royal jelly contains water 65-70% , protein 15-20%, carbohydrate 10-15%, fat 6-7.1%, rare elements such as sodium, potassium, iron, copper, magnesium, and manganese as well as vitamins including thiamine, riboflavin, pyridoxine, nicotinic acid, biotin, folic acid, inositol, pantothenic acid, ascorbic acid, and vitamins A and D (7).

Royal jelly has stimulatory effects on different body organs and could improve their function. In addition, some works have reported antibacterial and antitumor properties for royal jelly (6-8). Royal jelly has estrogenic properties and contributes to wound restoration, wrinkles improvement, and osteoporosis prevention (9,10). Royal jelly exerts anti-inflammatory effects through inhibiting production of pro-inflammatory cytokines by activated macrophages (11). Royal jelly properties have been demonstrated in treatment of oral mucocytes due to chemotherapy and decline of negative, harmful biochemical effects of sodium fluoride (1,12,13).

Thanks to therapeutic effects and low side effects, royal jelly seems to be an alternative drug for wound healing. Therefore, the purpose of this study was to investigate the effect of royal jelly on sterile skin cut repair in Balb/C mice.

Materials and Methods

In an experimental study 60 female Balb/C mice with 8-week old were selected and randomly assigned to 6 groups of 10 each. The animals were maintained in natural living conditions with water, food and light available.

The study protocol complied with international regulations for use of laboratory animals and was approved by Shahrekord University of Medical Sciences Ethics Committee.

The mice were anesthetized by ether. After shaving the hairs and disinfecting the skin with alcohol, a 10 mm long full skin-thickness incision was performed on the back of animals with a scalpel no. 20. First group (negative control), only saline was applied on the skin cut daily.

Royal jelly was mixed with saline and the concentration was adjusted to acquired doses in 100 µL of the solution. And test groups were treated as follows: Second group (positive control) treated with 2.0% nitrofurazone ointment. Third group received 200 mg/kg royal jelly daily, and forth group received 200 mg/kg royal jelly every other day. Fifth group 300 mg/kg royal jelly was applied daily, and sixth group, 300 mg/kg royal jelly was used every other day.

To assess wound recovery, all mice were physically examined every other day and parameters including the rate of wound shrinking, changes in wound length, and duration of recovery were used (14).

To measure the length of wounds, Vernier caliper with 0.01-mm precision was used. The wounds were examined for 17 days. On the day 17, all mice wounds recovered completely. The collected data were analyzed by SPSS using Kruskal-Wallis test.

Results

The wound length was measured each day. On the 3rd and 4th days of examination, there was a significant difference in wound length among different groups of study ($p<0.05$). But, no significant difference was seen on other days.

As illustrated in Figure 1, on days 3 and 4 of examination significant difference was observed between negative controls and the group receiving royal jelly daily ($p<0.05$). Therefore, daily application of royal jelly at any dose used in this study had a considerable effect on wound healing compared with negative control. In addition, there was a significant difference between mean score of positive controls and that of the groups receiving royal jelly every other day on the days 3 and 4 ($p<0.05$); this shows that daily application of royal jelly caused a better recovery of the wounds compared to nitrofurazone ointment.

The results also indicated that on the day 3 of examination there was a significant difference in mean score between the two group receiving royal jelly at 200 mg/kg daily and every other day ($p<0.05$). Daily use of same dose of royal jelly, had a better effect on wound recovery compared to every other-day. As illustrated in Figure, on the day 3, a significant difference was noted between the two group receiving royal jelly at 300 mg/kg daily and every other day ($p<0.05$).

In addition, on the days 3 and 4 the mean score of the group receiving royal jelly at 300 mg/kg daily was significantly different from that of the group receiving royal jelly at 200 mg/kg every other day. This could be due to frequent use of royal jelly at higher doses. However, no significant difference was seen between doses of 200 and 300 mg/k.

Discussion

In this study we investigated the effect of royal jelly

on sterile skin cut repair in Balb/c mice. Todays, more attention is being paid to therapeutic approaches to using natural and biological substances. Honey and other bee products including royal jelly, gum, and bee venom are used for stimulating not only immune system but also chronic wounds treatment (15,16). Royal jelly has been reported to have therapeutic in laboratory animals (4,15,17). The results of this study indicated a pronounced recovery of wounds and decreased duration of sterile skin cur repair in the mice treated with royal jelly. In addition, compared with nitrofurazone ointment which used as positive control for wounds healing in many researches, royal jelly had a better effect on wound healing. In this study, royal jelly was used at different doses. In addition to comparing different doses, applying royal jelly every day and every other day was also investigated. Results indicated further healing of wounds due to daily application of this substance. No significant difference was seen in days 1 and 2 of examination. The effect of royal jelly was seen on the days 3 and 4. This could represent better effect of frequent use of royal jelly on wound recovery acceleration. Suemaru et al investigated the effect of royal jelly topical application on oral mucocytes. The mucocytes were developed through five fluorouracils and mild rubbing of inside of the mice mouth and royal jelly, honey, and wax were applied on oral mucosa at three different concentrations in separate groups. Then, the process of recovery was assessed by measuring mucocyte size. This finding indicated that honey and wax did not decrease mucocyte size in comparison to Vaseline which used as control group, while royal jelly recovered mucocyte significantly in a dose-dependent manner. They concluded that topical application of royal jelly was likely to contribute to recovery of severe oral mucocytes induced by chemotherapy (17). In the present study, royal jelly had similar effect on wound restoration. The discrepancy between our study and Suemaru et al study is that no significant difference was noted among the doses in our study.

In another study the efficacy of Pedyphar ointment (a new ointment composed of natural royal and panthenol) was investigated in treatment of diabetic foot infections. 60 patients with threatening diabetic foot infections were assigned to three groups based on the severity of lesion; group 1: full-thickness skin ulcer; group 2: deep tissue infection and suspected osteomyelitis; and group 3: gangrenous lesions. All patients' wounds were washed and cleansed with saline and then underwent treatment with Pedyphar ointment. The wounds were assessed on weeks 3, 9, and 24 after the ointment was applied. 96% of the patients in groups 1 and 2 completely recovered at the end of week 9 and all patients in group 3 healed after surgical excision, debridement of necrotic tissue, and conservative treatment with Pedyphar ointment (18). The positive effect of royal jelly was obvious on sterile wound recovery. In Fuji et al study, the effect of royal jelly was investigated on hypoglycemia response and wound recovery acceleration in chronically diabetic mice that were under intravenous administration of streptozotocin. Oral administration of royal jelly at doses of 10, 100, and 1000 mg/kg body weight per day exhibited no insulin-like activity but shortened the duration of skin wounds recovery (5). The present study results are in consistent with Fuji et al's, and showed a decrease in duration of wounds recovery due to royal jelly application. The discrepancy was the method of royal jelly administration.

Taniguchia et al indicated that oral administration of royal jelly prevented the progression of dermatitis-like skin lesions in NC/Nga mice (19). Similarly to our results royal jelly administration prevented the progression of skin lesions.

In view of above findings, royal jelly as a natural product could be effective on wound restoration in mice, which indicates that this substance could be a good candidate for human wounds, as well. However, further human studies are needed to offer royal jelly as a main alternative to effective drugs used for wound restoration. Therefore, similar complementary works should be conducted on human to make use of royal jelly as an alternative possible.

Acknowledgements

This work was financially supported by Shahrekord University of Medical Sciences. Authors gratefully thank to staff of Medical Plants Research Center and Cellular and Molecular Research Center of Shahrekord University of Medical Sciences. Also many thanks go to Mrs. Drees and Mrs. A'zam Asgari for their collaborating in this work.

Authors' contributions

All the authors wrote the manuscript equally.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

None.

References

1. Cohen K, Robert F, Dorne D, Yagar R, Isaac L, Wornum III, *et al*. Crosslamd wound care and wound healing/Schwartz, Shires, SPENCER Daly. Fischer, galloway/ principle of surgery. 7th ed. New York: MC Graw-hill;2005. p. 263-6.
2. Thomas K, Hunt MD, Reid V, Mueller MD, William H. Goodson III. Wound healing. In: Lawrence W. Current surgical diagnosis & treatment. 11th ed.

USA:Prentic-Hall International Inc;2003. p. 80-5.
3. Okumura M, Okuda T, Nakamura T, Yajima M. Effect of basic fibroblast growth factor on wound healing in healing-impaired animal models. Arzneimittelforschung 1996; 47(2):222.
4. Jamnik P, Goranovic D, Raspor P. Antioxodative action of royal jelly in the yeast cell. University of Ljubljana, Biotechnical Faculty, Food Science and Technology department, Jamnikarjeva 101 Ljubljana Slovenia 2007; 42(7):594-600.
5. Fuji A, Kobayashi S, Kuboyama N. Augmantation of wound healing by royal jelly in streptozocin-diabetic rats. J Pharmacol 1990; 53(3): 331-7.
6. Jannuzzi J. Royal jelly mystery food. J American Bee 1990; 8: 532-4.
7. Orsolic SL, Tadic Z, Njari B, Valpotic I, Basic I. A royal jelly as a new potential immunomodulator in rats and mice comp. Immunol Microbial Infect Dis 1996; 19(1): 31-6.
8. Levy B, Deeken JF, Holt G, Morshal JL. Immunologic therapies for gastrointestinal cancrrs. Clin Colorectal Cancer 2005; 5(1):37-49.
9. Mishima S, Suzuki KM, Isohoma Y, Kuratsu N, Araki Y, Inoue M, *et al*. Royal jelly has estrogenic effects in vitro and in vivo. J Ethnopharmacol 2000; 101(1-3):215.
10. Hidaka S, Okamoto Y, Uchiyama S, Nakatsuma A, Hashimoto K, OHhnishi T, *et al*. Royal jelly prevents osteoporesis in rats. Bone Tissue 2006; 3(3):339-48.
11. Kohno K, Okamoto I, Sano O, Arai N, Iwaki K, Ikeda M, *et al*. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophage. Biosci Biotechnol Biochem 2004; 68(1);138-45.
12. Suemaru K, Cui R, Li B, Watanabe S, Okihara K, Hashimoto K, *et al*. Topical application of royal jelly has a healing effect for 5-fluorouracil-induced experimental oral mucositis in hamsters. Methods Find Exp Clin Pharmacol 2008; 30(2):103-6.
13. Kanbur M, Eraslan G, Silici S, Karabacak M. Effects of sodium fluoride exposure on some biochemical parameters in mice: Evaluation of the ameliorative effect of royal jelly applications on these parameters. Food and Chemical Toxicology 2009; 47:1184–9.
14. Asadi SY, Parsaei P, Karimi M, Ezzati S, Zamiri A, Mohammadizadeh F, *et al.* Effect of green tea (Camellia sinensis) extract on healing process of surgical wounds in rat. Int J Surg. 2013;11(4):332-7.
15. Majtán J. Apitherapy-the role of honey in the chronic wound healing process. Epidemiol Mikrobiol Imunol 2009; 58(3): 137-40.
16. Majtan J, Kumar P, Majtan T, Walls AF, Klaudiny J. Effect of honey and its major royal jelly protein 1 on cytokine and MMP-9 mRNA transcripts in human keratinocytes. Exp Dermatol 2010;19(8):e73-9.
17. Suemaru K, Cui R, Li B, Watanabe S, Okihara K, Hashimoto K, *et al*. Topical application of royal jelly has a healing effect for 5-fluorouracil induced experimental oral mucositis in hamsters. Exp Clin Pharmacol 2008; 30(2):103-6.
18. Abdelatif M, Yakoot M. Safety and efficacy of a new honey ointment on diabetic foot ulcers: a prospective pilot study. Wound Care 2008; 17(3):108-10.
19. Taniguchia Y, Kohnoa K, Inoue S, Koya-Miyata S, Okamoto I, Arai N, *et al*. Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int Immunopharmacol 2003; 3(9):1313-24.