Effects of Royal Jelly on Bone Healing in Rabbit Model: Radiological and Histopathological Evaluation

Abstract

**Background & Aim:** Today, bone grafts are used to stimulate fracture healing and restoration of bone defects in orthopedic surgeries. However, there is a higher tendency toward allograft and xenograft due to the complications associated with autograft. These transplants are not limited in the amount of extraction and contain cells and protein substances that stimulate bone healing. A white gelatinous substance produced by worker bees excreted by their subpharyngeal glands, Royal jelly (RJ) is exclusively made for the queen bee and has antibacterial, antioxidant, anti-tumor and anti-inflammatory properties. The present study aimed to evaluate the effects of RJ on bone healing and restoration in the rabbit model.

**Materials and Methods:** This experimental research was performed in the school of veterinary medicine of the Shahrekord University in December 2018. The samples included 10 New Zealand white rabbits with an approximate weight of 2 kg. The bone fragment was removed from the radius in a longitudinal shape twice the width of the radius bone. The samples were divided into two groups of five, one receiving RJ at the defect site while the other received no treatment. The radiological assessments were carried out on days 14th, 28th, and 42nd after the operation, and the results were analyzed.

**Results and Conclusion:** The histopathological samples were assessed on the 42nd postoperative day. According to the results, there was no significant difference between the groups in terms of radiological results on the 14th and 42nd days (P>0.05). However, a significant difference was observed on the 28th postoperative day in this regard (P=0.01), when more healing was detected in the RJ group, compared to the control group. Furthermore, no significant difference was found between the groups regarding histopathological results (P>0.05).

**Keywords:** Bone Healing, Royal jelly, Bone Grafting, Rabbit

**Introduction**

Today, bone grafts are used in veterinary and human orthopedics to heal fractures, accelerate joint connection, and repair bone defects. Autografts have been recognized as a golden standard for comparison with other factors stimulating bone formation. In addition to healing stimulants, autografts contain cells that stimulate immune responses, thereby preventing the transmission of communicable diseases \(^1\). In small animals, the inner surface of the upper side of the tibia and the upper end of the humerus are used to collect autografts from iliac crest. Samples are collected from the same place in humans. However, this process has consequences such as pain, infection, fracture, blood loss, increased surgical stages, and a limited amount of bone removed \(^2\). Given the problems of autograft, orthopedic surgeons seek more efficient alternatives with fewer damages and complications so that there is no bone removal limitation and the method could involve cells and proteins that promote bone healing \(^3\).

All larvae are fed royal jelly (RJ) for the first three days of life; however, only the queen bee is fed RJ throughout her life. Generally, RJ is rich in nutrients such as proteins, sugars, vitamins, free amino acids, fatty acids, and minerals \(^4\).
In addition, RJ has been reported to have various health benefits such as anti-inflammatory, anti-allergic, anti-coagulant, anti-tumor, anti-hypertensive, and stimulant properties (4-7).

Moreover, RJ consumption eliminates age-related diseases including postmenstrual syndrome. Studies on animals have also shown that RJ causes vascular dilatation and can increase blood supply. While increasing cell growth rate, the compound can improve cell differentiation and perform anti-tumor activities (5, 7, 8).

In rats that experienced osteoporosis induced by ovariectomy, RJ consumption relatively eliminated osteoporosis (9, 10). In the culture medium, osteoblast has a similar effect to estrogen (11-13). Limited research has been conducted on the effect of RJ on bone metabolism and related cellular activities. In a research, rats were fed RJ orally to assess the healing effects of the compound on the skull bone defects (14). In another study, researchers evaluated the effect of RJ combined with chitosan on bone defect healing (15). The present study aimed to assess the culture of powdered RJ on bone defect in the rabbit animal model in terms of ossification speed based on radiological and histopathological assessments.

**RJ Powder Preparation**

In this study, we used powdered RJ using the freeze-drying technique. In this regard, 20 gr of RJ was placed inside a freezer dryer for 48 hours. Afterwards, RJ became a solid mass, which was turned into powder using a mortar. First, 10 rabbits were prepared from the animal house of the school of veterinary injected with anti-parasitic drugs subcutaneously. The animals were kept in the new environment for 15 days and received standard plate food to get used to the situation. Afterwards, the animals were divided into two groups of control (N=5) and RJ (N=5). In the latter, one mg of powdered RJ was placed on the defect site. Ketamine (30 mg/kg) and acepromazine (0.2 mg/kg) were intramuscularly injected to provide anesthesia in the rabbits. Afterwards, the right hand of the animals was shaved to prepare for the surgery. A cut was made on the anterior-internal surface of the radius bone, which was revealed after removing soft tissues and muscles. A piece of bone was removed twice its width (approximately 10 mm), and the compound was placed on the defective site, as mentioned before. After the graft, the muscles were sutured and the skin was sutured subcutaneously with vicryl 2-0 suture. The rabbits were released into the cage without external stabilization after full recovery. For two days, the animals were injected intramuscularly with penicillin (40,000 international unit) and streptomycin (mg/kg) once a day. The rabbits were observed every day and information related to their hand use and weight gain was recorded. Any localized lesions, inflammation, or non-repair were considered, and radiographs were taken after surgery and on the 14th, 28th, and 42nd days in the lateral view. The film distance from the X-ray source was about 70 cm and the radiography device was set at 45 kV and 20 mAs. In addition, the modified Lane and Sandhu scoring system was applied to assess and score the radiographs prepared (Table 1).

**Method**

The present research was performed in the surgery and radiography department of the school of veterinary of Shahrekord University in Fall, 2018. It is notable that the study received approval from the ethics committee of the university and adhered to animal rights throughout the research project. In addition, the study was carried out based on the basics of working with animals approved by the research council of the university with the code of 170.996. In addition, RJ was naturally prepared in beehives by working bees and was purchased from bee products sales centers.
Effects of Royal Jelly on bone healing

Histopathological Evaluation
The rabbits were kept for six weeks and then euthanized humanely, followed by taking samples from the animals for histopathological evaluation. The samples were placed in 10% formalin and demineralized in formic acid and placed in paraffin molds. Afterwards, histological slides were prepared with a cut at five µ and hematoxylin-eosin staining. Moreover, skeletal healing scoring technique was used in the histopathological evaluation, as follows: the score is zero in case of an empty defect; the score is one when the defect is filled only with fibrous connective tissue; the score is two when there is a combination of fibrous tissue and fibrocartilage with the predominance of fibrous tissue; the score is three when there is a combination of fibrous tissue and fibrocartilage with the predominance of fibrocartilage; the score is four when there is only fibrocartilage tissue; the score is five when the fibrocartilage tissue is accompanied by bone tissue with the predominance of fibrocartilage tissue; the score is six when the fibrocartilage tissue is accompanied by bone tissue with the predominance of bone tissue, and the score is seven when there is only bone tissue at the defect site (16).

Statistical Analysis
In this research, data analysis was performed in SPSS version 24 using the nonparametric Kruskal-Wallis test (to assess radiological and histopathological data) and Mann-Whitney U (to compare the results). It is notable that a P-value of less than 0.05 was considered statistically significant.

Table 1. Evaluation and scoring of radiographs of samples using the modified Lane and Sandhu scoring system

<table>
<thead>
<tr>
<th></th>
<th>Max score</th>
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<tbody>
<tr>
<td>Ossification</td>
<td>4</td>
</tr>
<tr>
<td>Upper union</td>
<td>2</td>
</tr>
<tr>
<td>Lower union</td>
<td>2</td>
</tr>
<tr>
<td>Remodelling</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. Radiography results (max-min) median

<table>
<thead>
<tr>
<th>Postoperative days</th>
<th>Control group</th>
<th>RJ group</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>2 (1-4)</td>
<td>4 (2-7)</td>
<td>0.024</td>
</tr>
<tr>
<td>28</td>
<td>4 (2-5)</td>
<td>8 (5-10)</td>
<td>0.033</td>
</tr>
<tr>
<td>42</td>
<td>3 (2-7)</td>
<td>3 (2-7)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis non parametric ANOVA P*=0.01(compared with group 2 by Mann-Whitney U test)
The bone samples were extracted 42 days after the surgery and were delivered to the laboratory for histopathological assessment, carried out by Emery’s scoring system. Despite insignificant results obtained in this area, the observations were indicative of much more regular, better, and more extensive bone healing in the RJ group (Table 3, figures 3 & 4).

### Table 3. Histopathological results in the second, fourth and sixth postoperative weeks median (max-min)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>RJ group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathological</td>
<td>3 (2-4)</td>
<td>5 (3-6)</td>
<td>0.1</td>
</tr>
<tr>
<td>assessment based on</td>
<td></td>
<td></td>
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<tr>
<td>Emery’s scoring system</td>
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*Kruskal-Wallis non parametric ANOVA

### Discussion

In the present research, powdered RJ was used on rabbits and the selected bone was the radius since there is no need for an external or internal stabilizer due to the connection between ulna and radius in the rabbit by removing part of the radius. Therefore, the bone healing process could be assessed without the need to consider the impact of a stabilizer. The defect was created in the middle part of the radius, and the length of the removed piece was twice the width of the bone at that site to create a clear, unconnected empty space \(^{(17)}\).
In 1990, Fujii et al. evaluated the effect of RJ on wound healing of streptozotocin-induced hyperglycemic rats, concluding that while RJ had no insulin-like properties in the samples, it had anti-inflammatory effects through the formation of collagen in granulation tissue, thereby reducing the wound healing process (18). In similar research, Siavash et al. (2013), evaluated the effect of 5% RJ on foot ulcers of diabetes patients. While insignificant results were obtained, it was revealed that the topical use of RJ was associated with no side effects (19). In 2005, Mishima et al. proved RJ’s estrogenic activity during interaction with estrogen receptors (11). Moreover, Narita et al. (2006) marked that adding RJ to the food of female rats for nine weeks significantly increased tibia ash. According to the mentioned research, RJ as a whole or some of its components is able to increase collagen III and ossification activity by exerting an impact on osteoblasts (20). In 2008, Suzuki et al. evaluated the effect of fatty acids of RJ on rats, concluding that oral administration of RJ partially relieved osteoporosis in rats with osteoarthritis due to ovariectomy and exhibited estrogenic effects (13). Nevertheless, in a recent study by Shimizu et al. (2018) on ovariectomized rats, the results were indicative of a lack of RJ’s ability to prevent bone destruction while improving bone strength (21). Assessing rat bone maxilla, Ozan et al. (2015) found that RJ played an effective role in the improvement of bone regeneration quality (14).

Figure 3. Woven bone formation (porous structure consisting of scattered cells and i strands) angiogenesis, and fibrous tissue deficiency or absence in the RJ group
Various studies have confirmed the favorable effects of RJ on restoration by inducing collagen production. In 2011, Hye Min Park et al. documented that RJ could protect the skin against UVB rays by inducing collagen production. In 2012, the same scholars proved that RJ could prevent skin aging by increasing collagen production in rats with estrogen deficiency due to ovarian resection \(^{(22, 23)}\). While a significant difference between the groups was only observed in the fourth week, the numerical assessments showed that repair was faster and reconstruction was more efficient in the RJ group. In addition, it was concluded that RJ had favorable effects on ossification due to estrogenic activities \(^{(21)}\). Moreover, this compound was able to speed up the recovery process owing to its anti-inflammatory effects through affecting the active macrophages and inhibiting the production of inflammatory cytokines in the defect site \(^{(24)}\). Overall, the radiology and histopathology results demonstrated that ossification was considerably lower in the control group, compared to the RJ group, and the defect site was mostly filled by fibrous tissue and rarely cartilage instead of bone tissue. However, unfavorable bone
reconstruction at the defect site due to the absence of inductive repair factors was expected in the control group. In the absence of cartilage, the defect site is replaced by granular fibrosis made by fibroblasts. This discontinuity in the cartilage formed in the site and formation of fibrocartilage centers between broken parts prevent full recovery (25) and lead to the formation of a w structure. The numerical assessment showed that the tissue formed at the defect site in the RJ group was mostly fibrocartilage, cartilage, and bone tissue. Nevertheless, the difference between the two groups was insignificant, which was not unexpected since recovery occurred in the control group after 42 day.

This complicated the evaluation of histopathological results.

**Conclusion**

This was the first research to use solely powdered RJ on the defect site directly to promote bone healing. According to the results, this compound could be used for bone defects. Given its proper effect on ossification, its use along with grafts could yield more favorable results.

**Conflicts of Interest**

None declared.

**References**


