Orthopedic Research and Reviews

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Red Yeast Rice Increases Bone Morphogenetic Protein-2 Expression and Enhances Fracture Healing Process in Delayed Union Models of Sprague-Dawley Rats: A Preclinical Study

Aryadi Kurniawan[1], Felais Hediyanto Pradana[1], Evelina Kodrat[2], Anissa Feby Canintika[1], Aufa Kunti Riona Aryandhani[3]

[1]Department of Orthopedics and Traumatology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia; [2]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia; [3]Radboud University, Nijmegen, Netherland

Correspondence: Aryadi Kurniawan, Department of Orthopedics and Traumatology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, Email aryadi.kurniawan@ui.ac.id

Orthopedic Research and Reviews downloaded from https://www.dovepress.com/
For personal use only.

Introduction: Delayed union management is both challenging and expensive. Statins, which are HMG-CoA reductase inhibitors, induce bone morphogenetic protein-2 (BMP-2) gene expression, which in turn increases bone formation. Red yeast rice contains monacolin, which has a similar activity to lovastatin as an HMG-CoA reductase inhibitor. Red-yeast rice is readily available and inexpensive. This study aimed to demonstrate the effectiveness of red yeast rice in enhancing fracture healing in a delayed union model through increased expression of BMP-2.

Materials and Methods: This experimental animal study was conducted using 24 delayed union Sprague-Dawley (SD) rats divided into four groups (n=6 each), consisting of; the 4-weeks-given-red yeast rice extract group (4RYR), 2-weeks-given-red yeast rice extract group (2RYR), 4-weeks-control group (4C), and 2-weeks-control group (2C). The animals were euthanized in the second and fourth week. Immunohistochemical staining was used to assess BMP-2 expression using an Immunoreactive Score (IRS). The fracture healing process was evaluated using histomorphometry by measuring the fibrous area, cartilage area, and woven bone area percentage.

Results: In the 2RYR group, there was significantly higher BMP-2 expression (p=0.03), less fibrous area (p=0.05), and larger cartilage area (p= 0.04) than in the 2C group. In the 4RYR group, there was significantly higher expression of BMP-2 (p=0.011), larger woven bone area (p=0.01), and less fibrous area (p = 0.03) than in the 4C group. There was also a significantly smaller fibrous area (p=0.02), a larger cartilage area (p=0.05), and a larger woven bone area (p=0.04) in the 4RYR group than in the 2RYR group. The BMP-2 expression in the 2RYR group was as high as that in the 4RYR group.

Conclusion: Red yeast rice effectively improved fracture healing by increasing BMP-2 expression in a delayed-union model in SD rats.

Keywords: delayed union model, BMP-2 expression, red yeast rice, fracture healing

Introduction

Delayed union can occur after fracture healing, which often leads to decreased quality of life, substantial medical expenses, and impaired ability to work. This condition remains a challenging problem for orthopaedic surgeons because there is no consensus regarding the best treatment. Various surgical approaches have been used to treat delayed union of long-bone fractures; however, surgery often has significant drawbacks such as the requirement for multiple surgeries and postoperative infection.

Bone morphogenetic protein-2 (BMP-2), which is the most potent osteoinductor, has shown promising results. Interventions using recombinant human BMP-2 (rhBMP-2) have been shown to accelerate bone healing and reduce

Received: 3 October 2024
Accepted: 2 January 2025
Published: 14 February 2025

© 2025 Kurniawan et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

the risk of nonunion, delayed union, and infection.[1] Garrison et al[1] found that administering rhBMP-2 in conjunction with conventional bone grafts resulted in significantly more healing than administering conventional bone grafts alone. However, rhBMP-2 is expensive and requires a supraphysiological dose, which can lead to complications.

Red yeast rice (RYR) is a traditional Chinese medicine and food supplement that is popular in East Asian countries such as China, Japan, Korea, and Thailand. This rice is produced by the fermentation of cooked rice kernels with a Monascaceae mold, Monascus purpureus, which turns rice into reddish-purple kernels because of its pigmentation capability.[2] This rice contains monacolins, which lower cholesterol by inhibiting HMG-CoA reductase. It also has antioxidant and potential anti-inflammatory properties. Phedy et al[3] found that red yeast rice accelerated the healing process in fractures with perfusion problems, which was marked by an increase in Allen score. Red yeast rice contains monacolin, a compound with activity comparable to that of lovastatin.[4] Nefihancoro et al[5] found that a RYR dose of 100 mg/kg significantly increased BMP-2 levels in male rats (Rattus norvegicus).

To date, there have been no studies on the effect of monacolin derived from red yeast rice in a delayed union model using an increased BMP-2 pathway, according to our review of the literature. We hypothesized that monacolin in red yeast rice would increase BMP-2 expression and thus accelerate fracture healing in a Sprague-Dawley rat delayed union model. This study could be a pioneer in providing cost-effective nutritional management to patients with fractures, particularly those with delayed union.

Materials and Methods

This was an experimental study that used a randomized post-test control group design with the assumption that the research group was drawn from the same population, requiring no initial measurement. The aim of this study was to determine the efficacy of monacolin in red yeast rice by increasing BMP-2 expression and healing in a delayed union fracture model in Sprague-Dawley white rats. Rats are the most commonly used fracture model in experimental animals. The fracture healing stage in mice is similar to that in humans but occurs twice as fast.

Red Yeast Rice Administration

Red yeast extract was obtained from Cholestimax® (Pharmanex, Provo, UT, USA) and was distributed in Indonesia by Nu Skin. Cholestimax® was selected because it has raw content. One cholestimax capsule® contained 600 mg of red yeast rice extract dissolved in distilled water to ensure that every 0.2 mL solution contained red yeast at a dose of 25 mg/kg body weight.36 A 1 mL syringe probe was used to add the solution to the animal's mouth.

Animal Intervention

The 2–3 year old male white Sprague-Dawley rats, with 250–350 gram weight were obtained from the Research and Development Center of the Department of Health. The estimated sample size was 24 rats using Federer's formula, which consisted of 6 rats per group and had 4 groups in total.

Rats in the 2C and 4C groups had a delayed union model and were euthanized after two and four weeks, respectively. Rats in the 2RYR and 4RYR groups were administered red yeast rice extract at a dose of 25 mg/kg body weight after two and four weeks, respectively.

Experimental Animal Anesthesia

Anesthesia was induced by administering ketamine 80 mg/kg body weight (Ilium Ketamil Injection®, Troy laboratories PTY Limited, Australia) and xylazine 10 mg/kg body weight (Ilium Xylazil-100 Injection®, Troy laboratories PTY Limited, Australia).

Delayed Union Fracture Model and Internal Fixation

After the animal was sedated, the hair of the mice was shaved on the left thigh in an oblique position on the left side under aseptic conditions using 10% povidone-iodine and 70% alcohol. A 2 cm incision was made on the anterolateral side of the femur while separating the vast lateral muscle from the biceps femoris. The vastus lateralis and biceps femoris muscles were elevated to keep the periosteum intact along the femoral surface.

A saw was used to perform osteotomy of the diaphysis, resulting in a simple transverse fracture. In this study, the delayed union model refers to the model published by Kasman in the form of mechanical treatment with circular stripping of the periosteum using scalpels, 5 mm from the fracture line in the proximal and distal directions.[6] The procedure was followed by intramedullary reaming with a 21G needle and internal fixation with an intramedullary k-wire measuring 1.2–1.4 mm retrograde. Suturing the soft tissue with catgut 3.0, and the skin with Silk 3.0, were used to close the surgical wound.

Maintenance of Experimental Animals

Rats were housed in separate cages at 25–28°C room temperature. All rats were fed orally with a special food called Guyofeed (contains moisture 12%, protein 15%, fat 15%, calcium 1.33%, and phosphorous 0.7%), with access to tap water. For analgesics, the rats were orally administered 100 mg/kg body weight paracetamol, and antibiotics were administered at a dose of 100 mg/kg body weight for up to 1 week postoperatively.

Animal Sacrification

During the second and fourth weeks post-treatment, the rats were euthanized by intraperitoneal administration of phenobarbital (75 mg/kg body weight).[7] After the femur samples were separated, histomorphometry and immunohistochemical analyses were performed at the Department of Anatomic Pathology by an expert in the musculoskeletal pathology division.

Histomorphometry-Fracture Healing Evaluation

Femur tissue specimens were placed in a labeled cassette and fixed in 10% formalin solution for 48 h. Tissue specimens were examined by histomorphometry and immunohistochemistry at the Department of Anatomical Pathology, Faculty of Medicine, University of Indonesia. After fixation, the tissue specimens were dehydrated with increasing alcohol concentrations (70%, 80%, 95%, and 100%) for 3 h consecutively. The procedure continued with a clearing process in which the tissue specimens were submerged twice in xylol solution for 3 h. Afterwards, the tissue specimens were paraffinized with warmed paraffin at 45°C, in which the paraffinized tissue specimens were placed on a cooling plate for the paraffin to solidify. Subsequently, the tissue specimens were cut transversely using a microtome (sectioning) at a thickness of 5 μm and placed on microscope slides. To stain the tissue specimens using Hematoxylin and Eosin (HE) staining, the tissue specimens were cleared with xylol for de-paraffinization and rehydration using a decreased concentration of alcohol as follows: 100%, 95%, 80%, and 70%. The procedure followed with rinsing the tissue specimens using demineralized water two times and continue with hematoxylin solution. Afterwards, the tissue specimens were rinsed in running tap water and stained with an eosin solution. The tissue specimens were then dehydrated using the procedure described above and sealed using a coverslip. The stained and coverslipped tissue specimens were evaluated under a light microscope. Each slide was imaged using a Leica digital microscope camera (Leica ICC50 hD Series). In order to form one digital file approximately 100–150 photos of xx slides. The larger size of the bone and callus led to more photos being taken to capture the entire specimen. All pictures were combined using PTGUI Pro 9.1 software.

The total callus area, immature bone area, cartilage, and fibrous area were measured using ImageJ software. The essential structure of the sample was calculated based on the nomenclature and recommendation unit from the American Society of Bone and Mineral Research (ASBMR) as follows: (i) total callus area [CAr(mm2)], (ii) mineralization area [TOTAr (mm2)/(%)], (iii) fibrous area [FTAr(mm2)/(%)], and (iv) cartilage area [CgAr(mm2)(%)]. In this study, the parameters for mineralization, fibrous area, and cartilage area were presented as the percentage of total calluses for homogenization resulting from different bone callus areas.

Immunohistochemichemistry-BMP-2 Expression Evaluation

Semi-quantitative immunohistochemistry was performed. The evaluation was performed manually, with an Immunoreactive Score (IRS) ranging from 0 to 12 (Table 1). The IRS was calculated using the following formula:

$$\text{IRS} = \text{Positive cell proportion points x Staining intensity points}$$

Table 1 Immunoreactive Score Calculation

Points	Positive Cell Proportion	Intensity of Staining
0	0	No Staining
1	<20	Weak
2	20–50	Moderate
3	50–80	Strong
4	>80	–

Note: The results can be interpreted as negative (0–1), mild (2–3), moderate (4–8), or strongly positive (9–12).

IRS was used for the broad-spectrum expression of IHC markers (BMP and its receptor, VEGF, vWF) in a study by Koerdt et al[41]. Another way to interpret the results was described by Kloen et al[42] with an evaluation scheme that included: (+): positive staining in 25% cells, (++): positive staining in 25–50% cells, (+++): positive staining in 50–75% cells, (++++): positive staining in more than 75% cells; and (−): no cell staining.

Data Analysis

The data were processed using SPSS version 21.0 for windows computer program. Before the analysis, the Shapiro–Wilk test was used for each group. The *T* test was used for data with a normal distribution, and the Mann–Whitney *U*-test was used for data with an abnormal distribution.

Results

Characteristics of the Research Object

This study was conducted in 24 Sprague-Dawley rats. All rats were characterized by homogeneity with age 2–3 months, male sex, body weight of 250–300 grams, no congenital abnormalities, and a mature musculoskeletal system. The study was conducted between June and September 2020. After acclimatization, a delayed union model was created for each animal. Rats were divided into four groups. Histomorphometric assessment was performed using ImageJ software, and immunohistochemical assessment was performed to assess BMP-2 expression using the IRS score.

Demographic Data

In this study (Table 2), the highest mean was found in the fourth-week weight of the control sample (263.16 ± 1.35 mg) and the lowest was in the fourth-week treatment sample weight (261.33 ± 1.33 mg). For the IRS score variable, the lowest median was in the control sample in the second week (4), while the highest median was in the fourth weekend sample (12). The lowest percentage of immature bone area was observed in the control sample in the second week (6.93%), while the highest mean was observed in the treatment sample in the fourth week (65.5 ± 6.33%). The lowest median cartilage area percentage was observed in the fourth-week treatment sample (4.10), whereas the highest mean was observed in the second-week treatment sample (20.5 ± 6.39%). In terms of the percentage of fibrous tissue area, the lowest median was observed in the fourth-week treatment group (28.70 ± 5.09%), while the highest mean was observed in the second-week control group sample (80.51 ± 5.27%).

Histomorphometry and Immunohistochemistry Parameter Evaluation on Second Week

Three of the four parameter mean differences were significant (Table 3). Significant differences were found in the 2-weeks-given-red yeast rice extract group, namely, increased cartilage area percentage (p 0.04), reduced fibrous area percentage (p 0.05), and increased BMP-2 expression (p 0.03), compared with the two-weeks-control group. Thus, the difference in the means of the two variables affects the outcomes of this study.

Table 2 Demographic Tables of All Intervention and Control Groups Compared with Parameters of Weight, Total Callus Area, Woven Bone Area, Cartilage Area, Fibrous Tissue Area and IRS Score

Variable	Intervention Group	N	Mean/Median	±SD/Min-Max	p value
Sample Weight (mg)	2C	6	261.33	0.61	0.212
	4C	6	263.16	1.35	0.220
	2RYR	6	261.33	1.30	0.772
	4RYR	6	261.33	1.33	0.091
Woven Bone Area (%)	2C	6	6.93	(2.48–28.61)	0.058*
	4C	6	32.73	4.1	0.356
	2RYR	6	28.30	7.76	0.625
	4RYR	6	65.5	6.33	0.559
Cartilage Area* (%)	2C	6	6.8	2.66	0.190
	4C	6	12.2	4.12	0.739
	2RYR	6	20.5	6.39	0.368
	4RYR	6	4.10	(2.10–16.17)	0.013*
Fibrous Tissue Area (%)	2C	6	80.51	5.27	0.799
	4C	6	55.07	4.30	0.550
	2RYR	6	51.20	2.33	0.206
	4RYR	6	28.70	5.09	0.164
IRS Score*	2C	6	4	(3–6)	0.093
	4C	6	7.5	0.67	0.004*
	2RYR	6	9.33	0.55	0.008
	4RYR	6	12	(9–12)	0.058*

Note: *Abnormal data distribution.

Table 3 Histomorphometry and Immunohistochemistry Evaluation on Second Week

Parameter	Comparison	Mean±SD/Median (Min-Max)	Statistical Test	p value
Woven Bone Area (%)	2C	14.29 (11.66–51.61)	Mann–Whitney	0.15
	2RYR	28.30±7.75		
Cartilage Area (%)	2C	6.8±2.66	*T*-Test	0.04*
	2RYR	20.5±6.39		
Fibrous Tissue Area (%)	2C	80.51±5.27	*T*-Test	0.05*
	2RYR	51.20±2.33		
IRS Score	2C	4 (3–6)	Mann–Whitney	0.03*
	2RYR	9.33 ±0.55		

Note: *Significant if p value <0.05.

Histomorphometry and Immunohistochemistry Parameter Evaluation on the Fourth Week

Three of the four parameter mean differences were significant (Table 4). Significant differences were found in the 2-weeks-given -red yeast rice extract group, namely, increased woven bone area percentage (p=0.01), decreased fibrous tissue area percentage (p=0.03), and increased BMP-2 expression (p=0.011) compared with the four-weeks-control group. Four evaluations carried out in the fourth week indicated significant differences that could have affected the outcome of this study.

Table 4 Histomorphometry and Immunohistochemistry Evaluation of the Fourth Week

Parameter	Comparison	Mean±SD/Median (Min-Max)	Statistical Test	p value
Woven Bone Area (%)	4C 4RYR	32.73±4.1 65.5±6.33	*T*-Test	0.01*
Cartilage Area (%)	4C 4RYR	12.2±4.12 4.10 (2.10–16.17)	Mann–Whitney	0.42
Fibrous Tissue Area (%)	4C 4RYR	55.07±4.30 28.70±5.09	*T*-Test	0.03*
IRS Score	4C 4RYR	7.5±0.67 12 (9–12)	Mann–Whitney	0.011*

Note: *Significant if p value <0.05.

Table 5 Histomorphometric and Immunohistochemistry Evaluation of 2-Weeks-Given-Red Yeast Rice Extract and 4-Weeks-Given-Red Yeast Rice Extract Group

Parameter	Comparison	Mean±SD/Median (Min-Max)	Statistical Test	p value
Woven Bone Area (%)	2RYR 4RYR	28.30±7.75 65.5±6.33	*T*-Test	0.04*
Cartilage Area (%)	2RYR 4RYR	20.5±6.39 4.10 (2.10–16.17)	Mann–Whitney	0.05*
Fibrous Tissue Area (%)	2RYR 4RYR	51.20±2.33 28.70±5.09	*T*-Test	0.02*
IRS Score	2RYR 4RYR	9.33 ±0.55 12 (9–12)	Mann–Whitney	0.075

Note: *Significant if p value <0.05.

Histomorphometry and Immunohistochemical Parameters Evaluation Between 2RYR and 4RYR

Three of the four parameter mean differences were significant (Table 5). Significant differences were found in the 4-weeks-given-red yeast rice extract group: increased immature bone area percentage (p = 0.04), decreased fibrous tissue area percentage (p = 0.02) and decreased cartilage area percentage (p = 0.5). This result indicated that the mean difference between the three areas affected the outcome of this study.

Discussion

In our study, fracture healing followed endochondral ossification, which generally followed four steps: hematoma formation, fibrocartilaginous callus formation, woven bone callus formation, and bone remodeling. The euthanasia and tissue sample collection schedules in the second and fourth weeks were designed to depict the transition between fibrocartilaginous and woven bone formations.

Red yeast rice is widely used in Asian cuisine as a flavoring agent, preservative, and in rice wine.[8] It can decrease the blood lipid levels in animals and humans.[9,10] There are 14 types of monacolin-active agents found in red yeast rice. Monacolin and lovastatin are effective hypocholesterolemic agents.[4] The mechanism by which monacolin lowers blood lipid concentration is complex. It is obtained through various components and pathways from human blood. The main lipid-lowering mechanisms involve decreasing endogenous lipid synthesis, increasing exogenous lipid absorption, and promoting lipid transport and excretion.[11,12] It was also stated that when compared to statins, red yeast rice extract has fewer associated adverse events.[11]

Mundy et al[13] reported that orally administered lovastatin stimulates bone formation via the BMP-2 signaling pathway. Clinical applications have reported an increase in bone fracture and dentistry section.[14] The anabolic effects have caused mesenchymal cell differentiation into osteoblasts via the upregulation of BMP-2 and protection of osteoblasts from apoptosis. Additionally, lovastatin has been suggested to exert anti-osteoclastic effects by reducing osteoclast differentiation and activity.[15] Ibrahim et al evaluated the biomechanical properties of calli during fracture healing in osteoporotic Sprague-Dawley rats. The group that received lovastatin injection showed better callus biomechanics and a higher callus volume than the control group.[16] In another study, Garret et al[17] delivered lovastatin locally in a femoral rat fracture model, and computed tomography revealed an increased healing rate and a decreased fracture gap. Bleedorn et al[18] also found improved fracture healing in the injected lovastatin group using serial radiography and histological parameters. Lovastatin was administered percutaneously at 6 mg/kg body weight in a canine tibial osteotomy model with external fixation. Phedy et al[3] showed improved fracture healing in Sprague Dawley rats administered red yeast rice extract compared to that in the control group by histology and radiographic parameters.

Our findings confirmed that fracture healing was improved in a delayed union animal model. Because histomorphometry and BMP-2 expression in immunohistochemical staining were more quantitative, we preferred them. We also confirmed that increased BMP-2 expression improved fracture healing.

According to Gutierrez et al, RYR has strong bone anabolic effects both in vitro and in vivo, implying that it could be used as a therapy for bone loss in conditions such as osteoporosis.[19] However, Gutierrez et al[19] did not specify the pathways contributing to this result. Wong et al showed that, using histologic parameters, the RYR group showed more bone formation in parietal bone defects than the control group.[20] In comparison to our study, Wong et al[20] conducted an animal experimental study by making defects in the rabbit's parietal bone; whereas, our study used the rat's delayed union model in the femur bone. Another difference is that they implanted the red yeast rice extract, whereas our rats received it orally. Moreover, our observation time was also longer than the study conducted by Wong et al.[20] The same anabolic effects in the Gutierrez study were observed in our study when histological evaluation was performed. We found increased bone formation in the RYR group compared with that in the control group. Gutierrez et al[19] performed in vivo and in vitro studies with larger samples and compared them with those of the lovastatin group.

When comparing the RYR group to the controls, it was discovered that fracture healing increased with BMP-2 expression. According to Wang et al.[4] BMP-2 concentration increases in osteoporotic ovariectomized rats. This is consistent with an in vitro study by Alam et al[21] who reported an increase in BMP-2 immunohistochemical expression in the statin-treated group. Despite producing similar results, the method used by Alam et al[21] was not the same. The experimental animals were twelve rabbit samples.

In addition, Alam et al[21] implanted statins in the nasal bones of rabbits. Four defects were made 4 defects in the nasal bone of Each rabbit. In this study, the samples used were numerous and longer for evaluation. We chose the femur because it is a common fracture in humans. Rats had a longer femur column than rabbits, indicating a habit of standing upright more often. Another in vitro study conducted by Ayukawa et al[22] also showed an increase in BMP-2 expression in the statin-treated group of Wistar rats compared to that in the control group.

A limitation of this study is that the mouse samples used were not the same between the second and fourth weeks because they had to be harvested. The inclusion and exclusion criteria, on the other hand, were used to reduce bias.

Conclusion

Through increased BMP-2 expression, red yeast rice effectively improved fracture healing in a delayed union model in Sprague-Dawley rats. Further clinical studies regarding red yeast rice consumption and fracture healing are warranted in the future.

Ethical Approval

Ethical approval was obtained from the Ethics Committee Board Faculty of Medicine, Universitas Indonesia (protocol number 20-01-0008). All animal procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All animal care and use procedures were performed under strict ethical and regulatory standards.

Funding

The authors report no external funding sources during the writing of this manuscript.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Garrison KR, Shemilt I, Donell S, et al. Bone morphogenetic protein (BMP) for fracture healing in adults. *Cochrane Database Syst Rev*. 2010. doi:10.1002/14651858.CD006950.pub2
2. Zhu B, Qi F, Wu J, et al. Red yeast rice: a systematic review of the traditional uses, chemistry, pharmacology, and quality control of an important Chinese folk medicine. *Front Pharmacol*. 2019;10:1449. doi:10.3389/fphar.2019.00010
3. Phedy P, Kurniawan A, Siregar NC. Peran Beras Angkak dalam Mempercepat Penyembuhan Fraktur dengan Gangguan Vaskularisasi pada Rattus novergicus. *eJournal Kedokteran Indonesia*. 2016;3(3). Indonesian.doi:10.23886/ejki.3.5650.
4. Li YG, Zhang F, Wang ZT, Hu ZB. Identification and chemical profiling of monacolins in red yeast rice using high-performance liquid chromatography with photodiode array detector and mass spectrometry. *J Pharm Biomed Anal*. 2004;35(5):1101–1112. doi:10.1016/j.jpba.2004.04.004
5. Nefihancoro UH, Hartono H, Indarto D, Kurniawan A. The effect of red yeast rice on delayed union fracture in animal model: a molecular study of IL-6, BMP-2, VEGF, BALP, and N-Mid-OC in fracture healing. *F1000Res*. 2022;11:1398.
6. Kasman D, Kurniawan A. Histomorphometric analysis of fracture healing using ImageJ software in Sprague-Dawley rat models of fractures with mechanical force to the bone only and to the bone and periosteum. *J Phys*. 2018;1073:042036.
7. Close B, Banister K, Baumans V, et al. Recommendations for euthanasia of experimental animals: part 2. *Lab Anim*. 1997;31(1):1–32. doi:10.1258/002367797780600297
8. Heber D, Yip I, Ashley JM, Elashoff DA, Elashoff RM, Go VLW. Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. *Am J Clin Nutr*. 1999;69(2):231–236. doi:10.1093/ajcn/69.2.231
9. Li C, Zhu Y, Wang Y, Zhu JS, Chang J, Kritchevsky D. Monascus purpureus-fermented rice (red yeast rice): a natural food product that lowers blood cholesterol in animal models of hypercholesterolemia. *Nutr Res*. 1998;18(1):71–81. doi:10.1016/S0271-5317(97)00201-7
10. Wang J, Lu Z, Chi J, et al. Multicenter clinical trial of the serum lipid-lowering effects of a Monascus purpureus (red yeast) rice preparation from traditional Chinese medicine. *Curr Ther Res Clin Exp*. 1997;58(12):964–978. doi:10.1016/S0011-393X(97)80063-X
11. Cicero AFG, Fogacci F, Banach M. Red Yeast Rice for Hypercholesterolemia. *Methodist Debakey Cardiovasc J*. 2019;15:192.
12. Xiong Z, Cao X, Wen Q, et al. An overview of the bioactivity of monacolin K/lovastatin. *Food Chem Toxicol*. 2019;131:110585.
13. Mundy G, Garrett R, Harris S, et al. Stimulation of bone formation in vitro and in rodents by statins. *Science*. 1999;286(5446):1946–1949. doi:10.1126/science.286.5446.1946
14. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol*. 2010;160:1577. doi:10.1111/j.1476-5381.2010.00687.x
15. Oryan A, Kamali A, Moshiri A. Potential mechanisms and applications of statins on osteogenesis: current modalities, conflicts and future directions. *J Control Release*. 2015;215:12–24.
16. Ibrahim NI, Khamis MF, Yunoh MFM, Abdullah S, Mohamed N, Shuid AN. Targeted delivery of lovastatin and tocotrienol to fracture site promotes fracture healing in osteoporosis model: micro-computed tomography and biomechanical evaluation. *PLoS One*. 2014;9(12):e115595. doi:10.1371/journal.pone.0115595
17. Garrett IR, Gutierrez GE, Rossini G, et al. Locally delivered lovastatin nanoparticles enhance fracture healing in rats. *J Orthop Res*. 2007;25(10):1351–1357. doi:10.1002/jor.20391
18. Bleedorn JA, Sullivan R, Lu Y, Nemke B, Kalscheur V, Markel MD. Percutaneous lovastatin accelerates bone healing but is associated with periosseous soft tissue inflammation in a canine tibial osteotomy model. *J Orthop Res*. 2014;32(2):210–216. doi:10.1002/jor.22502
19. Gutierrez GE, Mundy B, Rossini G, Garrett IR, Chen ST, Mundy GR. Red yeast rice stimulates bone formation in rats. *Nutr Res*. 2006;26(3):124–129. doi:10.1016/j.nutres.2006.02.006
20. Wong RWK, Rabie B. Chinese red yeast rice (Monascus purpureus-fermented rice) promotes bone formation. *Chin Med*. 2008;3:1–6. doi:10.1186/1749-8546-3-3
21. Alam S, Ueki K, Nakagawa K, et al. Statin-induced bone morphogenetic protein (BMP) 2 expression during bone regeneration: an immunohistochemical study. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2009;107(1):22–29. doi:10.1016/j.tripleo.2008.06.025
22. Ayukawa Y, Yasukawa E, Moriyama Y, et al. Local application of statin promotes bone repair through the suppression of osteoclasts and the enhancement of osteoblasts at bone-healing sites in rats. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2009;107(3):336–342. doi:10.1016/j.tripleo.2008.07.013

Orthopedic Research and Reviews

Dovepress
Taylor & Francis Group

Publish your work in this journal

Orthopedic Research and Reviews is an international, peer-reviewed, open access journal that focusing on the patho-physiology of the musculoskeletal system, trauma, surgery and other corrective interventions to restore mobility and function. Advances in new technologies, materials, techniques and pharmacological agents are particularly welcome. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/orthopedic-research-and-reviews-journal