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RESEARCH ARTICLE

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 [version 1; peer review: 1 not approved]

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Abstract

Background

As serious fracture complications, delayed union and non-union are parts of complications from fracture healing. Growth factors such as BMP-2, VEGF, proinflammatory cytokines including IL-6 and bone formation BALP, N-Mid-OC are important regulators of the fracture healing process. Red yeast rice (RYR), produced by fermenting *Monascus purpureus* rice, monacolin K, which is the main ingredient in RYR, was found to play a major role in the anti-inflammatory process and increasing the proliferation of osteoblast in osteoporosis cases. This study aims to examine the effect of RYR in the fracture healing process in delayed union rats through molecular studies of levels of IL-6, BMP- 2, VEGF, BALP, and N-Mid-OC.

Methods

This study was experimental research that used male rats (*Rattus novergicus*) which were divided into a control and 3 treatment groups using a random sampling method. Group 1 was given orally 25 mg/kg, Group 2 was 50 mg/kg, Group 3 was 100 mg/kg, and the control group was given a placebo. The rats were then subjected to a delayed union fracture model. Observations were made for two periods on the 14th and 28th days.

Results

There were no significant differences in serology examination between days 0 and 14 between groups. However, there were significant differences between groups on day 28. IL-6, BMP-2, VEGF, BALP, and N-Mid-OC on day 28 between groups ($p<0.001$). The group

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with 100 mg/kg RYR extract was found to be the most influencing serology marker level. RYR 100 mg/kg significantly decreased IL-6, and increased BMP-2, VEGF, BALP, and N-Mid-Osteocalcin, thus enhancing the fracture healing process in the delayed union rats model.

Conclusion

A red yeast rice dose of 100 mg/KgBW significantly reduced IL-6, increased BMP-2, VEGF, BALP, N-Mid-OC, and RUST Score so as to improve the fracture healing process in delayed union rats.

Keywords

Red Yeast Rice, Delayed Union, ELISA, IL-6, BMP-2, VEGF, Fracture Healing

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Introduction

Fractures are the type of trauma that most require hospitalization. In 2018 the prevalence of fractures due to accidents ranked third out of all non-natural disasters, 31.4% occurring on roads and the most in the 15–24-year age group at 49.5% (Litbangkes, 2019). Not all fractures can heal completely, some have complications such as delayed union or non-union. Delayed union and non-union account for 5–10% of fracture healing (Kostenuik and Mirza, 2017). Impaired fracture healing significantly influences the quality of life, financial condition, and functional and psychological disorders of patients (Stewart, 2019).

The diamond concept, which includes biological chamber, mechanical stability, osteogenic cells, osteoinductive mediators (growth factors, cytokines), and osteoconductive matrix, needs to be applied in the treatment of fracture healing disorders (Andrzejowski and Giannoudis, 2019). Growth factors such as bone morphogenetic protein (BMP) are important regulators of the fracture healing process. BMP-2 functions for the differentiation of mesenchymal stem cells into osteoblast cells (Wu et al., 2020). Proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and, interleukin-6 (IL-6), help initiate the fracture healing cascade, and may also play a key role in the remodeling phase (Hartono et al., 2022; Ding et al., 2018).

Red yeast rice, produced by fermenting *Monascus purpureus* rice, has been used as a traditional medicine in East Asian countries such as China, Japan, Korea, and Thailand (Patel, 2016; Zhu et al., 2019). Red yeast rice has been reported to have many biological properties with hypolipidemic, anti-atherosclerotic, anti-cancer, neurocytoprotective, hepatoprotective, anti-osteoporosis, anti-fatigue, anti-diabetic, anti-obesity, immunomodulatory, anti-inflammatory, antihypertensive, and anti-inflammatory, and antibiotic properties (Zhang et al., 2018). Monacolin K, which is the main ingredient in red yeast rice, was found to play a major role in the fracture healing process by increasing the proliferation of osteoblasts (Wu et al., 2020; Song et al., 2019).

The researchers examined the effect of giving red yeast rice which is easily available and widely known to the public in Indonesia on the fracture healing process in delayed union rats through molecular studies of levels of IL-6, BMP- 2, and vascular endothelial growth factor (VEGF) as the predictor of the healing process, also N-Mid-Osteocalcin and bone alkali phosphatase (BALP) as the predictor of osteoblast activity.

Methods

Research design

This is experimental research with a completely randomized pre- and post-test control group. The experimental animals/male rats that met the inclusion and exclusion criteria were taken in as many as eight animals/group (three treatment groups and one control group) using a random sampling method. The Sprague Dawley rat model was chosen because the model rat has been widely used in previous studies so genetic data is easy to obtain and it can provide research results with a high level of validity. In addition, the stages of fracture healing in rats also resemble humans even though they occur twice as fast, genetic similarity, lamellar bone architects also resemble humans, and have a relatively short life with a fast bone turnover rate, making it easier to carry out multigenerational research. Care and maintenance are relatively inexpensive, and have the ability to adapt to a laboratory environment. Rats are also considered more suitable than other four-legged animal species to study the morphology of the femur bone (Gutierreza et al., 2006). The sample in each group was randomly chosen by giving each trial animal a tag number. Following that, the researcher randomly chose the tag numbers. The animal experiment and examination were carried out at the experimental animal husbandry in the Inter-University Center Building, Universitas Gadjah Mada (PAU-UGM) Yogyakarta. Identification and analysis of IL-6, BMP-2,VEGF, BALP and N-Mid-Osteocalcin were carried out at the Biomedical Laboratory, Faculty of Medicine, Gadjah Mada University. This study had been approved by The Ethic Committee No.513/IV/HREC/2021.

The population in this study were male rats (*Rattus norvegicus*) Sprague Dawley strain aged 12 weeks with a body weight of 150–200 grams which were developed and maintained at PAU-UGM totaling 8 individuals each group with 3 treatment groups and 1 control group so that a total of 32 animals. male rats (*Rattus novergicus*) were calculated using the Steel & Torry (1980) formula:

$$(n-1)(k-1) > 15$$

n = total samples

k = number of groups

This study used four groups so,

$$(n-1)(4-1) > 15$$

Then, the animals should also meet the inclusion criteria (healthy, active, good appetite, 12 weeks old, and weighs 150–200 grams) and exclusion criteria (dead during the study, unwilling to eat, and infection in the operating area). All experimental procedures involving animals were carried out in keeping with guidelines from the National Institutes of Health Guide for the Care and Use of Laboratory Animals to ameliorate any suffering of animals (Tan, 2004). Expected and unexpected adverse events were recorded to identify deficiencies in procedures or study design.

Procedures

Before the intervention, the experimental animals were kept for 1 week for acclimatization. The animal models were acclimatized for a week at a temperature of 21–23°C with controlled humidity (50±5%) in a 12-hour artificial light cycle (08:00 h to 20:00 h) to help them to adapt to the same conditions as their various origins. All rates were located individually in polycarbonate cages (0.90×0.60×0.60 m). Every animal model was fed with a standard pellet and water was provided *ad libitum* with the husk replaced every three days. All animal models were routinely inspected and observed regarding their food consumption and fecal characteristics. After being anesthetized by administering Ketamine (Dexa Medica, Tangerang) 35 mg/kg body weight (BW) and xylazine (Inter Chemie, Holland) 5 mg/kg BW intramuscularly, the animals were then subjected to a delayed union fracture model by antisepsis of the right lower leg. Perform a 2-cm long incision on the posterolateral side of the femur, the vastus lateral muscle is separated from the biceps femoris, then the vastus lateral and biceps femoris muscles are elevated while maintaining the periosteum intact along the surface of the femur bone, performed osteotomy/fracture in the diaphysis of the femur with a 1-mm manual saw to eliminate the effects of heat when using a chainsaw, the delayed union rat model in this study refers to the research of Kasman and Kurniawan (2018). It is in the form of stripping the periosteum/damaging the periosteum in a circular manner with a surgical blade as far as 5 mm from the fracture line towards the proximal and distal according to the Kokubu et al. (2003) and Utvåg et al. (1996) method.

After the fracture and periosteal stripping procedure, we performed intramedullary reaming using a 23G needle followed by fixation. Internally, using intramedullary k-wire measuring 1.2-1.4 mm retrograde, the surgical wound was closed using catgut 3.0 and the skin with silk 3.0. Blood samples were taken through puncture of the orbital vein and analyzed for cellular, IL-6, BMP 2, VEGF, N-Mid-Osteocalcin, and BALP by using the ELISA method. Randomization was performed with 1 control group and 3 treatment groups, each consisting of 8 experimental animals.

Treatment of experimental animals on the same day by giving red yeast rice (Monacolin K/Cholestimax®, Jakarta) one capsule of Cholestimax containing 600 mg of red yeast rice dissolved in 150 mL of distilled water so that every 1 mL of solution contains 4 mg of red yeast rice. The solution was diluted with distilled water according to the required dose for each experimental animal (25, 50, and 100 mg/kg), assuming a 50 mg dose was the optimum dose for delayed union cases and for a 25 mg dose which is half of the optimum dose assessed whether it is still effective. in accelerating the healing of delayed union cases, while the dose of 100 mg is the maximum dose (twice the optimum dose) assessed whether it is still safe/lethal and does not cause side effects. Then the solution was probed into the mouth of the experimental animal using a 1 ml syringe. It is given at the same time as meals, to avoid side effects of digestive system disorders. General observations for signs of pain or suffering in the animal were conducted daily as needed. The moribund condition was used as a humane endpoint (Tan, 2004).

Evaluation

All groups were observed on the 14th and 28th days for blood sampling through a puncture in the orbital vein and cellular analysis including, IL-6, BMP 2, VEGF, BALP, and N-Mid-Osteocalcin with the ELISA method. In this study, observations were made for 2 periods, namely on the 14th and 28th days, to be able to assess any changes that occurred during the fracture healing process.

5 mL of blood were collected through a puncture in the orbital vein under anaesthesia, using a serum separator tube (SST). Samples were centrifuged at 3,500 rpm (~1,000 ×g) for 20 min. Blood serum is separated into a sterile 1.5-mL microcentrifuge tube, immediately tested, or stored in a deep freezer at −80°C until the analysis is carried out. A commercially available research ELISA kit was used to measure serum concentrations of IL-6, BMP 2, VEGF, BALP, and N-Mid-Osteocalcin (FineTest, Wuhan).

Statistical analysis

To determine whether there is a difference in the results of the examination between the four groups of treatment preparations in this study, an unpaired difference test was carried out using the ANOVA test. The test results are considered significant if the p-value <0.05. This research uses the SPSS for Windows Release program (IBM).

Table 1. Changes in serological levels in treatment group P1 (red yeast rice 25 mg/kg).

	Treatment group P1 (read yeast rice 25 mg/kg) (*mean*)				
	IL- 6	**VEGF**	**BALP**	**N mid OC**	**BMP2**
Day 0	27.05	22.06	0.87	6.07	12.66
Day 14	79.97	35.17	14.21	13.02	5.94
Day 28	60.85	30.84	5.11	10.75	8.46
	p-value				
Day 0–Day 14	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Day 14–Day 28	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

*Significant p<0.05.

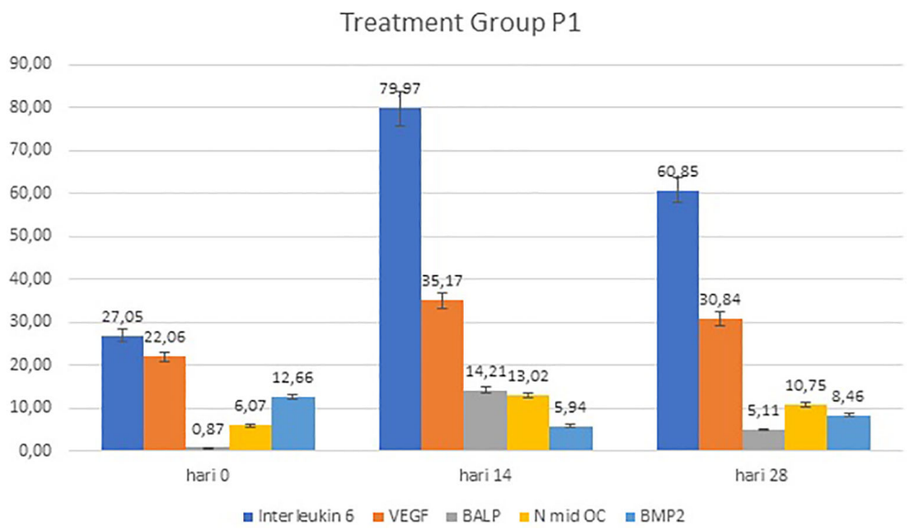

Figure 1. Histogram of changes in serological levels in group P1 (red yeast rice 25 mg/kg).

Results

Result in each treatment groups

Changes in serological levels of each observation in each treatment were found from day 0 to day 14 and day 14 to day 28 (Table 1).

Based on Table 1 and Figure 1 above, it can be seen that in treatment group P1 (red yeast rice 25 mg/kg) the IL-6 level experienced a significant increase ($p \leq 0.001$) between day 0–14, then there was a statistically significant decrease ($p \leq 0.001$) on days 14–28. Treatment group P1 (red yeast rice 25 mg/kg) VEGF level experienced a significant increase ($p \leq 0.001$) between days 0-14, then a statistically significant decrease ($p=0.001$) on days 14–28. Treatment group P1 (red yeast rice 25 mg/kg) BALP level experienced a significant increase ($p \leq 0.001$) between days 0–14, then there was a statistically significant decrease ($p \leq 0.001$) on days 14–28. Treatment group P1 (red yeast rice 25 mg/kg) N-mid-OC level experienced a significant increase ($p \leq 0.001$) between days 0–14, then a statistically significant decrease ($p \leq 0.001$) on days 14–28. Treatment group P1 (red yeast rice 25 mg/kg) BMP2 level experienced a significant decrease ($p \leq 0.001$) between days 0–14, then there was a statistically significant increase ($p \leq 0.001$) on days 14–28.

Based on the Table 2 and Figure 2 below, it can be seen that the treatment group P2 (red yeast rice 50 mg/kg) The IL-6 level experienced a significant increase ($p \leq 0.001$) between days 0–14, then there was a statistically significant decrease ($p \leq 0.001$) on day 14–28. Treatment group P2 (red yeast rice 50 mg/kg) VEGF level experienced a significant increase ($p \leq 0.001$) between days 0–14, then a statistically significant decrease ($p \leq 0.001$) on days 14–28. Treatment group P2 (red yeast rice 50 mg/kg) BALP level experienced a significant increase ($p \leq 0.001$) between days 0–14, then there was a statistically significant decrease ($p \leq 0.001$) on days 14–28. The P2 group (red yeast rice 50 mg/kg) N-mid-OC level

Table 2. Changes in serological levels in the treatment group P2 (red yeast rice 50 mg/Kg).

	Treatment group P2 (red yeast rice 50 mg/kg) (*mean*)				
	IL-6	**VEGF**	**BALP**	**N mid OC**	**BMP2**
Day 0	27.94	22.35	0.94	6.15	12.70
Day 14	80.14	35.28	15.08	13.07	5.95
Day 28	49.18	29.85	2.92	7.91	10.01
	p-value				
Day 0–Day 14	<0.001*	<0.001*	<0.001*	0.012*	<0.001*
Day 14–Day 28	<0.001*	<0.001*	<0.001*	0.018*	<0.001*

*Significant p<0.05.

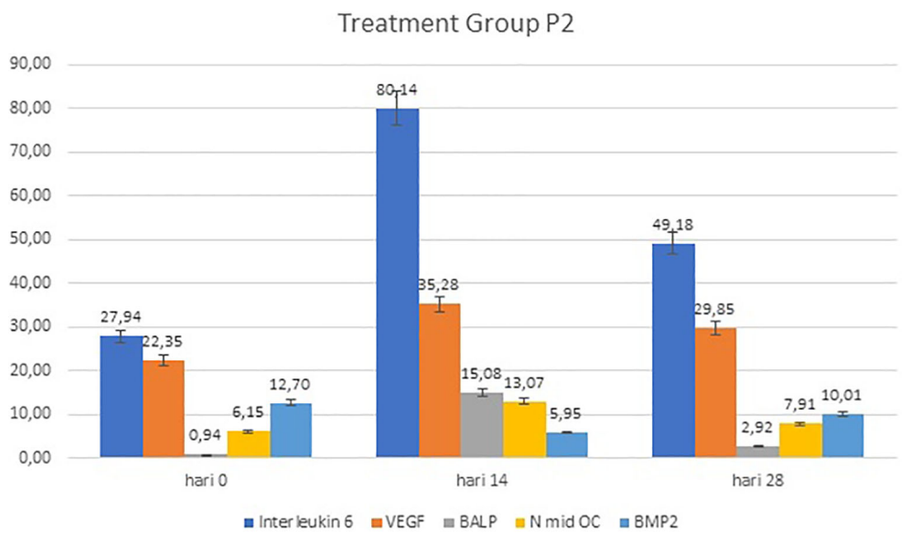

Figure 2. Histogram of changes in serological levels in treatment group P2 (red yeast rice 50 mg/kg).

Table 3. Changes in serological levels in the treatment group P3 (red yeast rice 100 mg/kg).

	Treatment group P3 (red yeast rice 100mg/kg) (*mean*)				
	Interleukin 6	**VEGF**	**BALP**	**N mid OC**	**BMP2**
Day 0	28.36	22.06	0.92	6.11	12.67
Day 14	80.04	35.25	14.74	13.14	5.92
Day 28	32.75	25.47	1.57	6.82	11.35
	p-value				
Day 0–Day14	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Day 14–Day 28	<0.001*	<0.001*	<0.001*	0.001*	<0.001*

*Significant p<0.05.

experienced a significant increase (p=0.012) between days 0–14, then a statistically significant decrease (p=0.018) on days 14–28. The P2 group (red yeast rice 50 mg/kg) BMP2 level experienced a significant decrease ($p \leq 0.001$) between days 0–14, then there was a statistically significant increase ($p \leq 0.001$) on days 14–28.

Based on the Table 3 and Figure 3 above, it can be seen that the treatment group P3 (red yeast rice 100 mg/kg) The IL-6 level experienced a significant increase ($p \leq 0.001$) between days 0–14, then there was a statistically significant decrease

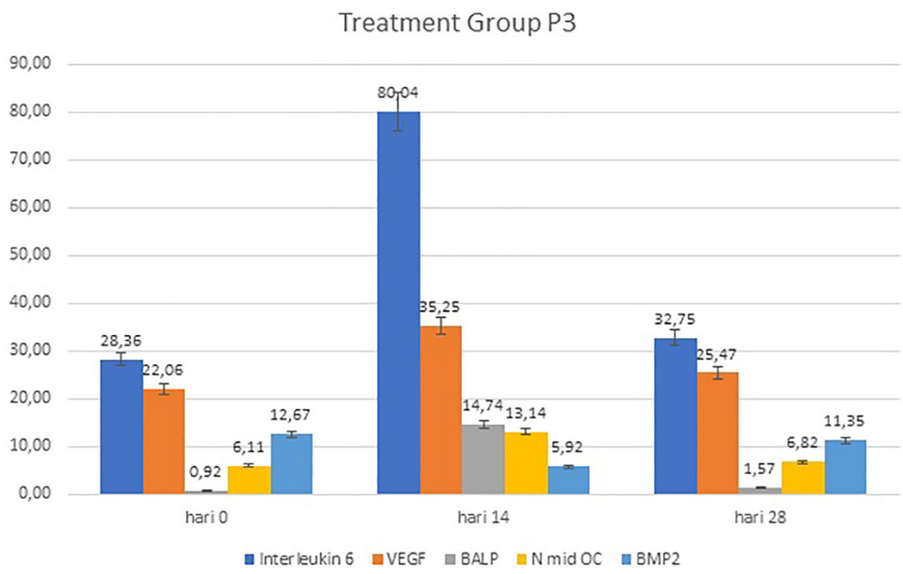

Figure 3. Histogram of changes in serological levels in treatment group P3 (red yeast rice 100 mg/kg).

($p \leq 0.001$) at day 14–28. Treatment group P3 (red yeast rice 100 mg/kg) VEGF level experienced a significant increase ($p \leq 0.001$) between days 0–14, then a statistically significant decrease ($p \leq 0.001$) on days 14–28. Treatment group P3 (red yeast rice 100 mg/kg) BALP level experienced a significant increase ($p \leq 0.001$) between days 0–14, then there was a statistically significant decrease ($p \leq 0.001$) on days 14–28. Treatment group P3 (red yeast rice 100 mg/kg) N-mid-OC level experienced a significant increase ($p \leq 0.001$) between days 0–14, then there was a statistically significant decrease ($p=0.001$) on days 14–28. Treatment group P3 (red yeast rice 100 mg/kg) BMP2 level experienced a significant decrease ($p \leq 0.001$) between days 0–14, then there was a statistically significant increase ($p \leq 0.001$) on days 14–28. The treatment group P3 (red yeast rice 100 mg/kg).

Discussion

From the experiment of the animal population sample rats that as many as 8 animals/group (3 treatment groups and 1 control group) using a random sampling method. All groups were periosteal damaged which made the model of delayed union. The Sprague Dawley rat as an experimental animal was used because they have been used in several studies so that the required data is easy to obtain, and standard strains with uniform genetic backgrounds are available, that this type of research can produce data with high validity and the treatment can be regulated by researchers (Sastroasmoro and Ismael, 2015). In addition, the stages of fracture healing in rats also resemble humans even though they occur twice as fast, have genetic similarities to humans, have similar lamellar bone architecture, relatively short life cycle with a fast bone turnover rate so that multigenerational research can be carried out, care and maintenance is relatively inexpensive, can adapt in a laboratory environment. Rats are also considered more suitable than four-legged animal species to study femur morphology (Gutierreza et al., 2006). The selection of experimental animals was male rats with the reason to minimize the biased influence of the hormone estrogen on the process of bone remodeling.

Based on a study by Einhorn and Gerstenfeld in 2015, the fracture healing period on day 14 is the peak of cellular proliferation in the intramembranous fracture healing process, as well as bone formation from periosteal osteoprogenitor cells and an increase in cartilage tissue. On day 28, there was a mineralization process, the formation of woven bone, and the change of callus into the lamellar bone by osteoclasts so that there was a combination of calcified cartilage and woven bone and lamellar bone. Due to the calcification process, the cartilage area becomes smaller, and also due to the remodeling process in the callus, the total callus area begins to decrease.

Analysis of changes in serological levels in the treatment group was evaluated. In all treatment groups P1, P2, and P3 all serological levels: IL-6, VEGF, BALP, and N-Mid-Osteocalcin, experienced a significant increase on day 0 to day 14. This is consistent with the previous discussion that the early phase or endochondral ossification of bone grafting requires the role of IL-6 as an inflammatory response, VEGF as growth factor for angiogenesis and osteogenesis, while BALP and N-Mid-Osteocalcin as markers of metabolism and osteoblast activity (Dong et al., 2014; Einhorn and Gerstenfeld, 2015; Cunningham et al., 2017). The administration of red yeast rice in this experimental animal model was

able to significantly increase the serological levels. The BMP-2 which decreased significantly on day 0 to day 14 is in accordance with *in vivo* studies conducted on dogs showing the same decrease in the initial week which is the initial phase of bone grafting (Rady et al., 2020). Furthermore, there was an increase in BMP-2 levels the following week. This happens because the inflammatory process is in accordance with research conducted on previous experimental animals that increased inflammation will reduce BMP-2 levels (Huang et al., 2014). Although theoretically, BMP-2 is indispensable in the early phase of fracture healing (Street et al., 2002), increased levels of TNF-α and IL-1β show a suppressive effect on BMP-2 levels (Huang et al., 2014). Thus, in the early or inflammatory phase, BMP-2 levels will decrease first and the process of osteogenesis in this phase is induced by VEGF (Rady et al., 2020). This indicates that the serological increase is significant because it is evidenced by an increase in callus formation and fracture union.

On day 14 to day 28, there was a significant decrease in levels of IL-6, VEGF, BALP, and N-Mid-Osteocalcin. As explained in the previous discussion, this decrease indicates that the union process has been completed (Dong et al., 2014; Einhorn and Gerstenfeld, 2015; Cunningham et al., 2017). Meanwhile, BMP-2 levels should always increase until the final phase of fracture healing because it is needed in the process of ossification and remodeling (Halloran et al., 2020).

Some limitations of this study is that we did not conduct the histological examination to see directly the union histologically or use immunohistochemistry directly to see the expression of IL-6, BMP-2, VEGF, BALP, and N-Mid-Osteocalcin on the fracture site. However, the union process was not clearly seen by using laboratory markers because the evaluation interval period was longer than the union process provided by the administration of red yeast rice unpredictably faster. Hopefully, this finding can provide the information that red yeast rice is a promising, safe, and effective therapeutic option for delayed union. However, further research on the effect on humans should be conducted in translational studies or further clinical trials.

Conclusion

Red yeast rice can decrease the IL-6 and increase BMP-2, VEGF, BALP, N-Mid-Osteocalcin, and enhance fracture healing in the delayed union Sprague Dawley rat model. However further studies to see the histopathology using immunohistochemistry should be conducted to make sure the expression of IL-6, BMP-2, VEGF, BALP, and N-Mid-Osteocalcin, directly on the fracture site. Moreover, the red yeast rice can be a promising and safe option for treatment option for delayed union fracture cases and can proceed to the next translational study or clinical trial to see the effect on patients.

Author contributions

U.H.N.: research concept, literature search, data analysis, manuscript preparation, drafting the manuscript, reviewing and editing the manuscript; H.: research concept, data analysis, manuscript preparation, reviewing and editing the manuscript; D.I.: literature search, data analysis, manuscript preparation, reviewing and editing the manuscript; A.K.: literature search, data analysis, manuscript preparation, reviewing and editing the manuscript.

Data availability

Underlying data

- Dataset available at: Nefihancoro, Udi Heru, Hartono, Indarto, Dono, & Kurniawan, Aryadi. (2022). Statistic Dataset (SPSS) [Data set]. Zenodo. https://doi.org/10.5281/zenodo.7042279 (Nefihancoro et al., 2022).

Reporting guidelines

- ARRIVE author checklist: Nefihancoro, Udi Heru. (2022). ARRIVE author checklist. https://doi.org/10.5281/zenodo.7042336.

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References

Andrzejowski P, Giannoudis PV: **The 'Diamond Concept' for Long Bone Non - Union Management.** *J. Orthop. Trauma.* 2019; **20**(21): 1–13.

(Litbangkes) Badan Penelitian dan Pengembangan Kesehatan Kementrian Republik Indonesia: *Laporan Nasional RISKESDAS 2018.* Kementrian Kesehatan Republik Indonesia; 2019.

Cunningham BP, Brazina S, Morshed S, *et al.*: **Fracture healing: A review of clinical, imaging and laboratory diagnostic options.** *Injury-international Journal of the Vare of the Injured.* 2017.
Publisher Full Text

Ding ZC, Lin YK, Gan YK, *et al.*: **Molecular Pathogenesis of Fracture Nonunion.** *J. Orthop. Translat.* 2018; **14**: 45–56.
PubMed Abstract | Publisher Full Text

Dong L, Yin H, Wang C, *et al.*: **Effect of the timing of surgery on the fracture healing process and the expression levels of vascular endothelial growth factor and bone morphogenetic protein-2.**

Exp. Ther. Med. 2014; **8**: 595–599.
PubMed Abstract | Publisher Full Text

Einhorn TA, Gerstenfeld LC: **Fracture healing: mechanisms and interventions.** *Nat. Rev. Rheumatol.* 2015; **11**(2): 45–54.

Gutierreza GE, Mundya B, Rossini G, *et al.*: **Red Yeast rice Stimulates bone formation in rats.** *Nutr. Res.* 2006; **26**: 124–129.
Publisher Full Text

Halloran D, Durbano HW, Nohe A: **Bone Morphogenetic Protein-2 in Development and Bone Homeostasis.** *J. Dev. Biol.* 2020; **8**(3): 19. Published 2020 Sep 13.
PubMed Abstract | Publisher Full Text

Hartono SB, Sari Y, Novika RGH, *et al.*: **The Effect of Curcumin and Virgin Coconut Oil Towards Cytokines Levels in COVID-19 Patients at Universitas Sebelas Maret Hospital, Surakarta, Indonesia.** *Pharm. J.* 2022; **14**(1).

Huang RL, Yuan Y, Tu J, *et al.*: **Exaggerated inflammatory environment decreases BMP-2/ACS-induced ectopic bone mass in a rat model: implications for clinical use of BMP-2.** *Osteoarthr. Cartil.* 2014; **22**: 1186–1196.
PubMed Abstract | Publisher Full Text

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.: Conf. Ser.* 2018; **1073**(2018): 042036.
Publisher Full Text

Kokubu T, Hak DJ, Hazelwood SJ, *et al.*: **Development of an atrophic nonunion model and comparison to a closed healing fracture in rat femur.** *J. Orthop. Res.* 2003; **21**: 503–510.

Kostenuik P, Mirza FM: **Fracture Healing Physiology and the Quest for Therapies for Delayed Healing and Nonunion.** *J. Orthop. Res.* 2017; **35**(2): 213–223.
Publisher Full Text

Nefihancoro UH, Hartono, Indarto D, *et al.*: Statistic Dataset (SPSS). [Data set]. *Zenodo.* 2022.
Publisher Full Text

Patel S: **Functional Food Red Yeast Rice (RYR) for Metabolic Syndrome Amelioration: A Review on Pros and Cons.** *World J. Microbiol. Biotechnol.* 2016; **32**(5): 87–91.
PubMed Abstract | Publisher Full Text

Rady AAM, Hamdy SM, Abdel-Hamid MA, *et al.*: **The role of VEGF and BMP-2 in stimulation of bone healing with using hybrid bio-composite scaffolds coated implants in animal model.** *Bull. Natl. Res. Cent.* 2020; **44**(131).

Song J, Luo J, Ma Z, *et al.*: **Quality and Authenticity Control of Functional Red Yeast Rice-A Review.** *Molecules (Basel, Switzerland).* 2019; **24**(10): 1944.
PubMed Abstract | Publisher Full Text

Spatuzza C, Postiglione L, Covelli B, *et al.*: **Effects of berberine and red yeast on proinflammatory cytokines IL-6 and TNF-α in peripheral blood mononuclear cells (PBMCs) of human subjects.** *Front. Pharmacol.* 2014; **5**: 230.

Stewart SK: **Fracture Non-Union: A Review of Clinical Challenges and Future Research Needs.** *Malays. Orthop. J.* 2019; **13**(2): 1–10.

Street J, Bao M, de Guzman L, *et al.*: **Vascular endothelial *growth factor* stimulates bone repair by promoting angiogenesis and bone turnover.** *Proc. Natl. Acad. Sci. U S A.* 2002; **99**(15): 9656–9661.

Tan B: **Guidelines on the Care and Use of Animals for Scientific Purposes.** *Naional Advis. Comm. Lab. Anim.* 2004.
Reference Source

Utvåg SE, Grundnes O, Reikeraos O: **Effects of periosteal stripping on healing of segmental fractures in rats.** *J. Orthop. Trauma.* 1996; **10**(4): 279–284.
PubMed Abstract | Publisher Full Text

Wang YF, Liu WT, Chen CY, *et al.*: **Anti-osteoporosis Activity of Red Yeast Rice Extract on Ovariectomy-induced bone loss in rats.** *Genet. Mol. Res.* 2015; **14**(3): 8137–8146.
PubMed Abstract | Publisher Full Text

Wu B, Huang JF, He BJ, *et al.*: **Promotion of Bone Formation by Red Yeast Rice in Experimental Animals: A Systematic Review and Meta-Analysis.** *Biomed Res. Int.* 2020; **2020**: 7231827–7231828.
PubMed Abstract | Publisher Full Text

Zhang BB, Xing HB, Jiang BJ, *et al.*: **Using millet as substrate for efficient production of monakolin K by solid-state fermentation of Monascus ruber.** *J. Biosci. Bioeng.* 2018; **125**(3): 333–338.
PubMed Abstract | Publisher Full Text

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**: 1–27.

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✗ **K.N.S. Sirajudeen**
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The authors studied the effect of red yeast rice on the different biochemical bone markers in a delayed union fracture animal model. Although they have reported the positive effect of the red yeast rice, there are concern regarding this manuscript in terms of the title, dose of red yeast rice administration, presentation of the results and statistical analysis among groups and the language.

With regards to the title, the authors mentioned as molecular studies of IL-6,BMP-2,VEGF,BALP etc. But I couldn't find any gene expression study results in the manuscript. Only the serum levels of these markers estimated by ELISA technique.

In terms of the dose of red yeast rice used in this study there is no scientific reference for the selected dose of administration.

In the results, there is no mention about how they express the results. Does it mean±SD? Its not clear. The comparison only done within each group with respect to the number of days of experimental period.

To identify the efficacy of the treated doses, the comparison should be done among the various groups studied and state which is the best dose to provide the expected results. The authors didn't compare it with the control animals although they have the control in their experimental design.

Although the authors mentioned as limitation with regards to the histological study, either the x-ray image or histological results are required to prove the delayed union and healing.

Overall English editing of the manuscript is also required.

So, with this current submission, it is not at acceptable scientific standard.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Wound healing

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 24 Mar 2023

Udy Herunefy

Thank you for the review,

We consider its molecular because we studied the level of molecule IL-6, BMP-2, VEGF, and BALP in the serum. We learned that molecular study not only gene expression or nucleic acid. However, we will consider to change our title. Thank you

Actually we have scientific reference for the selected dose of administration and we put on reference. However, we will make it more clear and mention about the scientific reference of our dosing.

Our results are the comparison between groups. We hope you may help us to tell which expression/result that you want us to deliver.

Actually, we have control group, and we will add and mention the control as you wish in our revision. Thank you

Thank you for your review,

We will revise it immediately and we hope you may change your recommendation.

Competing Interests: no competing interest to disclose

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