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Original Research Article

Effects of Vitamin C Supplementation on Histology of Callus Diameter and Osteoblast Number in Male Wistar Rats with Complete Femur Bone Fracture

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Abstract

Background: Although the role of nutrition, especially vitamin C, in bone repair has been widely researched, its specific impact on fracture healing remains unclear with conflicting findings.

Objective: This study aimed to investigate the effects of different doses of vitamin C on callus formation and osteoblast proliferation in a rat femur fracture model.

Methods: A post-test-only control group design was employed in this study, involving 27 male Wistar rats that were randomly divided into three groups. The first and second groups received daily intramuscular injections of vitamin C at doses of 200 mg/kg body weight (BW) and 500 mg/kg BW, respectively, following femur bone fracture and fixation. The control group did not receive vitamin C and underwent no fixation. After 14 days, all rats were euthanized, and their femur bones were histologically examined for callus diameter and osteoblast count.

Results: Vitamin C supplementation significantly increased the callus diameter in rats with complete femoral fractures. Both the 200 mg and 500 mg doses proved effective, demonstrating a clear dose-response relationship. Additionally, vitamin C significantly elevated the number of osteoblasts, which play a crucial role in bone formation. However, there was no statistically significant difference in osteoblast count between the 200 mg and 500 mg doses.

Conclusion: In conclusion, vitamin C supplementation has been shown to positively influence bone fracture healing in rats by promoting an increase in callus diameter and enhancing osteoblast proliferation. This study indicates that vitamin C could serve as a beneficial adjunct therapy for facilitating bone fracture healing, particularly by improving callus formation. Physicians should consider integrating vitamin C into treatment plans for patients with fractures, using doses similar to those applied in this study, adjusted appropriately for human use.

Keywords: *bone fracture healing, callus formation, osteoblast, vitamin C*
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INTRODUCTION

A bone fracture-defined as a disruption in the structural integrity of bone-most commonly results from traumatic events or physical stress that exceeds the bone's capacity to withstand force.[1,2] The ensuing physiological response involves an increased demand for calcium, which may be attributed to the metabolic stress of injury and the requirements of bone repair. Consequently, interventions such as calcium supplementation are recognized for their potential to optimize fracture healing.[3] The natural healing cascade progresses through distinct phases: hematoma formation, cellular proliferation, callus formation, consolidation, and eventual remodeling.[4,5] The initial inflammatory phase of bone healing involves immune cell recruitment, including neutrophils, to the fracture site.

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These cells signal mesenchymal stem cells (MSCs) to differentiate into osteoblasts (responsible for bone formation) and osteoclasts (responsible for bone resorption).[6,7] However, this inflammatory response can also generate reactive oxygen species (ROS) near the fracture.[8,9] Bone fractures are classified as orthopaedic injuries; their global impact is significant, as demonstrated by studies on vertebral and facial fractures. Moreover, these fractures show increased prevalence in specific populations, such as people living with human immunodeficiency virus (HIV).[10–14] In Indonesia, bone fractures constitute a significant public health issue, with a notably high prevalence. Traffic accidents-especially those involving motorcycles and predominantly affecting young men-are the primary cause. The legs and hands are most frequently involved, and data from H. Adam Malik Hospital in Medan indicate a considerable number of femur fractures resulting from traffic incidents.[15,16] The complex process of bone fracture healing involves a coordinated sequence of inflammation, cartilaginous callus formation, calcification, and remodelling, with osteoblasts and osteoclasts playing essential roles in bone formation and resorption.[17,18]

Vitamin C is recognized as a powerful antioxidant that plays a critical role in collagen synthesis, a major component of the extracellular matrix in bones. It enhances trabecular bone formation by influencing osteoblast gene expression and regulating skeletal development.[19] As an antioxidant, vitamin C scavenges free radicals that can adversely affect bone health.[20] Additionally, research indicates that vitamin C can transform mesenchymal stem cells into osteoblasts through various pathways. These include type I collagen synthesis, alpha2- and beta1-integrin interactions, activation of the mitogen-activated protein kinase pathway, and phosphorylation of osteoblast-specific transcription factors.[21] Furthermore, vitamin C serves as an essential cofactor for prolyl and lysyl hydroxylases, which are key enzymes in collagen biosynthesis. The potentially beneficial role of vitamin C in preventing low bone mineral density (BMD) has also been documented.[22]

The study of vitamin C's role in bone healing presents contrasting findings. Sarisözen et al.[23] reported an increase in new bone formation in rats administered vitamin C. Specifically, intraperitoneal injections of high-dose vitamin C (200 mg/kg body weight per day for three consecutive days) prior to and following bone surgery resulted in a significant increase in the formation of new bone tissue during the second and third weeks of healing, as determined by radiological and histological analyses. In contrast, Giordano et al.[24] found no significant differences in the bone healing process between vitamin C-treated and control rats. Their histological and histomorphological analyses indicated that there were no notable differences between the treatment and control groups at any of the three stages of the study. Notably, all experimental animals achieved complete bone union by six weeks post-fracture, suggesting that intraperitoneal vitamin C supplementation does not accelerate the bone consolidation process in the experimental tibia after fracture.

Research on the effects of vitamin C on bone healing has produced inconsistent findings. Further investigation is necessary to elucidate the role of vitamin C in bone fracture healing and to identify the optimal dosage and duration of supplementation. Consequently, this study aims to assess the increase in callus diameter and the number of osteoblasts in the histological analysis of femur bones subjected to complete fractures following vitamin C administration.

MATERIALS AND METHODS

Study Design

This research employs a laboratory experiment utilizing a post-test-only control group design. The study was conducted over a two-month period, from February to March 2024. All treatment activities and the maintenance of experimental animals (rats) were performed at the Integrated Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara. Histopathological examinations, integral to data analysis, were conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. Ethical approval for this study was obtained from the Health Research Ethics Committee at Universitas Sumatera Utara (No: 376/KEPK/USU/2024).

Sampling

The subjects of this study consisted of male Wistar strain white rats (Rattus norvegicus) weighing between 300-400 grams. Test animals were sourced from the Integrated Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. The research sample was randomly selected based on statistical calculations, resulting in a total of 27 rats that met the inclusion and exclusion criteria. These rats were divided into three groups. All subjects underwent thorough medical examinations prior to the experiment. The first and second groups received daily intramuscular injections of vitamin C following femur bone fractures and fixation. In this study, the vitamin C used was a generic 200mg ascorbic acid. The primary distinction between these two groups was the dosage of vitamin C administered: the first group received 200 mg/KgBW, while the second group received 500 mg/KgBW. Fixation commenced one-hour post-fracture and lasted for 14 days. The third group, serving as the control group, did not receive vitamin C injections, nor was fixation performed on their fractured femurs. On the fourteenth day, all rats from the three experimental groups were euthanized, and their femur bones were subjected to histological examination.

Femur Bone Fracturing and Fixation

Prior to surgery, rats were anesthetized using ketamine (40 mg/kg) and midazolam (2 mg/kg) via intramuscular injection. A trichotomy was performed on both femurs. Experimental animals received maintenance fluids in the form of normal saline (NaCl 0.9%) intravenously at a dose of 10 ml/kg/hour. To deepen anesthesia, propofol (5 mg/kg) was administered intravenously, followed by intubation and sedation with isoflurane in 100% oxygen. Five minutes before surgery, tramadol (5 mg/kg) and gentamicin (4 mg/kg) were given

Table 1. Histopathological parameters of the samples

Histological Parameters	Treatment Group	Mean ± SD
Callus Diameter	Control	323.87 ± 113.86
	Vit C 200 mg	874.11 ± 121.81
	Vit C 500 mg	1163.85 ± 160.19
Number of Osteoblasts	Control	30.33 ± 9.73
	Vit C 200 mg	102.56 ± 40.23
	Vit C 500 mg	98.89 ± 35.40

subcutaneously; gentamicin administration continued for five days post-surgery.

After positioning the animal in lateral decubitus using sterile techniques, a 1 cm long incision was made through a lateral approach on the right femur. The muscular septum between the vastus lateralis and biceps was identified, followed by a deeper incision to expose the femoral bone. The femoral bone was completely fractured using a scalpel. The incision was then sutured layer-by-layer with Vicryl 4.0 and silk 4.0 sutures. The procedure concluded with a radiographic examination of the femur. On day 14, animals were injected with ketamine (200 mg/KgBW) before femurs were removed for callus diameter measurement and histopathological analysis to assess callus thickness and osteoblast count.

Histopathology Preparation

Bone tissue sections were fixed using 10% neutral formalin buffer and decalcified for 7-10 days. A longitudinal histological examination was performed using a microtome. Tissue sections were affixed to glass slides and stained with hematoxylin-eosin (H&E) using Hematoxylin Scytek (1L) ® and a 2% weight/volume (w/v) eosin solution (125 ml).

Preparation Staining Procedure

Once flexible, bone tissue was thinly sliced and dehydrated using alcohol (Onemed®) at various concentrations (70%, 80%, 95%, and absolute alcohol). Subsequently, bone tissue was immersed in xylene for clearing before being infiltrated with liquid paraffin to form blocks. These blocks were sectioned using a microtome at a thickness of 5 µm. The thinly sliced bone tissue underwent deparaffinization with xylene followed by rehydration using alcohol. It was then dipped in hematoxylin solution for 15 minutes, followed by acid alcohol to ensure color differentiation without fading. Afterward, it was treated with ammonium and lithium carbonate solutions before being incubated in eosin solution for 2 minutes. The bone tissue preparations were dehydrated again with varying alcohol concentrations before being mounted on cover glass using an entelan adhesive for microscopic examination.

Data Analysis

Data regarding the success of rat samples treated with vitamin C at doses of 200 mg, 500 mg, or without treatment will be statistically analyzed using SPSS® version 15 from IBM®. The analysis will begin with a normality test using the Shapiro-Wilk test to determine data distribution characteristics. If data are normally distributed, ANOVA will be employed to compare average callus diameter, osteoblast count, and histopathological results across the three treatment groups. Conversely, if data are not normally distributed, the Kruskal-Wallis non-parametric test will be utilized. Test results will be deemed significant if the p-value is less than 0.05, indicating notable differences among treatment groups.

RESULTS

This research aims to evaluate the effects of vitamin C on callus diameter formation and osteoblast quantity in histological images of completely fractured femur bones. As shown in Table 1, vitamin C supplementation significantly increased both callus diameter and osteoblast numbers in rats. Notably, rats receiving 200 mg and 500 mg doses of vitamin C demonstrated a marked increase in callus diameter compared to the control group. Additionally, the quantity of osteoblasts—cells responsible for bone formation—was significantly elevated in the vitamin C-treated groups relative to controls.

Table 2. Differences in callus diameter in experimental animals that experienced complete femur bone fracture after treatment

Treatment Group	Mean ± SD	*p-value*
Control	323.87 ± 113.86^{bc}	
Vitamin C 200 mg	874.11 ± 121.81^{ac}	<0.001
Vitamin C 500 mg	1163.85 ± 160.19^{ab}	

The p value for callus diameter was determined based on the results of post hoc analysis. a: significant difference from the control group; b: significant difference from the vitamin C 200 group; c: significant difference from the vitamin C 500 group.

Table 2 illustrates that the callus diameter significantly increased in experimental animals with complete femur bone fractures following treatment with Vitamin C. After a monitoring period of 14 days, the callus diameter was measured at 1163.85 ± 160.19 mm in the vitamin C 500 mg group and 874.11 ± 121.81 mm in the Vitamin C 200 mg group, compared to just 323.87 ± 113.86 mm in the control group. The one-way ANOVA test revealed a statistically significant difference in average callus diameter between the vitamin C groups and the control group after 14 days of monitoring (p-value < 0.001). These findings indicate that administration of Vitamin C at both 200 mg and 500 mg doses significantly enhances callus diameter compared to the control group.

Post-hoc analysis revealed that both treatment groups (200 mg and 500 mg of vitamin C) exhibited significant differences compared to the control group ($p < 0.001$). Furthermore, the group receiving 500 mg of vitamin C showed a significant difference from the group receiving

200 mg. The results illustrated a clear dose-response relationship, indicating that increasing the vitamin C dosage from 200 mg to 500 mg was associated with a more pronounced increase in callus diameter. This finding suggests that higher doses of vitamin C have a stronger effect on enhancing callus formation.

Table 3. Differences in the number of osteoblasts in animals with complete femur bone fracture after treatment

Treatment Group	Mean ± SD	*p-value*
Control	30.33 ± 9.73^{bc}	
Vitamin C 200 mg	102.56 ± 40.23^{a}	<0.001
Vitamin C 500 mg	98.89 ± 35.40^{a}	

The p value for the number of osteoblasts was determined based on the results of post hoc analysis. a: significant difference from the control group; b: significant difference from the vitamin C 200 group; c: significant difference from the vitamin C 500 group.

Table 3 indicates a significant increase in the number of osteoblasts in experimental animals with complete femur bone fractures following treatment with vitamin C. After a 14-day monitoring period, the highest number of osteoblasts was observed in the vitamin C 200 mg group (102.56 ± 40.23), followed by the vitamin C 500 mg group (98.89 ± 35.40) and the control group (30.33 ± 9.73). The results of the one-way ANOVA test revealed a significant difference in the average number of osteoblasts between the vitamin C groups and the control group (p-value <0.001). This study demonstrates that the administration of vitamin C at both 200 mg and 500 mg doses significantly enhances the number of osteoblasts compared to the control group, suggesting that vitamin C stimulates bone formation by increasing the population of bone-forming cells.

Although both doses of vitamin C resulted in an increase in osteoblast numbers, the post hoc test did not indicate a statistically significant difference between the groups receiving 200 mg and 500 mg of vitamin C (p-value >0.05). This finding suggests that both doses were equally effective in promoting osteoblast proliferation.

DISCUSSION

The results of this study indicate that vitamin C supplementation significantly increases callus diameter in experimental animals with femoral fractures. The observed dose-response relationship suggests that vitamin C may serve as a promising therapeutic agent in fracture management. Higher doses appear to meet the increased metabolic demands associated with the bone healing process, thereby enhancing collagen production and improving overall healing outcomes. Additionally, vitamin C offers protection against oxidative damage and supports the health of cells essential for callus formation.[19] This finding aligns with research conducted by Wiyastha[25], which demonstrated that vitamin C administration can increase callus diameter in the femur fractures of white rats subjected to alcohol exposure. According to Sarisözen et al.[23], vitamin C accelerates bone matrix mineralization by promoting collagen synthesis within the bone matrix.

Vitamin C is essential for bone healing and tissue regeneration. It serves as a crucial cofactor in collagen synthesis, which is the primary protein constituting the extracellular matrix and providing the structural framework for bone and connective tissues.[19,26] Specifically, vitamin C is necessary for the hydroxylation of proline and lysine, amino acids integral to collagen's structure.[27] Collagen imparts strength and structural integrity to the callus formed during fracture healing. A deficiency in vitamin C can hinder collagen production, thereby delaying the healing process and reducing callus size.[2,28] In addition to its role in collagen synthesis, vitamin C aids tissue regeneration and repair through its antioxidant properties. It mitigates oxidative damage to cells and tissues, facilitates calcium absorption, and contributes to the synthesis of other bone components like proteoglycans. These actions collectively enhance callus formation and repair damaged bone structures.[26,29]

The results of this study demonstrated that the administration of vitamin C at both 200 mg and 500 mg doses significantly accelerated fracture healing, as evidenced by histological images showing an increased number of osteoblasts compared to the control group. The selection of these two dosages was based on prior literature.[23,30–33] Additionally, a notable difference was observed in the mean increase in osteoblast numbers between the vitamin C-treated groups and the control after a 14-day monitoring period. This indicates that vitamin C supplementation substantially enhances osteoblast proliferation in experimental animals with femoral fractures, underscoring its critical role in bone formation and fracture healing.

Vitamin C is essential for osteoblast differentiation and bone formation through various mechanisms. It upregulates EB1, a microtubule-binding protein that stabilizes β-catenin, thereby promoting osteoblast differentiation.[34] Furthermore, vitamin C activates the Wnt/β-catenin/ATF4 signaling pathway, which enhances osteoblastogenesis while simultaneously inhibiting osteoclastogenesis.[35] It also modulates the expression of bone matrix genes in osteoblasts and positively influences trabecular bone formation.[19] Additionally, vitamin C exerts epigenetic control over osteogenesis by influencing chromatin accessibility and DNA hydroxymethylation near bone-specific genes, which are vital for osteoblast differentiation and bone formation.[36] Vitamin C plays a vital role in the healing of connective tissue as a cofactor for the enzymes prolyl hydroxylase and lysyl hydroxylase.[37] These enzymes are crucial for the hydroxylation of proline and lysine residues in procollagen, which is essential for the formation of collagen's stable triple helix structure. In addition to its significance in collagen production, vitamin C serves as a powerful antioxidant, neutralizing harmful ROS that can lead to cellular apoptosis during inflammation.[38] Furthermore, vitamin C has been shown to mobilize tendon-derived stem cells, stimulate the growth and differentiation of osteoblasts, and enhance fibroblast activity.[37,39]

An imbalance between ROS and antioxidants is recognized as oxidative stress, which creates an unfavorable environment for healing. This condition adversely affects the viability and growth of cells responsible for collagen production, ultimately leading to programmed cell death.[40] Vitamin C, functioning as an antioxidant, can neutralize ROS through redox reactions,

thereby alleviating oxidative stress associated with inflammation. Research conducted prior to clinical trials focusing on oxidative stress has shown that vitamin C effectively mitigates such stress following injury. This is accomplished by reducing ROS generated from both internal and external sources. The beneficial effects of vitamin C are evidenced by improvements in the structural composition of bones, tendons, and ligaments.[22,26]

The results indicated that vitamin C supplementation at doses of 200 and 500 mg/kg significantly accelerated the fracture repair process in an experimental animal model. This was evidenced by an increase in callus diameter and osteoblast density observed histologically. Notably, the 200 mg/kg dose demonstrated considerable effectiveness in mediating the fracture healing process, suggesting it could serve as a foundational dose for future human clinical trials. The role of experimental animal models in preclinical pharmacotherapy is crucial, particularly for determining initial dosing parameters. A body surface area-based scalometric approach may be a valuable tool for dose extrapolation between species.[41] Animal studies represent an essential step in the development of new drugs, including vitamin C; however, converting doses from animals to humans presents numerous challenges. Research indicates that the dose of vitamin C derived from animal studies—32.4 mg/kg body weight—should only be regarded as an initial reference point. The physiological and metabolic differences between species complicate direct applicability of this dose conversion to humans.[42,43] To determine the equivalent human dosage, the literature recommends using a conversion formula. For example, assuming an average human body weight of 60 kg and a body surface area of 1.62 m², the K_m factor for humans is calculated by dividing 60 by 1.62, resulting in a value of approximately 37 mg/m².[42] The recommended upper intake limit for adults is ≤2,000 mg (2 grams) per day to avoid these side effects.[44]

CONCLUSION

Vitamin C appears to enhance fracture healing in animal models by improving callus formation and osteoblast activity. While our study shows significant effects at 200 and 500 mg/kgBW in rats, further research is needed to confirm these benefits and determine optimal dosage for human use, as well as to fully understand the underlying mechanisms.

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REFERENCES

1. Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. Nat Rev Rheumatol. 2015 Jan 30;11(1):45–54. https://doi.org/10.1038/nrrheum.2014.164
2. Sheen JR, Mabrouk A, Garla V V. Fracture healing overview. Treasure Island (FL): StatPearls Publishing; 2023.
3. Blom A, Warwick D, Whitehouse M, editors. Apley & Solomon's System of Orthopaedics and Trauma 10th Edition. 10th ed. Florida: CRC Press; 2017.
4. Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: The cellular picture. Semin Cell Dev Biol. 2008 Oct;19(5):459–66. https://doi.org/10.1016/j.semcdb.2008.07.004
5. Szczęsny G. Fracture healing and its disturbances. A literature review. Ortop Traumatol Rehabil. 2015 Oct 16;17(5):437–54. https://doi.org/10.5604/15093492.1186809
6. Su P, Tian Y, Yang C, Ma X, Wang X, Pei J, et al. Mesenchymal stem cell migration during bone formation and bone diseases therapy. Int J Mol Sci [Internet]. 2018 Aug 9;19(8):2343. Available from: https://www.mdpi.com/1422-0067/19/8/2343 https://doi.org/10.3390/ijms19082343
7. Gori JL, Butler JM, Chan YY, Chandrasekaran D, Poulos MG, Ginsberg M, et al. Vascular niche promotes hematopoietic multipotent progenitor formation from pluripotent stem cells. J Clin Invest [Internet]. 2015 Mar 2;125(3):1243–54. Available from: http://www.jci.org/articles/view/79328 https://doi.org/10.1172/jci79328
8. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive Oxygen Species in inflammation and tissue injury. Antioxid Redox Signal. 2014 Mar;20(7):1126–67. https://doi.org/10.1089/ars.2012.5149
9. Nugroho F, Prasetyo A, Hasan M. Analisis jumlah sel osteoblas pada fraktur femur tikus wistar jantan yang diberi ekstrak etanol daun bayam merah (Amaranthus tricolor L.). J Agromediciine Med Sci. 2019;5(1):45–9.
10. Cordero DM, Miclau TA, Paul A V., Morshed S, Miclau T, Martin C, et al. The global burden of musculoskeletal injury in low and lower-middle income countries. OTA Int Open Access J Orthop Trauma. 2020 Jun;3(2):e062. https://doi.org/10.1097/oi9.0000000000000062
11. Dong Y, Peng R, Kang H, Song K, Guo Q, Zhao H, et al. Global incidence, prevalence, and disability of vertebral fractures: a systematic analysis of the global burden of disease study 2019. Spine J. 2022 May;22(5):857–68. https://doi.org/10.1016/j.spinee.2021.12.007
12. 12. Frankel VH, Kaplan DJ, Egol KA. Biomechanics of fractures. J Orthop Trauma. 2016 Aug;30(2):S2–6. https://doi.org/10.1097/bot.0000000000000579

13. Lalloo R, Lucchesi LR, Bisignano C, Castle CD, Dingels Z V, Fox JT, et al. Epidemiology of facial fractures: incidence, prevalence and years lived with disability estimates from the Global Burden of Disease 2017 study. Inj Prev. 2020 Oct;26(Suppl 2):i27–35. https://doi.org/10.1136/injuryprev-2019-043297
14. Pramukti I, Lindayani L, Chen YC, Yeh CY, Tai TW, Fetzer S, et al. Bone fracture among people living with HIV: A systematic review and meta-regression of prevalence, incidence, and risk factors. Blank RD, editor. PLoS One. 2020 Jun 4;15(6):e0233501. https://doi.org/10.1371/journal.pone.0233501
15. Sembiring TE, Rahmadhany H. Karakteristik penderita fraktur femur akibat kecelakaan lalu lintas di RSUP Haji Adam Malik Medan pada tahun 2016-2018. Ibnu Sina J Kedokt dan Kesehat. 2022 Jan 1;21(1):123–8. https://doi.org/10.30743/ibnusina.v21i1.244
16. Kementerian Kesehatan Republik Indonesia. Riset Kesehatan Dasar 2018 (2018 Basic Health Research). Jakarta; 2018.
17. Marsell R, Einhorn TA. The biology of fracture healing. Injury. 2011 Jun;42(6):551–5. https://doi.org/10.1016/j.injury.2011.03.031
18. Sarifah N, Epsilawati L, Azhari A, Satari MH, Priosoeryanto BP, Hatta I, et al. Analysis of osteoblast, osteoclast levels and radiographic patterns in the healing process of bone fractures (preliminary research). J Radiol Dentomaksilofasial Indones. 2021 Dec 31;5(3):106. https://doi.org/10.32793/jrdi.v5i3.740
19. Aghajanian P, Hall S, Wongworawat MD, Mohan S. The roles and mechanisms of actions of vitamin C in bone: New developments. J Bone Miner Res. 2015 Nov 1;30(11):1945–55. https://doi.org/10.1002/jbmr.2709
20. Chin KY, Ima-Nirwana S. Vitamin C and bone health: Evidence from cell, animal and human studies. Curr Drug Targets. 2018 Mar 19;19(5):439–50. https://doi.org/10.2174/1389450116666150907100838
21. Barrios-Garay K, Toledano-Serrabona J, Gay-Escoda C, Sánchez-Garcés MÁ. Clinical effect of vitamin C supplementation on bone healing: A systematic review. Vol. 27, Medicina Oral Patologia Oral y Cirugia Bucal. 2022. p. 205–15. https://doi.org/10.4317/medoral.24944
22. Hung KC, Chiang MH, Wu SC, Chang YJ, Ho CN, Wang LK, et al. A meta-analysis of randomized clinical trials on the impact of oral vitamin C supplementation on first-year outcomes in orthopedic patients. Sci Rep. 2021;11(1):1–10. https://doi.org/10.1038/s41598-021-88864-7
23. Sarisözen B, Durak K, Dinçer G, Bilgen OF. The effects of vitamins E and C on fracture healing in rats. J Int Med Res. 2002;30(3):309–13.
24. Giordano V, Giordano M, Glória RC, de Souza FS, di Tullio P, Lages MM, et al. General principles for treatment of femoral head fractures. J Clin Orthop Trauma. 2019;10(1):155–60. https://doi.org/10.1016/j.jcot.2017.07.013
25. Wiyastha PM. Pemberian vitamin C pada fraktur femur tikus putih yang terpapar alkohol memiliki diameter kalus lebih tebal serta jumlah osteoblas dan ekspresi osteocalcin lebih banyak dibandingkan tanpa pemberian vitamin C. Universitas Udayana; 2016.
26. DePhillipo NN, Aman ZS, Kennedy MI, Begley JP, Moatshe G, LaPrade RF. Efficacy of vitamin C supplementation on collagen synthesis and oxidative stress after musculoskeletal injuries: A systematic review. Orthop J Sport Med. 2018 Oct 25;6(10):232596711880454. https://doi.org/10.1177/2325967118804544
27. Pullar J, Carr A, Vissers M. The Roles of Vitamin C in Skin Health. Nutrients. 2017 Aug 12;9(8):866. https://doi.org/10.3390/nu9080866
28. Besio R, Maruelli S, Battaglia S, Leoni L, Villani S, Layrolle P, et al. Early Fracture Healing is Delayed in the Col1a2+/G610C Osteogenesis Imperfecta Murine Model. Calcif Tissue Int. 2018 Dec 3;103(6):653–62. ttps://doi.org/10.1007/s00223-018-0461-x
29. Gref R, Deloménie C, Maksimenko A, Gouadon E, Percoco G, Lati E, et al. Vitamin C–squalene bioconjugate promotes epidermal thickening and collagen production in human skin. Sci Rep. 2020 Oct 9;10(1):16883. https://doi.org/10.1038/s41598-020-72704-1
30. Barker T, Leonard SW, Hansen J, Trawick RH, Ingram R, Burdett G, et al. Vitamin E and C supplementation does not ameliorate muscle dysfunction after anterior cruciate ligament surgery. Free Radic Biol Med. 2009 Dec;47(11):1611–8. https://doi.org/10.1016/j.freeradbiomed.2009.09.010
31. Duygulu F, Yakan B, Karaoglu S, Kutlubay R, Karahan OI, Ozturk A. The effect of zymosan and the protective effect of various antioxidants on fracture healing in rats. Arch Orthop Trauma Surg. 2007 Sep 17;127(7):493–501. https://doi.org/10.1007/s00402-007-0395-7
32. 32. Ekrol I, Duckworth AD, Ralston SH, Court-Brown CM, McQueen MM. The Influence of Vitamin C on the Outcome of Distal Radial Fractures. J Bone Jt Surg. 2014 Sep 3;96(17):1451–9. https://doi.org/10.2106/jbjs.m.00268
33. Giordano V, Albuquerque RP, do Amaral NP, Chame CC, de Souza F, Apfel MIR. Supplementary vitamin C does not accelerate bone healing in a rat tibia fracture model. Acta Ortop Bras. 2012;20(1):10–2.
34. Pustylnik S, Fiorino C, Nabavi N, Zappitelli T, da Silva R, Aubin JE, et al. EB1 Levels Are Elevated in Ascorbic Acid (AA)-stimulated Osteoblasts and Mediate Cell-Cell Adhesion-induced Osteoblast Differentiation. J Biol Chem. 2013 Jul;288(30):22096–110. https://doi.org/10.1074/jbc.m113.481515

35. Choi HK, Kim GJ, Yoo HS, Song DH, Chung KH, Lee KJ, et al. Vitamin C Activates Osteoblastogenesis and Inhibits Osteoclastogenesis via Wnt/β-Catenin/ATF4 Signaling Pathways. Nutrients. 2019 Feb 27;11(3):506. https://doi.org/10.3390/nu11030506
36. Thaler R, Khani F, Sturmlechner I, Dehghani SS, Denbeigh JM, Zhou X, et al. Vitamin C epigenetically controls osteogenesis and bone mineralization. Nat Commun. 2022 Oct 6;13(1):5883. https://doi.org/10.1038/s41467-022-32915-8
37. D'Aniello C, Cermola F, Patriarca EJ, Minchiotti G. Vitamin C in stem cell biology: Impact on extracellular matrix homeostasis and epigenetics. Stem Cells Int. 2017;2017:1–16. https://doi.org/10.1155/2017/8936156
38. Katariya C, Jayakumar ND. Evaluation of vitamin C as an adjunct to periodontal therapy. Int J Health Sci (Qassim). 2022 Aug 11;7842–62. https://doi.org/10.53730/ijhs.v6ns5.11696
39. Hadzir SN, Ibrahim SN, Abdul Wahab RM, Zainol Abidin IZ, Senafi S, Ariffin ZZ, et al. Ascorbic acid induces osteoblast differentiation of human suspension mononuclear cells. Cytotherapy. 2014 May;16(5):674–82. https://doi.org/10.1016/j.jcyt.2013.07.013
40. Ahmed OM. Relationships between oxidative stress, cancer development and therapeutic interventions. J Cancer Sci Res. 2018;2(1). https://doi.org/10.4172/2576-1447.1000e104
41. Jacob S, Nair AB, Morsy MA. Dose conversion between animals and humans: A practical solution. Indian J Pharm Educ Res. 2022 Jun 30;56(3):600–7. https://doi.org/10.5530/ijper.56.3.108
42. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27. https://doi.org/10.4103/0976-0105.177703
43. Hickey AJ, Maloney SE, Kuehl PJ, Phillips JE, Wolff RK. Practical Considerations in Dose Extrapolation from Animals to Humans. J Aerosol Med Pulm Drug Deliv. 2024 Apr 1;37(2):77–89. https://doi.org/10.1089/jamp.2023.0041
44. Hathcock JN, Azzi A, Blumberg J, Bray T, Dickinson A, Frei B, et al. Vitamins E and C are safe across a broad range of intakes1,2. Am J Clin Nutr. 2005 Apr;81(4):736–45. https://doi.org/10.1093/ajcn/81.4.736