JOURNAL LA MEDIHEALTICO

VOL. 05, ISSUE 01 (056-060), 2024
DOI: 10.37899/journallamedihealtico.v5i1.1049

Aloin Extract on Histopathological Features of Osteogenesis in Fractures of Rattus Norvegicus Wistar Strains

Dessy Abdullah[1], Nadilla Nurrifdah[1*], Budi Yulhasfi Febrianto[1], Ade Teti Vani[1], Nadia Purnama Dewi[1]

[1]*Faculty of Medicine, Baiturrahmah University, Padang*

**Corresponding Author: Nadilla Nurrifdah*

Article Info

Article history:
Received 24 December 2023
Received in revised form 15 January 2024
Accepted 30 January 2024

Keywords:
Aloin Extract
Features of Osteogenesis
Fractures Rattus Norvegicus
Wistar Strains

Abstract

The process of bone repair after trauma or fracture will involve cells, signalling molecules, and processes that have been active during the remodelling process. The new study found that Aloin can promote the expression of osteogenic differentiation regulators, such as Bmp-1 (Bone morphogenetic protein 1) and Runx1 (Runt-related transcription factor 1) in MC3T3–E1 cells, where Runx1 is a transcription factor in osteogenic differentiation—knowing and analyzing aloin extract on the histopathological picture of osteogenesis in Wistar strain rattus norvegicus fractures. This type of research is a laboratory experimental test with a post-test-only control group design. The population number will be divided into four groups, namely positive control group (+), negative control (-), treatment group 1, and treatment group 1, with 14 samples using the Purposive Sampling technique. Univariate data analysis is presented as mean and standard deviation and one-way ANOVA statistical test. Data processing using computerized IBM version 15.0 of the SPSS program. Histopathological picture of osteogenesis in fractures of male rats (Rattus norvegicus) based on osteoblast values at least at K(-), which is 30.64, and K(+), which is 31.90. In the P1 group, the highest average osteoblast value was 39.58, and P1's was 38.14. The osteoclast value is obtained at least at K(-) and 8, and K(+) is 19.01. In the P1 group, the highest average osteoclast value was 16.73, and P1 was 11.70. Aloin extract did not affect the histopathological picture of osteogenesis in fractures of male rats (Rattus norvegicus) with a sig of >0.05.

Introduction

Bones are an essential part of the human body. Bones have various uses, especially supporting tools for body movement, forming a skeleton to look proportional, a source for mineral accumulation, and a protective agent for vital internal organs (Amelia, 2018; Busman & Novera, 2019; Evelyn, 2009). An imbalance between bone resorption and formation in the remodelling process will decrease bone density, which can lead to metabolic disease or involve elements of fragility, thereby triggering fracture incidents. This condition can occur due to various factors, ranging from accidents, repeated stress, and other pathological conditions. The process of bone repair after trauma or fracture involves the role of cells, signalling molecules, and active processes during the bone remodelling process (Satiti et al., 2020). The remodelling process is estimated to occur within three months (Mescher, 2011).

There is one plant that is quite familiar, namely Aloe vera, which has many benefits, such as as an antibiotic, anti-inflammatory, anti-fungal function and as an ingredient which is a biogenic stimulator to stimulate bone graft to accelerate bone growth (Kresnoadi et al., 2014; Walsh, 2018). Commercially, Aloin is often known as barbaloin, which is an anthraquinone

glycoside compound with a bitter brownish-yellow colour from aloe vera extract or what is known as the aloe vera plant. Aloin has a typical glycoside group arranged in a chain, namely carbon hydroxybenzene, with the molecular formula C12H22O9. The hydroxyl group found in phenolic compounds has distinctive properties, including anti-bacterial, viral, and anti-carcinogenic (Purba, 2020).

The new study found that Aloin can promote the expression of osteogenic differentiation regulators, such as Bmp-1 and Runx1, in MC3T3 –E1 cells, where Runx1 is a transcription factor in osteogenic differentiation (Li et al., 2019; Septiani et al., 2020). This research aims to understand whether aloin extract affects the histopathological features of osteogenesis in fractures of male rats (Rattus norvegicus).

Methods

This research refers to laboratory experimental testing along with a post-test-only control group design research design scheme. Meanwhile, the specific animal involved in the testing process is the male Rattus norvegicus, carried out in the pharmacology laboratory at the Faculty of Pharmacy, Andalas University. The tests on animals involved reached 24 animals, with each group having six mice in a fractured condition, with an age range of 2 to 3 months and a body weight of around 150-250 grams.

Data Collection

Making Aloin Extract Preparations

Making this preparation involves peeling, washing and cutting the aloe vera gel. This process involves a unique freeze-drying technique to change the sample's shape to the powder's specifications. The samples involved underwent a dilution process where the ratio was 1:1, namely 0.1 for acetic acid in methanol. Then, it will be centrifuged for 3 minutes at 5000 rpm. The sample will also go through an injection stage, which refers to HPLC, in which there is an element of mobile phase involvement of 0.1 acetic acid in water that has passed the filtration action of a 0.2µm nylon filter.

Grouping Test Animals

Test animals will be grouped into four groups, each with six male Rattus norvegicus. Then, the first group refers to the control group (-), and the second group refers to the control group (+), namely by administering 500mg calcium lactate, treatment group 1 (40% aloin extract), and treatment 2 (80% aloin extract). The preparation is given orally for one week, and then the bone will be taken to make a histology preparation to see osteoblasts and osteoclasts.

Data Analysis

Data analysis in this research was carried out using statistical tests involving the help of software called SPSS. Further initial testing was attempted by applying a normality test, which at this stage involved the Shapiro-Wilk test to find results quickly. Next, a data homogeneity test was carried out. After the data was proven to be homogeneous with a value of sig>0.05, meaning it was proven to be homogeneous in all groups, then one-way ANOVA testing was carried out to see the effect of aloin extract on the histopathological picture of osteogenesis in fractures of male rats (Rattus norvegicus). The test will be meaningful if the sig is >0.05.

Result and Discussion

Respondent's Characteristics

Histopathological Picture of Osteoblasts in Fractures that Have Been Given Aloin Extract

Rattus norvegicus fractures are based on the lowest osteoblast value at K(-) 30.64 and K(+) 31.90. In group P1, the mean osteoblast value was 39.58, and P2's was 38.24.

Table 1. Average histopathological features of osteogenesis in fractures of male rats (*Rattus Norvegicus*) after being given aloin extract

Group	Osteoblasts	
	Mean	Std. Deviation
K (-) Negating control	30.64	8.00
K (+) Positive Control	31.90	19.01
P1 (Concentration 40%)	39.58	12.70
P2 (Concentration 80%)	38.24	16.73

During fracture healing, bone remodelling occurs, where the old or damaged bone is removed by osteoclasts and replaced with new bone formed by osteoblasts (Zhu et al., 2019). This aligns with research conducted by Machmud et al. (2019 The study's results found an increase in the number of osteoblast cells in both the treatment and control groups, but the value in the treatment group was more excellent (Machmud et al., 2019).

Histopathological Features of Ostoeclasts in Fractures that Have Been Given Aloin Extract

Table 2 shows the histopathological picture of osteogenesis in fractures of male rats (*Rattus Norvegicus*) based on the osteoclast value obtained at least at K(-) 0.96 and the value at K(+) 1.32. In the P1 group, the highest mean osteoclast value was 1.24, and P1 was 1.08.

Table 2. Average histopathological features of osteogenesis in fractures of male rats (Rattus norvegicus) after being given aloin extract.

Group	Osteoclasts	
	Mean	Std. Deviation
K (-)	0.96	,522
K(+)	1.32	1.00
P1	1.24	0.75
P2	1.08	0.59

Aloin is a potential antioxidant, where aloin extract has an inhibition level of 11.1 ± 0.55, higher than the inhibition level of vitamin C, namely 23.7 ± 0.50. The aloin content of aloe vera gel is 0.099 mg - 3.1 mg per 100 g in fresh conditions (Septiani et al., 2020). The research showed that the mean number of osteoclasts was lower in the treatment group compared to the control group. This shows an increase in the number of osteoblasts and suppression of the number of osteoclasts after being given aloin extract. Osteoclasts, bone-resorbing cells, originate from hematopoietic stem cells (HSCs) and degrade bone through the secretion of acids and proteolytic enzymes, such as cathepsin K (CTSK), which dissolve collagen and others. Matrix proteins during bone resorption (Sánchez et al., 2020).

Several studies found that isolated *Aloe vera compounds aimed at studying potential protective effects on bone pathogenesis.* Aloe-emodin induces chondrogenic differentiation in clonal mouse chondrogenic ATDC5 cells associated with bone formation via BMP-2 and activation of the MAPK signalling pathway. In addition, Aloin has produced benefits in osteoporosis and osteopenia disorders by suppressing receptor activator of NFκB ligand (RankL) induced through NF-κB inhibition in rat RAW 264.7 macrophage cells (Sánchez et al., 2020).

Rattus Norvegicus Fractures

Table 3 presents the results of statistical tests using a specific test called one-way ANOVA. The results were obtained if the sig value was > 0.05. It can be concluded that aloin extract had no effect on the histopathological picture of osteogenesis in fractures of male rats (*Rattus norvegicus*).

Table 3. Statistical results of the effect of aloin extract on the histopathological features of osteogenesis in fractures of male rats (Rattus Norvegicus)

Group	Sig	Sig Limit	Information
Osteoblasts	0.713	0.05	Not significant
Osteoclasts	0.870	0.05	Not significant

It was seen in the research that although there was no significant effect of giving aloin extract on the histopathological osteogenesis in fractures of male rats (*Rattus norvegicus*), by comparison of the average values, it was found that after being given aloin extract, there was an increase in osteoblast levels and a decrease in osteoclast values. This can be caused by Aloin's vitamin C content, which can trigger osteoblast growth. According to Carinci's research, the content of Vit. C shows that it can induce mesenchymal stem cells to transform into osteoblasts. The mechanism by which vitamin C induces osteoblast proliferation is mediated through the synthesis of type I collagen, the interaction between alpha2- and beta1-integrins, activation of the mitogen-activated protein kinase pathway, as well as phosphorylation of osteoblast-specific transcription factors (Carinci et al., 2005).

A recent study demonstrated that osteoblastogenesis and osteoclastogenesis in mouse osteoporosis models are regulated by shared serine/threonine kinase and mitogen-activated protein kinase (MAPK) signalling pathways, which are well handled by vitamin C. By Brzezinska's opinion, vitamin C can stimulate bone formation by inducing the maturity and mineralization phenotype of osteoblasts. The same group has shown that Vitamin C prevents bone resorption by osteoclasts in an inflammatory environment, indicating the reasonable efficiency of vitamin C supplementation as a therapeutic intervention in postmenopausal osteoporosis and bone loss associated with chronic inflammatory conditions. Vitamin C deficiency is also associated with increased expression of tumour necrosis factor ligand superfamily member 11 (RANKL), which acts as a critical inducer of osteoclasts and bone resorption but also as an inflammatory mediator (Brzezińska et al., 2020; Choi et al., 2019).

Conclusion

Based on the results of research on the effect of aloin extract on the histopathological picture of osteogenesis in fractures of male rats (Rattus norvegicus), it can be concluded that it is proven that all groups of data on osteoclasts and osteoblasts are proven to be regular and homogeneous. The one-way ANOVA statistical test is rejected, or aloin extract has no effect on the histopathological picture of osteogenesis in fractures of male rats (Rattus norvegicus). The absence of a significant impact of aloin extract on the histology of fractures could be because oral administration was considered less efficient and effective in influencing osteoblasts and osteoclasts in mice with fractures.

References

Amelia, P. (2018). Buku Ajar Biologi Reproduksi. In *Buku Ajar Biologi Reproduksi*. https://doi.org/10.21070/2018/978-602-5914-12-6

Brzezińska, O., Łukasik, Z., Makowska, J., & Walczak, K. (2020). Role of vitamin C in osteoporosis development and treatment—A literature review. In *Nutrients*. https://doi.org/10.3390/nu12082394

Busman, B., & Novera, Y. N. Y. (2019). Efektifitas Ekstrak Kulit Buah Delima (Punicagranatum) Secara Topikal Terhadap Proses Pembentukan Kembali (Remodelling) Pada Fraktur Tulang Paha Tikus …. *Menara Ilmu*. http://repository.lldikti10.id/id/eprint/122/1/Jurnal efektifitas ekstrak kulit delima pdf.pdf

Carinci, F., Pezzetti, F., Spina, A. M., Palmieri, A., Laino, G., De Rosa, A., Farina, E., Illiano,

F., Stabellini, G., Perrotti, V., & Piattelli, A. (2005). Effect of Vitamin C on pre-osteoblast gene expression. *Archives of Oral Biology*. https://doi.org/10.1016/j.archoralbio.2004.11.006

Choi, H. K., Kim, G. J., Yoo, H. S., Song, D. H., Chung, K. H., Lee, K. J., Koo, Y. T., & An, J. H. (2019). Vitamin C activates osteoblastogenesis and inhibits osteoclastogenesis via Wnt/β-catenin/ATF4 signaling pathways. *Nutrients*. https://doi.org/10.3390/nu11030506

Evelyn, P. C. (2009). Anatomi dan Fisiologi untuk mahasiswa. *Alfabeta: Jakarta*.

Li, P., Kong, J., Chen, Z., Huang, S., Lv, G., Wei, B., Wei, J., Jing, K., Quan, J., & Chu, J. (2019). Aloin promotes osteogenesis of bone-marrow-derived mesenchymal stem cells via the ERK1/2-dependent Runx2 signaling pathway. *Journal of Natural Medicines*. https://doi.org/10.1007/s11418-018-1249-z

Machmud, E., Jubhari, E. H., & Ardiansyah, M. (2019). Histometry analysis of osteoclasts, osteoblasts and fibroblasts in immediate placement implants with addition of aloe vera extract. *Pesquisa Brasileira Em Odontopediatria e Clinica Integrada*. https://doi.org/10.4034/PBOCI.2019.191.74

Mescher, A. L. (2011). Histologi Dasar JUNQUEIRA: Teks & Atlas Edisi 12. In *Suparyanto dan Rosad (2015*.

Purba. (2020). *UJI EFEKTIVITAS PEMBERIAN EKSTRAK GEL LIDAH BUAYA (Aloe vera) 90 % TERHADAP WAKTU PENYEMBUHAN LUKA PASCA ODONTEKTOMI DI*. Universitas Sumatera Utara.

Sánchez, M., González-Burgos, E., Iglesias, I., & Gómez-Serranillos, M. P. (2020). Pharmacological update properties of aloe vera and its major active constituents. In *Molecules*. https://doi.org/10.3390/molecules25061324

Septiani, Fatimah-muis, S., & Anjani, G. (2020). Aktivitas Antioksidan dan Kadar Aloin pada Lidah Buaya. *Jurnal Medika Indonesia*, *1*(2), 17–24.

Kresnoadi, U. Retno, P. Rahayu, & Eha, D. (2014). Aktivitas Expressi Kolagen Ii Dan Osteocalsin Tulang Alveol Akibat Preservasi Soket Pencabutan Gigi Dengan Campuran Aloe Vera Dan Graft 0,5%. *Dentika: Dental Journal*, *18*(1), 80–86. https://doi.org/10.32734/dentika.v18i1.2005

Walsh, J. S. (2018). Normal bone physiology, remodelling and its hormonal regulation. In *Surgery (United Kingdom)*. https://doi.org/10.1016/j.mpsur.2017.10.006

Satiti, W. R. D., Sahputra, R. E., & Silvia, R. (2020). Profil Kejadian Fraktur Humerus Pada Anak Di RSUP Dr. M. Djamil Padang. *Jurnal Ilmu Kesehatan Indonesia*. https://doi.org/10.25077/jikesi.v1i2.54

Zhu, Y., Liu, S., Chen, W., Liu, B., Zhang, F., Lv, H., Ji, C., Zhang, X., & Zhang, Y. (2019). Epidemiology of low-energy lower extremity fracture in Chinese populations aged 50 years and above. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0209203