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THE EFFECT OF *HIBISCUS SABDARIFFA* LINN ON TRABECULAR BONE MICROARCHITECTURE IN AN AGING MODEL DUE TO D-GALACTOSE INDUCTION: A HISTOMORPHOMETRIC STUDY

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Abstract

The aging process can cause an imbalance in bone remodeling and changes in trabecular bone microarchitecture. D-galactose induction is used as an experimental aging model because it can increase oxidative stress, while Hibiscus sabdariffa Linn. contains antioxidant compounds that have the potential to provide a protective effect on bone tissue. This study aims to analyze changes in trabecular bone microarchitecture in aging models due to D-galactose induction and the effect of Hibiscus sabdariffa Linn. This experimental study involved 22 samples divided into young normal (NM; n = 6), old normal (NA; n = 4), aging due to D-galactose induction (AA; n = 6), and aging due to D-galactose induction given by Hibiscus sabdariffa Linn. (AH; n = 6). Femur bone tissue was fixed, decalcified using formic acid, processed into paraffin blocks, and stained with hematoxylin-eosin. Trabecular bone images were taken in five fields of view using a 20× objective lens. Histomorphometric examination was performed to assess trabecular thickness (Tb.Th) and bone volume per tissue volume (BV/TV). Data met the assumptions of normality and homogeneity so they were analyzed using one-way analysis of variance followed by Tukey's post hoc test if there were significant differences. The significance level was set at $p < 0.05$. The mean Tb.Th in the NM, NA, AA, and AH groups were 71.61 ± 15.90 µm; 130.98 ± 11.31 µm; 120.61 ± 37.79 µm; and 100.48 ± 20.92 µm, respectively. There was a significant difference in Tb.Th values between groups with $F(3,18) = 5.975$ and $p = 0.005$. Post hoc test showed a significant difference between the NM and NA groups ($p = 0.008$) and between the NM and AA groups ($p = 0.014$). There was no significant difference between the AA and AH groups ($p = 0.509$). The mean BV/TV in the NM, NA, AA, and AH groups were 55.06 ± 11.06%, respectively.

Keywords: aging;BV/TV;Tb.Th;D-galactose;HibiscussabdariffaLinn.;histomorphometry.

INTRODUCTION

Bone is a dynamic tissue that continuously undergoes remodeling to maintain structural integrity, mineral homeostasis, and mechanical competence. Bone remodeling occurs through the coordinated activity of osteoclasts, which resorb old bone tissue, and osteoblasts, which form new bone matrix. A balance between bone resorption and formation is necessary to maintain bone mass and quality throughout life.[1] The aging process causes progressive changes in the bone microenvironment. These changes include a decrease in the number and function of osteoblasts, increased bone marrow adipogenesis, accumulation of senescent cells, altered immune responses, impaired mitochondrial function, and increased bone resorption activity.[2,3] This imbalance causes the amount of bone formed in each remodeling cycle to be lower than the amount of bone tissue resorbed, resulting in a gradual decrease in bone mass and deterioration of bone microarchitecture. Trabecular bone has a relatively high surface area and metabolic activity, making it sensitive to changes in the remodeling balance. Deterioration of trabecular microarchitecture can include thinning, perforation, changes in orientation and connectivity, and a reduced proportion of bone tissue to total tissue. These changes can reduce the bone's ability to distribute loads and increase its susceptibility to fracture . 2 Oxidative stress is an important mechanism in bone aging. Increased reactive oxygen species can cause mitochondrial damage, inhibit osteoblast differentiation and function, increase apoptosis of bone-forming cells, and amplify osteoclastogenesis signals. Activation of inflammatory pathways and an increased response to receptor

activator of nuclear factor-κB ligand can further shift bone remodeling toward resorption. D-galactose is widely used as an experimental aging model. Chronic and excessive D-galactose administration can increase the formation of advanced glycation end products, AGE-RAGE activation, inflammatory free radical production, mitochondrial dysfunction, and cellular senescence. The D-galactose model has been reported to result in decreased bone density, microarchitectural disruption, decreased biomechanical strength, and changes in bone remodeling markers.[5] Experimental research by Imerb et al. showed that D-galactose administration increased systemic oxidative stress, expression of bone inflammatory mediators, RANKL expression, and bone resorption markers. D-galactose administration also caused a decrease in BV/TV and changes in Tb.Th, which were influenced by the duration of induction and the metabolic state of the experimental animals.[6] This indicates that the effects of D-galactose on bone are complex and can be influenced by dose, duration, age, gender, and biological condition of the subjects. Increased oxidative stress in aging encourages the development of antioxidant-based interventions. One potential plant is Hibiscus sabdariffa Linn or roselle. This plant contains various bioactive compounds, including anthocyanins, flavonoids, phenolic acids, and organic acids. These compounds are known to have antioxidant and anti-inflammatory activities and have the potential to reduce lipid peroxidation and maintain the endogenous antioxidant defense system. [7,8] The antioxidant activity of Hibiscus sabdariffa Linn. can theoretically reduce osteoblast damage due to oxidative stress and inhibit the stimulation of osteoclastogenesis. However, evidence regarding the direct effect of Hibiscus sabdariffa Linn. on trabecular bone microarchitecture in the aging model induced by D-galactose is still limited. Therefore, this study aims to analyze changes in trabecular bone microarchitecture in the aging model induced by D-galactose and evaluate the effect of Hibiscus sabdariffa Linn administration based on Tb.Th and BV/TV parameters.

METHOD

Research design

This study is an experimental laboratory study that evaluates trabecular bone microarchitecture in young normal, old normal, aging due to D-galactose induction, and aging due to D-galactose induction given Hibiscus sabdariffa Linn. The study was conducted at [name of laboratory and institution] in [month and year of study]. All animal husbandry and treatment procedures have obtained approval from the Research Ethics Committee of [name of institution] with ethics approval number [ethics approval number].

Research subjects

The study involved 22 male Sprague-Dawley rats. The animals were housed in cages with controlled environmental conditions, including light-dark cycles, temperature, humidity, access to food, and drinking water, all in accordance with laboratory animal care standards.

The subjects were divided into four groups as follows:

1. The young normal group or NM consists of six animals.
2. The normal old group or NA consisted of four animals.
3. The aging group due to D-galactose or AA induction consisted of six animals.
4. The aging group due to D-galactose induction given Hibiscus sabdariffaLinn or AH consisted of six animals.

The AA group was given D-galactose at a dose of 200 mg/kgBW/day orally for 8 weeks. The AH group received D-galactose induction using the same procedure and was given Hibiscus sabdariffa Linn extract at a dose of 400 mg/kgBW/day for 8 weeks.

Tissue retrieval and fixation

After the treatment period was completed, the experimental animals were euthanized in accordance with ethical procedures for animal research. The femur of the left posterior limb was carefully removed. The attached muscle and soft tissue were removed without damaging the bone structure. The femur samples were placed in a 10% neutral formalin solution to preserve the tissue structure and prevent autolysis. The fixation duration was carried out for [fixation duration] according to the laboratory's standard operating procedures.

Decalcification and paraffin block preparation

After the fixation process was completed, the samples were washed using running water to remove any remaining formalin. The bone tissue was then decalcified using TBD solution for three weeks. The decalcification solution was changed periodically until the bone tissue was soft enough for further processing. After decalcification was completed, the samples were washed to remove any remaining acid solution. The tissue then underwent gradual dehydration using alcohol with increasing concentrations, followed by a clearing process using xylene and paraffin infiltration. The samples were embedded in paraffin blocks with a longitudinal orientation so that the epiphysis and metaphysis of the proximal femur could be observed in the same section plane. The paraffin blocks were serially sectioned using a microtome at a thickness of approximately 5 µm. The tissue sections were placed on glass slides, dried, and prepared for staining.

Hematoxylin-Eosin Staining

Bone tissue preparations were stained using the hematoxylin-eosin method. H&E staining was used to demonstrate the general structure of bone tissue, bone matrix, bone marrow, osteocytes, and trabecular distribution. The use of H&E on decalcified bone tissue allowed for morphological evaluation and histomorphometric analysis of trabecular bone.[9,10] The staining process began with deparaffinization of the preparations using xylene. The preparations were then rehydrated gradually using alcohol at decreasing concentrations until they reached water. The preparations were immersed in hematoxylin to stain the nuclei, then washed with running water. Differentiation and bluing processes were carried out according to standard laboratory operating procedures. The slides were then immersed in eosin solution to stain the cytoplasm, extracellular matrix, collagen, and bone tissue. After eosin staining, the slides were dehydrated again using increasing concentrations of alcohol, clarified with xylene, and then covered with mounting medium and a coverslip. All slides were processed using the same steps, reagent concentrations, and staining times to maintain consistency in staining intensity between samples.

Determination of observation area

Observations were made on the trabecular bone in the metaphyseal region of the proximal femur, specifically in the secondary spongiosa area. The observation area was determined approximately 0.46 mm from the epiphyseal plate towards the metaphysis. The primary spongiosa area located directly below the growth plate was not included in the analysis because it still contains primary trabeculae and remnants of growth cartilage. The region of interest was determined at an area relatively equidistant from the medial and lateral cortex. The cortical area, epiphyseal plate, tissue folds, slide tears, uneven staining, and processing artifacts were not included in the analysis. Consistency in location determination, staining, and measurement methods is necessary to minimize variation in histomorphometric results.[9]

Microscopic image capture

The preparations were observed using an Olympus light microscope equipped with a digital camera. Initial observations were made at low magnification to identify the epiphyseal plate, proximal metaphysis, cortex, and secondary spongiosa area. After the observation area was determined, images of the trabecular network were taken using a 20x objective lens.1⁰ Five fields of view were taken from each sample. Fields of view were systematically selected in the medial, mediocentral, central, laterocentral, and lateral areas of the secondary spongiosa. The five fields of view did not overlap. The lighting, contrast, focus, image size, and camera configuration settings were maintained the same for all samples. Images were saved in TIFF format or uncompressed digital format to maintain the quality of the tissue structure. Before measurements were taken, images were calibrated using a stage micrometer on a 20x objective lens so that pixel size could be converted to micrometers.

Histomorphometric analysis

Digital images were analyzed using ImageJ software [software version]. Images were converted to 8-bit images, then segmented or thresholded to distinguish trabecular bone tissue from bone marrow and non-bone tissue. Threshold values were applied using a consistent method across all samples.

The parameters analyzed are :

Trabecular thickness

Tb.Th describes the average thickness of the trabecular structure and is expressed in micrometers. Measurements are made on trabeculae within a predetermined region of interest.

Bonevolumepert issue volume

BV/TV describes the proportion of trabecular bone tissue to the total tissue within the region of interest. In two-dimensional image analysis, this value is obtained by comparing the area of trabecular bone to the total tissue area and is expressed as a percentage. Values from five fields of view in each animal were averaged to obtain one Tb.Th value and one BV/TV value for each biological sample. The average per animal was used as the unit of statistical analysis to avoid the assumption that each field of view is an independent sample.

Statistical analysis

Data were analyzed using IBM SPSS Statistics software version [SPSS version]. Numerical data are presented as mean ± standard deviation. Normality was tested using the Shapiro–Wilk test and homogeneity of variance was assessed using Levene's test. Since the data met the assumptions of normality and homogeneity, the mean differences between the four groups were analyzed using one-way ANOVA. If the ANOVA results showed significant differences, the analysis was continued using the Tukey post hoc test to identify different pairs of groups. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Characteristics of the research group

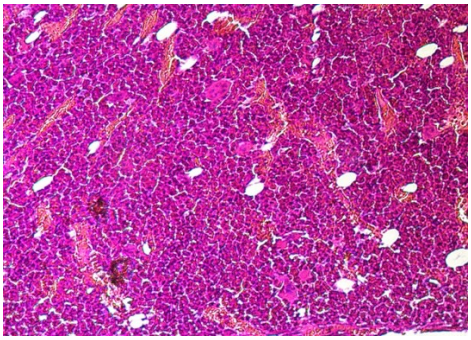
A total of 22 samples were analyzed, consisting of six samples from the young normal group (NM), four samples from the old normal group (NA), six samples from the aging group due to D-galactose induction (AA), and six samples from the aging group due to D-galactose induction given by Hibiscus sabdariffa Linn. (AH).

The Shapiro–Wilk test results showed that the trabecular thickness (Tb.Th) and bone volume per tissue volume (BV/TV) data were normally distributed. Levene's test showed that the data variance between groups was homogeneous. Therefore, the mean differences between groups were analyzed using one-way ANOVA.

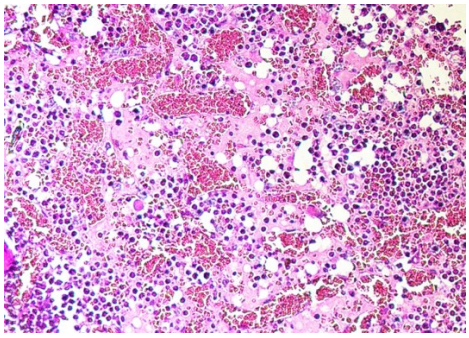
Histological picture of trabecular bone

The histological appearance of trabecular bone in hematoxylin-eosin staining was observed using a 20× objective lens. Visually, there were variations in the thickness, distribution, and area of the trabecular network among the study groups. The NM group showed a trabecular image with a relatively even distribution. The NA and AA groups showed trabeculae that visually appeared thicker in some areas, while the AH group showed a trabecular network that appeared to fill the observation area more widely than the AA group. However, this visual interpretation must be confirmed through quantitative histomorphometric measurements.

A. NM



B. NA



C. AA

D. AH

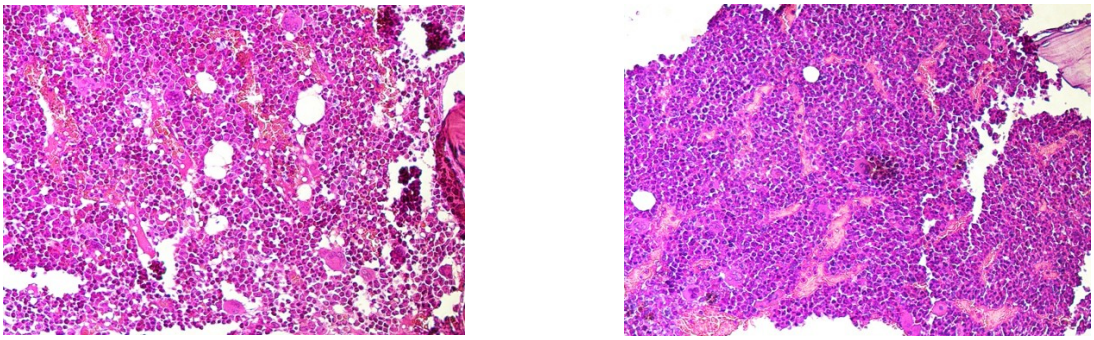


Figure 1. Histological image of the trabecular bone of the femur in each research group with hematoxylin-eosin staining and a 20× objective lens.
(A) Young normal group (NM); (B) old normal group (NA); (C) aging group due to D-galactose induction (AA); and (D) aging group due to D-galactose induction given Hibiscus sabdariffaLinn. (AH). The arrow indicates the trabecular bone tissue. Scale bar = 100 µm.

Trabecular thickness examination results

The NM group had an average Tb.Th of 71.61 ± 15.90 µm. The NA group had an average of 130.98 ± 11.31 µm, the AA group 120.61 ± 37.79 µm, and the AH group 100.48 ± 20.92 µm.

The highest mean Tb.Th was found in the NA group, while the lowest mean was found in the NM group. The results of one-way ANOVA showed a significant difference in Tb.Th values between groups with $F(3,18) = 5.975$ and $p = 0.005$.

Tukey's post hoc test showed a significant difference between the NM and NA groups with $p = 0.008$ and between the NM and AA groups with $p = 0.014$. There was no significant difference between the AA and AH groups with $p = 0.509$. The distribution of the mean Tb.Th values in each group is presented in Figure 2.

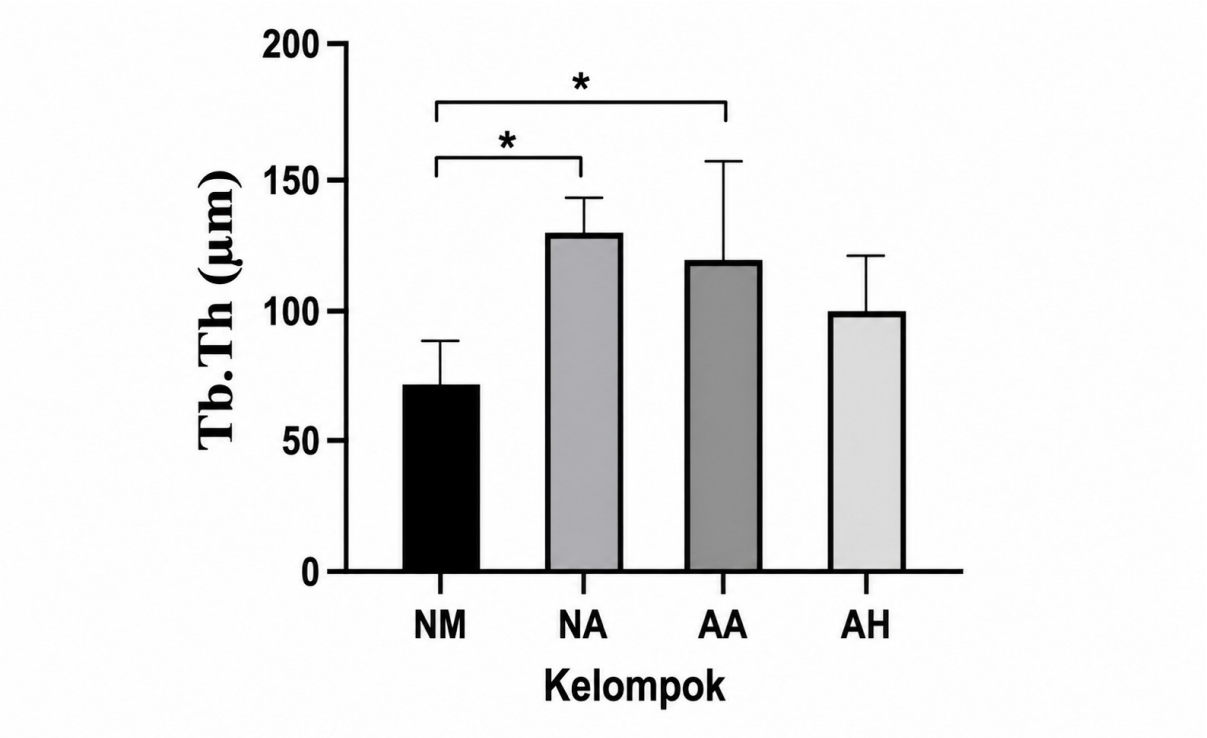


Figure 2. Comparison of average trabecular thickness in the study groups. Data are presented as mean ± standard deviation. NM: young normal; NA: old normal; AA: aging due to D-galactose induction; AH: aging due to D-galactose induction given by Hibiscus salvia Linn. Analysis using one-way ANOVA and Tukey post hoc test. *p <

0.05 compared to the NM group. NM compared to NA: p = 0.008; NM compared to AA: p = 0.014; AA compared to AH: p = 0.509

Table 1. Results of the Tukey *posthoc test* on the Tb.Th parameter

Group comparison	Mean difference (µm)	p value	Interpretation
NM versus NA	−59.37	0.008*	Meaningful
NM vs AA	-49.00	0.014*	Meaningful
AA vs AH	20.13	0.509	Meaningless

*Statistically significant at < 0.05.

Bone volume pert issue volume examination results

The NM group had an average BV/TV of 55.06 ± 11.06%. The NA group had an average of 61.74 ± 11.37%, the AA group 52.37 ± 10.00%, and the AH group 62.95 ± 6.67%.

The lowest mean BV/TV was found in the AA group, while the highest mean was found in the AH group. Although there were differences in descriptive values, the one-way results

ANOVA showed that there was no significant difference in BV/TV values between groups with F(3,18) = 1.542 and p = 0.238. The distribution of the mean BV/TV values in each group is presented in Figure 3.

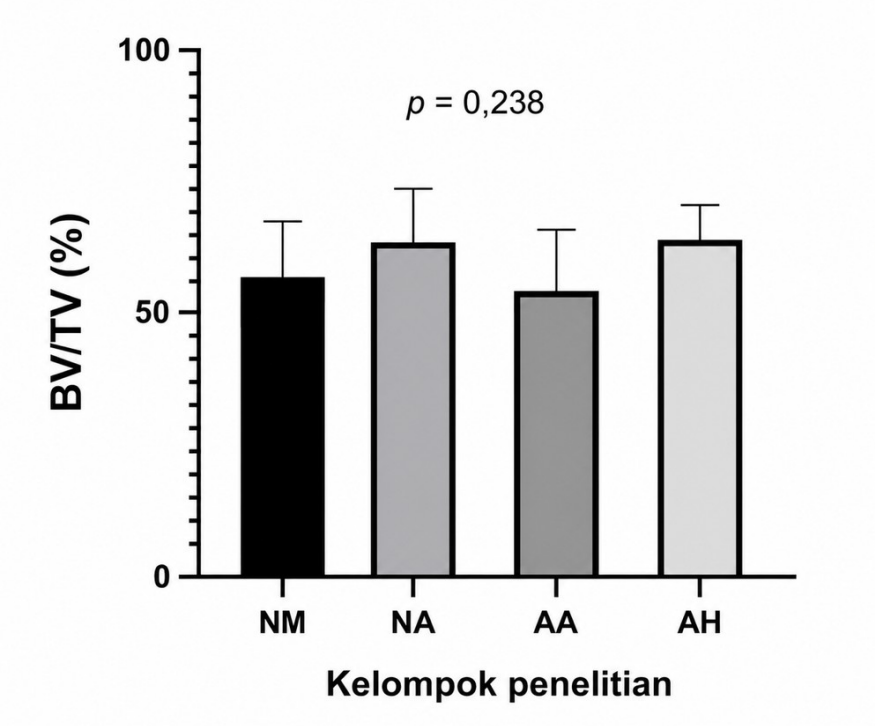


Figure 3. Comparison of mean bone volume per tissue volume in the study groups. Data are presented as mean ± standard deviation. NM: young normal; NA: old normal; AA: aging due to D-galactose induction; AH: aging due to D-galactose induction given by Hibiscus sabdariffa Linn. There was no significant difference between groups based on one-way ANOVA, F(3,18) = 1.542; p = 0.238.

Group	Average BV/TV(%)	Standard deviation
NM	55.06	11.06
NA	61.74	11.37
A A	52.37	10.00
AH	62.95	6.67

Table 2. Results of BV/TV% trabecular bone examination

Parameters	NM (n= 6)	NA(n = 4)	AA(n = 6)	AH(n = 6)	F(3,18)	p
Tb.Th (µm)	71.61 ± 15.90	130.98 ± 11.31	120.61 ± 37.79	100.48 ± 20.92	5.975	0.005*
BV/TV(%)	55.06 ± 11.06	61.74 ± 11.37	52.37 ± 10.00	62.95 ± 6.67	1.542	0.238

Table 3. Results of histomorphometric examination of trabecular bone

Data are presented as mean ± standard deviation. NM: young normal; NA: old normal; AA: aging due to D-galactose induction; AH: aging due to D-galactose induction given by Hibiscus sabdariffa Linn.; Tb.Th: trabecular thickness; BV/TV: bone volume per tissue volume. *Statistically significant at $p < 0.05$.

CONCLUSION

Histological picture of trabecular bone

The histological images in Figure 1 demonstrate variations in trabecular meshwork morphology among the study groups. The NM group exhibited a relatively even distribution of trabeculae, while the NA and AA groups exhibited some trabecular structures that appeared thicker. In the AH group, the trabecular meshwork visually appeared to fill more of the observed area than in the AA group. However, visual assessment cannot be used solely to conclude that microarchitectural improvement has occurred because the two-dimensional histological image is influenced by the section orientation and the location of the visual field.[9]

Changes in trabecular thickness between groups

The results showed significant differences in Tb.Th values between the four groups. The young normal group had the lowest mean Tb.Th, while the old normal group had the highest mean. Significant differences were found between the NM and NA groups and between the NM and AA groups. In general, the aging process is associated with remodeling imbalance, decreased osteoblast function, increased resorptive activity, changes in skeletal stem cell function, and the accumulation of senescent cells.[2,3] These changes can lead to decreased bone mass, perforation and disconnection of trabeculae, increased porosity, and decreased mechanical strength of bone. However, microarchitectural changes due to aging do not always occur uniformly in all parts of the bone.

The higher Tb.Th values in the NA group compared to the NM group indicate that physiological aging in this study did not result in detectable trabecular thinning based on the mean Tb.Th values. However, an increase in Tb.Th values cannot be directly interpreted as an increase in bone quality. Bone remodeling during aging can occur heterogeneously. Thinner and more vulnerable trabeculae may undergo resorption or perforation first. Consequently, trabeculae remaining in the observation area may be dominated by relatively thicker residual structures. This condition can result in an increase in the mean Tb.Th even though the tissue has experienced decreased connectivity or partial loss of trabecular structure Two-dimensional histomorphometric measurements are also influenced by the orientation of the tissue cut. Trabeculae cut transversely, obliquely, or longitudinally can produce different thickness profiles. Furthermore, the location of the region of interest, decalcification stage, slice thickness, staining, threshold determination, and inter-examiner variation can affect measurement results. Therefore, methodological standardization is necessary in histomorphometric interpretation.[9]

The AA group had a mean Tb.Th of 120.61 ± 37.79 µm, significantly higher than the NM group. This finding differs from several D-galactose studies that reported a decrease in Tb.Th. Recent studies on D-galactose-induced osteoporosis models have shown that the effects of D-galactose can vary based on species, sex, age, dose, route of administration, and duration of induction.[5] Imerbetal. reported that D-galactose administration can reduce Tb.Th, BV/TV, and other microarchitectural parameters. The study also showed an increase in oxidative stress, bone inflammation, RANKL expression, and bone resorption.[6] The differences in the results of this study may be related to differences in induction protocols, animal characteristics, anatomical areas analyzed, and histomorphometric measurement methods.

The AA group had a Tb.Th standard deviation of 37.79 µm, which was higher than the other groups. The large standard deviation indicates heterogeneity in the response between samples to D-galactose induction. This variation may stem from differences in biological responses, levels of oxidative stress, imaging position, trabecular structure, or technical variations in image segmentation. Therefore, higher Tb.Th values in the AA group cannot be directly interpreted as better bone condition. Interpretation of Tb.Th requires consideration of BV/TV values and the overall trabecular meshwork distribution. The AH group had a mean Tb.Th of 100.48 ± 20.92 µm, descriptively lower than the AA group of 120.61 ± 37.79 µm. This decrease resulted in a value closer to the NM group. However, the difference between the AA and AH groups was not statistically significant with $p = 0.509$.

These results indicate that the study has not been able to prove the effect of Hibiscus sabdariffa Linn. on trabecular thickness. Descriptive differences cannot be considered an intervention effect if they are not supported by statistical significance because they can be influenced by biological variation and random error.

The change in bone volume per tissue volume between BV/TV groups reflects the proportion of trabecular bone tissue to the total tissue within the analyzed area. This parameter provides information on the amount of trabecular bone tissue that fills a region of interest.[9] The AA group had the lowest mean BV/TV, at 52.37 ± 10.00%. This value was descriptively lower than the NM group at 55.06 ± 11.06%, the NA group at 61.74 ± 11.37%, and the AH group at 62.95 ± 6.67%. The decrease in BV/TV in the AA group is consistent with the hypothesis that D-galactose induction can disrupt bone homeostasis. D-galactose can increase the formation of AGEs, oxidative stress, inflammation, and cellular senescence.[5,6]Increased ROS can inhibit osteoblast differentiation and function and increase the response of osteoclast precursors to lRANKL signals.[4] This condition can shift remodeling towards resorption and reduce the proportion of trabecular bone.

Imerb et al. showed that D-galactose induction caused a decrease in BV/TV, an increase in RANKL expression, an increase in resorption markers, and disruption of bone microarchitecture.[6] These results support the tendency for a decrease in BV/TV in the AA group in this study, although the difference did not reach statistical significance. The AH group had a mean BV/TV of 62.95±6.67%, or 10.58 percentage points higher than the AA group. Descriptively, these results suggest the possibility that administration of Hibiscus sabdariffa Linn. helps maintain the proportion of trabecular bone tissue. However, the ANOVA results showed $p = 0.238$ so the difference cannot be declared statistically significant. Hibiscus sabdariffa Linn contains anthocyanins, flavonoids, phenolic acids, and various organic acids that have antioxidant activity.[7,8] These compounds have the potential to capture free radicals, reduce lipid peroxidation, and maintain the endogenous antioxidant system. Theoretically, reduced oxidative stress may help maintain osteoblast function and suppress the stimulation of osteoclastogenesis.

However, the effects of Hibiscus sabdariffa Linn extract can be influenced by the composition of the plant material, the part of the plant used, the extraction method, the solvent, the temperature, the storage time, the dose, the duration of administration, and the bioavailability of the active compounds.[8] The same extract dose may not necessarily produce the same levels of active compounds if the preparation and standardization processes are different. The absence of significant differences indicates that the antioxidant effects of Hibiscus sabdariffa Linn may not be large enough to produce detectable bone structural changes at the doses and duration of this study. Molecular or biochemical changes may also occur earlier than microarchitectural changes because the bone remodeling process takes a relatively long time.

Relationship between Tb.Th and BV/TV results

The results showed that changes in Tb.Th and BV/TV did not entirely move in the same direction. The AA group had relatively high Tb.Th values but the lowest BV/TV values. A combination of high Tb.Th and low BV/TV can occur if some trabeculae have undergone resorption or perforation, while the remaining trabeculae are relatively thicker. Thus, the tissue can have a lower proportion of bone despite a higher average thickness of the visible structures. This interpretation remains a possibility because this study no longer included trabecular number parameters and did not evaluate trabecular connectivity. Therefore, the results cannot be used to directly conclude that the AA group has fewer trabeculae. The AH group showed lower Tb.Th values but higher BV/TV values compared to the AA group. Descriptively, this pattern may indicate that bone tissue in the AH group filled a larger area of observation, although the individual trabecular profiles measured were not thicker. However, because the intergroup differences were not significant, this interpretation cannot be considered evidence of microarchitectural improvement. Two-dimensional histomorphometry has limitations in depicting the spatial relationships and connectivity of three-dimensional trabecular structures.[9] Micro-CT examination can provide additional information regarding bone volume, thickness, distribution, connectivity, orientation, and separation of trabeculae without being influenced by a single histological plane.

Factors that influence research results

The sample size in this study was relatively limited and unbalanced, especially in the NA group, which consisted of only four samples. A small sample size can reduce statistical power to detect differences, especially when within-group variation is relatively large. The large variation in Tb.Th in the AA group can widen the data distribution, reducing the likelihood of finding a significant difference between the AA and AH groups. Increasing the sample size can provide more stable mean estimates and increase the power of the statistical analysis. The histological process can also influence the results. The use of acid-based decalcifying agents can accelerate the loss of mineral components, but excessively long decalcification duration can potentially affect tissue quality and staining. Block orientation, cutting angle, slice thickness, H&E intensity, field selection, and threshold setting can result in measurement variations. [9,10] The use of five fields aims to obtain a better representation of the trabecular area. However, five fields still depict only a small portion of the overall metaphyseal structure. Therefore, each field should be selected systematically and not based on the area that appears best or most damaged. The dose and duration of Hibiscus sabdariffa Linn administration can also affect the results. The effects of antioxidants at the molecular level do not necessarily immediately result in histologically detectable microarchitectural changes. Evaluation of markers of oxidative stress, inflammation, bone formation, and bone resorption can complement the histomorphometric results.

Research limitations

This study has several limitations. First, the sample size was relatively small and unbalanced between groups. Second, the microarchitectural examination was conducted using two-dimensional histomorphometry, which cannot depict the connectivity and three-dimensional arrangement of trabeculae. Third, the analysis only used Tb.Th and BV/TV parameters, thus not being able to evaluate the overall microarchitectural characteristics. Fourth, the study did not link histomorphometric parameters with bone biomechanical strength or micro-CT examination. Further research is needed with a larger sample size, balanced groups, standardization of extract content, variation in dosage and duration, and micro-CT and biomechanical examination.

CONCLUSION

Physiological aging and D-galactose induction result in microarchitectural changes in trabecular bone. Significant differences were found in Tb.Th parameters, particularly between the young and old normal groups and between the young and aging groups due to D-galactose induction. The D-galactose-induced aging group had the lowest BV/TV values, although the difference in BV/TV between groups was not statistically significant. Administration of Hibiscus sabdariffa Linn resulted in lower Tb.Th values and higher BV/TV descriptively compared to the D-galactose-induced aging group. However, the difference between the AA and AH groups did not reach statistical significance. Therefore, administration of Hibiscus sabdariffa Linn. at the dose and duration of this study has not been proven to significantly improve trabecular bone microarchitecture based on Tb.Th and BV/TV parameters.

REFERENCES

Al-Daghestani H, Qaisar R, Al Kawas S, Ghani N, Rani KGA, Azeem M, et al. Pharmacological inhibition of endoplasmic reticulum stress mitigates osteoporosis in a mouse model of hindlimb suspension. *Sci Rep.* 2024;14:4719. doi:10.1038/s41598-024-54944-7.

Bolamperti S, Villa I, Rubinacci A. Bone remodeling: an operational process ensuring survival and bone mechanical competence. *Bone Res.* 2022;10(1):48. doi:10.1038/s41413-022-00219-8.

Chandra A, Rajawat J. Skeletal aging and osteoporosis: mechanisms and therapeutics. *Int J Mol Sci.* 2021;22(7):3553. doi:10.3390/ijms22073553.

Chavassieux P, Chapurlat R. Interest of bone histomorphometry in bone pathophysiology investigation: foundation, present, and future. *Front Endocrinol (Lausanne).* 2022;13:907914. doi:10.3389/fendo.2022.907914.

Soto *C* , Expósito-Almellón *Foods.* 2023;12(5):963. doi:10.3390/foods12050963.

Iantomasi T, Romagnoli C, Palmini G, Donati S, Falsetti I, Miglietta F, et al. Oxidative stress and inflammation in osteoporosis: molecular mechanisms involved and the relationship with microRNAs. *Int J Mol Sci.* 2023;24(4):3772. doi:10.3390/ijms24043772.

Imerb N, Thonusin C, Pratchayasakul W, Arunsak B, Nawara W, Ongnok B, et al. D-galactose-induced aging aggravates obesity-induced bone dyshomeostasis. *Sci Rep.* 2022;12(1):8580. doi:10.1038/s41598-022-12206-4.

Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, et al. Organic acids from roselle (*Hibiscus sabdariffa* L.): a brief review of its pharmacological effects. *Biomedicines.* 2020;8(5):100. doi:10.3390/biomedicines8050100.
Sfeir JG, Drake MT, Khosla S, Farr JN. Skeletal aging. *Mayo Clinic Proc.* 2022;97(6):1194–1208. doi:10.1016/j.mayocp.2022.03.011.
Wang Y, Goh LL, Chin KY. D-galactose-induced osteoporosis in rodents—development models, skeletal characteristics, and optimal dosing regimens: a scoping review. *Biomol Biomed.* 2026;Advance online publication. doi:10.17305/bb.2026.13914.