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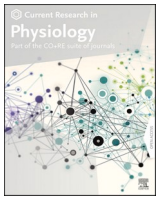

Effects of cosmos caudatus (Kenikir) antioxidant properties on bone metabolism marker in rat

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ABSTRACT

Cosmos caudatus leaves are one of around 7500 types of plants that are known to have herbal or medicinal plant properties in Indonesia. This research determines the effectiveness of Cosmos caudatus as an antioxidant agent against cells, biomolecules, and bone density. Forty-three male rat aged 3–4 months were divided into four groups.Group P0 was only given distilled water. Group P1 was given kenikir leaf extract at a dose of 0.91 mg/kg. Group P2 was given kenikir leaf extract at a dose of 1.82 mg/kg. And group P3 was given kenikir leaf extract at 3.64 mg/kg ad libitum once a day for 28 days. The highest average SOD level was in the 1.82 mg/bb P2 conversion dose group (1.09 ± 1.76). The lowest mean CTX level was in the P2 group (8.30 ± 1.10). There was a significant increase in mean trabecular bone in the P2 group (43.33 ± 5.32). The number of osteoblast cells increased significantly at P2 (103.94 (SD 38.14)). The number of osteoclasts decreased from the control group (P0) to 0.60 (SD 0.43) at P2. Indicate that the Cosmos caudatus extract may have advantages as an antioxidant support agent for bone metabolism.

1. Introduction

Cosmos caudatus leaves are one of around 7500 types of plants that are known to have herbal or medicinal plant properties in Indonesia (Zamroni Salim and Ernawati Munadi, 2017). A study conducted by Mustafa et al. on the potential antioxidant content of 21 plants using the DPPH (2,2-diphenyl-1-picrylhydrazyl) examination method with graphical calculations of the percentage of radical scavenging activity for the extract concentration given 50% inhibitory power (IC 50) states that Cosmos caudatus leaf extract has high antioxidant activity with an IC50 value of 21.3 μg/mL (Mustafa et al., 2010). Another study conducted by Abdul Rahman et al. and Tan K. Lee et al. using the same method also stated the results of high antioxidant activity in Cosmos caudatus leaf extract (24.88 ± 1.06 μg/mL and 22.82 ± 0.05 μg/mL) (Lee and Vairappan, 2011; Abdul Rahman et al., 2017). Antioxidant activity plays a very important role in overcoming free radicals. Antioxidants are able to inactivate the development of oxidation reactions by preventing the formation of free radicals through electron donors, which can have a bad impact on the body (Ardies, 2014)

Research on the properties of Cosmos caudatus leaves has been carried out several times, including stating the benefits of Cosmos caudatus leaf extract as antidiabetic, antioxidant, anti-inflammatory, antibacterial, antifungal, anti-osteoporosis, anti-hyperlipidemic, anticancer, antihypertensive, anti-hepatoprotective, and overcoming fertility problems. The effect of Cosmos caudatus extract on bones was carried out in 2012 and 2013, with the conclusion that administering Cosmos caudatus extract can be an alternative treatment that is quite effective in recovering bone damage that may occur in postmenopausal women. Previous studies done in 2012 and 2013 have shown the effect of Cosmos caudatus extract on bone, the conclusion that the administration of Cosmos caudatus extract can be a fairly effective alternative treatment to restore bone damage that may occur in postmenopausal women. (Mohamed et al., 2012; Mohamed, Chai Mei Yin et al., 2013b; Mohamed, Sahhugi, et al., 2013). Mohamed

Bone regeneration is balanced by combining bone formation and resorption in a continuous remodeling process. Bone regeneration markers are grouped into two categories based on the metabolic phase during their production: bone formation markers and bone resorption markers (Shetty et al., 2016). One of the markers of bone formation is osteocalcin (OC), which is a hydroxyapatite-binding protein that is exclusively synthesized by osteoblasts, odontoblasts, and hypertrophic chondrocytes as a final marker of osteoblastic activity. In the

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reabsorption process, one of the markers according to the recommendations of the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine is serum CTX-1 (sCTX), which is a degradation product of Type 1 bone collagen produced by osteoclasts during bone resorption (Shetty et al., 2016).

Free radicals will affect bone metabolism, creating an imbalance in the matrices that make up bones, which results in bone damage. Superoxide dismutase (SOD) is a potential endogenous antioxidant enzyme in the body, a primary antioxidant that also has an important role in reducing the formation of free radicals. SOD catalyzes the dismutation of superoxide anions into hydrogen peroxide, which is then detoxified into oxygen and water by catalase or glutathione peroxidase with the catalase process. The expression and activity of SOD have a profound influence and response on vascular cells experiencing acute and chronic oxidative stress (Wahjuni, 2015). Research by Sanaz et al. concluded that higher SOD activity in postmenopausal women was associated with a significantly lower risk of osteoporosis (Malekian et al., 2023). Based on data from the Indonesian Ministry of Health, the number of osteoporosis patients in Indonesia is much larger and is the country with the 2nd largest osteoporosis sufferer after China, with a population over 60 years old at higher risk of osteoporosis (Leopoldini et al., 2011; Dirjen Pelayanan Kesehatan, 2022). The number of DXA scans for osteoporosis detection in Indonesia is still very low (<5 million population), so osteoporosis cases are mostly found when the condition is already severe and requires pharmacological treatment with greater costs (Lauralee Sherwood, 2016). Prevention of bone disease will be better done as early as possible by utilizing and optimising natural resources, one of which is the use of Cosmos caudatus leaves.

This study was done in order to find out the effect of administering several doses of Cosmos caudatus leaf extract on bone metabolism carried out ad libitum. Using experimental animals with a final total of 43 samples with each number of P0 12 samples, P1 12 samples, P2 10 samples, and P3 9 samples. treatment was carried out for 28 days, then blood serum analysis was carried out to determine the levels of SOD, OC, and CTX, as well as histopathological examination of the number of cells and trabecular bone. The hypothesis of this study is the discovery of the most effective effect of one dose of Cosmos caudatus leaf extract on bone remodeling through the measurement of bone metabolism biomarkers, so that the use of kenikir leaves can be known and applied.

Bone metabolism is a continual process of remodeling that plays a crucial role in preserving bone health. It entails a careful equilibrium between the creation and resorption of bone tissue. Bone metabolism markers are essential for evaluating this equilibrium and are classified into two categories: markers for bone production and markers for bone resorption. The production of osteocalcin (OC) by osteoblasts serves as a crucial marker for bone formation and serves as a conclusive indicator of osteoblastic activity. The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine have recommended serum CTX-1 (sCTX) as a marker for bone resorption. This marker serves as an indicator of the degradation of Type 1 bone collagen by osteoclasts during the process of bone resorption (Shetty et al., 2016).

The detrimental effects of free radicals on bone metabolism are well-documented, as they induce disruptions in the bone matrix, ultimately resulting in bone destruction. Antioxidants play a crucial role in mitigating this harm by inhibiting the formation of free radicals. According to Zamroni Salim and Ernawati Munadi, 2017, Cosmos caudatus, often known as kenikir leaves, is acknowledged as a powerful natural antioxidant within the vast array of roughly 7500 medicinal plant species found in Indonesia(Zamroni Salim and Ernawati Munadi, 2017). Previous research has demonstrated that kenikir leaves have significant antioxidant activity, as indicated by their low IC50 values in DPPH experiments. These findings suggest that kenikir leaves possess a robust capacity to counteract detrimental free radicals (Mustafa et al., 2010; Lee and Vairappan, 2011; Ardies, 2014; Abdul Rahman et al., 2017).

Prior studies have indicated that the extract of Cosmos caudatus may have an impact on bone metabolism, thereby presenting a potentially efficacious alternative therapy for bone fragility, particularly among postmenopausal women who are more susceptible to conditions such as osteoporosis (Mohamed et al., 2012; Mohamed, Chai Mei Yin et al., 2013b; Mohamed, Sahhugi, et al., 2013). Osteoporosis is a notable health issue in Indonesia, particularly among individuals aged 60 and above. The underdiagnosis of osteoporosis is frequently observed until it reaches a severe stage, primarily attributed to the limited utilization of DXA scans (Lauralee Sherwood, 2016; Dirjen Pelayanan Kesehatan, 2022).

The primary objective of our research is to investigate the effectiveness of different dosages of Cosmos caudatus leaf extract in influencing bone metabolism. Our objective is to determine the optimal dosage that impacts bone remodeling, as evidenced by indicators of bone metabolism such as SOD, OC, and CTX. The research will employ a total of 43 experimental animals, which will be divided into control and treatment groups. The treatment intervention will span a duration of 28 days. The amounts of these indicators, as well as the number of cells and trabecular bone integrity, will be evaluated through further blood serum analysis and histological examination. This study has the potential to establish a foundation for utilizing kenikir leaves as a natural resource for the prevention and treatment of bone-related ailments.

2. Materials and methods

2.1. Animals and treatments

A total of forty three male rats, aged between 3 and 4 months, were chosen from the experimental animal laboratory at the Faculty of Veterinary Medicine, Universitas Airlangga. In order to reduce the likelihood of death, two extra samples were added to each group. The rats were randomly assigned to four separate groups: a control group (P0) that received distilled water, and three experimental groups (P1, P2, P3) that were given different amounts of Cosmos caudatus leaf extract.

2.2. Extract preparation

The task of making Cosmos caudatus leaf extract was carried out in the laboratory of the Faculty of Pharmacy, Universitas Airlangga. The dried leaves of C. caudatus were ground into powder. Percolation of the powder (200g) was carried out at 40 °C using 70% ethanol (plant: solvent, 1:10, w/v). The extract was evaporated under vacuum at 30 °C using a rotary evaporator (Büchi, Switzerland) to get a dry extract of C. caudatus leaves. Results: The yield of the extraction process was 9,4%.

2.3. Animal nutrition and treatment administration

The researchers actively oversaw the diet and application of the extract to the rats. The animals were provided with uniform feeding (Table 1), and the extract dosages were accurately measured and supplied in accordance with the experimental design. The P0 control was only given aquadest; P1 was subjected to a given Cosmos caudatus extract orally at a dose of 0.91 mg/kg BW; P2 was subjected to a given

Table 1
Composition of food ingredients (511-Bravo).

Composition	Amount/percentage
Water	11–12 %
Crude protein	21–23 %
Crude fat	5–8 %
Crude fibre	3–5 %
Abu	4–7 %
Calsium min	0.9 %
Phospor min	0.6 %
Kalory	2.800–3.100 kcal/kg

Cosmos caudatus orally at a dose of 1.82 mg/kg BW; and P3 was subjected to a given Cosmos caudatus orally at a dose of 3.64 mg/kg BW according to the conversion formula for experimental animals (Diah Kusumawati dan Nunung Prajarto and Prajarto, 2016). All groups were treated for 28 days.

2.4. Sample collection

After the 28-day therapy period, an independent party was tasked with gathering blood and bone samples. Blood serum was collected to analyze biomarkers, and the right femur bone was processed for histomorphometric examination.

2.5. Laboratory assays

An independent party conducted ELISA assays to detect serum levels of SOD1, osteocalcin, and CTX. Additionally, they performed bone cell counting and measured the % area of trabecular bone. The implementation of this separation method guaranteed impartial processing and analysis of the samples.

2.6. Bone histomorphometry

After 28 days of treatment, the right femur bone was taken from the experimental animals, he steps in making bone preparations were fixation of rats femur bone tissue with 10% formalin, then decalcification in 8% HCl. Furthermore, it was processed into paraffin blocks, cut with a microtome with a thickness of 4 μm, and placed on the slide. The slides were deparaffinized with xylene for 2 × 5 minutes, then were rehydrated with graded alcohol starting with ethanol 100%, 96%, 70%, and distilled water for 5 min per solution. The slides were then stained with hematoxylin for 8 min and rinsed with distilled water for 10 min. Subsequently, the slides were dehydrated with 70% alcohol for 5 min and 96% alcohol for 5 min and immersed in eosin solution for 2 min. Then, the slides were rinsed in ethanol 96% and 100% for 5 min each. Lastly, the slides were cleared in xylene for 2 × 5 min and mounted in the deck glass with entellant for examination under a microscope (Liu et al., 2017).

2.7. Data analysis

The principal researchers were solely responsible for data analysis. They performed exploratory data analysis and variance analysis using R Studio (R) version 4.3.2. The Kolmogorov-Smirnov test was utilized to evaluate the normality of the data, hence evaluating the appropriateness of using either ANOVA or the Kruskal-Wallis test for subsequent analysis. Irrespective of the importance of the initial findings, a post-hoc test was prearranged to thoroughly examine the interactions.

2.8. Ethical consideration

The study obtained clearance from the Unit Animal Ethics Committee of the Fakultas Kedokteran Hewan, Universitas Airlangga, under the approval code: 2.KEH.116.07.2023, guaranteeing that all procedures were conducted ethically and in accordance with applicable norms.

3. Results

Initially, the rat had an average weight ranging from 173.3 g to 194.9 g. By the end of the trial, their average weight had increased to a range of 197.00 g–220.60 g (Table 2). On the whole, there appears to be little variations in the weight of each group, which should mitigate the impact of weight as a confounding variable.

In this work, the researcher measured the amounts of C-terminal telopeptide (CTX), osteocalcin (OC), and superoxidase dismutase 1 (SOD1) in four different rat groups (P0, P1, P2, P3) in order to examine the impact of various therapies on bone metabolism. The ELISA results demonstrated a distinct and varying reaction to the interventions. More precisely, the concentrations of SOD1 exhibited a consistent pattern of increase throughout the groups starting from the initial level in P0 (0.33 ± 0.09), followed by P1 (0.53 ± 0.42) and P2 (1.09 ± 1.76). However, there was a modest reduction in P3 (0.65 ± 0.47). On the other hand, the levels of OC showed clear variations. They started at a baseline of 6.65 ± 4.34 in P0, increased to 7.91 ± 3.11 in P1, decreased to 5.61 ± 2.56 in P2, and then rose again to 7.25 ± 2.46 in P3. The CTX marker exhibited an opposite trend to SOD1, with levels showing a modest decrease from P0 (9.26 ± 0.50) to P1 (9.03 ± 1.13), a more significant decrease in P2 (8.30 ± 1.10), and a slight increase in P3 (8.41 ± 1.20) (See Table 3).

Table 2
Weight comparision table.

Group	Before Treatment	After Treatment
	Mean (SD)	
P0	194.90 (12.39)	220.60 (13.70)
P1	173.30 (22.40)	197.00 (21.39)
P2	185.88 (23.27)	205.55 (18.99)
P3	185.25 (21.59)	211.75 (23.98)

The measurement of bone trabecular area percentage reveals intriguing results (Table 4). In the control group (P0), the average trabecular area percentage was found to be 40.32 (9.73). Compared to P0, the P1 group showed a marginal reduction in trabecular area, with an average of 39.53 and a standard deviation of 8.82. Notably, there was an apparent rise in the P2 group, with the trabecular area % reaching an average of 43.33 and a lower standard deviation of 5.32, indicating a more uniform response within this group. On the other hand, the P3 group had an average trabecular area percentage of 41.65. Although this value was higher than the original control measures, it did not reach the elevated levels found in P2. Additionally, the P3 group had a much higher standard deviation of 13.89. This implies a greater variability in the response within P3 and demonstrates that although the intervention resulted in a general rise in trabecular area relative to P0, the extent of this increase was less uniform than in P2.

The cell counting results from the study provides more insights into the dynamics of antioxidants in bone metabolism, as reflected by the counts of osteoblasts, osteoclasts, and osteocytes. The general trend observed across the patient groups indicated a decrease in osteoclast count, suggesting a potential reduction in bone resorption activity (Table 5). Specifically, osteoclast counts decreased from a mean of 0.88 (SD 2.99) in the control group (P0) to 0.60 (SD 0.43) in P2, with a slight increase to 0.82 (SD 0.63) in P3. This pattern may imply a modulation of bone resorption processes due to the interventions.

In the case of osteoblasts, which are responsible for bone formation, the cell counts demonstrated more variability. While there was a decrease from P0's mean count of 97.79 (SD 37.13) to 84.58 (SD 30.06) in P1, a notable increase was seen in P2, with a count of 103.94 (SD 38.14). However, this increase was not sustained in P3, where the count dropped to 90.53 (SD 32.70). This fluctuation in osteoblast numbers may reflect changes in the anabolic phases of bone metabolism in response to the treatments applied in each group.

For osteocytes, the data showed a more uniform trend of decrease across the groups, although not as pronounced as with osteoclasts. Osteocytes, being the chief cells responsible for the maintenance of bone

Table 3
ELISA's results.

Concentration	P0	P1	P2	P3
	Mean (SD)			
SOD1	0.33 (0.09)	0.53 (0.42)	1.09 (1.76)	0.65 (0.47)
OC	6.65 (4.34)	7.91 (3.11)	5.61 (2.56)	7.25 (2.46)
CTX	9.26 (0.50)	9.03 (1.13)	8.30 (1.10)	8.41 (1.20)

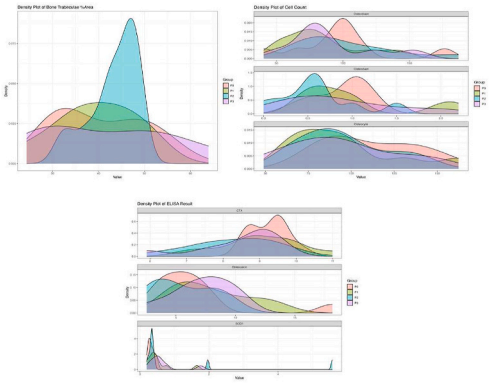
Table 4
Trabecular histology results.

Percentage	P0	P1	P2	P3
	Mean (SD)			
% Trabecular Area	40.32 (9.73)	39.53 (8.82)	43.33 (5.32)	41.65 (13.89)

Table 5
Cell counting results.

Cell Count	P0	P1	P2	P3
	Mean (SD)			
Osteoblast	97.79 (37.13)	84.58 (30.06)	103.94 (38.14)	90.53 (32.70)
Osteoclast	0.88 (2.99)	1.01 (0.64)	0.60 (0.43)	0.82 (0.63)
Osteocytes	111.14 (26.91)	95.61 (32.71)	94.93 (25.80)	93.92 (31.88)

tissue, decreased from 111.14 (SD 26.91) in P0 to 95.61 (SD 32.71) in P1, followed by a marginal decrease to 94.93 (SD 25.80) in P2, and then to 93.92 (SD 31.88) in P3. The consistent reduction in osteocyte counts across the interventions could suggest a potential influence of the treatments on bone maintenance and signaling functions of these cells.



The density plots of bone cell populations exhibited a significant degree of overlap among the various experimental groups. The count of osteoclasts displayed a wide range of values, suggesting significant variability in the measures within the group. Similarly, the number of osteoblasts and osteocytes showed significant overlap between the groups, indicating a varied response to the intervention. Although there was some variation, there was a clear trend indicating higher numbers of osteoblasts and osteocytes in the intervention groups as compared to the control groups.

The ELISA results for SOD1, CTX, and osteocalcin exhibited density distributions that overlapped between the control and intervention groups, suggesting a subtle impact of the treatment. The intervention groups showed a moderate reduction in SOD1 levels, which is a marker for oxidative stress. This suggests that the medication may have an antioxidative effect. On the other hand, the treated groups exhibited a decrease in CTX, a marker that indicates the breakdown of bone tissue. This suggests that there may be a reduction in bone loss. The intervention groups had greater levels of osteocalcin, which is indicative of bone production, and this aligns with the reported rise in osteoblast numbers.

The histology counts revealed a significant overlap in density plots for the bone trabecular area percentage among all groups. Nevertheless, there was a collective augmentation in the trabecular area among the groups who received treatment. This discovery is consistent with the enhanced biomarker profiles and indicates a beneficial impact of the intervention on the structure of the bone.

The density plots, when considered together for all assessed parameters, suggest an intricate relationship between the intervention and bone metabolism. Although there is some overlap and apparent variation in the data, the overall trend indicates that the intervention may have a positive effect on bone health. The positive signs are defined by an elevation in markers of bone production and a reduction in markers of bone resorption.

The Kruskal-Wallis test, a non-parametric statistical approach, was used to evaluate the variances among groups because the data did not follow a normal distribution, as shown by the Kolmogorov-Smirnov normality test and the density plots.

The results indicate that there are no statistically significant variations across the groups for all the outcome measures (Table 6). The variables examined were the concentrations of C-Terminal Telopeptide, Osteocalcin, and Superoxidase Dismutase 1, as well as the Mean Bone Trabecular % area, and the cell counts of Osteoblasts, Osteoclasts, and Osteocytes.

The Eta Squared values, serving as an estimate of effect size, were predominantly minimal, suggesting that the observed distinctions between groups are of minor magnitude. The C-Terminal Telopeptide concentration (0.139) and Superoxidase dismutase 1 concentration (0.137) had the greatest Eta Squared values, indicating a tiny effect size. Conversely, the Mean Bone Trabecular percent area exhibited a negligible impact size (0.013), and none of the effect sizes were moderate or large to suggest a substantial distinction between the groups.

The post-hoc comparisons revealed that most of the pairwise group differences were not statistically significant. This aligns with the previous findings from the Kruskal-Wallis test, which indicated a lack of significant differences when evaluating all groups together. This absence of significant changes was consistent with the patterns seen in the mean and density plots (Table 7).

Nevertheless, specific comparisons revealed noteworthy deviations from this overall pattern. Group P3 exhibited a substantial reduction in the concentration of Superoxidase dismutase 1 compared to group P0, with a mean difference of -2.22 ($p < 0.05$). These findings show that the intervention could potentially have a beneficial impact in the long run, resulting in a decrease in oxidative stress as evidenced by this biomarker.

Similarly, there was a notable rise in the concentration of C-Terminal Telopeptide between groups P0 and P2, with an average difference of 2.02 ($p < 0.05$). This discovery suggests that during the initial stages of the intervention, there might be a rise in bone resorption, which might either be a temporary consequence or a manifestation of a more intricate reaction to the treatment.

The Dunn post-hoc test confirms the substantial pairwise differences and further validates the previous discovery of a consistent favourable impact of the intervention. Further inquiry is necessary to completely comprehend the consequences of the intervention on bone health due to the notable changes in biomarkers related to bone metabolism, which include both good and unfavourable outcomes.

The intervention demonstrates potential, but the variation in response, as indicated by both the statistically significant and non-significant outcomes, emphasises the intricate nature of the biological mechanisms at play.

Table 6
The Kruskal-Wallis test.

Variable	Test statistic	df	p	Eta squared
C-Terminal Telopeptide concentration	5.13	3	0.163	0.139
Osteocalcin concentration	3.60	3	0.308	0.097
Superoxidase dismutase 1 concentration	5.06	3	0.167	0.137
Mean Bone Trabecular percent area	0.481	3	0.923	0.013
Osteoblast cell count	1.87	3	0.600	0.050
Osteoclast cell count	3.09	3	0.378	0.083
Osteocyte cell count	2.23	3	0.527	0.060

Table 7
The Dunn post-hoc test.

Variable	P0 – P1	P0 – P2	P1 – P2	P0 – P3	P1 – P3
Conc SOD	−1.33	−0.99	0.31	−2.22*	−0.96
Conc OC	−1.33	0.2	1.49	−1.17	0.09
Conc CTX	0.52	2.02*	1.51	1.52	1.03
Mean	0.16	−0.51	−0.67	−0.21	−0.37
Oclast Avg	0.09	1.54	1.45	0.78	0.69
Ocyt Avg	1.25	1.2	−0.06	1.18	−0.03
Oblast Avg	1.1	−0.06	−1.16	0.66	−0.41

4. Discussion

Bone metabolism is a dynamic and intricate process of remodeling, which comprises resorption and creation stages. This process is tightly regulated by the cellular actions of osteoclasts, osteoblasts, and osteocytes, as well as biomolecular factors (Rowe et al., 2023). The first stage of bone remodeling starts with osteoclasts reacting to signals for resorption, which is subsequently accompanied by a reversal phase wherein these cells vanish, paving the way for osteoblastic cells to initiate the creation phase. The cycle ends with the final differentiation of osteoblasts, preparing for the subsequent phase of remodeling (Fig. 1) (Siddiqui and Partridge, 2016; Rowe et al., 2023). Biomarkers, such as osteoblast-derived peptides like BALP, osteocalcin (OC), and chemicals generated during bone synthesis (P1NP) or osteoclastic resorption (such as TRAP5b, urine or serum CTX-1), play a crucial role in assessing bone metabolic activity (Burr and Allen, 2013; Shetty et al., 2016).

Antioxidants are crucial in this process, as enzymes such as superoxide dismutase (SOD) operate as a protective barrier against free radicals. Endogenous antioxidants are synthesized internally in the body, but Cosmos caudatus leaf extract serves as an exogenous source of antioxidants, distinct from endogenous SOD. While the exact connection between the consumption of natural antioxidants and the enhancement of endogenous antioxidant enzymes is not completely proven, several studies, as Wrasiati et al. (2011), have noted an elevation in SOD levels after the application of natural antioxidant extracts (Wrasiati et al., 2011). Some studies have also shown that plants' antioxidant content is efficacious in reducing SGOT and SGPT levels and significantly repairing damaged hepatocytes in diabetic rats, especially in swollen, hydropic, and necrotizing cells. The presence of antioxidants also enhances open wound healing in diabetic rats (Husen et al., 2019; Winarni et al., 2022). The coexistence of Cu/Zn-SOD and Fe-SOD in chloroplasts implies that the amounts of superoxide dismutase (SOD) could be influenced by natural antioxidants (Pilon et al., 2011; Juszczak and Baier, 2012; Zhang et al., 2015). This study found that the group receiving increasing dosages of the extract up to P2 showed higher values of SOD.

Antioxidants are believed to influence the process of bone remodeling by counteracting reactive oxygen species (ROS), which can promote the formation of osteoclasts and inhibit the formation of osteoblasts through the activation of antioxidant enzymes (Ardies, 2014; Dudarić et al., 2015; Torre, 2017). The effects of Cosmos caudatus leaf extract on bone metabolism were assessed using osteocalcin and CTX-1 in this study. Osteocalcin is a crucial protein found in bone that is not made up of collagen. It acts as a marker in the blood to indicate the production of bone by osteoblasts. Nevertheless, the levels of it may not exhibit a direct correlation with the quantities of osteoblasts. This study observed a disparity in the P2 group, where, despite a significantly elevated average number of osteoblast cells, the levels of osteocalcin were comparatively lower than those in the other groups (Mohamed, Chai Mei Yin et al., 2013b).

Research on the effects of antioxidants on bone thickness has been carried out several times using various sources of antioxidants and quite diverse methods. Several studies have proven the effect of antioxidants on bone cells and bone density (Mohamed, Chai Mei Yin et al., 2013b; Mohamed, Sahhugi, et al., 2013; Liu et al., 2017; Juwita and Fatma, 2021). The aim of this research is to look at another perspective regarding the effect of antioxidant protection through Cosmos caudatus leaf extract on normal body conditions without special conditioning. This is answered by the synchronized increase between the average percentage of trabecular bone (Table 4) and the treatment group dose of kenikir leaf extract, highest at P2.

The ability of antioxidants in the process of catalyzing or converting superoxide anions (O2-) into hydrogen peroxide and oxygen will inhibit or stop free radical reactions, which may trigger increased RANK regulation in osteoclasts, which leads to increased RANKL-RANK interactions, which increases the risk of bone resorption and damage. Antioxidant juga mempengaruhi sel osteoblas sebagai kofaktor pada proses hidroksilasi untuk sintesis kolagen yang memiliki peran penting menyusun martriks tulang dan mendukung proses mineralisasi tulang (Fig. 2) (Ardies, 2014).

The study's conclusions are limited due to the presence of uncontrolled circumstances during its implementation. Nevertheless, this particular study on the impacts of Cosmos caudatus leaf extract could serve as a benchmark for the use of this plant. The study's findings, combined with prior research, indicate that the extract may have advantages as a support agent. This is evident from the observed patterns of higher SOD levels and lower CTX levels at higher doses, compared to previous studies. Additionally, histopathological findings related to

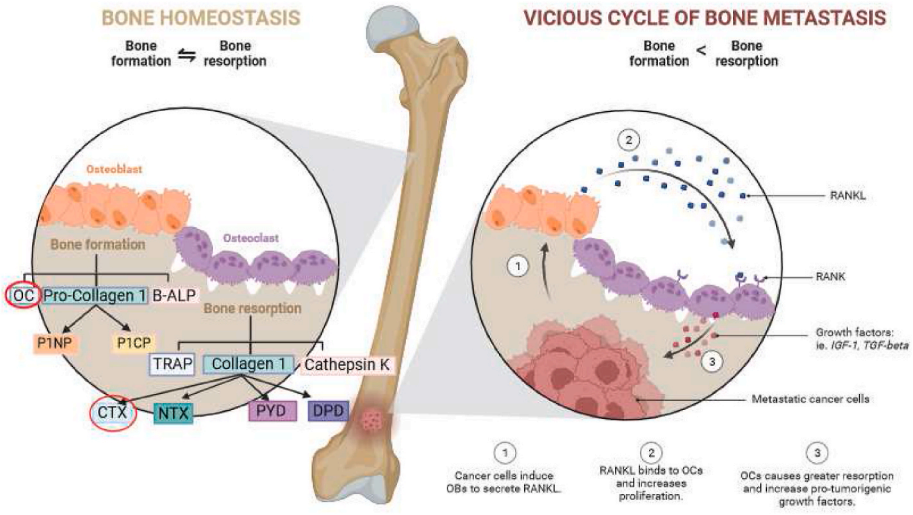


Fig. 1. Bone metabolism.

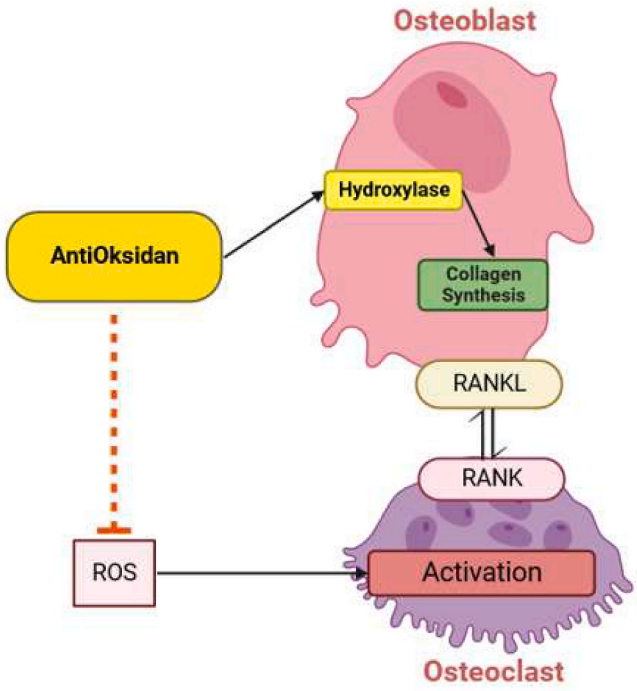


Fig. 2. Antioxidant mechanisms in cells.

osteoblast and osteoclast cell counts in the medium dose group (P2:1.82 mg/bw) further support this conclusion. Future research is still required to explore and validate the antioxidant potential of kenikir (Cosmos caudatus) leaf extract.

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CRediT authorship contribution statement

Gadis Meinar Sari: Methodology, Formal analysis, Writing – original draft. **Idha Kusumawati:** Conceptualization, Methodology, Formal analysis, Resources. **Yoga Akbar Arifandi:** Data collection, Treatment of experimental animals, Writing draft research reports. **Julian Benedict Swannjo:** Data collection, Treatment of experimental animals, Analysis data, Writing draft research reports.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gadis Meinar Sari reports financial support was provided by Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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