Kocatepe Veterinary Journal

https://doi.org/10.30607/kvj.1895818

**Research Article**

# The Effects of Alendronate and Vitamin D Administration on Biochemical Parameters in Rats Treated with Dexamethasone

## Deksametazon Uygulaması Yapılan Ratlarda Alendronat ve Vitamin D Uygulamalarının Biyokimyasal Parametrelere Etkileri

**Beyza SUVARIKLI ALAN[1*✉], Abdullah Murat ALTINSOY[1], Adnan ÇITIRIK[1], Seyfullah HALİLOĞLU[1]**

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye

**Corresponding Author:**
Beyza SUVARIKLI ALAN
✉beyza.alan@selcuk.edu.tr

**ORCID:**
BSA: 0000-0003-4698-9291
AMA:0000-0002-9724-0021
AÇ: 0009-0009-6913-7439
SH: 0000-0001-8269-9668

Received: 23.02.2026
Accepted: 10.06.2026
Published: 15.06.2026

**Citation:**
*Alan BS. Altınsoy AM. Çıtırık A. Haliloğlu S. The Effects of Alendronate and Vitamin D Administration on Biochemical Parameters in Rats Treated with Dexamethasone. Kocatepe Veterinary Journal (2026) 19(2):229-238*



**Abstract**

Glucocorticoids, frequently preferred for treatment due to their immunosuppressive and anti-inflammatory effects, are known to have significant side effects on many systems, such as the musculoskeletal and cardiovascular systems, depending on their use. This study investigated the effects of dexamethasone administration in rats and the effects of alendronate and vitamin D, administered alone or in combination to reduce its potential effects, on biochemical parameters. A total of 40 rats were divided into 5 groups and administered 7.5 mg·kg$^{-1}$ dexamethasone subcutaneously once a week for 6 weeks, in all groups except for the control group. No treatment was administered to the control group. The remaining three groups received treatment with alendronate (0.9 mg·kg$^{-1}$ 5 days per week), vitamin D (10.000 IU/500 µL), and alendronate/vitamin D combination administered at the same doses via gavage, respectively. With dexamethasone administration, LDH activity, triglyceride, magnesium and iron levels significantly increased compared to the control group, while chloride levels lowered. The increase in LDH activity and iron levels also continued with alendronate administration. Triglyceride levels were significantly higher in all treatment groups. The decrease in albumin levels observed in all groups compared to the control group was significant with alendronate and combined use, while the increase in urea levels was significant only in the vitamin D group. In conclusion, it is thought that further studies are needed in which alendronate and vitamin D are administered at different doses and durations to eliminate the effects of dexamethasone on biochemical parameters.

**Keywords:** Alendronate, Biochemical parameters, Dexamethasone, Rat, Vitamin D

**Öz**

İmmünosüpresif ve antienflamatuar etkileriyle tedavi amacıyla sıklıkla tercih edilen glukokortikoidlerin kullanıma bağlı olarak kas-iskelet ve kardiyovasküler sistem gibi birçok sistemde önemli yan etkilerinin olabileceği bilinmektedir. Bu çalışma ile ratlarda deksametazon uygulaması ve bunun olası etkilerini azaltmak amacı ile alendronat ve vitamin D'nin tek başına ya da kombine uygulamalarının biyokimyasal parametrelere etkileri araştırılmıştır. Toplam 40 adet rat 5 gruba ayrılarak 6 hafta sürdürülen çalışmada kontrol grubu dışındaki tüm gruplara haftada bir kez deri altı 7.5 mg/kg deksametazon uygulandı. Kontrol grubuna herhangi bir uygulama yapılmadı. Kalan son üç gruba tedavi amacıyla sırasıyla alendronat (haftada 5 gün 0.9 mg·kg$^{-1}$), vitamin D (10.000 UI/500 µL) ve aynı dozlarda alendronat /vitamin D kombinasyonu gavaj yolu ile verildi. Deksametazon uygulamasıyla LDH aktivitesi, trigliserid, magnezyum ve demir düzeylerinin kontrol grubuna göre önemli düzeyde arttığı, klor düzeylerinin ise düştüğü gözlendi. LDH aktivitesi ve demir düzeylerindeki artış alendronat uygulamasıyla devam etti. Tüm tedavi gruplarında trigliserid düzeyleri anlamlı derecede daha yüksek bulundu. Tüm gruplarda kontrol grubuna göre gözlenen albümin düzeylerindeki düşüşün ise alendronat ve kombine kullanımda anlamlı olduğu, üre düzeylerindeki artışın ise sadece vitamin D grubunda anlamlı olduğu bulundu. Sonuç olarak deksametazonun biyokimyasal parametrelere olan etkilerinin giderilmesinde farklı doz ve sürelerde alendronat ve vitamin D uygulamalarının yapıldığı yeni çalışmalara gereksinim duyulduğu düşünülmüştür.

**Anahtar Kelimeler:** Alendronat, Biyokimyasal parametreler, Deksametazon, Rat, Vitamin D

## Introduction

Dexamethasone is a synthetic glucocorticoid receptor agonist that mimics the effects of natural glucocorticoids. It is a corticosteroid derivative with a 21-carbon steroid skeleton similar to hydrocortisone (Pierre-Louis, 2010). It has been reported that glucocorticoids, which directly affect osteoblasts and osteoclasts, reduce Ca absorption and increase their excretion by the kidneys, and leads to significant bone loss even at pharmacological doses by disrupting vitamin D metabolism when administered for longer periods (Adler & Rosen, 1994, Mazziotti et al., 2006). In fact, glucocorticoid use has been associated with the development of secondary osteoporosis (Mazziotti et al., 2007).

Bisphosphonates are among the most commonly used drugs in treatment due to their ability to reduce fracture risk along with increasing bone mineral density (Tamura et al., 2004). These drugs function by inducing osteoclast apoptosis and inhibiting bone resorption (Rogers, 2003). Alendronate, in particular, is an effective drug in preventing disorders that may occur in glucocorticoid-induced bone metabolism and provides long-term protection in terms of bone mineral density (Adachi et al., 2001, Okada et al., 2008). However, experimental studies on alendronate (Rizzoli et al., 2010), which reduces the remodeling rate by about 60%, have shown that it may damage gastric mucosa and may have various side effects (Amagase et al., 2011).

Vitamin D is an essential vitamin for bone mineralization, supporting calcium absorption in the intestines and playing a role in bone growth and remodeling by osteoblasts and osteoclasts (Bouillon et al., 2019). It has been observed that intermittent or daily administration of standard doses of vitamin D reduces the risk of fractures in adults over 50 years of age who do not have vitamin D deficiency (Cranney et al., 2007, Yao et al., 2019).

Considering the efficacy of alendronate and vitamin D, it has been suggested that the combination of the two agents may be a more effective treatment method than either agent alone, particularly in bone metabolism (Zhang et al., 2015). Combined treatments have been reported to be more effective than monotherapy in terms of bone mineral density in the femoral neck in postmenopausal women (Frediani et al., 1998), and similar findings have been observed in rats (Nakamura et al., 2008).

Considering the success rates of drugs used to minimize bone loss or increase bone development, there is a need to develop more effective drugs for the treatment of bone diseases and to implement rational treatment procedures. Currently, studies on the combined use of bisphosphonates and vitamin D are increasing. Therefore, the primary aim of this study is to investigate in detail the effects of sodium alendronate and vitamin D on biochemical parameters in rats treated with dexamethasone.

## Materials and Methods

### *Animal Material*

A total of 40 female rats of the Wistar Albino breed, aged 2-2.5 months (210-230 g), obtained from the Selçuk University Experimental Medicine Application and Research Center, were used in the study. During the study period, the animals were housed in polycarbonate cages at 24±10°C, with 60% atmospheric humidity, and a 12-hour light/12 hour-darkness circle. Their feed and water requirements were met ad libitum.

### *Method*

The rats were divided into 5 groups based on balanced live weights, and the following applications were performed for 6 weeks.

Group 1 (C, Control Group, n: 8): Rats in this group served as the control group and received no treatment.

Group 2 (Dexamethasone (Dekort injectable solution in ampoule), n: 8): 7.5 mg·kg$^{-1}$ dexamethasone (Ferreira-Júnior et al., 2008) was administered subcutaneously once a week.

Group 3 (Dexamethasone + Alendronate (Fosamax), n: 8): 7.5 mg·kg$^{-1}$ dexamethasone (Ferreira-Júnior et al., 2008) was administered subcutaneously once a week and 0.9 mg·kg$^{-1}$ alendronate (Sabry et al., 2013) was administered via gavage 5 days a week.

Group 4 (Dexamethasone+Vitamin D (Devit-3), n: 8): 7.5 mg·kg$^{-1}$ dexamethasone (Ferreira-Júnior et al., 2008) was administered subcutaneously once a week and 10.000 IU/500 μL vitamin D (Queiroz Júnior et al., 2022) was administered via gavage once a week.

Group 5 (Dexamethasone + Alendronate + Vitamin D, n: 8): Once a week, 7.5 mg/kg dexamethasone (Ferreira-Júnior et al., 2008) was administered subcutaneously, and 0.9 mg·kg$^{-1}$ alendronate (Sabry et al., 2013) was administered via gavage 5 days a week, in combination with 10.000 IU/500 μL vitamin D once a week.

Twenty-four hours after the completion of the experiment, blood samples were collected from rats euthanized with xylazine (8 mg·kg$^{-1}$, IP, Xylazin Bio 2% inj., Bioveta, Ankara, Turkiye) + ketamine (75 mg·kg$^{-1}$, IP, Ketosol 10% inj., Interhas, Ankara, Turkiye) anesthesia using the intracardiac method. Alanine Aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase activities, triglycerides, cholesterol, HDL, glucose, total protein, albumin, total bilirubin, creatinine, urea, sodium, potassium, chloride, magnesium, phosphate, calcium, and iron levels (Alinity c8000) were determined from the obtained serum samples.

### *Ethical Approval*

This study ethical approval (Approval no: 2025/119, date 30.12.2025) was obtained from Selcuk University Experimental Medicine And Application Center Ethics Committee (SÜDAM).

### *Statistical Analysis*

The data were exposed to normality test using Shapiro-Wilk test. The biochemical parameters ALT, amylase, AST, cholesterol, creatinine, iron, potassium, and total bilirubin did not show a normal distribution. Parameters outside the normal range were evaluated using the Kruskal-Wallis and Mann-Whitney U tests. The values of parameters showing statistical differences were added to the tables as medians. Parameters within the normal range were evaluated using the One-Way ANOVA statistical test (SPSS Statistics 25.0, Corporation, Armonk, New York, USA). When there was a difference between the groups, the post hoc Tukey multiple comparison test was used to examine the difference, and $p<0.05$ was accepted as the statistical significance threshold. Values are presented as Mean ± SE.

## Results

In the present study, compared to the control group, dexamethasone administration was found to cause an increase in LDH, triglyceride, magnesium, and iron levels in rats ($p<0.05$). It was determined that the increased LDH and iron levels in the groups administered vitamin D approached those of the control group. It was found that the increased magnesium levels resulting from dexamethasone administration approached control group levels only with the alendronate/vitamin D combination. Compared to the control group, dexamethasone administration caused a decrease in chloride levels ($p<0.05$), and this decrease continued only in the alendronate group, while vitamin D administration caused chloride levels to return to concentrations that were not statistically different from the control group.

Decreases in serum albumin levels ($p<0.05$) were observed in the groups treated with alendronate, while high glucose and urea levels ($p<0.05$) and decreases in total protein levels were found to be statistically significant ($p<0.05$) in the groups treated with vitamin D.

In the inter-group examination, no statistical difference was determined in AST, ALT, ALP, amylase activities, cholesterol, HDL, total bilirubin, creatinine, sodium, potassium, calcium and phosphate levels ($p>0.05$).

**Table 1.** Effects of Alendronate and Vitamin D on Biochemical Parameters in Rats Treated with Dexamethasone (mean±SE).

| Biochemical Parameters | Control (n=8) | Dexamethasone (n=8) | Dexamethasone + Alendronate (n=8) | Dexamethasone + Vitamin D (n=8) | Dexamethasone + Alendronate + Vitamin D (n=8) |
|---|---|---|---|---|---|
| **AST (U/L)** | 159,75±15,61 [a] | 211,37±15,09 [a] | 244,25±30,52 [a] | 202,50±18,24 [a] | 209,25±23,39 [a] |
| **ALT (U/L)** | 76,00±6,44 [a] | 81,00±9,40 [a] | 99,50±12,85 [a] | 83,00±5,59 [a] | 84,75±7,71 [a] |
| **ALP (U/L)** | 254,87±15,09 [ab] | 293,75±19,59[ab] | 221,87±18,78 [b] | 315,62±23,13[a] | 231,12±17,67 [b] |
| **LDH (U/L)** | 362,50±104,24 [b] | 1647,00±204,84 [a] | 1593,62±143,12 [a] | 770,87±68,22 [b] | 784,25±130,11 [b] |
| **Amylase (U/L)** | 1360,01±278,19 [a] | 1216,96±433,62 [a] | 1369,02±1860,90 [a] | 1228,49±396,38 [a] | 1189,99±427,08 [a] |
| **Triglyceride (mg·dL$^{-1}$)** | 48,75±4,68 [b] | 84,12±4,30 [a] | 78,37±4,49 [a] | 69,75±3,88 [a] | 70,87±3,77 [a] |
| **Cholesterol (mg·dL$^{-1}$)** | 91,50±1,68 [a] | 92,12±3,40 [a] | 94,75±3,40 [a] | 88,87±7,029 [a] | 93,25±2,74 [a] |
| **HDL** | 66,5±10,25 [a] | 66,8±31,33 [a] | 68,8±31,40 [a] | 63,7±47,41 [a] | 67,3±22,77 [a] |
| **Glucose (mg·dL$^{-1}$)** | 193,12±6,13[c] | 226,75±16,20 [bc] | 228,62±13,37[bc] | 268,62±12,21 [ab] | 282,62±12,18 [a] |
| **T. Protein (mg·dL$^{-1}$)** | 67,79±8,10 [a] | 63,41±14,18 [ab] | 63,40±7,60 [ab] | 62,77±12,43 [b] | 61,04±14,21 [b] |
| **Albumin (g·dL$^{-1}$)** | 32,35±4,20 [a] | 30,00±9,71 [ab] | 28,89±6,72[b] | 29,47±6,84 [ab] | 28,65±9,54[b] |
| **Total Bilirubin** | 0,10±0,00 [a] | 0,11±0,12 [a] | 0,11±0,12 [a] | 0,10±0,00 [a] | 0,10±0,00 [a] |
| **Creatinine (mg·dL$^{-1}$)** | 0,47±0,87 [a] | 0,46±1,48 [a] | 0,48±2,95 [a] | 0,47±1,33 [a] | 0,46±1,14 [a] |
| **Urea (mg·dL$^{-1}$)** | 50,37±1,68 [b] | 55,12±2,76 [ab] | 55,37±1,91 [ab] | 61,00±1,53 [a] | 52,25±1,26 [b] |
| **Sodium (mmol·L$^{-1}$)** | 154,62±0,68 [a] | 152,25±1,40 [a] | 153,12±1,34 [a] | 153,75±0,70 [a] | 151,37±0,98 [a] |
| **Potassium (mmol·L$^{-1}$)** | 4,54±1,37 [a] | 4,41±1,07 [a] | 4,62±1,62 [a] | 4,41±1,29 [a] | 4,45±0,92 [a] |
| **Chloride (mmol·L$^{-1}$)** | 116,00±0,42 [a] | 111,00±1,00 [b] | 111,87±1,02 [b] | 113,87±0,40 [ab] | 113,3750±0,50 [ab] |
| **Magnesium (mg·dL$^{-1}$)** | 2,30±0,50[c] | 2,61±0,67 [ab] | 2,70±1,00 [a] | 2,61±0,85 [ab] | 2,39±0,51 [bc] |
| **Calcium (mg·dL$^{-1}$)** | 11,64±0,80 [ab] | 10,96±1,90 [b] | 11,40±2,76 [ab] | 11,80±1,03 [a] | 11,31±2,42 [ab] |
| **Phosphate (mg·dL$^{-1}$)** | 7,10±0.22 [a] | 6,61±0.23 [a] | 6,49±0.26 [a] | 6,55±0.23 [a] | 6,06±0.36 [a] |
| **Iron (ug·dL$^{-1}$)** | 291,00±21,39[b] | 430,00±28,74 [a] | 474,37±35,27 [a] | 350,00±41,25 [b] | 356,00±26,61 [b] |
| **(Median)** | (297) | (415.5) | (499) | (330) | (335.5) |

[a,b,c] Different letters in the same row indicate statistical significance ($p<0.05$).

## Discussion

Among biochemical parameters, calcium and phosphorus are the most important laboratory markers of bone metabolism. In the study, it was thought that the lack of change in Ca concentrations in the dexamethasone group could be related to increased Ca release from bone tissue, and the results were found to be consistent with Elshal et al., (2013) studies. It has been reported that glucocorticoids may cause phosphaturia by directly affecting the kidney and indirectly reducing tubular phosphate reabsorption in the absence of paratyroid hormones (Durasin et al., 1984). In the present study, the statistically insignificant ($p>0.05$) decreases in

phosphate levels observed in all groups treated with dexamethasone compared to the control group can be explained by this mechanism.

A study conducted on male Wistar rats revealed that dexamethasone caused an increase in LDH activity depending on the administered dose, suggesting that increased serum activity may be related to damage occurring in liver tissue (Arab Dolatabadi & Mahboubi, 2015). Increases observed in the parameters such as AST, ALT, and ALP were not significant despite significant increases in LDH activity in the present study. This situation was thought to be due to LDH being an enzyme widely distributed in various tissues and the possible damage that could occur in non-liver tissues following dexamethasone administration. Similarly, it was suggested that alendronate may also cause increases in LDH activity due to damage that may occur in various tissues, and it was observed that mucosal inflammation developing in the lungs following its administration in male Wistar rats elevated LDH activity in bronchoalveolar fluid (Katsumi et al., 2010). Likewise, LDH activities increased compared to control group in the study. In contrast, it has been demonstrated that vitamin D administration in osteoporosis cases can reverse this cycle and lower MDA levels, a biomarker of oxidative stress developing in various tissues (Abdlkarem & Zainulabdeen, 2024). Thus, the current study also showed that LDH activity elevated by dexamethasone administration decreased with vitamin D administration, approaching control group levels.

Although the mechanism of the hyperlipidemic effect caused by dexamethasone has not been fully elucidated (Moghadam-Kia & Werth, 2010), it has been reported that intraperitoneal administration of 2 and 4 mg/kg dexamethasone for one month in rats has a hyperlipidemic effect, with increased triglyceride levels observed at high doses (Razzaq et al., 2020). In the same direction, the present study also observed that dexamethasone administration could lead to hyperlipidemia associated with an increase in triglyceride levels. Furthermore, it was found that the therapeutic agents used were insufficient in reversing this effect.

Glucocorticoids cause hyperglycemia by decreasing glucose utilization and increasing hepatic glucose production. It has been reported that glucocorticoid administration exacerbates hyperglycemia in patients with pre-existing diabetes or glucose intolerance, and this increase is proportional to the patient's previous glucose tolerance (Moghadam-Kia et al., 2010). Although this is a known side effect of glucocorticoids, overt diabetes rarely develops in healthy individuals. In this study, dexamethasone administration caused a numerical increase in glucose levels; however, the increase was not significant compared to the control group; significant increases ($p<0.05$) were only detected in the groups treated with vitamin D. In this with line, Rateb et al., (2021) reported that HOMA-B values increased in groups treated with vitamin D compared to the dexamethasone group in male Wistar rats.

Vitamin D and alendronate administration have been found to cause various changes in protein metabolism. Albumin has an anabolic effect on bone calcification and bone components (Yamaguchi et al., 2003). It has been determined that hypoalbuminemia may directly stimulate osteoclast formation and inhibit osteogenesis and may affect the metabolism of parathyroid hormone and vitamin D-binding protein (Kunutsor et al., 2019). In this context, it was observed that vitamin D administered via gavage to rats caused significant decreases in serum total protein levels and increased gastrocnemius muscle tissue protein levels in dexamethasone-induced muscle damage (Rateb et al., 2021). While vitamin D is transported by vitamin D-binding protein, ◎-globulin, and albumin, the main protein to which alendronate binds is albumin (Karamustafa & Çelebi, 2006). This study showed a significant decrease in albumin levels in groups treated with alendronate. Furthermore,

it was considered that the decrease in total protein levels ($p<0.05$) observed in the groups treated with vitamin D could be due to changes in vitamin D-binding protein metabolism, as reported by Kunutsor et al., (2019). Furthermore, the significant increases in urea levels observed in the group receiving only vitamin D in the present study suggest that these changes may be due to similar variable effects of vitamin D on protein metabolism.

It was reported that the excretion of chloride in urine increased in male Sprague-Dawley rats depending on the dose of dexamethasone administered, but no change in plasma chloride levels was observed (Zhao et al., 2024). Okon et al., (2022) also determined that dexamethasone administration did not cause any change in the levels of chloride and other electrolytes in rats. In the present study, significant decreases ($p<0.05$) were observed in serum chloride levels in rats treated with dexamethasone; this difference was thought to be due to the very small changes in chloride levels and differences in administration. Furthermore, it was determined that alendronate administration in mice with developing acidosis caused a decrease in chloride levels in the (day 0) initial phase (Moody et al., 2023). The fact that the decrease in chloride levels resulting from dexamethasone administration persisted only in the alendronate-treated group and levels approaching those of the control group with vitamin D administration suggested that alendronate is an effective drug on serum chloride levels.

It has been reported that hypomagnesemia generally develops in rats treated with dexamethasone. It has been determined that administration of dexamethasone at a dose of 2.5 mg/kg for 5 days in female rats causes decreases in magnesium levels (Wang et al., 2022). Similar decreases were observed in another study in which 0.1 mg/kg dexamethasone was administered to female rats (Huo et al., 2022). However, Kenyon et al., (1990) found significant increases in magnesium levels with low-dose dexamethasone treatment in rats and suggested that these findings could be due to the profound catabolic effects of glucocorticoids. In this study, in which dexamethasone was administered at 7.5 mg/kg once a week for 6 weeks, serum magnesium levels elevated, similar to the findings of Kenyon et al. (1990). The difference observed in other studies may be related to the stabile levels of calcium, phosphorus, and potassium in the current study, as well as the dose and duration of administration. Furthermore, it was observed that the elevated magnesium levels resulting from dexamethasone administration only reached levels similar to those in the control group when the agents were administered in combination.

It has been demonstrated that dexamethasone can cause oxidative stress on osteocytes (Zhang et al., 2024), and it has been stated that it depletes glutathione stores via glucocorticoid receptors, leaving cells vulnerable to ferroptosis (von Mässenhausen et al., 2022). In parallel, in the present study, increases were observed in iron levels upon dexamethasone administration, and a similar pattern was observed after alendronate administration. However, iron levels approached those of the control group with vitamin D administration. This suggests that dexamethasone and alendronate administered at the current doses and regimens may cause oxidative stress in various cells.

## Conclusion

Studies have shown that the variable effects of dexamethasone are largely dependent on the dose administered, the duration of administration, and the animal species. Accordingly, the results of the study showed that alendronate, administered as a therapeutic agent at the specified dose and regimen, had a rather insufficient therapeutic effect on biochemical parameters and could even worsen the existing condition. On

the other hand, Vitamin D was found to cause undesirable changes in several parameters, while its therapeutic effect remained limited. Consequently, it is thought that further studies are needed, including different dosages and duration of administration of alendronate and vitamin D, to against the harmful effects of dexamethasone on various biochemical parameters.

**Conflict of interest:** No conflict of interest declared.
**For Studies with Ethics Committee Approval:** This study ethical approval (Approval no: 2025/119, date 30.12.2025) was obtained from Selcuk University Experimental Medicine and Application Center Ethics Committee (SÜDAM).
**Artificial Intelligence Usage Declaration Statement:** Artificial intelligence was not used at any stage of the article.
**Data Availability Statement:** The data supporting the findings of this study are available from the authors upon reasonable request after publication of the article. Corresponding Author Contact: [beyza.alan@selcuk.edu.tr].
**CRediT author statement:** BSA: Conceptualization, Methodology, Software BSA, AMA: Data curation, Writing- Original draft preparation. BSA, AMA, AÇ: Visualization, Investigation. BSA, AMA, AÇ, SH *:* Supervision.: BSA, AMA, AÇ, SH: Software, Validation.: BSA, AMA, AÇ, SH: Writing- Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.
**Acknowledgements:** The authors declare that there are no acknowledgements.
**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## References

Abdlkarem, H. A., & Zainulabdeen, J. A. (2024). A comparative study of vitamin D level and lactate dehydrogenase activity in relation to oxidative stress in women with osteoporosis. Journal of the Faculty of Medicine Baghdad, 66(1), 110-115. https://doi.org/10.32007/jfacmedbagdad.6612255

Adachi, J. D., Saag, K. G., Delmas, P. D., Liberman, U. A., Emkey, R. D., Seeman, E., Lane, N. E., Kaufman, J. M., Poubelle, P. E., Hawkins, F., Correa-Rotter, R., Menkes, C. J., Rodriguez-Portales, J. A., Schnitzer, T. J., Block, J. A., Wing, J., McIlwain, H. H., Westhovens, R., Brown, J., Melo-Gomes, J. A., Gruber B. L., Yanover M. J., Odette M. O. R., Siminoski K. G., Nevitt M. C., Sharp J. T., Malice M. P., Dumortier T., Czachur M., Carofano W., & Daifotis, A. (2001). Two-year effects of alendronate on bone mineral density and vertebral fracture in patients receiving glucocorticoids: a randomized, double-blind, placebo-controlled extension trial. Arthritis and rheumatism, 44(1), 202–211. https://doi.org/10.1002/1529-0131(200101)44:1<202::AID-ANR27>3.0.CO;2-W

Adler, R. A., & Rosen, C. J. (1994). Glucocorticoids and osteoporosis. Endocrinology and metabolism clinics of North America, 23(3), 641–654. https://doi.org/10.1016/S0889-8529(18)30090-2

Amagase, K., Inaba, A., Senta, T., Ishikawa, Y., Nukui, K., Murakami, T., & Takeuchi, K. (2011). Gastric ulcerogenic and healing impairment effects of risedronate, a nitrogen-containing bisphosphonate in rats. Comparison with alendronate and minodronate. Journal of physiology and pharmacology, 62(6), 609–618.

Arab Dolatabadi, A., & Mahboubi, M. (2015). A study of the influence of dexamethasone on lipid profile and enzyme lactate dehydrogenase. Journal of medicine and life, 8(Spec Iss 3), 72–76.

Bouillon, R., Marcocci, C., Carmeliet, G., Bikle, D., White, J. H., Dawson-Hughes, B., Lips, P., Munns, C. F., Lazaretti-Castro, M., Giustina, A., & Bilezikian, J. (2019). Skeletal and extraskeletal actions of vitamin D: Current evidence and outstanding questions. endocrine reviews, 40(4), 1109–1151. https://doi.org/10.1210/er.2018-00126

Cranney, A., Horsley, T., O'Donnell, S., Weiler, H., Puil, L., Ooi, D., Atkinson, S., Ward, L., Moher, D., Hanley, D., Fang, M., Yazdi, F., Garritty, C., Sampson, M., Barrowman, N., Tsertsvadze, A., & Mamaladze, V.

(2007). Effectiveness and safety of vitamin D in relation to bone health. Evidence report/technology assessment, (158), 1–235.

Durasin, I., Frick, A., & Neuweg, M. (1984). Glucocorticoid-induced inhibition of the reabsorption of inorganic phosphate in the proximal tubule in the absence of parathyroid hormone. Renal physiology, 7(2), 115-123. doi: 10.1159/000172930

Elshal, M. F., Almalki, A. L., Hussein, H. K., & Khan, J. A. (2013). Synergistic antiosteoporotic effect of Lepidium sativum and alendronate in glucocorticoid-induced osteoporosis in Wistar rats. African journal of traditional, complementary, and alternative medicines, 10(5), 267–273. https://doi.org/10.4314/ajtcam.v10i5.8

Ferreira-Júnior, D. B., Pinto, A. S., Del Carlo, R. J., Fonseca, C. C., Oliveira, T. T., Cardoso, C. A., Amaral, G.G., & Lima, L. R. P. (2008). Effects of bisphosphonates and statins on tibial bone healing in rats with osteoporosis induced by dexamethasone treatment. Revista de Ciências Farmacêuticas Básica e Aplicada, 29(2), 203-209.

Frediani, B., Allegri, A., Bisogno, S., & Marcolongo, R. (1998). Effects of combined treatment with calcitriol plus alendronate on bone mass and bone turnover in postmenopausal osteoporosis two years of continuous treatment. Clinical drug investigation, 15(3), 235-244. https://doi.org/10.2165/00044011-199815030-00008

Huo, J., Ding, Y., Wei, X., Chen, Q., & Zhao, B. (2022). Antiosteoporosis and bone protective effect of nimbolide in steroid-induced osteoporosis rats. Journal of biochemical and molecular toxicology, 36(12), e23209. https://doi.org/10.1002/jbt.23209

Karamustafa, F., & Çelebi, N. (2006). Bisphosphonates and alendronate. FABAD Journal of Pharmaceutical Sciences, 31(1), 31-42.

Katsumi, H., Nakatani, M., Sano, J., Abe, M., Kusamori, K., Kurihara, M., Shiota, R., Takashima, M., Fujita, T., Sakane, T., Hibi, T., & Yamamoto, A. (2010). Absorption and safety of alendronate, a nitrogen-containing bisphosphonate, after intrapulmonary administration in rats. International journal of pharmaceutics, 400(1-2), 124–130. https://doi.org/10.1016/j.ijpharm.2010.08.041

Kenyon, C. J., Brown, W. B., Fraser, R., Tonolo, G., McPherson, F., & Davies, D. L. (1990). Effects of dexamethasone on body fluid and electrolyte composition of rats. Acta endocrinologica, 122(5), 599–604. https://doi.org/10.1530/acta.0.1220599

Kunutsor, S. K., Voutilainen, A., Whitehouse, M. R., Seidu, S., Kauhanen, J., Blom, A. W., & Laukkanen, J. A. (2019). Serum albumin and future risk of hip, humeral, and wrist fractures in caucasian men: new findings from a prospective cohort study. Medical principles and practice, 28(5), 401–409. https://doi.org/10.1159/000499738

Mazziotti, G., Angeli, A., Bilezikian, J. P., Canalis, E., & Giustina, A. (2006). Glucocorticoid-induced osteoporosis: an update. Trends in endocrinology and metabolism, 17(4), 144–149. https://doi.org/10.1016/j.tem.2006.03.009

Mazziotti, G., Giustina, A., Canalis, E., & Bilezikian, J. P., (2007). Glucocorticoid-induced osteoporosis: clinical and therapeutic aspects. Arquivos brasileiros de endocrinologia e metabologia, 51(8), 1404-1412. doi: 10.1590/s0004-27302007000800028

Moghadam-Kia, S., & Werth, V. P. (2010). Prevention and treatment of systemic glucocorticoid side effects. International journal of dermatology, 49(3), 239–248. https://doi.org/10.1111/j.1365-4632.2009.04322.x

Moody, M., Schmidt, T. A., Trivedi, R., & Deymier, A. (2023). Administration of alendronate exacerbates ammonium chloride-induced acidosis in mice. Plos one, 18(9), e0291649. https://doi.org/10.1371/journal.pone.0291649

Nakamura, Y., Naito, M., Hayashi, K., Fotovati, A., & Abu-Ali, S. (2008). Effect of combined treatment with alendronate and calcitriol on femoral neck strength in osteopenic rats. Journal of orthopaedic surgery and research, 3(1), 51. https://doi.org/10.1186/1749-799X-3-51

Okada, Y., Nawata, M., Nakayamada, S., Saito, K., & Tanaka, Y. (2008). Alendronate protects premenopausal women from bone loss and fracture associated with high-dose glucocorticoid therapy. The Journal of rheumatology, 35(11), 2249–2254. https://doi.org/10.3899/jrheum.080168

Okon, I. A., Beshel, J. A., & Owu, D. U. (2022). Antioxidant status, antihyperlipidemic activity, and serum electrolyte levels in dexamethasone-induced oxidative stress in Wistar rats treated with vitamin E. Thai Journal of Pharmaceutical Sciences, 46(4), 441–448.

Pierre-Louis, T. (2010). Anti-inflammatory agents. Synthia M. K. Editor (Eds), Mercks Veterinary Manual (10th ed., pp. 2313-2328). Merck and Co., Inc.

Queiroz Júnior, J. R. A., Cartaxo, M. F. S., Paz, S. T., Tenório, F. D. C. Â. M., Lemos, A. J. J. M., & Maia, C. S. (2022). Histomorphometry of bone microarchitecture in rats treated with vitamin d and bisphosphonate in the management of osteoporosis. Revista brasileira de ortopedia, 57(2), 267–272. https://doi.org/10.1055/s-0041-1741023

Rateb, E., Khowailed, A., Rashed, L., Eesa, A., & Mohammed, M. (2021). Effect of vitamin D on dexamethasone ınduced metabolic disturbance and gastric ulcer. Annals of the Romanian Society for Cell Biology, 15200-15221.

Razzaq, S. A., Jaber, I. J., Kadhim, S. A., & Abbas, A. A. (2020). Pharmacological effects of dexamethasone in rats. Indian Journal of Forensic Medicine & Toxicology, 14(3), 1002–1006. https://doi.org/10.37506/ijfmt.v14i3.10503

Rizzoli, R., Laroche, M., Krieg, M. A., Frieling, I., Thomas, T., Delmas, P., & Felsenberg, D. (2010). Strontium ranelate and alendronate have differing effects on distal tibia bone microstructure in women with osteoporosis. Rheumatology international, 30(10), 1341–1348. https://doi.org/10.1007/s00296-010-1542-y

Rogers M. J. (2003). New insights into the molecular mechanisms of action of bisphosphonates. Current pharmaceutical design, 9(32), 2643–2658. https://doi.org/10.2174/1381612033453640

Sabry, L., Sattar, M. A., Amin, H. A., & Sattar, E. A. (2013). Antiosteoporotic effect of some herbal extracts versus alendronate on an animal model of osteoporosis. Life Science Journal, 10(4), 177-187.

Tamura, Y., Okinaga, H., & Takami, H. (2004). Glucocorticoid-induced osteoporosis. Biomedicine & pharmacotherapy, 58(9), 500–504. https://doi.org/10.1016/j.biopha.2004.08.018

von Mässenhausen, A., Zamora Gonzalez, N., Maremonti, F., Belavgeni, A., Tonnus, W., Meyer, C., Beer, K., Hannani, M. T., Lau, A., Peitzsch, M., Hoppenz, P., Locke, S., Chavakis, T., Kramann, R., Muruve, D. A., Hugo, C., Bornstein, S. R., & Linkermann, A. (2022). Dexamethasone sensitizes to ferroptosis by glucocorticoid receptor-induced dipeptidase-1 expression and glutathione depletion. Science advances, 8(5), eabl8920. https://doi.org/10.1126/sciadv.abl8920

Wang, H., Yang, L., & Chao, J. (2022). Antiosteoporosis and bone protective effect of dieckol against glucocorticoid-induced osteoporosis in rats. Frontiers in endocrinology, 13, 932488. https://doi.org/10.3389/fendo.2022.932488

Yamaguchi, M., Igarashi, A., Misawa, H., & Tsurusaki, Y. (2003). Enhancement of albumin expression in bone tissues with healing rat fractures. Journal of cellular biochemistry, 89(2), 356–363. https://doi.org/10.1002/jcb.10510

Yao, P., Bennett, D., Mafham, M., Lin, X., Chen, Z., Armitage, J., & Clarke, R. (2019). Vitamin D and calcium for the prevention of fracture: a systematic review and meta-analysis. JAMA network open, 2(12), e1917789. https://doi.org/10.1001/jamanetworkopen.2019.17789

Zhang, X., Yang, Z., Xu, Q., Xu, C., Shi, W., Pang, R., Zhang, K., Liang, X., Li, H., Li, Z., & Zhang, H. (2024). Dexamethasone ınduced osteocyte apoptosis in steroid-ınduced femoral head osteonecrosis through ROS-Mediated oxidative stress. Orthopaedic surgery, 16(3), 733–744. https://doi.org/10.1111/os.14010

Zhang, Z. L., Liao, E. Y., Xia, W. B., Lin, H., Cheng, Q., Wang, L., Hao, Y. Q., Chen, D. C., Tang, H., De Peng, Y., You, L., He, L., Hu, Z. H., Song, C. L., Wei, F., Wang, J., Zhang, L., & Santora, A. C. (2015). Alendronate sodium/vitamin D3 combination tablet versus calcitriol for osteoporosis in Chinese postmenopausal women: a 6-month, randomized, open-label, active-comparator-controlled study

with a 6-month extension. Osteoporosis international, 26(9), 2365–2374. https://doi.org/10.1007/s00198-015-3141-y

Zhao, P., Higashijima, Y., Sonoda, H., Morinaga, R., Uema, K., Oguchi, A., Matsuzaki, T., & Ikeda, M. (2024). Glucocorticoid-induced acute diuresis in rats in relation to the reduced renal expression of sodium-dependent cotransporter genes. Journal of pharmacological sciences, 156(2), 115–124. https://doi.org/10.1016/j.jphs.2024.07.005