Indonesian Journal of Pharmacy

VOL 35 (2) 2024: 250–258 | RESEARCH ARTICLE

Enhancement of Vitamin D_2 Levels in *Pleurotus ostreatus* Using Ultraviolet Irradiation and Assessing Its Effect on Dexamethasone-Induced Osteoporosis in Mice

Atina Hussaana[1], Fitria Revoni[2], Indri Aulia[2], Amade Dea[2], Denny Pradana[2], Tiara Agustina[2] and Hudan Taufiq[3]

[1.] Department of Pharmacology, Faculty of Medicine, Universitas Islam Sultan Agung, Indonesia
[2.] Faculty of Medicine, Universitas Islam Sultan Agung, Indonesia
[3.] Department of Pharmacy, Faculty of Medicine, Universitas Islam Sultan Agung, Indonesia

Article Info

Submitted: 24-05-2023
Revised: 06-11-2023
Accepted: 19-11-2023

*Corresponding author
Atina Hussaana

Email:
atinahussaana@unissula.ac.id

ABSTRACT

Mushrooms are a dietary source of vitamin D_2 due to their high ergosterol content, which can be converted into vitamin D_2 after exposure to ultraviolet (UV) irradiation. Several reports have shown that regular consumption of UVB-irradiated mushrooms is effective in increasing bone calcification and modulation of host immunity. Therefore, this study aimed to enhance vitamin D_2 formation in oyster mushrooms (*Pleurotus ostreatus*) using UVB irradiation and assess its effect on dexamethasone-induced osteoporosis in mice. Oyster mushrooms were irradiated with a UVB lamp at doses ranging from 8.01 J/cm^2 - 24.03 J/cm^2. Vitamin D_2 levels were then measured using high-performance liquid chromatography (HPLC) with calciferol as the standard. The powder of vitamin D-enriched oyster mushrooms powder was then referred to as Oyster-D. Osteoporosis in BALB/c mice was induced using Dexamethasone at a dose of 0.0029 mg/20 g BW, orally for 30 days. The number of osteoclasts, osteoblasts, and osteocytes in the femur was assessed using hematoxylin-eosin staining, while the serum calcium levels were measured with spectrophotometry. The results showed that UVB irradiation with a dose of 48.06 J/cm^2 yielded the highest vitamin D_2 concentration at 11.333 µg/g. Furthermore, there was a significant increase in the number of osteocytes, osteoblasts, and serum calcium levels, while a reduction was observed in the number of osteoclasts. Based on these findings, UVB irradiation increased vitamin D_2 levels, and vitamin D-enriched oyster mushrooms had potential anti-osteoporosis effects.

Keywords: Vitamin D; *Pleurotus ostreatus*; UV irradiation; Osteoporosis.

INTRODUCTION

Vitamin D deficiency is a significant global health concern (Holick, 1996), with prevalence rates of 24%, 37%, and 40% in the United States, Canada, and Europe, respectively. Several studies have identified various factors contributing to its occurrence, including insufficient sun exposure due to the use of sunscreens that inhibit the nutrient's synthesis in the skin. Furthermore, apart from this condition, prolonged or excessive consumption of dexamethasone, a glucocorticoid, can lead to decreased bone density. The expression of insulin-like growth factor-I has been reported to be inhibited by glucocorticoids, directly and indirectly reducing the activity of osteoblasts (Cashman, 2020; Cashman *et al*., 2016; Sarafin *et al*., 2015; Schleicher *et al*., 2016). This inhibition stimulates bone resorption, initiating bone loss in response to exposure to these compounds. The impediment of bone formation can disrupt bone remodeling, leading to an increased risk of fractures and decreased bone density (Canalis, 2003). Osteoporosis is a common metabolic disease characterized by decreased bone density, thereby necessitating careful attention (Giustina *et al*., 2018).

Mushrooms are edible macrofungi containing several vitamins and are often used in various traditional medicines (Valverde *et al*., 2015; Hasanah *et al*., 2023). These microfungi contain ergosterol, which can be converted into vitamin D_2 when exposed to ultraviolet (UV)

radiation (Sánchez, 2017). UVB irradiation has been proven to be effective in augmenting vitamin D_2 levels in *Agaricus bisporus* and other cultivated mushrooms, including the white oyster variant. The use of UVB-irradiated mushrooms is an essential and cost-effective method for preventing vitamin D deficiency (Roberts *et al*., 2008). Exposure to UVB breaks the bonds in ergosterol, specifically in C9-C10, leading to the formation of an unstable intermediate "pre-vitamin D_2". Subsequently, a thermally catalyzed mechanism converts pre-vitamin D_2 to vitamin D_2 (Chauhan *et al*., 2023). Alshammaa reported that white oyster mushrooms contained up to 48.27% more ergosterol per gram than button mushrooms, with only 27.6% (Alshammaa, 2017). This study suggested that white oyster mushrooms (*Pleurotus ostreatus*) could produce more vitamin D2 after exposure to UV radiation. Ergosterol conversion is also determined by the UV exposure, the UV spectrum, the UV position of fungi, and the moisture content. When exposed to UVB, the gill of the fungus exhibits the highest conversion to vitamin D_2 (Jasinghe & Perera, 2005; Roberts *et al*., 2008).

The duration of UVB exposure is a major factor influencing vitamin D_2 formation in mushrooms. When a fungus is exposed to UV radiation for an extended period, it increases the amounts of vitamin D_2 (Chauhan *et al*., 2023).

Vitamin D levels in the body have been reported to have a significant effect on calcium metabolism (Holick, 1996). Calcium, as the main component of bones, is essential for maintaining bone strength and density (Giustina *et al*., 2018). Furthermore, consuming oyster mushrooms enriched with vitamin D_2 and calcium can be an effective strategy for minimizing the risk of osteoporosis (Valverde *et al*., 2015). Vitamin D plays a key role in the absorption of calcium from the digestive tract into the bloodstream. Several studies have shown that inadequate levels can impair calcium absorption from the food calcium irrespective of the adequacy of Ca (Chauhan *et al*., 2023). According to a previous study, vitamin D also helps to regulate Ca balance in the body. When calcium levels in the blood are too low, it stimulates Ca absorption from the small intestine, and vice versa (Cashman, 2020).

Calcium is the main component of bones and teeth (Giustina *et al*., 2018), and adequate levels in the blood are crucial for maintaining bone strength and density. When calcium levels in the blood are too low, the body resorts to drawing Ca from the bones to meet its physiological needs. This can lead to decreased bone density and an increased risk of osteoporosis (Cashman *et al*., 2016). Previous studies have also shown that blood Ca levels affect the production and release of parathyroid hormone. Furthermore, parathyroid hormone plays a crucial role in regulating blood Ca levels, and vitamin D influences the body's response to the hormone (Canalis, 2003).

Based on these findings, there is a close link between vitamin D and calcium levels in the blood, underscoring their concerted role in maintaining healthy bones, muscle function, and a well-functioning nervous system (Roberts *et al*., 2008). A deficiency in either vitamin D or calcium can lead to bone health problems and affect overall bodily function. This shows that it is important to evaluate blood calcium levels in relation to the anti-osteoporosis effect of vitamin D-enriched oyster mushroom powder. Therefore, this study aimed to enhance the vitamin D level of oyster mushrooms (*Pleurotus ostreatus*) using UVB irradiation and examine its effect on osteoclasts, osteoblasts, osteocytes, and serum calcium levels in dexamethasone-induced BALB/c mice.

MATERIALS AND METHODS

UVB Irradiation of Oyster Mushrooms

Fresh oyster mushrooms were purchased from a local farm located in Semarang, Indonesia. The cleaned oyster mushrooms were chopped into small pieces and placed on a tray (flat container). The samples were divided into four groups and exposed to UVB radiation (Narrowband UVB lamp TL-F72, 100W/12, length 106 cm). Furthermore, groups 1, 2, 3, and 4 were exposed to doses of 8.01, 16.02, 24.03, and 48.06 J/cm2, respectively. Mushrooms that were treated with UVB radiation were dried in a drying cupboard at a temperature of 40°C for 24 hours. The dried samples were ground with a grinder into a powder and sieved with a 120 mesh sieve.

HPLC analysis of vitamin D_2 levels

The vitamin D_2 content of UVB-irradiated oyster mushrooms was determined using a method described by Koyyalamudi, *et al*. (2009) with a few modifications. The sample was then saponified and the residue was dissolved in 10 ml ethanol (95%), followed by filtration through a 0.45 nm filter. A total of 20 microliters of the filtered sample with a concentration of 100 µg /mL was injected into the HPLC system (Waters 2690; Waters Corp., USA).

Furthermore, the HPLC system used the following components, including a UV-486 detector, a C18 column (250 × 2.00 mm, Waters Corp., United States), a mobile phase of acetonitrile/methanol (75:25, v/v) at a flow rate of 1.0 mL/minute, UV detection performed at 264 nm, a 10 µl injection, and a temperature of 30°C. The vitamin D_2 content was determined by comparing the retention times with those of calciferol standards (abcam, ab143588, UK) and quantified based on a calibration curve with analyte concentrations ranging from 5 to 40 µg/mL of vitamin D_2 (Koyyalamudi *et al*., 2009). Oyster mushroom powder with the highest vitamin D content was then referred to as Oyster-D.

Animals and treatment

Male BALB/c mice (2-3-month-old, 30 g) were obtained from the Integrated Research and Testing Laboratory (LPPT) (Yogyakarta, Indonesia). All mice were housed in cages at a room temperature of 23°C ± 1°C. °C, with 55% ± 5% relative humidity, 12 h illumination period, and alternating light and dark periods. Furthermore, the animal samples received water and a standard diet with libitum. The sample size was determined using the Federer formula $(t-1)\ (n-1) \geq 15$ with group numbers (t) of 6, leading to a minimum sample size of ≥4 for each group (n). The mice underwent 2 weeks of acclimatization before diet-based grouping was carried out. A total of 25 mice were randomly divided into 5 groups, namely a) Normal, b) Negative control induced with dexamethasone and treated with aquades as a placebo, c) induced with dexamethasone, and treated with Oyster-D powder (19.5 IU/30g BW), d) induced with dexamethasone, and treated with Oyster-D powder (31.2 IU / 30 g BW), and e) induced with dexamethasone, and treated with Oyster-D powder (39 IU/30g BW). All groups were fed with standard diet ad libitum, and dexamethasone induction was given at a dose of 0.0029 mg/20 g BW, orally for 30 days.

The study protocol was approved by the bioethical committee of Universitas Islam Sultan Agung (No. 428/XII/2021/bioethics committee).

Serum Calcium Analysis

Whole blood (approximately 1 ml) was collected in a collection tube and the serum was separated by centrifugation at 15,000 rpm for 10 min at 4 °C. All collected samples were subjected to a spectrophotometry analysis, and serum calcium levels were analyzed using a Microlab 300 spectrophotometer at a wavelength of 587 nm.

Histological analysis of the femur

The separated femur of each mouse was fixed in 10% neutral buffered formalin, femur trochlea head regions were longitudinally trimmed, embedded in paraffin, sectioned in 3–4 µm, and stained with hematoxylin and eosin (HE). In each prepared histological sample, the histological profiles were interpreted. Furthermore, the osteoporosis parameters, including osteoclast, osteoblast, and osteocyte were estimated for bone.

Statistical Analysis

The statistical tests were performed using statistical software (SPSS, version 25.0, IBM Corporation, Armonk, NY, USA). Furthermore, the qualitative data were expressed as mean ± standard deviation (SD), and one-way ANOVA was used for multiple groups' comparison. The least significant difference (LSD) test was used for comparison of the mean value of each group, and a $P< 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Vitamin D2 level Enhancement of UVB-irradiated oyster mushrooms

The determination of vitamin D_2 levels in oyster mushrooms was carried out using High-Performance Liquid Chromatography (HPLC) with standard calciferol (vitamin D2) from ABCAM. The standard curve equation $Y = 29463.0142x - 12708.6676$ was obtained, and the value of R was 0.9999. Furthermore, the results showed that UVB treatment led to an increased content of vitamin D_2 in the samples. Level observed in the 4 groups treated with different doses of UVB radiation was significantly higher compared to that of the normal (Table I). The highest level of 11.333 µg/g was found in the samples treated with 48.060 J/cm^2 of UVB-radiation.

Serum Calcium Levels

The highest serum Ca was found in the group treated with 39.0 IU of Oyster-D. Furthermore, the higher the dose of Oyster D, the higher the serum Ca level (Table II).

Histological analysis of the femur

The microscopic appearance of the femoral biopsy of dexamethasone-induced mice after treatment with Oyster-D (Figure 1).

Table I. Mean of Vitamin D_2 levels of oyster mushroom at various doses of UVB-radiation.

	Without UVB-radiation	**Dose of UVB-radiation (J/cm²)**			
		8.01	16.02	24.03	48.06
Vitamin D_2 levels ± SD (µg/g) n=3	1.666±0.901	6.099±1.731	8.144±0.619	9.107±1.953	11.333±0.830

Table II. LSD Post Hoc of mean differences of Vitamin D_2 levels of oyster mushroom between various doses of UVB-radiation.

Between group		**p-value**
Without UVB-radiation	UVB-radiation 8.01 J/cm²	0.007*
	UVB-radiation 16.02 J/cm²	0.001*
	UVB-radiation 24.03 J/cm²	0.000*
	UVB-radiation 48.06 J/cm²	0.000*
UVB-radiation 8.01 J/cm²	UVB-radiation 16.02 J/cm²	0.110
	UVB-radiation 24.03 J/cm²	0.031*
	UVB-radiation 48.06 J/cm²	0.001*
UVB-radiation 16.02 J/cm²	UVB-radiation 24.03 J/cm²	0.444
	UVB-radiation 48.06 J/cm²	0.024*
UVB-radiation 24.03 J/cm²	UVB-radiation 48.06 J/cm²	0.031*

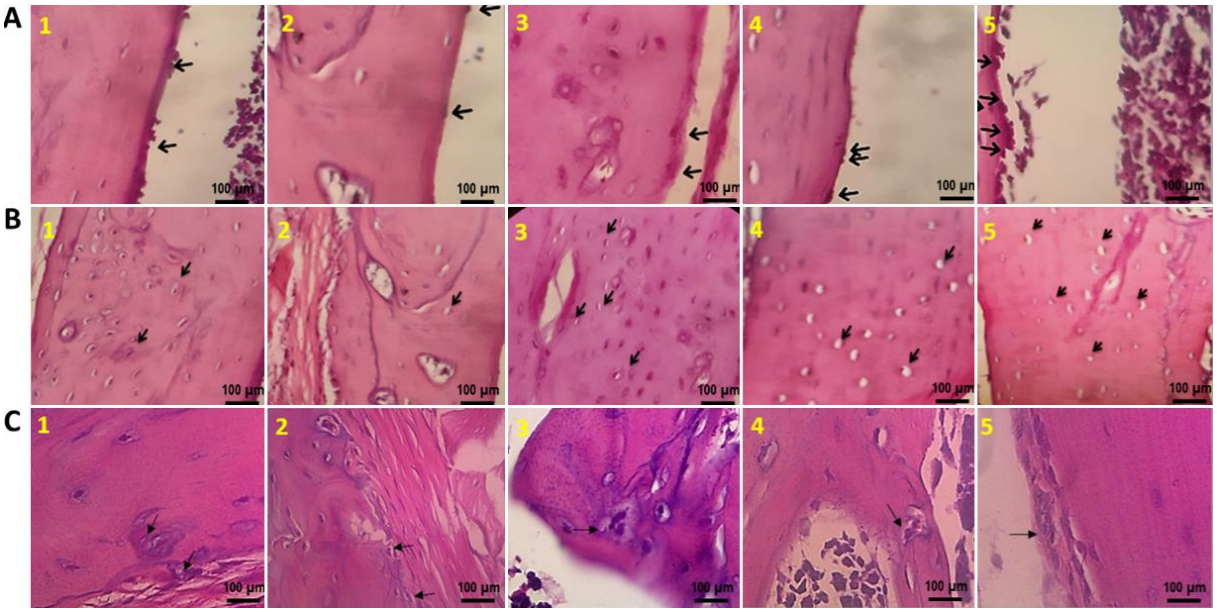

Figure 1. Microscopic image of dexamethasone-induced mouse femoral biopsy with Hematoxylin-Eosin staining at 400x magnification. (A) osteoblasts; (B) osteocytes; (C) osteoclasts. (1). normal control, (2) without Oyster-D treatment, (3) 19.5 IU/30g BW Oyster-D treatment, (4) 31.2 IU/30g BW Oyster-D treatment, and (5) 39 IU/30g BW Oyster-D treatment.

The mean number of osteoclasts, osteoblasts, and osteocytes (Table III). According to the data, the dexamethasone-induced mice group without Oyster-D treatment had the largest number of osteoclasts compared to others. Furthermore, the Oyster-D treatment group receiving 39.0 IU had the highest number of osteoblasts compared to the untreated group (Table IV).

Table III. Calcium serum levels, the number of osteoclasts, osteoblasts, and osteocytes of dexamethasone-induced mice after treatment with Oyster-D.

	Normal control	Without Oyster-D treatment	Dose of Oyster-D treatment (IU/30g BW)		
			19.5	31.2	39.0
	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
	Mean ± SD				
Number of osteoblasts	3.5±0.5	3.2±0.8	4.6±0.5	4.7±0.7	5.7±1.2
Number of osteocytes	31.2±5.9	25.4±5.8	41.1±2.9	41.9±4.1	31.2±5.9
Number of osteoclasts (x0.1)	6.4±3.2	8.4±1.6	3.6±3.2	4.0±3.1	2.4±2.6
Ca serum level (mg/dl)	12.5±1.8	11.1±0.9	13.7±2.1	16.2±1.9	16.1±2.0

Table IV. LSD Post Hoc of Mean differences of Calcium serum levels, the number of osteoclasts, osteoblasts, and osteocytes.

Between group		p-value			
		Number of osteocytes	Number of osteoblasts	Number of osteoclasts	Ca serum level
without Oyster-D treatment	19.5 IU Oyster-D	0.000*	0.016*	0.016*	0.015
	31.2 IU Oyster-D	0.000*	0.008*	0.025*	0.000*
	39.0 IU Oyster-D	0.000*	0.000*	0.004*	0.000*
19.5 IU Oyster-D	31.2 IU Oyster-D	0.779	0.753	0.828	0.010*
	39.0 IU Oyster-D	0.252	0.037*	0.516	0.014*
31.2 IU Oyster-D	39.0 IU Oyster-D	0.381	0.07*	0.389	0.882

Note: *statistically difference ($p < 0.05$)

This study enhanced vitamin D_2 formation in edible mushrooms using UV irradiation. Furthermore, the assessment of the effect of enhanced vitamin D mushrooms in dexamethasone-induced osteoporosis in mice was also carried out.

HPLC was used to determine the level of the nutrient in white oyster mushrooms (*Pleurotus ostreatus*) with calciferol (vitamin D_2) as the standard. The findings showed a linear association between the UVB exposure period and vitamin D_2 levels. UVB irradiation at a high rate significantly increased the levels of the nutrient (Wu & Ahn, 2014). The levels in *Pleurotus ostreatus* increased because its ergosterol was converted into vitamin D_2 when exposed to ultraviolet (UV) radiation (Sánchez, 2017; Phillips *et al*., 2011). The observed differences in vitamin D_2 levels were due to the distinction of cultivars, moisture contents, UVB exposure period, temperature, mushroom preparation, and operating conditions.

According to Heo *et al*. (2020), mushroom powder irradiated by UVB rays contained up to 8.19 μg/g of vitamin D_2. The results showed that group 4 had the highest levels at 9.1074 μg/g, with extended irradiation of 45 minutes. Based on these findings, UVB irradiation affected the reaction of ergosterol related to the formation of vitamin D_2. Ergosterol was the most abundant content, and when irradiated with UVB, it underwent photolysis to produce provitamin, which slowly isomerized to vitamin D (Phillips *et al*., 2011; Villares *et al*., 2014). Furthermore, before becoming vitamin D_2, system heating was performed using a reflux (Ahlborn *et al*., 2018; Zhang *et al*., 2018). Vitamin D_2 levels in white oyster mushrooms increased because ergosterol was exposed to UV radiation. When the compound was exposed to UV radiation, it underwent a bond breaking at C_9-C_{10} accompanied by an isomerization reaction to form provitamin D_2. The steric interaction between the methyl group's C6 and C19 rings made provitamin D_2 unstable, leading to single and s-trans bonds. The more the white oyster mushroom was exposed to UVB, the more the amount of cis and trans isomers produced, leading to an increase in vitamin D_2 (Chauhan *et al*., 2023).

The longer the UVB irradiation exposure time, the greater the energy exerted on oyster mushrooms, thereby affecting vitamin D_2 levels. A

high energy of 48,06 J/cm^2 caused a vitamin D_2 level of 11.333 ± 0.830 µg/g. After UVB irradiation, high levels caused the amount of ergosterol in fungal cell walls to decrease, impairing their function. Several studies had shown that ergosterol played a role in membrane fluidity and permeability (Iwaki *et al*., 2008). Vitamin D_2 levels in the present study were higher compared to the values reported by Heo *et al*. (2020) using superfine white button mushroom powder (7.1 ± 0.3 µg/g) (Bikle, 2014). In 94.28 minutes of UVB exposure, Keflie *et al*., 2019 obtained 315.5±8.2 µg/g of vitamin D_2 from white oyster mushroom (*Pleurotus ostreatus*).

According to the analysis of the number of osteocytes, osteoblasts, osteoclasts, and serum calcium levels, the Oyster-D treatment of 39.0 IU had a significant effect on the growth of osteoblasts compared to the untreated group. Glucocorticoids or corticosteroids could reduce osteoblasts and osteocytes through apoptosis, which was caused by caspase-3 activation. The anti-anabolic effect of glucocorticoids reduced IGF2, which functioned as a local regulator of osteoblast function. Glucocorticoids reduced the expression of OPG, causing the osteoclast lifespan to be elongated compared to osteoblasts and increasing the resorption of the bone matrix (Briot & Roux, 2015).

The presence of ergocalciferol (vitamin D_2) in white oyster mushrooms stimulated the growth of osteoblasts. Ergocalciferol was metabolized and transformed to 1,25-dihidroxyvitamin D_3 ($1,25(OH)_2D_3$), which bound to receptors in the bone to regulate mineralization (Pike *et al*., 2017). $1,25(OH)_2D_3$ bound to VDR (Vitamin D Receptors) near each tissue through different genome genes, leading to various effects (Bikle, 2014; Pike *et al*., 2017). Previous studies had reported the effect of vitamin D_2-fortified mushrooms on circulating levels of 25(OH)D and parathyroid hormone (PTH). Distal tomographic microcomputer (micro-CT) analysis of the femur was used to assess the ability of the mushrooms to support bone growth, and histological evidence of soft tissue calcification was assessed to ensure the safety of high vitamin D2 levels of white button mushrooms given to mice daily for ten weeks (Calvo *et al*., 2013).

Osteoblasts differentiated when transcription factors, such as osterix (Osx), runt-related transcription factor 2 (RUNX-2), and the WNT pathway were activated (Berardi *et al*., 2021; Vega *et al*., 2017). When 1,25-dihydroxy vitamin D bound to VDR at the RUNX-2 binding site on bone cells, it induced osteoblast transcription and differentiation (Bikle, 2014; Corrado *et al*., 2017). Osteoblasts produced OPG (osteoprotegerin), which served as a competitive inhibitor of RANK, preventing RANKL from binding to RANK. Osteoclastogenesis was a process that often led to a reduction in the number of osteoclasts. Vitamin D, calcitonin, estrogen, parathyroid hormone (PTH), serotonin, and leptin played essential role in osteoblast OPG expression. Furthermore, decreased osteoclastogenesis caused the production of osteoclast cells to reduce (Christakos *et al*., 2015).

Increasing the dosage of Oyster-D to 19.5-39.0 IU, led to an increase in the number of osteoblasts, osteocytes, and calcium serum levels, as well as a decrease in osteoclasts. The findings showed that exposing white oyster mushrooms to UVB radiation led to osteoporosis repair. This result had the potential to be expanded to prevent osteoporosis and continued into clinical studies to determine effectiveness, tolerability, and quality of life in osteoporosis patients.

In this study, there was evidence that osteocyte cells affected not only bone remodeling but could also improve bone structure. Osteocytes were located in areas close to the micro-damage where these processes occurred through apoptosis or were caused by other pathological factors. When increased bone remodeling due to pathological or physiological conditions that affected the bone structure occurred, osteocytes often increased the release of RANKL by the sclerotin protein, leading to the formation of osteoclasts. Several studies had shown that both substances had an essential role in bone remodeling (Kular *et al*., 2012; Prideaux *et al*., 2016). In this study, dexamethasone was used to induce osteoporosis in mice, which could be recognized in normal mice by transverse bending lumbar vertebrae. This was because mice had already developed osteoporosis or bone loss (Kim *et al*., 2021). Decreased blood calcium in osteoporosis was associated with the regulation of parathyroid and thyroid hormones that affected bone production and resorption. Calcium and vitamin D deficiency often affected the hormones regulating calcium. Uncontrolled calcium levels promoted parathyroid hormone secretion, leading to excessive bone remodeling that caused a loss in bone density and an increased risk of fracture (Felsenfeld *et al*., 2014).

The limitation of this study was the lack of bone density quantification, which led to inaccuracy in describing osteoporosis repair. However, measurements of the number of

osteoblasts, osteocytes, osteoclasts, and serum calcium levels were sufficient to represent a repair in the symptoms of osteoporosis.

CONCLUSION

In conclusion, this study showed the effect of 4 different doses of UVB irradiation on the vitamin D content of oyster mushrooms. The UVB irradiation significantly increased D_2 levels of oyster mushrooms up to 11.333 µg/g. Furthermore, the administration of vitamin D_2 19.5 – 39.0 IU significantly increased serum calcium, the number of osteocytes and osteoblasts, as well as decreased the number of osteoclasts. Further studies were needed to ascertain the effects of UVB-irradiated mushrooms on bone density.

ACKNOWLEDGMENTS

This study was supported by grants No : 02/P-Kel/VIII/2022, from the Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahlborn, J., Calzolari, N., Spielmeyer, A., Avci, S. S., Zimmer, M., & Rühl, M. (2018). Enrichment of vitamin D2 in mycelium from submerged cultures of the agaric mushroom Pleurotus sapidus. *Journal of Food Science and Technology*, *55*(9), 3833–3839. https://doi.org/10.1007/s13197-018-3290-z

Alshammaa, D. (2017). Phytochemical investigation and quantitative comparison of ergosterol between *Agaricus bisporus* and *Pleurotus ostreatus* by HPLC and GC-MS methods. *International Journal of Pharmaceutical Sciences Review and Research*, *2*(44), 215–220.

Berardi, S., Corrado, A., Maruotti, N., Cici, D., & Cantatore, F. P. (2021). Osteoblast role in the pathogenesis of rheumatoid arthritis. *Molecular Biology Reports*, *48*(3), 2843–2852. https://doi.org/10.1007/s11033-021-06288-y

Bikle, D. D. (2014). Vitamin D metabolism, mechanism of action, and clinical applications. *Chemistry and Biology*, *21*(3), 319–329. https://doi.org/10.1016/j.chembiol.2013.12.016

Briot, K., & Roux, C. (2015). Glucocorticoid-induced osteoporosis. *RMD Open 2015*, *1*(1), e000014. https://doi.org/10.1136/rmdopen-2014-000014

Calvo, M. S., Babu, U. S., Garthoff, L. H., Woods, T. O., Dreher, M., Hill, G., & Nagaraja, S. (2013). Vitamin D2 from light-exposed edible mushrooms is safe, bioavailable and effectively supports bone growth in rats. *Osteoporosis International*, *24*(1), 197–207. https://doi.org/10.1007/s00198-012-1934-9

Canalis, E. (2003). Mechanisms of glucocorticoid-induced osteoporosis. *Current Opinion in Rheumatology*, *15*(4), 454–457. https://doi.org/10.1097/00002281-200307000-00013

Cashman, K. D. (2020). Vitamin D deficiency: defining, prevalence, causes, and strategies of addressing. *Calcified Tissue International*, *106*(1), 14–29. https://doi.org/10.1007/s00223-019-00559-4

Cashman, K. D., Dowling, K. G., Škrabáková, Z., Gonzalez-Gross, M., Valtueña, J., De Henauw, S., Moreno, L., Damsgaard, C. T., Michaelsen, K. F., Mølgaard, C., Jorde, R., Grimnes, G., Moschonis, G., Mavrogianni, C., Manios, Y., Thamm, M., Mensink, G. B. M., Rabenberg, M., Busch, M. A., … Kiely, M. (2016). Vitamin D deficiency in Europe: pandemic? *American Journal of Clinical Nutrition*, *103*(4), 1033–1044. https://doi.org/10.3945/ajcn.115.120873

Chauhan, K., Shahrokhi, M., & Huecker, M. R. (2023). Vitamin D. In *StatPearls [Internet]* (pp. 4–7). https://www.ncbi.nlm.nih.gov/books/NBK441912/

Christakos, S., Dhawan, P., Verstuyf, A., Verlinden, L., & Carmeliet, G. (2015). Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiological Reviews*, *96*(1), 365–408. https://doi.org/10.1152/physrev.00014.2015

Corrado, A., Maruotti, N., & Cantatore, F. P. (2017). Osteoblast role in rheumatic diseases. *International Journal of Molecular Sciences*, *18*(6), 1272. https://doi.org/10.3390/ijms18061272

Giustina, A., Adler, R. A., Binkley, N., Bouillon, R., Ebeling, P. R., Lazaretti-Castro, M., Marcocci, C., Rizzoli, R., Sempos, C. T., & Bilezikian, J. P.

(2018). Controversies in vitamin D: summary statement from an international conference. *Journal of Clinical Endocrinology and Metabolism*, *104*(2), 234–240. https://doi.org/10.1210/jc.2018-01414

Hasanah, S. N., Chodidjah, C., & Hussaana, A. (2023). The potential of UV-B irradiated oyster mushroom (*Pleurotus ostreatus*) powder on plasma glucose and TNF-a levels in type 2 diabetic rats. *Tropical Journal of Natural Product Research*, 7(12). http://www.doi.org/10.26538/tjnpr/v7i12.7

Heo, T.-Y., Kim, Y.-N., Park, I. B., & Lee, D.-U. (2020). Amplification of vitamin D2 in the white button mushroom (*Agaricus bisporus*) by UV-B irradiation and jet-milling for its potential use as a functional ingredient. *Foods*, *9*(11), 1713. https://doi.org/10.3390/foods9111713

Holick, M. F. (1996). Vitamin D and bone health. *The Journal of nutrition*, 126, 1159S-1164S. SUPPL.), 1159–1164. https://doi.org/10.1093/jn/126.suppl_4.1159s

Iwaki, T., Iefuji, H., Hiraga, Y., Hosomi, A., Morita, T., Giga-Hama, Y., & Takegawa, K. (2008). Multiple functions of ergosterol in the fission yeast *Schizosaccharomyces* pombe. *Microbiology*, *154*(3), 830–841. https://doi.org/10.1099/mic.0.2007/011155-0

Jasinghe, V. J., & Perera, C. O. (2005). Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D2 by UV irradiation. *Food Chemistry*, *92*(3), 541–546. https://doi.org/10.1016/j.foodchem.2004.08.022

Keflie, T. S., Nölle, N., Lambert, C., Nohr, D., & Biesalski, H. K. (2019). Impact of the natural resource of UVB on the content of vitamin D2 in oyster mushroom (*Pleurotus ostreatus*) under subtropical settings. *Saudi Journal of Biological Sciences*, 26(7), 1724-1730.https://doi.org/10.1016/j.sjbs.2018.07.014

Kim, M. Y., Lee, K., Shin, H. I., Lee, K. J., & Jeong, D. (2021). Metabolic activities affect femur and lumbar vertebrae remodeling, and anti-resorptive risedronate disturbs femoral cortical bone remodeling. *Experimental and Molecular Medicine*, *53*(1), 103–114. https://doi.org/10.1038/s12276-020-00548-w

Koyyalamudi, S. R., Jeong, S. C., Song, C. H., Cho, K. Y., & Pang, G. (2009). Vitamin D2 formation and bioavailability from *Agaricus bisporus* button mushrooms treated with ultraviolet irradiation. *Journal of Agricultural and Food Chemistry*, *57*(8), 3351–3355. https://doi.org/10.1021/jf803908q

Kular, J., Tickner, J., Chim, S. M., & Xu, J. (2012). An overview of the regulation of bone remodelling at the cellular level. *Clinical Biochemistry*, *45*(12), 863–873. https://doi.org/10.1016/j.clinbiochem.2012.03.021

Levine, B. S., Rodríguez, M., & Felsenfeld, A. J. (2014). Serum calcium and bone: effect of PTH, phosphate, vitamin D and uremia. *Nefrología* (English Edition), 34(5), 658-669.

Phillips, K. M., Ruggio, D. M., Horst, R. L., Minor, B., Simon, R. R., Feeney, M. J., Byrdwell, W. C., & Haytowitz, D. B. (2011). Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. *Journal of Agricultural and Food Chemistry*, *59*(14), 7841–7853. https://doi.org/10.1021/jf104246z

Pike, J. W., Meyer, M. B., Lee, S. M., Onal, M., & Benkusky, N. A. (2017). The Vitamin D receptor: contemporary genomic approaches reveal new basic and translational insights. *Journal of Clinical Investigation*, *127*(4), 1146–1154. https://doi.org/10.1172/JCI88887

Prideaux, M., Findlay, D. M., & Atkins, G. J. (2016). Osteocytes: The master cells in bone remodelling. *Current Opinion in Pharmacology*, *28*, 24–30. https://doi.org/10.1016/j.coph.2016.02.003

Roberts, J. S., Teichert, A., & McHugh, T. H. (2008). Vitamin D2 formation from post-harvest UV-B treatment of mushrooms (*Agaricus bisporus*) and retention during storage. *Journal of Agricultural and Food Chemistry*, *56*(12), 4541–4544. https://doi.org/10.1021/jf0732511

Sánchez, C. (2017). Reactive oxygen species and antioxidant properties from mushrooms. *Synthetic and Systems Biotechnology*, *2*(1), 13–22. https://doi.org/10.1016/j.synbio.2016.12.001

Sarafin, K., Durazo-Arvizu, R., Tian, L., Phinney, K. W., Tai, S., Camara, J. E., Merkel, J., Green, E.,

Sempos, C. T., & Brooks, S. P. J. (2015). Standardizing 25-hydroxyVitamin D values from the Canadian health measures survey. *American Journal of Clinical Nutrition*, *102*(5), 1044–1050. https://doi.org/10.3945/ajcn.114.103689

Schleicher, R. L., Sternberg, M. R., Looker, A. C., Yetley, E. A., Lacher, D. A., Sempos, C. T., Taylor, C. L., Durazo-Arvizu, R. A., Maw, K. L., Chaudhary-Webb, M., Johnson, C. L., & Pfeiffer, C. M. (2016). National estimates of serum total 25-hydroxyvitamin D and metabolite concentrations measured by liquid chromatography-tandem mass spectrometry in the US population during 2007-2010. *Journal of Nutrition*, *146*(5), 1051–1061. https://doi.org/10.3945/jn.115.227728

Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: improving human health and promoting quality life. *International Journal of Microbiology*, *2015*, 14 pages. https://doi.org/10.1155/2015/376387

Vega, O. A., Lucero, C. M. J., Araya, H. F., Jerez, S., Tapia, J. C., Antonelli, M., Salazar-Onfray, F., Las Heras, F., Thaler, R., Riester, S. M., Stein, G. S., van Wijnen, A. J., & Galindo, M. A. (2017). Wnt/β-Catenin signaling activates expression of the bone-related transcription factor RUNX2 in select human osteosarcoma cell types. *Journal of Cellular Biochemistry*, *118*(11), 3662–3674. https://doi.org/10.1002/jcb.26011

Villares, A., Mateo-Vivaracho, L., García-Lafuente, A., & Guillamón, E. (2014). Storage temperature and UV-irradiation influence on the ergosterol content in edible mushrooms. *Food Chemistry*, *147*, 252–256. https://doi.org/10.1016/j.foodchem.2013.09.144

Wu, W. J., & Ahn, B. Y. (2014). Statistical optimization of ultraviolet irradiate conditions for vitamin D2 synthesis in oyster mushrooms (*Pleurotus ostreatus*) using response surface methodology. *PLoS ONE*, *9*(4), e95359. https://doi.org/10.1371/journal.pone.0095359

Zhang, Y., Zhou, W. E., Yan, J. Q., Liu, M., Zhou, Y., Shen, X., Ma, Y. L., Feng, X. S., Yang, J., & Li, G. H. (2018). A review of the extraction and determination methods of thirteen essential vitamins to the human body: an update from 2010. *Molecules*, *23*(6), 1–25. https://doi.org/10.3390/molecules23061484