rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Journal of Publlic Health in Africa: Decision on your manuscript

6 messages

ICPHS 2022 <scicommicphs2022@gmail.com> Thu, Dec 22, 2022 at 6:12 AM
To: rr-retno-w@ff.unair.ac.id

Ref: Submission ID ICPHS-13

Dear Dr. Retno Widyowati,

Your manuscript, "Effects of Eleutherine bulbosa (Mill.) Urb. Extract on Mice Glucocorticoid-induced Osteoporosis Models", has now been reviewed, and the reviewer comments are appended below. While the reviewers find your work of interest, you will see that they have raised points that need to be addressed.

We therefore invite you to revise your paper, taking into account the points raised. At the same time, we ask you to make sure your manuscript complies with our format by reviewing our guidelines for preparing your manuscript, as attached to this email. After revision, the manuscript should have a similarity index of less than 20%.

Once you have addressed each comment and completed each step listed below, the revised submission and final file can be uploaded via the link below.

https://forms.gle/XoiHYyMZGkwwvbor5

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This must be uploaded as a 'Point-by-point response to the reviewers' file. All changes to the manuscript must be highlighted or indicated using tracked changes.

At this stage, please also ensure that you have replaced your initial-submission image files with production quality figures. These should be supplied at 300 dpi resolution for .jpeg and .tiff or as .eps files. Figures should not include Figure number labels in the image.

Please ensure you conform to our authorship policies, also outlined here: https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html

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Please note that your revised manuscript will be subject to another round of quality checking before it is returned to the Editor for assessment.

Please note that we usually expect revisions to be returned within 14 days. Please request an extension by replying to this email if this does not apply to you.

Kind regards,

Tutik Sri Wahyuni
Andang Miatmoko

Guest Editors
Journal of Public Health in Africa (The thematic Issue of ICPHS 2022)

Reviewer Comments:

Reviewer 1
The article is well written. Suggestions to improve the clarity and quality of the manuscript (details attached):

1. Title: please include the part of the plant used
2. Introduction: Any traditional use related to osteoporosis?
3. There is a study on anti osteoporosis from E. bulbosa extract (Bahtiar A., Annisa R. Effects of Dayak onion Bulbs (Eleutherine bulbosa (Mill.) Urb) on bone development of the hypoestrogen model rat. Pharmacogn. J. 2018;10:299–303. doi: 10.5530/pj.2018.2.52.). What is the difference between your study and this?
4. Methods:
    a. The method used for drying the sample should be included. Please check on the term used.
    b. Use the common terms
    c. what is the dose for glucocorticoid used?
    d. How do you know that the animal has developed osteoporosis?
5. Results:
    a. Include the p-value for calcium measurement
    b. What is the value of bone density categorized as osteoporosis in animal models?
6. Discussion: Has any plant from Eleutherine been studied for osteoporosis?
7. Conclusion: Please clearly state the dosage in the last sentence
8. Table: Please add information on which one statistically differ
9. Grammar should be checked, and some sentences should be revised for clarity.

Reviewer 2
The manuscript describes the finding on the efficacy of E. bulbosa bulb extract as anti osteoporosis. The research design has been well described. The results have been adequately shown in the tables. There is sufficient discussion regarding the findings.
Minor notes are as follows.

1. Using mg/200 g BW as the dose unit is uncommon. The author may consider converting and replacing it with mg/kg.
2. It should be clearly shown in the table when the results statistically give a significant difference. The significant marks and further footnotes may be useful.
3. The method attribute for the spectrophotometer by Balai Besar Laboratory should be clearly stated in detail e.g., the wavelength.
4. What kind of glucocorticoids have been used? Please provide the manuscript's exact materials information, code, and manufacturer. Add the dose of it in the animal model part and abstract.
5. Additionally, the author needs to examine the grammatical matters in the whole manuscript.
6. "The treatment dose 3 group then demonstrated a rise in bone density percentage." This sentence should be rechecked.
7. The last paragraph of the discussion is puzzling. The comparison data is irrelevant. Further, which one is being the author's argument? Do the extract and drug increase the calcium level or not? There was an increase based on the conclusion, but the author further discussed that there is no correlation between Ca level and osteoporosis. The discussion structure should be improved at this point.
8. The use of "might" in the result part of the abstract should be reconsidered.

3 attachments

ICPHS-13-reviewed - ICPHS 2022.docx

Effects of *Eleutherine bulbosa* (Mill.) Urb. Extract on Mice Glucocorticoid-induced Osteoporosis Models

** 4/12/22 20.27
Comment [1]: part of the plant used should be stated

Fina Luthfiana[1], Riza Ambar Sari[1], Irawati Sholikhah[2], Katsuyoshi Matsunami[3], Retno Widyowati[4,5]*, Sukardiman[4,5]

[1]*Master Programe of Pharmaceutical Sciences, Faculty of Pharmacy, University of Airlangga, Surabaya, 60115 Indonesia*
[2]*Department of Chemistry, Faculty of Sains and Technology, University of Airlangga, Surabaya, 60115 Indonesia*
[3]*Department of Pharmacognosy, Graduate School of Biomedical & Health Sciences, Hiroshima University, Japan*
[4]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Airlangga, Surabaya, 60115 Indonesia*
[5]*Natural Products Drug Discovery and Development, Faculty of Pharmacy, Universitas Airlangga, 60115 Indonesia*

Corresponding author: Retno Widyowati, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Airlangga University, Surabaya, 60115. Tel: (+62) 81615886978. Email: rr-retno-w@ff.unair.ac.id

Acknowledgments: This research was supported by International Research Collaboration Top #500 of Airlangga University with the contract No. 1546/UN3.15/PT/2021

Keywords: *Eleutherine bulbosa*, Calcium, Osteoblast cell, Bone density, Osteoporosis

Authors' Contributions :

Fina Luthfiana : literature search, experimental studies, data analysis

Riza Ambar Sari : literature search, experimental studies, data acquisition

Irawati Sholihah : Statistical analysis, research materials

Katsuyoshi Matsunami : Manuscript editing and review

Sukardiman : Manuscript editing and review

Retno Widyowati : conception and design research, Manuscript editing and review

Conflict of Interests : The authors have declared that no conflict of interest exist.

Further information

Funding : This research was supported by International Research Collaboration Top #500 of Airlangga University with the contract No. 1546/UN3.15/PT/2021

Abstract:

Backgrounds: Low bone mass accompanied by microarchitectural alterations in the bone that cause fragility fractures is known as secondary osteoporosis and occurs when there is an underlying condition or medication present. *Eleutherine bulbosa* bulb extract has been shown to affect bone because of its content which can help osteoblast differentiation and inhibit osteoclast differentiation.

Objective: This study aimed to assess the effects of *E. bulbosa* bulbs extract (EBE) with 70% ethanol from from Pasuruan-East Java on blood calcium levels, osteoblast cell count, and bone density of trabecular femur in osteoporosis rats.

Methods: Six groups of 30 female Wistar rats were created. There were no test materials offered to the healthy group; the negative group received 0.5% CMC; the positive group received alendronate 0.18 mg/200 g BW; and the dose group received of 6, 12, 24 mg/200 g BW. Glucocorticoid induction was given to all groups except the healthy group to create osteoporosis rats for approximately four weeks. Then given oral therapy for approximately 28 days. Followed by the determination of blood calcium levels, the number of osteoblast cells, and bone density of the rat femur trabecular.

Results: The result showed that *E. bulbosa* bulbs extract might raise blood calcium levels and bone density percentage at doses of 12 and 24 mg/200 g BW, as well as raise osteoblast cell levels at doses of 24 mg/200 g BW.

Conclusions: The findings suggested that *E. bulbosa* bulbs extract could be used as an innovative drug for osteoporosis.

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Comment [2]: please rewrite this sentence

** 4/12/22 20.29
Comment [3]: This sentence should be rewrite for clarity

1. *Introduction*

A bone metabolic disorder called osteoporosis causes diminished bone mass, alteration of bone's microarchitecture, and enhanced bone fragility, all of which raise the chance of fracture.[1] Noteworthy is that about 300,000 hip fracture patients each year wind up in nursing homes, and half never restore their pre-injury function.[2] A variety of factors can impair bone metabolism, including a lack of nutritional deficiency and sedentary lifestyle,[3] use of alcohol,[4] smoking,[5] genetic factors,[6] medication,[7] hyperparathyroidisms,[8] rheumatoid arthritis,[9] diabetes mellitus,[10] dementia,[11] and cancer.[12,6]

The glucocorticoid group is a drugs in the first order that causes secondary osteoporosis, which affects adults more frequently than any other cause due to its side.[13,14] Adults on glucocorticoid often have a hunchback, back pain, height loss, or even fractures that may result in disability, creating a significant financial burden on families and society.[14] This is because glucocorticoids affect bone mineral homeostasis with the mechanism of action of vitamin D antagonists, stimulating renal calcium excretion, and inhibiting bone formation which causes an increase in osteoclast resorption resulting in a decrease in bone mass.[15]

Corticosteroids induce osteoporosis up to eight times greater than osteoporosis due to underlying disease.[16] Induction of dexamethasone for four weeks in mice is equivalent to induction for 3-4 years for humans.[17,18] Long-term (at least 3-6 months) use of the group compounds corticosteroids may slow the process of bone growth.[19]

The *E. bulbosa* bulbs are one of the plants that contain compounds with osteoporosis activity. It's from the Indonesian province of Central Kalimantan.[20] This plant is from the Iridaceae family and is used to treat breast cancer.[21] An in silico study published in 2014 found that *E. bulbosa* bulbs contain derivatives of the nafthoquinone compound, eleutherinol, which acts as an antagonism for mammary estrogen alpha receptors (ER-α).[20] These substances may be employed as a treatment option for postmenopausal conditions since they are anticipated to be selective agonists of

** 4/12/22 20.30
Comment [4]: do you mean antiosteoprosis activity?

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Comment [5]: Any traditional use related to osteoporosis?

estrogen receptors in different tissues, including bone and blood vessels. This extract also contains a liquiritigenin compound, which has a high affinity for selectively binding with estrogen beta receptors and can promote osteoblast differentiation while inhibiting osteoclast differentiation.[22]

As a consequence, more research is needed to scientifically prove the effects of *E. bulbosa* bulbs on osteoporosis treatment as seen by raising levels of serum calcium, the percentage of bone density, and the level of osteoblast cells.

2. *Materials and Methods*

2.1 Plant Materials

Eleutherine bulbosa (Mill.) Urb. was found and obtained from Pasuruan, East Java, Indonesia. Determined by UPT Laboratorium Herbal Materia Medica Batu, East Java, Indonesia (Certificate of Determination No. 074/722/102.7-A/2021).

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Comment [6]: removed this

2.2 Preparation of Extract

Ethanol 70% was used to extract the dry powder from *E. bulbosa* bulbs using the maceration method. A rotary evaporator was used to concentrate the extract. Ethanol extract of *E. bulbosa* bulbs was calculated as % w/w yield, which was 15,98%.

** 4/12/22 20.33
Comment [7]: Drying method should be detailed

** 4/12/22 20.34
Comment [8]: 15.98%

2.3 Ethical Considerations

This research was conducted using experimental animals which were female white rats (*Rattus norvegicus*) Wistar strain, obtained from the Animal Laboratory, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia. Research Ethics Commission (Animal Care and Use Committee) Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia, have carefully studied the proposed research design, rats with healthy conditions aged 3-4 months weighing 200-300 g. Food and water were available *ad libitum*. Acclimatized for 1 week. Placed in a room with a 12-hour light/dark cycle with controlled conditions of temperature and humidity in the Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga.

Then, hereby declare that ethically appropriate (No: 2.KEH.120.09.2022).

2.4 Animal model

Thirty rats were divided into six different groups (5 rats per group). The group was divided into (1) a healthy group, rats were not induced by glucocorticoids, (2) a negative group, rats were induced with glucocorticoids and given 0.5% CMC-Na therapy, (3) a positive group, rats were induced with glucocorticoids and given alendronate 0.18 mg /200 g BW/day, (4) dose 1, rats were induced with glucocorticoids and given 6 mg/200 g BW of extract, (5) dose 2, rats were induced with glucocorticoids and given 12 mg/200 g BW, (6) dose 3, rats were induced with glucocorticoids and given 24 mg/200 g BW of extract. Glucocorticoid induction was carried out for 4 weeks orally. After the animal developed osteoporosis, therapy was carried out for 4 weeks orally. Measurements of serum calcium levels, femoral trabecular bone density, and the number of osteoblasts were performed at week 4 after therapy.

** 4/12/22 20.36
Comment [9]: what is the dose for glucocorticoid used?

** 4/12/22 20.38
Comment [10]: change with EBE so that the same with previous paragraph

** 4/12/22 20.41
Comment [11]: How do you know that the animal have developed osteoporosis?

2.5 Evaluation of Parameters

2.5.1 The level of serum calcium

At the end of the treatment, the rats in all groups were sacrificed and blood samples were taken from the heart to determine the calcium serum level. Examination of blood calcium using a spectrophotometer at the Balai Besar Laboratory, Surabaya, Indonesia.

2.5.2 Histological analysis

When the procedure is over, the rats in all groups were sacrificed and the trabecular femur bone was taken. The femoral trabecular bone was prepared by fixing them with 10% formalin, decalcifying them, neutralizing them, washing them with water, and then rinsing them with 70% alcohol. After that, the bone was sealed with paraffin before being microtome-cut. Afterward, it was soaked in 70% alcohol and stained with Mallory Azan (MA). Olympus Cellsens software with a 200× zoom was used to analyze the observation slides. The microscope used for

observations was connected to a computer and *Matic image software*. Bone density and osteoblast cell were calculated at the Histology Laboratory, Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

2.5.3 Statistical analysis

SPSS was used to examine the data from the animal experiments. A one-way analysis of variance (ANOVA) and an analysis of the Least Significant Difference (LSD) was carried out to investigate the relationship between the treatment groups, with p-values of less than 0.05 considered to be significantly different.

3. Results

The level of serum calcium

The positive control group, EBE 12 mg/200 g BW, EBE 24 mg/200 g BW, healthy group, and negative control group all had average calcium levels that ranged from highest to lowest (Table 1).

There was a difference between the dose 1, 2, and 3 groups, as seen by the average calcium levels with dose variation. A substantial difference between EBE 6 and 12 mg/200 g BW, as well as EBE 6 and 24 mg/200 g BW, was revealed by statistical analysis. There was no significant difference between EBE 12 and 24 mg/200 g BW.

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Comment [12]: what is the p value?

Average values for the positive and negative control group were vastly different. According to statistical analyses, there is a significant difference between them. This is demonstrated by statistical tests that show a significant difference between the positive and negative groups. Moreover, statistical analysis revealed no significant difference in calcium levels between the positive control group and EBE (12 mg/200 g BW and 24 mg/200 g BW). The calcium levels of the positive control group and EBE 6 mg/200 g BW. High levels of calcium in the serum of the group receiving three doses of extract therapy demonstrated that the rise in the extract dose had an impact on the rise in calcium levels in the serum.[23]

Percentage of bone density

The positive control group, EBE 24 mg/200 g BW, the healthy group, EBE 12 mg/200 g BW, EBE 6 mg/200 g BW, and the negative control group had an average bone density from highest to lowest (Table 1). The healthy group's average bone density is significantly higher than that of the negative control group. Statistical tests that demonstrate a substantial difference in bone density between the healthy group and the negative control group serve as proof of this. This shows that the glucocorticoid induction process was successful and that rats were in fact experiencing osteoporosis.

** 4/12/22 20.47
Comment [13]: What is the value of bone density that is categorised as osteoporosis in animal model?

In comparison to the negative control group, the positive control group had an average bone density that was considerably higher. This is supported by statistical analyses that show a significant difference between the positive and negative control group. The level of bone density between of positive control group and EBE (12 and 24 mg/200 g BW) did not differ significantly, according to statistical analysis. Additionally, there was a statistically significant difference in bone density between the EBE 6 mg/200 g BW group and the positive control group.

Level of osteoblast cell

The positive control group, EBE 24 mg/200 g BW, EBE 12 mg/200 g BW, healthy group, EBE 6 mg/200 g BW, and negative control group had an average level of osteoblast cell from highest to lowest (Table 1). There was a difference in the number of osteoblast cells with the various dose changes in the extract dose treatment group. The statistical test results revealed that EBE 12 and 24 mg/200 g BW did not differ significantly from each other, while EBE 6 and 24 mg/200 g BW; EBE 6 and 12 mg/200 g BW did differ significantly from each other.

The average level of osteoblast cells was considerably greater in the positive control group than in the negative control group. Statistical tests demonstrating a significant difference between the positive control group and the negative control group serve as proof of this. Additionally, statistical analysis revealed no significant difference between EBE 24 mg/200 g BW and the positive control group's osteoblast cell levels. Moreover, there were significant differences between the

osteoblast cell levels of the positive control group and EBE (6 mg/200 g BW, 12 mg/200 g BW).

4. *Discussion*

Direct inhibition of osteoblast proliferation, hyperparathyroidism brought on by direct effects on the parathyroid gland, a rise in urinary calcium excretion linked to glucocorticoids, and direct stimulation or inhibition of osteoclast formation are some of the multiple ways that glucocorticoids affect bone metabolism.[24,25] Similar to glucocorticoid-induced osteoporosis, this condition is characterized by a raise in the angle of the spine or is called a state of kyphosis in animal models. Osteoporosis can also be proven in the trabecular femur by looking at the parameters of decreasing bone volume density (BV/TV) and bone mineral density (BMD).[26]

The percentage of bone density and osteoblast cell levels differed significantly between the healthy and negative groups ($p < 0.05$), it could be seen that the glucocorticoid can decrease the percentage of bone density and osteoblast cell levels. The treatment dose 3 group then demonstrated a rise in bone density percentage.

The chemicals included *E. bulbosa* bulbs enabled it to raise both the percentage of bone density and osteoblast cell level. *E. bulbosa* bulbs are reported to contain 2,4,7-Trihydroxy-9,10-dihydrophenanthrene (phenanthrene), cuspidatumin A (naphthoquinone), dendromoniliside E (glycoside), liquiritigenin (flavonoid), and natsudaidain (flavonoid).[27] In vitro studies have shown that liquiritigenin raises osteoblast activity and reduces osteoclast differentiation.[28] Furthermore, fish scales' natural bone metabolism can be preserved by liquiritigenin.[29] Liquiritigenin was found to be able to stimulate dose-dependent osteoblast development by working on the Smad1/5 pathway, boosting ALP activity, collagen synthesis, and mineralization in a study utilizing MC3T3-E1 cells.[30,22]

The average calcium level in the healthy group found no significant differences between the negative group and EBE 6 mg/200 g BW ($p > 0.05$). However, there were significant differences between the positive control, EBE 12 and 24 mg/200 g

** 4/12/22 21.02
Comment [14]: Any plant from the genus Eleutherine reported for antiosteoporosis?

BW ($p < 0.05$). This is because the bone density parameter does not show a significant linear relationship with the results of measuring serum calcium levels.[31] Calcium levels in serum are influenced by food intake and nutrients consumed so serum calcium levels are not specific to describe the condition of patients in osteoporosis therapy.[32]

Limitations

The study's limitations were recognized. No examination was performed when glucocorticoid induction was completed. However, from the previous reference, it was stated that it took about four weeks to obtain a state of osteoporosis after induction with a change in posture in the spine to a hunchback (kyphosis) (based on preliminary research).

5. *Conclusion*.

In summary, it is inferable that EBE bulbs have an effect on raising serum calcium levels, bone density (the best effect by EBE 12 and 24 mg/200 g BW), and raising the number of osteoblast cells (EBE 24 mg/200 g BW). According to these results, EBE at this dosage may be just as effective at treating osteoporosis as alendronate.

** 4/12/22 20.50
Comment [15]: which dosage? should be stated

References

1. Laven JS, Visser JA, Uitterlinden AG, *et al*. Menopause: genome stability as new paradigm. *Maturitas*. 2016;92:15-23. doi: 10.1016/j.maturitas.2016.07.006
2. Bone Health and Osteoporosis Foundation (BHOF). *Osteoporosis fast facts*. 251 18th Street S, Suite 630, Arlington, VA, 22202.
3. Cohen JE, Wakefield CE, Cohn RJ. Nutritional interventions for survivors of childhood cancer. *Cochrane Database Syst Rev*. 2016;CD009678. doi: 10.1002/14651858.CD009678.pub2
4. Paccou J, Edwards MH, Ward K, *et al*. Relationships between bone geometry, volumetric bone mineral density and bone microarchitecture of the distal radius and tibia with alcohol consumption. *Bone*. 2015;78:122-129. doi: 10.1016/j.bone.2015.05.002
5. Kline J, Tang A, Levin B. Smoking, alcohol and caffeine in relation to two hormonal indicators of ovarian age during the reproductive years. *Maturitas*. 2016;92:115-122. doi: 10.1016/j.maturitas.2016.07.010
6. Pouresmaeili F, Kamalidehghan B, Kamarehei M, Goh YM. A comprehensive overview on osteoporosis and its risk factors. *Therapeutics and Clinical Risk Management*. 2018;14:2029-2049. doi: 10.2147/TCRM.S138000
7. Buehring B, Viswanathan R, Binkley N, Busse W. Glucocorticoid-induced osteoporosis: an update on effects and management. *J Allergy Clin Immunol*. 2013;132:1019-1030. doi: 10.1016/j.jaci.2013.08.040
8. Wishart J, Horowitz M, Need A, Nordin BE. Relationship between forearm and vertebral mineral density in postmenopausal women with primary hyperparathyroidism. *Arch Intern Med*. 1990;150:1329-1331. doi: 10.1001/archinte.1990.00390180133024
9. Cooper C, Coupland C, Mitchell M. Rheumatoid arthritis, corticosteroid therapy and hip fracture. *Ann Rheum Dis*. 1995;54:49-52. doi: 10.1136/ard.54.1.49
10. Isidro ML, Ruano B. Bone disease in diabetes. *Curr Diabetes Rev*. 2010;6(3):144-155. doi: 10.2174/157339910791162970
11. Haasum Y, Fastbom J, Fratiglioni L, Johnell, K. Undertreatment of osteoporosis in persons with dementia? a population-based study. *Osteoporos Int*. 2012;23:1061-1068. doi: 10.1007/s00198-011-1636-8

12. Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. *Clin Cancer Res*. 2006;12:6243s-6249s. doi: 10.1158/1078-0432.CCR-06-0931
13. Licata A. Osteoporosis in men: suspect secondary disease first. *Cleveland Clinic Journal of Medicine*. 2003;70:247-254. doi: 10.3949/ccjm.70.3.247
14. Lin S, Huang J, Zheng L, *et al*. Glucocorticoid-induced osteoporosis in growing rats. *Calsif Tissue Int*. 2014;95:362-373. doi: 10.1007/s00223-014-9899-7
15. Katzung BG. *Basic & clinical pharmacology 12th edition volume 1*. San Fransisco: Mc Graw Hill Lange; 2012.
16. Cohen D, Adachi JD. The treatment of glucocorticoid-induced osteoporosis. *Journal Steroid Biochemical and Molecul Biology*. 2004;88:337-349. doi: 10.1016/j.jsbmb.2004.01.003
17. Noor Z. *Buku ajar: Osteoporosis patofisiologi dan peran atom mineral dalam manajemen terapi.* Jakarta: Salemba Medika; 2014.
18. Agil M, Ma'arif B, Aemi NY. Antiosteoporotic activity of n-hexane fraction from *Marcilea crenata* Presl. leaves in increasing trabecular vertebrae bone density of female mice. *Jurnal Tumbuhan Obat Indonesia*. 2018:11;1-7. doi: https://doi.org/10.22435/jtoi.v11i2.671
19. Laswati H, Agil M, Widyowati R. Effect of *Spilanthes acmella* and exercise on osteoblast cells femur in mice dexamethasone induced. *Media Penelitian dan Pengembangan Kesehatan*. 2015;25:43-50. doi: 10.22435/mpk.v25i1.4095.43-50
20. Amelia T, Pratiwi D, Tjahjono D. *In silico* study of the component of *Eleutherine americana* MERR. on human estrogen reseptor alpha as potential anti-breast cancer. *International Conference on Computation for Science and Technology*. 2014;3:6-9. doi: 10.2991/iccst-15.2015.2
21. Bianchi C, Ceriotti G. Chemical and pharmacological investigations of constituents of *Eleutherine bulbosa* (Miller) Urb. (Iridaceae). *Journal of Pharmaceutical Sciences*. 1975;64:1305-8. doi: 10.1002/jps.2600640809
22. Uchino K, Okamoto K, Sakai E, *et al*. Dual effects of liquiritigenin on the proliferation of bone cells: promotion of osteoblast differentiation and inhibition of osteoclast differentiation. *Phytotherapy Research*. 2015;29:1714-1721. doi: 10.1002/ptr.5416
23. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. *J Clin Invest*. 2006;116:1186-94. doi: 10.1172/JCI28550

24. Takahashi M, Ushijima K, Hayashi Y, *et al*. Dosing-time dependent effect of dexamethasone on bone density in rats. *Life Sciences*. 2009:86;24-29. doi: 10.1016/j.lfs.2009.10.020
25. Mirza F, Canalis E. Secondary osteoporosis: pathophysiology and management. *Eur J Endocrinal*. 2015;173:R131-R151. doi: 10.1530/EJE-15-0118
26. Saleem AN, Chen YH, Baek HJ, *et al*. Mice with alopecia, osteoporosis, and systematic amyloidosis due to mutation in *Zdhhc13*, a gene coding for palmitoyl acyltransferase. *PLoS Genet*. 2010;6:e1000985. doi: 10.1371/journal.pgen.100098
27. Bahtiar A, Dewi R. Antiosteoporosis effects of 70% ethanolic extract combination of dayak onion bulbs (*Eleutherine bulbosa* (Mill.) Urb) and cowpea (*Vigna unguiculata* (L.) Walp.) on the hypoestrogen rats. *Pharmacogn J*. 2019;11:632-638. doi: 10.5530/pj.2019.11.101
28. Choi EM. Liquiritigenin isolated from *Glycyrrhiza uralensis* stimulates osteoblast function in osteoblastic MC3T3-E1c. *International Immunopharmacology*. 2012;12:139-143. doi: http://dx.doi.org/10.1016/j.intimp.2011.11.003
29. Carnovali M, Luzi L, Terruzzi I, *et al*. Liquiritigenin reduces blood glucose level and bone adverse effects in hyperglycemic adult zebrafish. *Nutrients*. 2019;11:1042. doi: 10.3390/nu11051042
30. Choi EM, Suh KS, Lee YS. Liquiritigenin restores osteoblast damage through regulating oxidative stress and mitochondrial dysfunction. *Phytother Res*. 2014;28:880-886. doi: 10.1002/ptr.5071
31. Sun JY, Zhang H, Zhang Y, *et al*. Impact of serum calcium levels on total body bone mineral density: a mendelian randomization study in five age strata. *Clin Nutr*. 2021;40:2726-2733. doi: 10.1016/j.clnu.2021.03.012
32. Yusmiati SNH, Wulandari RE. Pemeriksaan kadar kalsium pada masyarakat dengan pola makan vegetarian. *Journal Sains Health*. 2017;1:43-49.

Acknowledgments: This research was supported by International Research Collaboration Top #500 of Airlangga University with the contract No. 1546/UN3.15/PT/2021

Tables and figures

TABLE 1. Analysis result of blood and bones

** 4/12/22 20.44
Comment [16]: Please add information which one statistically differ

Treatments	Average ± SD					
	Healthy group	Negative control (0.5% CMC-Na)	Positive control (Alendronat 0.18mg/200g BW)	EBE (6mg/200g BW)	EBE (12mg/200g BW)	EBE (24mg/200g BW)
Serum calcium level	10.0 ± 0.39	9.9 ± 0.13	11.0 ± 0.23	9.9 ± 0.17	10.8 ± 0.18	10.7 ± 0.23
Bone density Percentage	50.65 ± 6.42	37.70 ± 7.54	65.48 ± 7.61	39.69 ± 1.31	48.43 ± 14.49	60.67 ± 10.54
Osteoblast cells level	125.8 ± 16.99	49 ± 25.00	192.8 ± 3.27	96.4 ± 5.31	130.6 ± 15.70	146.6 ± 47.76

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Reviewer 2
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Author Response:

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Manuscript number:
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Author Response:

Title: Name of the Article
Manuscript number:
Revision Version: 3
Editor's Decision Received Date:
Revision Submission Date:

Author Response 3rd revision:

Reviewer 1
Reviewer Comments:

Author Response:

Reviewer 2
Reviewer Comments:

Author Response:

Response to Reviewers

Thank you for giving us the chance to submit a revised version of our manuscript, which is "Effects of *Eleutherine bulbosa* (Mill.) Urb. Bulb Extract on Mice Glucocorticoid-induced Osteoporosis Models" to *Journal of Public Health of Africa*. We appreciate the effort and time you and the other reviewers put into providing your informative comments on our manuscript. We have noted the changes to the manuscript and marked the change we made. We have to rewritten the text. Here is a point-by-point response to the reviewers' comments and inquiries.

Title	: Effects of *Eleutherine bulbosa* (Mill.) Urb. Bulb Extract on Mice Glucocorticoid-induced Osteoporosis Models
Manuscript number	: Submission ID ICPHS-13
Revision Version	: 1
Editor's Decision Received Date	: Thursday, December 22nd 2022
Revision Submission Date	: Thursday, January 5th 2022

Author Response 1st revision

Reviewer 1

Reviewer Comments:

The article is well written. Suggestions to improve the clarity and quality of the manuscript (details attached)

Comment 1: *Title: please include the part of the plant used*

Author Response: Thank you for the constructive comment you have given.

The part is a bulb. We change it to "Effects of *Eleutherine bulbosa* (Mill.) Urb. Bulb Extract on Mice Glucocorticoid-induced Osteoporosis Models."

Comment 2: *Introduction: Any traditional use related to osteoporosis?*

Author Response: Thank you for the question you have given.

"Traditional use as a treatment for sprained feet, anti-inflammatory, etc. Not said directly as anti-osteoporosis."

Comment 3: *There is a study on anti-osteoporosis from E. bulbosa extract (Bahtiar A., Annisa R. Effects of Dayak onion Bulbs (Eleutherine bulbosa (Mill.) Urb) on bone development of the hypoestrogen model rat. Pharmacogn. J. 2018;10:299–303. doi: 10.5530/pj.2018.2.52.). What is the difference between your study and this?*

Author Response: Thank you for the question you have given.

"There are differences in the osteoporosis induction method, animal type, dose, test parameters, and the origin of the *E.bulbosa.*"

Comment 4: *Methods:*

a. *The method used for drying the sample should be included. Please check on the term used.*

   Author Response: Thank you for the constructive comment you have given.

   "The *E.bulbosa* bulbs are air-dried before being crushed into powder."

b. *Use the common terms*

   Author Response: Thank you for the constructive comment you have given.

   We change it to "15.98%"

c. *What is the dose for glucocorticoid used?*

   Author Response: Thank you for the question you have given.

   "Indexon® contains 0.5 mg dexamethasone by Interbat, Indonesia (dose used 0.1015 mg/kg BW/day)."

d. *How do you know that the animal has developed osteoporosis?*

   Author Response: Thank you for the question you have given.

   "Kyphosis condition (a change in posture in the spine to a hunchback). Based on preliminary research"

Comments 5: *Results:*

a. Include the p-value for calcium measurement

Author Response: Thank you for the constructive comment you have given.

"*P*<0.05."

b. *What is the value of bone density categorized as osteoporosis in animal models?*

Author Response: Thank you for the question you have given.

"The value for a healthy group is 50.65 ± 6.42 and for a negative control is 37.70 ± 7.54."

Comments 6: *Discussion: Has any plant from Eleutherine been studied for osteoporosis?*

Author Response: Thank you for the question you have given.

"There have been no reports. However, there have been previous studies on *E.bulbosa* bulb for the treatment of osteoporosis."

Comments 7: *Conclusion: Please clearly state the dosage in the last sentence*

Author Response: Thank you for the constructive comment you have given.

"120 mg/kg BW."

Comments 8: *Table: Please add information on which one statistically differ*

Author Response: Thank you for the constructive comment you have given.

"TABLE 1. Analysis result of blood and bones. Mann-Whitney test was used for statistical comparison between treatment and healthy groups (n=5). *$P < 0.05$."

Treatments	Average ± SD					
	Healthy group	Negative control (0.5% CMC-Na)	Positive control (Alendronat 0.9 mg/kg BW)	EBE (30 mg/kg BW)	EBE (60 mg/kg BW)	EBE (120mg/kg BW)
Serum calcium level	10.0 ± 0.39	9.9 ± 0.13	11.0 ± 0.23*	9.9 ± 0.17	10.8 ± 0.18*	10.7 ± 0.23*
Bone density Percentage	50.65 ± 6.42	37.70 ± 7.54*	65.48 ± 7.61*	39.69 ± 1.31*	48.43 ± 14.49	60.67 ± 10.54
Osteoblast cells level	125.8 ± 16.99	49 ± 25.00*	192.8 ± 3.27*	96.4 ± 5.31*	130.6 ± 15.70	146.6 ± 47.76

Comments 9: *Grammar should be checked, and some sentences should be revised for clarity.*

Author Response: Thank you for the reminder. In addition to the above comments, all spelling and grammatical errors have been corrected.

Reviewer 2

Reviewer Comments:

The manuscript describes the finding on the efficacy of E. bulbosa bulb extract as anti-osteoporosis. The research design has been well described. The results have been adequately shown in the tables. There is sufficient discussion regarding the findings.

Minor notes are as follows.

Comments 1: *Using mg/200 g BW as the dose unit is uncommon. The author may consider converting and replacing it with mg/kg.*

Author Response: Thank you for the constructive comment you have given.

"We have changed all dosage units from mg/200 g BW to mg/kg BW"

Comments 2: *It should be clearly shown in the table when the results statistically give a significant difference. The significant marks and further footnotes may be useful.*

Author Response: Thank you for the constructive comment you have given.

"TABLE 1. Analysis result of blood and bones. Mann-Whitney test was used for statistical comparison between treatment and healthy groups (n=5). *$P < 0.05$."

Treatments	Average ± SD					
	Healthy group	Negative control (0.5% CMC-Na)	Positive control (Alendronat 0.9 mg/kg BW)	EBE (30 mg/kg BW)	EBE (60 mg/kg BW)	EBE (120mg/kg BW)
Serum calcium level	10.0 ± 0.39	9.9 ± 0.13	11.0 ± 0.23*	9.9 ± 0.17	10.8 ± 0.18*	10.7 ± 0.23*
Bone density Percentage	50.65 ± 6.42	37.70 ± 7.54*	65.48 ± 7.61*	39.69 ± 1.31*	48.43 ± 14.49	60.67 ± 10.54
Osteoblast	125.8 ± 16.99	49 ± 25.00*	192.8 ± 3.27*	96.4 ± 5.31*	130.6 ± 15.70	146.6 ± 47.76

cells level						

Comments 3: *The method attribute for the spectrophotometer by Balai Besar Laboratory should be clearly stated in detail e.g., the wavelength.*

Author Response: Thank you for the constructive comment you have given.

"Spectrophotometer (λ=570 nm) by Balai Besar Laboratorium Kesehatan (BBLK)."

Comments 4: *What kind of glucocorticoids have been used? Please provide the manuscript's exact materials information, code, and manufacturer. Add the dose of it in the animal model part and abstract.*

Author Response: Thank you for the constructive comment and question you have given.

"Indexon® contains 0.5 mg dexamethasone by Interbat, Indonesia (dose used 0.1015 mg/kg BW/day)"

Comment 5: *Additionally, the author needs to examine the grammatical matters in the whole manuscript.*

Author Response: Thank you for the reminder. In addition to the above comments, all spelling and grammatical errors have been corrected.

Comments 6: *"The treatment dose 3 group then demonstrated a rise in bone density percentage." This sentence should be rechecked.*

Author Response: Thank you for the constructive comment you have given.

We change it to: "The dose treatment group then demonstrated the opposite effect, increasing the percentage of bone density and osteoblast cell levels."

Comments 7: *The last paragraph of the discussion is puzzling. The comparison data is irrelevant. Further, which one is being the author's argument? Do the extract and drug increase the calcium level or not? There was an increase based on the conclusion, but the author further*

discussed that there is no correlation between Ca level and osteoporosis. The discussion structure should be improved at this point.

Author Response: Thank you for the constructive comment and question you have given.

We change it to: “The results of this study prove that EBE 60 and 120 mg/kg BW can increase serum calcium levels when compared to the healthy group. Increased calcium content in serum and plasma is a sign of a variety of diseases, one of which is primary hyperparathyroidism (pHPT). Secondary osteoporosis is caused by pHPT, which results in low bone mineral density (BMD).[33] BMD is primarily used to diagnose osteoporosis.[34]” It will be similar to the conclusion.

Comments 8: *The use of "might" in the result part of the abstract should be reconsidered.*

Author Response: Thank you for the constructive comment you have given.

We change it to “could.”

We look forward to hearing from you in due time regarding our submission and to responding to any further questions and comments you may have.

Sincerely,

Retno Widyowati

Corresponding author

Effects of *Eleutherine bulbosa* (Mill.) Urb. Bulb Extract on Mice Glucocorticoid-induced Osteoporosis Models

** 4/12/22 20.27
Comment [1]: part of the plant used should be stated

Fina Luthfiana[1], Riza Ambar Sari[1], Irawati Sholikhah[2], Katsuyoshi Matsunami[3], Retno Widyowati[4,5]*, Sukardiman[4,5]

[1]*Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, 60115 Indonesia*
[2]*Department of Chemistry, Faculty of Sains and Technology, Universitas Airlangga, 60115 Indonesia*
[3]*Department of Pharmacognosy, Graduate School of Biomedical & Health Sciences, Hiroshima University, Japan*
[4]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, 60115 Indonesia*
[5]*Natural Products Drug Discovery and Development, Faculty of Pharmacy, Universitas Airlangga, 60115 Indonesia*

Corresponding author: Retno Widyowati. Department of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, 60115 Indonesia. Phone number: +62816-1588-6978, and e-mail address: rr-retno-w@ff.unair.ac.id

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Keywords: *Eleutherine bulbosa*, calcium, osteoblast cell, bone density, osteoporosis

Authors' Contributions :

Fina Luthfiana : literature search, experimental studies, data analysis

Riza Ambar Sari : literature search, experimental studies, data acquisition

Irawati Sholihah : Statistical analysis, research materials

Katsuyoshi Matsunami : Manuscript editing and review

Sukardiman : Manuscript editing and review

Retno Widyowati : conception and design research, Manuscript editing and review

Conflict of Interests : The authors have declared that no conflict of interest exists.

Further information

Funding : This research was supported by International Research Collaboration Top #500 of Airlangga University with the contract No. 1546/UN3.15/PT/2021

Abstract:

Backgrounds: Low bone mass accompanied by microarchitectural alterations in the bone that cause fragility fractures is known as secondary osteoporosis and occurs when there is an underlying condition or medication present. *Eleutherine bulbosa* bulb extract has been shown to affect bone because of its content which can help osteoblast differentiation and inhibit osteoclast differentiation.

Objective: This study aimed to assess the effects of 70% ethanol extract of *E.bulbosa bulbs* (EBE) from Pasuruan-East Java on blood calcium levels, osteoblast cell count, and bone density of trabecular femur in osteoporosis rats.

Methods: Six groups of 30 female Wistar rats were created. There were no test materials offered to the healthy group; the negative group received 0.5% CMC; the positive group received alendronate 0.9 mg/kg BW; and the dose group received 30, 60, and 120 mg/kg BW. Glucocorticoid (Dexamethasone) 0.1015 mg/kg BW/day induction was given to all groups except the healthy group to create osteoporosis rats for approximately four weeks. Then given oral therapy for approximately 28 days. Followed by the determination of blood calcium levels, the number of osteoblast cells, and bone density of the rat femur trabecular.

Results: The result showed that *E. bulbosa* bulbs extract could raise blood calcium levels and bone density percentage at doses of 60 and 120 mg/kg BW, as well as raise osteoblast cell levels at doses of 120 mg/kg BW.

Conclusions: The findings indicate that *E.bulbosa* bulb extract is a potential complementary medicine for osteoporosis.

1. *Introduction*

A bone metabolic disorder called osteoporosis causes diminished bone mass, alteration of bone's microarchitecture, and enhanced bone fragility, all of which raise the chance of fracture.[1] Noteworthy is that about 300,000 hip fracture patients each year wind up in nursing homes, and half never restore their pre-injury function.[2] A variety of factors can impair bone metabolism, including a lack of nutritional deficiency and sedentary lifestyle,[3] use of alcohol,[4] smoking,[5] genetic factors,[6] medication,[7] hyperparathyroidisms,[8] rheumatoid arthritis,[9] diabetes mellitus,[10] dementia,[11] and cancer.[12,6]

The glucocorticoid group is a drug in the first order that causes secondary osteoporosis, which affects adults more frequently than any other cause due to its side.[13,14] Adults on glucocorticoid often have a hunchback, back pain, height loss, or even fractures that may result in disability, creating a significant financial burden on families and society.[14] This is because glucocorticoids affect bone mineral homeostasis with the mechanism of action of vitamin D antagonists, stimulating renal calcium excretion, and inhibiting bone formation which causes an increase in osteoclast resorption resulting in a decrease in bone mass.[15]

Corticosteroids induce osteoporosis up to eight times greater than osteoporosis due to underlying disease.[16] Induction of dexamethasone for four weeks in mice is equivalent to induction for 3-4 years for humans.[17,18] Long-term (at least 3-6 months) use of the group compounds corticosteroids may slow the process of bone growth.[19]

The *E. bulbosa* bulbs are one of the plants that contain compounds with antiosteoporosis activity. It's from the Indonesian province of Central Kalimantan.[20] Traditional use as a treatment for sprained feet.[21] This plant is from the Iridaceae family and is used to treat breast cancer and inflammatory diseases, including rheumatoid arthritis.[22,23] An in silico study published in 2014 found that *E. bulbosa* bulbs contain derivatives of the naphthoquinone compound, eleutherinol, which acts as an antagonism for mammary estrogen alpha receptors (ER-α).[20] These

** 4/12/22 20.30
Comment [2]: do you mean antiosteoprosis activity?

** 4/12/22 20.31
Comment [3]: Any traditional use related to osteoporosis?

substances may be employed as a treatment option for postmenopausal conditions since they are anticipated to be selective agonists of estrogen receptors in different tissues, including bone and blood vessels. This extract also contains a liquiritigenin compound, which has a high affinity for selectively binding with estrogen beta receptors and can promote osteoblast differentiation while inhibiting osteoclast differentiation.[24]

As a consequence, more research is needed to scientifically prove the effects of *E. bulbosa* bulbs on osteoporosis treatment as seen by raising levels of serum calcium, the percentage of bone density, and the level of osteoblast cells.

2. *Materials and Methods*

2.1 Plant Materials

Eleutherine bulbosa (Mill.) Urb. obtained from Pasuruan, East Java, Indonesia. Determined by UPT Laboratorium Herbal Materia Medica Batu, East Java, Indonesia (Certificate of Determination No. 074/722/102.7-A/2021).

2.2 Preparation of Extract

The *E.bulbosa* bulbs are air-dried before being crushed into powder. The dry powder was extracted using a maceration method with 70% ethanol. A rotary evaporator was used to concentrate the extract. Ethanol extract of *E. bulbosa* bulbs was calculated as % w/w yield, which was 15.98%.

** 4/12/22 20.34
Comment [4]: 15.98%

2.3 Ethical Considerations

This research was conducted using experimental animals which were female white rats (*Rattus norvegicus*) Wistar strain, obtained from the Animal Laboratory, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia. Research Ethics Commission (Animal Care and Use Committee) Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia, have carefully studied the proposed research design, rats with healthy conditions aged 3-4 months weighing 200-300 g. Food and water were available *ad libitum*. Acclimatized for 1 week. Placed in a

room with a 12-hour light/dark cycle with controlled conditions of temperature and humidity in the Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga. Then, hereby declare that ethically appropriate (No: 2.KEH.120.09.2022).

2.4 Animal model

Thirty rats were divided into six different groups (5 rats per group). The group was divided into (1) a healthy group, rats were not induced by glucocorticoids, (2) a negative group, rats were induced with glucocorticoids, dexamethasone (Interbat, Indonesia) 0.1015 mg/kg BW/day and given 0.5% CMC-Na therapy, (3) a positive group, rats were induced with glucocorticoids and given alendronate 0.9 mg /kg BW/day, (4) dose 1, rats were induced with glucocorticoids and given 30 mg/kg BW of EBE, (5) dose 2, rats were induced with glucocorticoids and given 60 mg/kg BW, (6) dose 3, rats were induced with glucocorticoids and given 120 mg/kg BW of extract. Glucocorticoid induction was carried out for 4 weeks orally. After the animal developed osteoporosis (kyphosis condition), therapy was carried out for 4 weeks orally. Measurements of serum calcium levels, femoral trabecular bone density, and the number of osteoblasts were performed at week 4 after therapy.

** 4/12/22 20.41
Comment [5]: How do you know that the animal have developed osteoporosis?

2.5 Evaluation of Parameters

2.5.1 The level of serum calcium

At the end of the treatment, the rats in all groups were sacrificed and blood samples were taken from the heart to determine the calcium serum level. Examination of blood calcium using a spectrophotometer (λ=570 nm) at the Balai Besar Laboratorium Kesehatan (BBLK), Surabaya, Indonesia.

2.5.2 Histological analysis

When the procedure is over, the rats in all groups were sacrificed and the trabecular femur bone was taken. The femoral trabecular bone was prepared by fixing them with 10% formalin, decalcifying them, neutralizing them, washing them with water, and then rinsing them with 70% alcohol. After that, the bone was sealed with paraffin before being microtome-cut. Afterward, it was soaked in 70%

alcohol and stained with Mallory Azan (MA). Olympus Cellsens software with a 200× zoom was used to analyze the observation slides. The microscope used for observations was connected to a computer and *Matic image software*. Bone density and osteoblast cell were calculated at the Histology Laboratory, Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

2.5.3 Statistical analysis

SPSS was used to examine the data from the animal experiments. A Kruskal-Wallis test and a Mann-Whitney test were carried out to investigate the relationship between the treatment groups, with p-values of less than 0.05 considered to be significantly different.

3. *Results*

The level of serum calcium

The positive control group, EBE 60 mg/kg BW, EBE 120 mg/kg BW, healthy group, and negative control group all had average calcium levels that ranged from highest to lowest (Table 1).

There was a difference between the dose 1, 2, and 3 groups, as seen by the average calcium levels with dose variation ($P < 0.05$). A substantial difference between EBE 30 and 60 mg/kg BW, as well as EBE 30 and 120 mg/kg BW, was revealed by statistical analysis. There was no significant difference between EBE 60 and 120 mg/kg BW.

** 4/12/22 20.43
Comment [6]: what is the p value?

Average values for the positive and negative control group were vastly different. According to statistical analyses, there is a significant difference between them. This is demonstrated by statistical tests that show a significant difference between the positive and negative groups. Moreover, statistical analysis revealed no significant difference in calcium levels between the positive control group and EBE (60mg/kg BW and 120 mg/kg BW). The calcium levels of the positive control group and EBE 30 mg/kg BW. High levels of calcium in the serum of the group receiving three doses of extract therapy demonstrated that the rise in the extract dose had an impact on the rise in calcium levels in the serum.[25]

Percentage of bone density

The positive control group, EBE 120 mg/kg BW, the healthy group, EBE 60 mg/kg BW, EBE 30 mg/kg BW, and the negative control group had an average bone density from highest to lowest (Table 1). The healthy group's average bone density is significantly higher than that of the negative control group. Statistical tests that demonstrate a substantial difference in bone density between the healthy group and the negative control group serve as proof of this. This shows that the glucocorticoid induction process was successful and that rats were experiencing osteoporosis. The value for a healthy group is 50.65 ± 6.42 and for a negative control is 37.70 ± 7.54 (mean ± SD).

** 4/12/22 20.47
Comment [7]: What is the value of bone density that is categorised as osteoporosis in animal model?

In comparison to the negative control group, the positive control group had an average bone density that was considerably higher. This is supported by statistical analyses that show a significant difference between the positive and negative control group. The level of bone density between of positive control group and EBE (60 and 120 mg/kg BW) did not differ significantly, according to statistical analysis. Additionally, there was a statistically significant difference in bone density between the EBE 30 mg/kg BW group and the positive control group.

Level of osteoblast cell

The positive control group, EBE 120 mg/kg BW, EBE 60 mg/kg BW, the healthy group, EBE 30 mg/kg BW, and the negative control group had an average level of osteoblast cells from highest to lowest (Table 1). There was a difference in the number of osteoblast cells with the various dose changes in the extract dose treatment group. The statistical test results revealed that EBE 60 and 120 mg/kg BW did not differ significantly from each other, while EBE 30 and 120 mg/kg BW; EBE 30 and 60 mg/kg BW did differ significantly from each other.

The average level of osteoblast cells was considerably greater in the positive control group than in the negative control group. Statistical tests demonstrating a significant difference between the positive control group and the negative control

group serve as proof of this. Additionally, statistical analysis revealed no significant difference between EBE 120 mg/kg BW and the positive control group's osteoblast cell levels. Moreover, there were significant differences between the osteoblast cell levels of the positive control group and EBE (30 mg/kg BW, 60 mg/kg BW).

4. *Discussion*

Direct inhibition of osteoblast proliferation, hyperparathyroidism brought on by direct effects on the parathyroid gland, a rise in urinary calcium excretion linked to glucocorticoids, and direct stimulation or inhibition of osteoclast formation are some of the multiple ways that glucocorticoids affect bone metabolism.[26,27] Similar to glucocorticoid-induced osteoporosis, this condition is characterized by a raise in the angle of the spine or is called a state of kyphosis in animal models. Osteoporosis can also be proven in the trabecular femur by looking at the parameters of decreasing bone volume density (BV/TV) and bone mineral density (BMD).[28]

The percentage of bone density and osteoblast cell levels differed significantly between the healthy and negative groups ($P < 0.05$), it could be seen that the glucocorticoid can decrease the percentage of bone density and osteoblast cell levels. The dose treatment group then demonstrated the opposite effect, increasing the percentage of bone density and osteoblast cell levels.

The chemicals included *E. bulbosa* bulbs enabled it to raise both the percentage of bone density and osteoblast cell level. *E. bulbosa* bulbs are reported to contain 2,4,7-Trihydroxy-9,10-dihydrophenanthrene (phenanthrene), cuspidatumin A (naphthoquinone), dendromoniliside E (glycoside), liquiritigenin (flavonoid), and natsudaidain (flavonoid).[29] In vitro studies have shown that liquiritigenin raises osteoblast activity and reduces osteoclast differentiation.[30] Furthermore, fish scales' natural bone metabolism can be preserved by liquiritigenin.[31] Liquiritigenin was found to be able to stimulate dose-dependent osteoblast development by working on the Smad1/5 pathway, boosting ALP activity, collagen synthesis, and mineralization in a study utilizing MC3T3-E1 cells.[32,24]

The average calcium level in the healthy group found no significant differences

** 4/12/22 21.02
Comment [8]: Any plant from the genus Eleutherine reported for antiosteoporosis?

between the negative group and EBE 30 mg/kg BW ($P > 0.05$). However, there were significant differences between the positive control, EBE 60 and 120 mg/kg BW ($P < 0.05$). The results of this study prove that EBE 60 and 120 mg/kg BW can increase serum calcium levels when compared to the healthy group. Increased calcium content in serum and plasma is a sign of a variety of diseases, one of which is primary hyperparathyroidism (pHPT). Secondary osteoporosis is caused by pHPT, which results in low bone mineral density (BMD).[33] BMD is primarily used to diagnose ostoeporosis.[34]

Limitations

The study's limitations were recognized. No examination was performed when glucocorticoid induction was completed. However, from the previous reference, it was stated that it took about four weeks to obtain a state of osteoporosis after induction with a change in posture in the spine to a hunchback (kyphosis) (based on preliminary research).

5. *Conclusion*.

In summary, it is inferable that EBE bulbs have an effect on raising serum calcium levels, bone density (the best effect by EBE 60 and 120 mg/kg BW), and raising the number of osteoblast cells (EBE 120 mg/kg BW). According to these results, EBE at this dosage of 120 mg/kg BW may be just as effective at treating osteoporosis as alendronate.

** 4/12/22 20.50
Comment [9]: which dosage? should be stated

References

1. Laven JS, Visser JA, Uitterlinden AG, *et al*. Menopause: genome stability as new paradigm. *Maturitas*. 2016;92:15-23. doi: 10.1016/j.maturitas.2016.07.006
2. Bone Health and Osteoporosis Foundation (BHOF). *Osteoporosis fast facts*. 251 18th Street S, Suite 630, Arlington, VA, 22202.
3. Cohen JE, Wakefield CE, Cohn RJ. Nutritional interventions for survivors of childhood cancer. *Cochrane Database Syst Rev*. 2016;CD009678. doi: 10.1002/14651858.CD009678.pub2
4. Paccou J, Edwards MH, Ward K, *et al*. Relationships between bone geometry, volumetric bone mineral density and bone microarchitecture of the distal radius and tibia with alcohol consumption. *Bone*. 2015;78:122-129. doi: 10.1016/j.bone.2015.05.002
5. Kline J, Tang A, Levin B. Smoking, alcohol and caffeine in relation to two hormonal indicators of ovarian age during the reproductive years. *Maturitas*. 2016;92:115-122. doi: 10.1016/j.maturitas.2016.07.010
6. Pouresmaeili F, Kamalidehghan B, Kamarehei M, Goh YM. A comprehensive overview on osteoporosis and its risk factors. *Therapeutics and Clinical Risk Management*. 2018;14:2029-2049. doi: 10.2147/TCRM.S138000
7. Buehring B, Viswanathan R, Binkley N, Busse W. Glucocorticoid-induced osteoporosis: an update on effects and management. *J Allergy Clin Immunol*. 2013;132:1019-1030. doi: 10.1016/j.jaci.2013.08.040
8. Wishart J, Horowitz M, Need A, Nordin BE. Relationship between forearm and vertebral mineral density in postmenopausal women with primary hyperparathyroidism. *Arch Intern Med*. 1990;150:1329-1331. doi: 10.1001/archinte.1990.00390180133024
9. Cooper C, Coupland C, Mitchell M. Rheumatoid arthritis, corticosteroid therapy and hip fracture. *Ann Rheum Dis*. 1995;54:49-52. doi: 10.1136/ard.54.1.49
10. Isidro ML, Ruano B. Bone disease in diabetes. *Curr Diabetes Rev*. 2010;6(3):144-155. doi: 10.2174/157339910791162970
11. Haasum Y, Fastbom J, Fratiglioni L, Johnell, K. Undertreatment of osteoporosis in persons with dementia? a population-based study. *Osteoporos Int*. 2012;23:1061-1068. doi: 10.1007/s00198-011-1636-8

12. Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. *Clin Cancer Res*. 2006;12:6243s-6249s. doi: 10.1158/1078-0432.CCR-06-0931
13. Licata A. Osteoporosis in men: suspect secondary disease first. *Cleveland Clinic Journal of Medicine*. 2003;70:247-254. doi: 10.3949/ccjm.70.3.247
14. Lin S, Huang J, Zheng L, *et al*. Glucocorticoid-induced osteoporosis in growing rats. *Calsif Tissue Int*. 2014;95:362-373. doi: 10.1007/s00223-014-9899-7
15. Katzung BG. *Basic & clinical pharmacology 12th edition volume 1*. San Fransisco: Mc Graw Hill Lange; 2012.
16. Cohen D, Adachi JD. The treatment of glucocorticoid-induced osteoporosis. *Journal Steroid Biochemical and Molecul Biology*. 2004;88:337-349. doi: 10.1016/j.jsbmb.2004.01.003
17. Noor Z. *Buku ajar: Osteoporosis patofisiologi dan peran atom mineral dalam manajemen terapi*. Jakarta: Salemba Medika; 2014.
18. Agil M, Ma'arif B, Aemi NY. Antiosteoporotic activity of n-hexane fraction from *Marcilea crenata* Presl. leaves in increasing trabecular vertebrae bone density of female mice. *Jurnal Tumbuhan Obat Indonesia*. 2018:11;1-7. doi: https://doi.org/10.22435/jtoi.v11i2.671
19. Laswati H, Agil M, Widyowati R. Effect of *Spilanthes acmella* and exercise on osteoblast cells femur in mice dexamethasone induced. *Media Penelitian dan Pengembangan Kesehatan*. 2015;25:43-50. doi: 10.22435/mpk.v25i1.4095.43-50
20. Amelia T, Pratiwi D, Tjahjono D. *In silico* study of the component of *Eleutherine americana* MERR. on human estrogen reseptor alpha as potential anti-breast cancer. *International Conference on Computation for Science and Technology*. 2014;3:6-9. doi: 10.2991/iccst-15.2015.2
21. Duke JA, Godwin MJB, Ottesen AR. *Duke's handbook of medicinal plants of Latin America*. London: CRC Press; 2008.
22. Bianchi C, Ceriotti G. Chemical and pharmacological investigations of constituents of *Eleutherine bulbosa* (Miller) Urb. (Iridaceae). *Journal of Pharmaceutical Sciences*. 1975;64:1305-8. doi: 10.1002/jps.2600640809
23. Hand PTB, Thao DT, Nga NT, *et al*. Toxicity and anti-inflammatory activities of an extract of the *Eleutherine bulbosa* rhizome on collagen antibody-induced arthritis in a mouse model. *Natural Product Communications*. 2018;13:883-886. doi: https://doi.org/10.1177/1934578X1801300725

24. Uchino K, Okamoto K, Sakai E, *et al*. Dual effects of liquiritigenin on the proliferation of bone cells: promotion of osteoblast differentiation and inhibition of osteoclast differentiation. *Phytotherapy Research*. 2015;29:1714-1721. doi: 10.1002/ptr.5416
25. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. *J Clin Invest*. 2006;116:1186-94. doi: 10.1172/JCI28550
26. Takahashi M, Ushijima K, Hayashi Y, *et al*. Dosing-time dependent effect of dexamethasone on bone density in rats. *Life Sciences*. 2009:86;24-29. doi: 10.1016/j.lfs.2009.10.020
27. Mirza F, Canalis E. Secondary osteoporosis: pathophysiology and management. *Eur J Endocrinal*. 2015;173:R131-R151. doi: 10.1530/EJE-15-0118
28. Saleem AN, Chen YH, Baek HJ, *et al*. Mice with alopecia, osteoporosis, and systematic amyloidosis due to mutation in *Zdhhc13*, a gene coding for palmitoyl acyltransferase. *PLoS Genet*. 2010;6:e1000985. doi: 10.1371/journal.pgen.100098
29. Bahtiar A, Dewi R. Antiosteoporosis effects of 70% ethanolic extract combination of dayak onion bulbs (*Eleutherine bulbosa* (Mill.) Urb) and cowpea (*Vigna unguiculata* (L.) Walp.) on the hypoestrogen rats. *Pharmacogn J*. 2019;11:632-638. doi: 10.5530/pj.2019.11.101
30. Choi EM. Liquiritigenin isolated from *Glycyrrhiza uralensis* stimulates osteoblast function in osteoblastic MC3T3-E1c. *International Immunopharmacology*. 2012;12:139-143. doi: http://dx.doi.org/10.1016/j.intimp.2011.11.003
31. Carnovali M, Luzi L, Terruzzi I, *et al*. Liquiritigenin reduces blood glucose level and bone adverse effects in hyperglycemic adult zebrafish. *Nutrients*. 2019;11:1042. doi: 10.3390/nu11051042
32. Choi EM, Suh KS, Lee YS. Liquiritigenin restores osteoblast damage through regulating oxidative stress and mitochondrial dysfunction. *Phytother Res*. 2014;28:880-886. doi: 10.1002/ptr.5071
33. Sun JY, Zhang H, Zhang Y, *et al*. Impact of serum calcium levels on total body bone mineral density: a mendelian randomization study in five age strata. *Clin Nutr*. 2021;40:2726-2733. doi: 10.1016/j.clnu.2021.03.012
34. Dalemo S, Eggertsen R, Hjerpe P, *et al*. Bone minral density in primary care patients related to serum calcium concentration: a longitudinal cohort study from Sweden. *Scandinavian Journal of Primary Health Care*.2018;36:198-206. doi: https://doi.org/10.1080/02813432.2018.1459430

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Tables and figures

TABLE 1. Analysis result of blood and bones. Mann-Whitney test was used for statistical comparison between treatment and healthy groups (n=5). *$P < 0.05$.

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Treatments	Average ± SD					
	Healthy group	Negative control (0.5% CMC-Na)	Positive control (Alendronat 0.9 mg/kg BW)	EBE (30 mg/kg BW)	EBE (60 mg/kg BW)	EBE (120mg/kg BW)
Serum calcium level	10.0 ± 0.39	9.9 ± 0.13	11.0 ± 0.23*	9.9 ± 0.17	10.8 ± 0.18*	10.7 ± 0.23*
Bone density Percentage	50.65 ± 6.42	37.70 ± 7.54*	65.48 ± 7.61*	39.69 ± 1.31*	48.43 ± 14.49	60.67 ± 10.54
Osteoblast cells level	125.8 ± 16.99	49 ± 25.00*	192.8 ± 3.27*	96.4 ± 5.31*	130.6 ± 15.70	146.6 ± 47.76

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