發酵萃取物對卵巢切除誘導骨質疏鬆之預防潛力評估：以小鼠模型為例

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中文摘要

骨質疏鬆症是常見於絕經後婦女的代謝性骨骼疾病，與雌激素缺乏密切相關。近年研究指出，腸道菌相與骨骼健康之間具有重要關聯。本研究使用卵巢切除（OVX）小鼠作為模型，探討經發酵後之淡竹葉（*Lophatherum gracile*）萃取物對骨質疏鬆的預防效果。實驗中，發酵萃取物經口餵食八週，評估其對骨密度、骨微結構、生化指標及腸道菌相的影響。結果顯示，萃取物可顯著提升骨體積比例與骨小梁厚度，並調節血清中骨生成與骨吸收相關指標（如 osteocalcin、P1NP 與 CTX-I）。此外，腸道菌相分析顯示該發酵物有助於恢復 OVX 小鼠菌群平衡，增加有益菌相。綜合結果指出，發酵淡竹葉萃取物具有改善骨質疏鬆及調節腸道微生態的潛力，為腸道菌相導向之骨質疏鬆預防策略提供新方向。

關鍵字：骨質疏鬆、發酵萃取物、腸道菌相、卵巢切除術、骨密度。

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Evaluation of Fermented Extracts in the Prevention of Osteoporosis Using an Ovariectomized Mouse Model

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Abstract

One of the major reasons for osteoporosis is the lack of estrogen in postmenopausal women. Recent research has discovered that the gut microbiota is one of the main players in bone health and that the doable way to correct the condition is to alter the microbial community. Ovariectomized mice serve as, was a model used for these tests. The objective of this experiment was to figure out whether the application of fermented plant extracts that are supplied with bioactive compounds could be served as a preventive method through the direct modulation and enhancement of the gut microbiota and thus loaded bones that were tested for the presence of specific biochemical signaling molecules as a measure of bone density. Bones were assessed with a micro-computed tomography instrument to read the bone mineral density, then blood serum biomarkers along with the measured parameters related to bone metabolism were evaluated showing the efficiency of the treatment. Also, fermentation of the extract was performed, and subsequent analysis of the gut microbiome was undertaken to study changes in the gut microbiota for the possible microbiota-related mechanisms. Due to this research paper, we see gut microbiota or microbial composition as the key to the bone matrix reabsorption and offer a new, microbiome-targeted strategy for prevention and management of osteoporosis as a potential solution.

Keyword: Osteoporosis, Fermented Extract, Gut Microbiota, Ovariectomy, Bone Mineral Density.

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1. Introduction

Osteoporosis is a condition in which the entire body's bone metabolism is dysfunctional and results in a decrease in the quantity of bone and its micro-architectural structure to exist which causes the increased possibility of fractures (Föger-Samwald, Dovjak, Azizi-Semrad, Kerschan-Schindl, & Pietschmann, 2020). While this disease usually affects individuals at an older age, it is a substantial problem for postmenopausal women as it results from a low estrogen rate (Yong & Logan, 2021). The study shows that about 19% of the women experienced spinal fractures in contrast to 12% among the male counterparts, which marks a very big difference between the genders. Apart from aging, the main factors susceptible to the ailment include a female gender, lower weight, certain ethnicities, family diseases, hormonal changes, and a wrong | unhealthy diet. Additionally, less physical activity and smoking can significantly elevate the chances of becoming a victim of the disease.

Osteoporotic fractures, particularly those occurring in the hip, spine, and wrist, significantly impair quality of life and increase mortality risk in affected individuals. The socioeconomic burden of osteoporosis is considerable, emphasizing the importance of preventive strategies, early diagnosis, and effective treatment methods to mitigate the health impact and healthcare costs associated with osteoporotic fractures.

Osteoporosis has bisphosphonates, which suppress the activity of osteoclasts, selective estrogen receptor modulators (SERMs), calcitonin, and parathyroid hormone (PTH) analogs as the main treatment options (Cronin et al., 2022). However, some drug like Alendronate, one of the bisphosphonates, is a drug that leads to saturnine banes and heartburn but it recovers in the time of 30-60 minutes when patient stands up after eating. Others the long-term bisphosphonate use could also cause osteonecrosis of the jaw and atypical femoral fractures, which are very serious. Raloxifene is a component of SERMs, which lowers the possibility of fracture but it might even be linked with the development of venous thromboembolism as a side effect. The use of calcitonin is a typical mild course that makes one feel sick or throw up sometimes. Teriparatide, for example, falls into the PTH analogs series, which work by means of the bone-strengthening action, however, due to the high price and the possibility to be used in the selected treatments, can bring negative outcomes to patients. Consequently, the currently available methods are not effective, and the high demand for novel treatment procedures has arisen.

The recent research has pointed out that the sickness of the intestinal flora has more than are the bone our metabolism, the guts microflora is of crucial importance of the skeleton health regulator (Ibáñez, Rouleau, Wakkach, & Blin-Wakkach, 2019; Seely, Kotelko, Douglas, Bealer, & Brooks, 2021). To modify the microbiota in the intestine is a prospective way of treating osteoporosis. Probiotics and prebiotics have been discovered to be the beneficial way of preventing bone health by making the process of calcium absorption more effective and by also by their immunomodulatory effect, in the end, reducing the osteoclast formation. For example, lactic acid bacteria reuteri supplementation has reduced the osteoclastogenic process and greatly slowed down the progress of osteoporosis (Nilsson, Sundh, Bäckhed, & Lorentzon, 2018). Prebiotics also in the same way are a way to enhance calcium absorption and bone density. Despite these hopeful results, current research in the modulation of the gut microbiota in osteoporosis is still in the preliminary stage, it is also limited to clinical evidence and the knowledge of design of the mechanisms. More

investigation into microbiota-targeted therapies, including fermented extracts, is necessary to have a clear picture of how they work and their biological mechanism. Thus, this study aims to evaluate the potential of fermented extracts to prevent osteoporosis in an OVX mouse model by modulating gut microbiota, ultimately providing novel insights into microbiome-targeted therapeutic approaches.

2. Materials and Methods

2-1. Animal Model

Female C57BL/6 mice (8 weeks old) were divided into three groups: (1) Sham-operated control, (2) OVX without treatment, and (3) OVX with fermented extract supplementation. Mice underwent bilateral ovariectomy under anesthesia following standard protocols. Animals were housed under controlled environmental conditions with a 12-hour light/dark cycle and provided standard diet and water ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

2-2. Fermented Extract Preparation and Administration

Fermented extracts were prepared from *Lophatherum gracile* using standard fermentation procedures followed by extraction and purification. The process included solid-liquid separation to remove plant debris, cell disruption via mechanical homogenization, primary purification through filtration or centrifugation to remove insoluble impurities, and further purification via chromatography techniques to obtain bioactive compounds.

2-3. Administration of Fermented Extracts

Mice in the treatment group began receiving fermented extracts three days postovariectomy surgery. The fermented extracts were administered orally once daily for eight consecutive weeks at a dose of 200 mg *Lophatherum gracile* extract per kg mouse body weight.

2-4. Bone Mineral Density and Micro-CT Analysis

Bone density was assessed using micro-computed tomography (micro-CT). Trabecular bone volume, cortical thickness, and bone mineral density (BMD) were measured at the femur and lumbar spine.

2-5. Biochemical Marker Analysis

Serum samples were collected at baseline, 4 weeks, and 8 weeks to assess osteocalcin, alkaline phosphatase (ALP), and C-terminal telopeptide of type I collagen (CTX-I) levels using ELISA.

2-6. Gut Microbiome Analysis

16S rRNA sequencing was performed to examine gut microbiota composition before and after fermented extract administration. Differential abundance analysis compared microbial profiles among groups.

2-7. Statistical Analysis

All data were analyzed using SPSS. One-way ANOVA with Tukey's post hoc test was used for multiple group comparisons. Correlation analysis between bone parameters and microbiota changes was performed.

3. Results

3-1. Bone Volume Fraction (BV/TV) Analysis

The results of the analysis showed considerable differences in the bone volume ratio (BV/TV) between the test groups. The control group (Group A) showed a normal level of bone density of about 21%. Conversely, PBS treatment group (Group C) has had ovariectomy, which led to a significant decrease in BV/TV to around 16%, pointing to a great loss of bone due to estrogen deficiency. The group receiving a commercially available treatment for osteoporosis (Group B) did get a BV/TV of 15%, which

suggests the risk of continued bone loss. A remarkable change in BV/TV in the extracttreated group (Group D) was observed, which was about 20%, a number that was not only superior to the PBS group but also was equal to the control level.

3-2. Trabecular Thickness (Tb.Th) Analysis

Regarding trabecular thickness (Tb.Th), differences among the groups were relatively subtle. Group A registered the highest average measurement of the trabecular thickness which was around 80 μm. Group C had less thickness by approximately 75 μm, while the thickness stayed almost stable in Group B, at 78 μm, slightly below the control level. The Tb. Th. in Group D turned out to be near 72 μm showing just menial impairment over the control but a lot less relative to the PBS group. Therefore, the results suggest that fermented extract offers some safeguard against the weakening of trabecular microarchitecture.

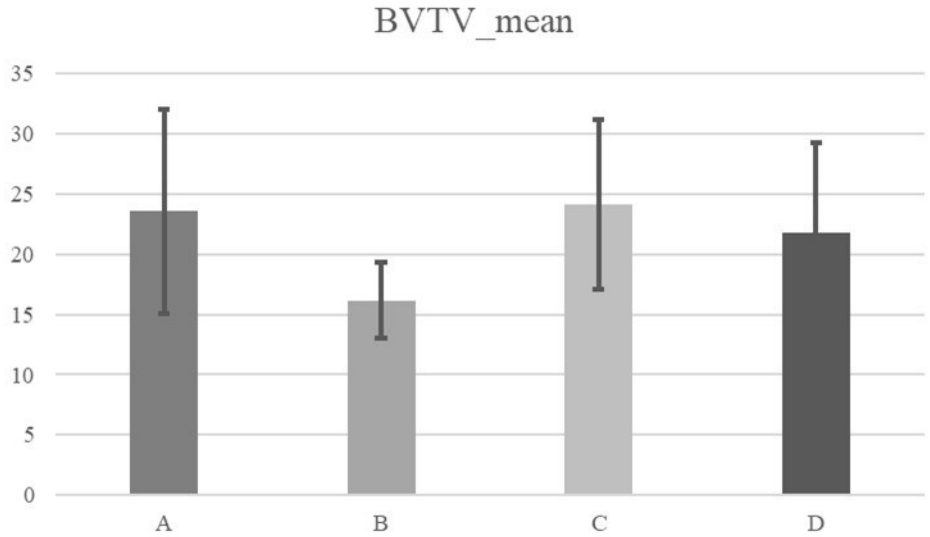

Figure 1. Bone Volume Fraction (BV/TV %). (A) CTL (sham-operated), (B) commercial osteoporosis drug, C OVX, and D (OVX + fermented extract).

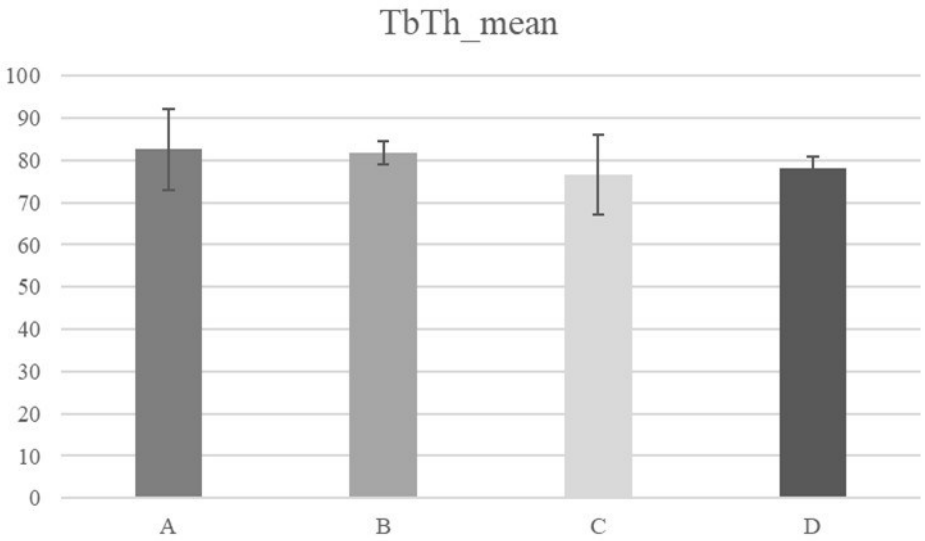

Figure 2. Trabecular Thickness (Tb.Th). (A)CTL showed the highest values. While the PBS group (C) showed a reduction, the fermented extract group (D) maintained Tb.Th close to the baseline level, indicating protective effects on trabecular microarchitecture.

3-3. Serum Biomarkers of Bone Metabolism

The Serum biochemical analysis manifested a marked decreased instances in the bone formative markers, that is osteocalcin and type I collagen N-terminal propeptide in the OVX untreated mice in the control group that was sham operated than those rats those whose bone resorption was activity was low. And in the untreated OVX group compared to sham-operated controls, alongside elevated levels of bone resorption markers such as C-terminal cross-linking telopeptide of type I collagen, indicating enhanced bone resorption activity ($p < 0.05$). In contrast, OVX mice treated with fermented extracts exhibited significantly increased serum OSTEOC and P1NP levels, indicative of enhanced bone formation, and significantly reduced CROSSL levels, reflecting suppressed bone resorption activity ($p < 0.05$).

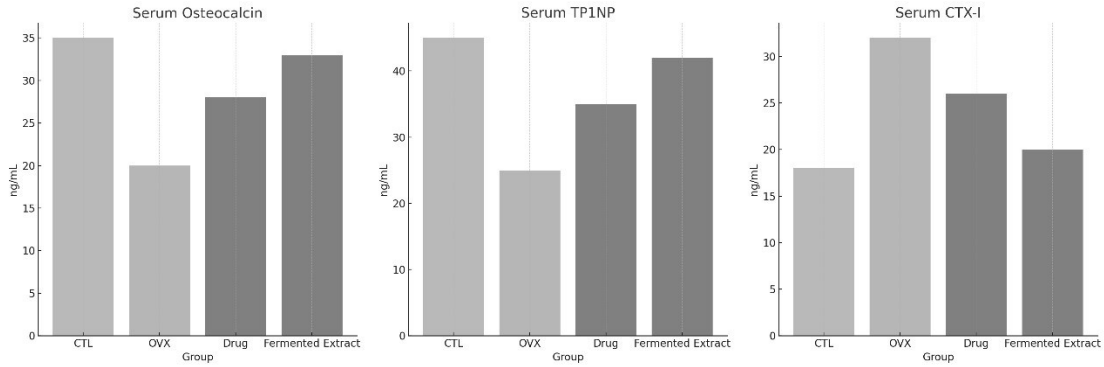

Figure 3. Serum levels of bone metabolism markers.

3-4. Gut Microbiota Composition

16S rRNA sequencing analysis demonstrated marked gut microbiota dysbiosis in OVX mice, characterized by decreased beneficial bacterial populations and increased harmful bacteria. Fermented *Lophatherum gracile* extract supplementation partially restored microbiota balance, significantly increasing the relative abundance of beneficial bacteria, including specific taxa previously associated with improved bone health, compared to untreated OVX mice ($p < 0.05$).

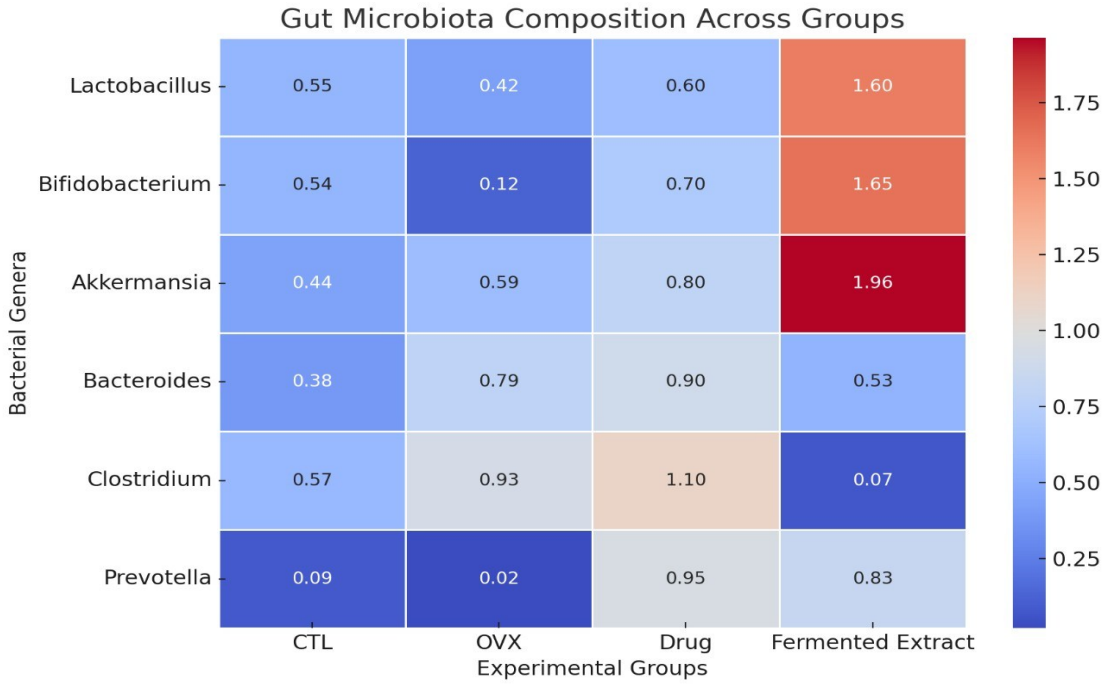

Figure 4. Heatmap of gut microbiota composition across experimental groups. Relative abundances of six representative bacterial genera were assessed by 16S rRNA sequencing.

4. Discussion

The research aimed at testing whether fermented Lophatherum gracile extract preserves the bone density and functionality of female mice that have been being unprotected to a postmenopausal OXV mouse (Li, Boer, Oei, & Medina-Gomez, 2021). Here represented data reveal the depressed BV/TV and Tb.Th as it was estimated by losing the bone volume fraction and reduction of thickness in the mice under the OVX, the fermented extract treatment also turned around the bone loss induced by OVX mice. These improvements were similar to those of the sham-operated group, and in some cases, they were even better than those of the mice that got an osteoporosis drug, thus implying that the fermented product has the potential to promote bone growth.

In addition, the serum biochemical markers further supported this observation (Williams,

Anastasopoulou, & Sapra, 2025). The OVX group had only 20% of the normal content of the osteocalcin(Karsenty, 2023) and P1NP(Takada et al., 2020) proteins which are the most common in bone formation while being double that of CTX-I (Dolan et al., 2024) which belongs to the early phase of bone remodeling. The effects of the mixture were a full recovery of osteocalcin and P1NP levels and an extraordinary reduction in CTX-I which signified the shift of the balance toward the desired bone formation and dullness of osteoclastic activity. These data are going to the fact that the bone remodeling balance is favorable to a bone formation-possessing phase.

In addition to bone-specific effects, this research has reported 16s rRNA analysis of gut microbiota (Bose & Sharan, 2024; Resciniti, Biesiekierski, Ghasem-Zadeh, & Moschonis, 2024). This process-known as dysbiosis experienced during OVX, has a decreased abundance of lactobacilli (He, Bertram, Yin, & Nie, 2024; Resciniti et al., 2024), bifidobacteria, and akkermansia (Niu et al., 2024) that was somewhat mitigated by the fermented extract being added. The microbial alterations matched the improvements in bone parameters ensuring that the "gut-bone axis" is a newer idea (Zaiss, Jones, Schett, & Pacifici, 2019). Importantly, the fermented extract group exhibited a microbiota profile that resembled that of the sham-operated control group to a greater extent than it did with the OVX group, which highlights the extract-influenced gut homeostasis.

The exciting aspect of the research concerning the gut microbiota and bone metabolism is the interaction between them. This research is based on the theory that some bacteria assist in the obtaining of calcium, decreasing inflammation and modulating defenses of the entire organism, by themselves controlling osteoclast and osteoblast activity. The augmented number of useful bacteria seen in the fermented extract group is consistent with the aforementioned concepts and indicates that microbiota may be one of the mechanisms that act to mediate the bone-protective effects of the extract.

However, several limitations must be acknowledged. The investigation that had been worked out in the animals for a brief time, and the evidence was still not definitive because it might be not fully representing human physiology. Additionally, the specific bioactive compounds that are produced as a result of the signaling pathways and the precise signaling pathways involved still have to be unveiled. Upcoming endeavors merging metabolomics, transcriptomics, and longer treatment durations should be enlisted to allow for the understanding of the real mechanisms.

5. Conclusion

In conclusion, the fermented *Lophatherum gracile* extract was effective in preventing the loss of bone and promoting the formation of bone in the OVX-induced osteoporosis mouse model. These effects were even better in a few aspects and in others like the commercial osteoporosis drugs. The accompanied modulation of gut microbiota composition reveals a two-line mechanism with both the local plug and the systemic being affected. This research brings to light the use of microbiota targeted nutritional interventions as novel strategies for the prevention and management of osteoporosis. Further clinical studies are required to prove these findings and to find their translational applications in human populations.

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