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Evaluation of *Ginkgo biloba* Leaves Extract Effect on Some Biochemical Factors in Osteoporotic Rats

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Abstract

Although *Ginkgo biloba* has been widely investigated for its therapeutic potential, its specific role in glucocorticoid-induced osteoporosis is poorly understood. This study aims to evaluate the effects *Ginkgo biloba* treatment on glucocorticoid-induced osteoporosis compared to alendronate as a standard therapy. The study included 40 male rats, which were divided into five groups (n=8). Except for the control group, the other four groups were induced with osteoporosis by receiving orally 10 mg/kg body weight of glucocorticoid three times a week for four weeks. Group 3 was treated with alendronate 70mg/kg body weight, while the fourth and fifth groups were given *Ginkgo biloba* extract orally in two doses (28 and 56 mg/kg body weight). The results showed significant increases in phosphorus, osteocalcin, and catalase levels in the treated groups compared to the osteoporotic group. Additionally, histological study confirmed the improvement of bone structure in the treated groups compared to the osteoporotic group. In conclusion, the most important finding of this study is that *Ginkgo biloba* extract may be proven to be a highly effective treatment for osteoporosis, even surpassing the effectiveness of the standard drug, alendronate.

Keywords: Bone tissue, Catalase, *Ginkgo biloba*, Osteocalcin, Osteoporosis, Phosphorus.

تقييم تأثير مستخلص أوراق الجنكة بيلوبا على بعض العوامل الكيميائية الحيوية في الفئران المصابة بهشاشة العظام

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الخلاصة

على الرغم من أن الجنكة بيلوبا قد خضعت لدراسات واسعة النطاق لإمكاناتها العلاجية، إلا أن دورها المحدد في هشاشة العظام الناجمة عن الجلوكوكورتيكويد غير مفهوم جيداً. تهدف هذه الدراسة إلى تقييم تأثيرات علاج الجنكة بيلوبا على هشاشة العظام الناجمة عن الجلوكوكورتيكويد مقارنةً بالألندرونات كعلاج

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قياسي. شملت الدراسة 40 فأراً ذكراً، قُسمت إلى خمس مجموعات (عدها 8). باستثناء المجموعة الضابطة، تم تحفيز هشاشة العظام في المجموعات الأربع الأخرى عن طريق تلقي 10 ملغم/كغم من وزن الجسم من الجلوكوكورتيكويد عن طريق الفم ثلاث مرات أسبوعياً لمدة أربعة أسابيع. عولجت المجموعة الثالثة بالأليندرونات بجرعة 70 ملغم/كغم من وزن الجسم، بينما أعطيت المجموعتان الرابعة والخامسة مستخلص الجنكة بيلوبا عن طريق الفم على جرعتين (28 و 56 ملغم/كغم من وزن الجسم). أظهرت النتائج زيادة كبيرة في مستويات الفوسفور والأوستيوكالسين والكاتاليز في المجموعات المعالجة مقارنةً بالمجموعة المصابة بهشاشة العظام. بالإضافة إلى ذلك، أكدت الدراسة النسيجية تحسن بنية العظام في المجموعات المعالجة مقارنةً بالمجموعة المصابة بهشاشة العظام. يمكن الاستنتاج، بأن أهم نتيجة توصلت إليها هذه الدراسة هي أن مستخلص الجنكة بيلوبا قد يكون علاجاً فعالاً للغاية لهشاشة العظام، متجاوزاً حتى فعالية الدواء القياسي، الأليندرونات.

1. Introduction

Plant extracts have garnered significant research interest for their therapeutic potential against diverse diseases, including diabetes [1], and tumors [2]. Osteoporosis, or "porous bones," is a systemic skeletal disorder that affects the entire skeleton. It is characterized by low bone mass and skeletal tissue microarchitecture degradation, which makes bones more brittle and prone to breaking [3]. Oral glucocorticoid steroids (GCS) are widely utilized due to their anti-inflammatory and immunosuppressive properties [4]. The use of glucocorticoids is often restricted due to their severe adverse effects, with osteoporosis being a common adverse outcome that exacerbates musculoskeletal issues and bone fractures. Glucocorticoid-induced osteoporosis leads to bone demineralization and considerable microscale alterations in the spatial heterogeneities of bone [5]. Alendronate, a type of bisphosphonate, has been clinically used for decades. The oral bisphosphonate (ALN) inhibits bone resorption by affecting osteoclasts. It has a potent and focused suppressing action [6]. However, because long-term systemic treatment can induce adverse effects like nausea, vomiting, gastrointestinal pain, and osteonecrosis of the jaws, the therapeutic use of ALN is still unclear [7]. Many plant extracts have been studied against pathogenic bacteria and oxidative stress and have shown high biological activities against oxidizing species and pathogenic bacteria [8,9]. Over the years, there has been intense interest in discovering new therapeutic approaches for treating osteoporosis. For instance, studies using phytoestrogens—chemicals derived from plants that have estrogen-like properties—showed promise as osteoporosis treatment alternatives [10]. *Ginkgo biloba*, a deciduous tree belonging to the Ginkgoaceae family, is widely planted around the globe. It grows primarily in China's east and south. The leaves can be harvested multiple times throughout the growing season, presenting a significant source of bioactivities [11]. Many natural plant-based formulations contain bioactive compounds that may aid in treating osteoporosis patients. Due to its antioxidant qualities, *Ginkgo biloba* Leaf extract has been used for generations to cure a variety of illnesses in both Chinese and Western countries. Flavonoids are one of the most important active ingredients in *Ginkgo biloba* leaf extract, which have anti-inflammatory, antioxidant, and free radical scavenging properties [12]. This study aims to investigate the effect of *Ginkgo biloba* Leaves on osteoporotic rats (glucocorticoid-induced osteoporosis in rats) and compare it with the standard drug (alendronate) in terms of the relevant biochemical factors and supported histological study.

2. Methodology

2.1. Plant material

Dried *Ginkgo biloba* leaves were purchased from the local market in Baghdad in August to October 2024. The plant components were ground and maintained in dark, airtight-lidded

containers until the extraction stage. Aqueous extraction was prepared with 30g/ 300 mL water using a Soxhlet apparatus and then dried with a lyophilizer for 24 hours.

2.2. Experimental design

Male Wistar rats weighing 175–250 g and aged 3 months were used in the experiments and maintained under identical conditions. Eight animals were assigned as a control group and treated orally with distilled water for a month, then terminated.

Osteoporosis was induced in the other 32 animals with prednisolone in a dose of 10mg/kg 3 times a month [13]. Then the osteoporotic rats were divided into 4 groups: osteoporotic group (n=8), alendronate-treated group (n=8) in a dose of 70 mg/kg body weight [14], Ginkgo biloba-treated group (EGb1) (n=8, oral aqueous extract of Ginkgo biloba (EGb1) for 30 days in a dose of 28 mg/kg body weight) [15], Ginkgo biloba-treated-group (EGb2) (n=8, oral aqueous extract of *Ginkgo biloba* (EGb2) for 30 days in a dose of 56 mg/kg body weight) [15].

After 30 days of the administration of alendronate and *Ginkgo biloba* extract, animals were sacrificed, and the femurs were removed from each rat and fixed in 10% formalin for histological examination.

2.3. Serum and tissue preparation

At the end of the treatment period, blood samples were collected via cardiac puncture from rats under light ether anesthesia. The sera were stored at -20°C after being separated by centrifugation at 2012.4 x g. The femurs were dissected, with all surrounding muscles and tissues removed. All femur samples were weighed, and some were saved for histological analysis.

2.4. Euthanasia method

Rats were euthanized after finishing the treatment protocol for each group by cervical dislocation; a well-known and ethically approved approach in experimental animal research. Cervical dislocation ensures a quick and painless termination, reducing the risk of suffering [16, 17].

2.5. Biochemical parameters

In this study, Serum was analyzed for bioassays (phosphorus), using a kit provided by Elabscience-China (Cat. No.:E-BC-K245-M), The colorimetric method was used to detect serum phosphorus. The colorimetric microplate reader method was used to measure the concentration of serum phosphorus (P). Serum osteocalcin (OT) concentration was determined by the sandwich ELISA method, using an ELISA kit for osteocalcin from SunLong-China company (Cat. No.:SL0543Ra). Serum catalase (CAT) was determined by using an ELISA kit of catalase (CAT) from SunLong-China company (Cat. No.:SL1084Ra), by applying the sandwich enzyme-linked immunosorbent assay (sandwich ELISA). Serum calcium (Ca^{+2}) was tested using a colorimetric method with a kit provided by Elabscience-China (Cat. No.: E-BC-K103-M), and its concentration was measured using a colorimetric microplate reader. Sandwich ELISA kit from SunLong-China (Cat. No.:SL0137Ra) was used to measure the levels of serum calcitonin. Sandwich ELISA kit from Fine Test-China (Cat. No.:EU0390) was used to measure the level of serum 17β -oestradiol.

The ELISA procedure involves immobilizing an antigen or antibody on a microplate, followed by a sequence of specific antibody binding steps and washes. Later, an enzyme-linked antibody is added. An enzyme substrate is then added, which is converted into a detectable color or fluorescent signal by the enzyme, allowing for the detection of the presence and concentration of the target molecule.

2.6. Inducing of osteoporosis in animals

Before initiating the full-scale therapeutic experiment, a pilot study was conducted to confirm the efficacy of the osteoporosis-inducing protocol. Rats were administered a standardised dose of the osteoporotic agent glucocorticoids three times per week for 4 weeks, consistent with established methodologies for inducing bone density loss by Banji et al [13]. After the end of treatment, they were anesthetized, and blood samples were collected by heart puncture. Chemical analysis was performed, which included estimation of Ca^{+2} , estrogen, phosphorus, calcitonin, and osteocalcin. After blood was collected, rats were euthanized by cervical dislocation, and then the femur bone was taken for histological study. The results of this pilot study revealed highly significant differences between the above-mentioned biomarkers in the control group and the osteoporotic rat group, as shown in Table 1. Additionally, histopathology analysis revealed decreased osteoblast activity and elevated osteoclast counts, consistent with glucocorticoid-induced bone pathology and increased porosity, as shown in Figure 1.

2.7. The hematoxylin/eosin (H&E) staining

For 24 hours at room temperature, the samples were submerged in brand-new 10% neutral formalin. A 15% formic acid solution was used to decalcify the femur specimens. Following the completion of the decalcification process, the decalcified specimens were progressively submerged in varying ethanol concentrations to achieve a gradient dehydration. After that, the bones were chopped into 5-mm pieces for hematoxylin and eosin (H&E) staining [12].

2.8. Statistical analysis

The data were analyzed using Minitab 18.1 software (Minitab Inc., MB) and expressed as mean $\pm$ standard deviation (mean $\pm$ SD); one-way ANOVA was used to assess statistical significance. The osteoporosis group was contrasted with the control group. P-values ≤ 0.05 were regarded as significant.

3. Results

3.1. Inducing of osteoporosis in animals

Table 1 shows the Ca^{+2} , estrogen, phosphorus, calcitonin, and osteocalcin levels in the osteoporotic and control groups. This experiment is designed to confirm the induction of osteoporosis in the affected rat group by glucocorticoids (prednisolone). The biochemical parameters analyzed and presented in Table 1 showed significant differences between the control and osteoporotic groups. These results confirm the development of osteoporosis rats treated with the specified glucocorticoid dose (prednisolone). Additionally, a histological investigation was performed, as indicated in Figure 1, revealed notable structural changes in osteoporotic rat bone, including increased porosity, bone resorption, and loss of trabecular connection. Consequently, the histopathology results support the study findings, which revealed significant structural changes in osteoporotic rat bone, including increased porosity, bone resorption, and loss of trabecular connection. Subsequently, the histological results support the biochemical factor results, indicating osteoporosis in the osteoporotic rat group treated with glucocorticoid (prednisolone).

Table 1: Serum levels of the Ca^{+2} , estrogen, phosphorus, calcitonin, and osteocalcin in the osteoporosis-induced groups and control group.

Parameters Groups	Calcium (mean±SD) mmol/L	Estrogen (mean±SD) ng/ml	Phosphorus (mean±SD) mmol/L	Calcitonin (mean±SD) pg/ml	Osteocalcin (mean±SD) nmol/L
Control group 5 rats (G1)	1.5221±0.1648 ^a	38.065±2.116 ^a	4.006±0.9782 ^a	59.786±6.221 ^a	1.335±0.142 ^a
Induced- osteoporosis group 5 rats (G2)	1.0430±0.1132 ^b	19.976±1.853 ^b	2.511±0.563 ^b	39.672±4.557 ^b	0.4976±0.0854 ^b
P-value	0.0002	0.00012	0.0003	0.00004	0.00026
LSD	0.1805	3.225	0.645	5.223	0.247

Different small letters mean significant differences at $p \leq 0.05$. The means having different alphabets (a) indicating the highest value and (b) denoting progressively lower values.

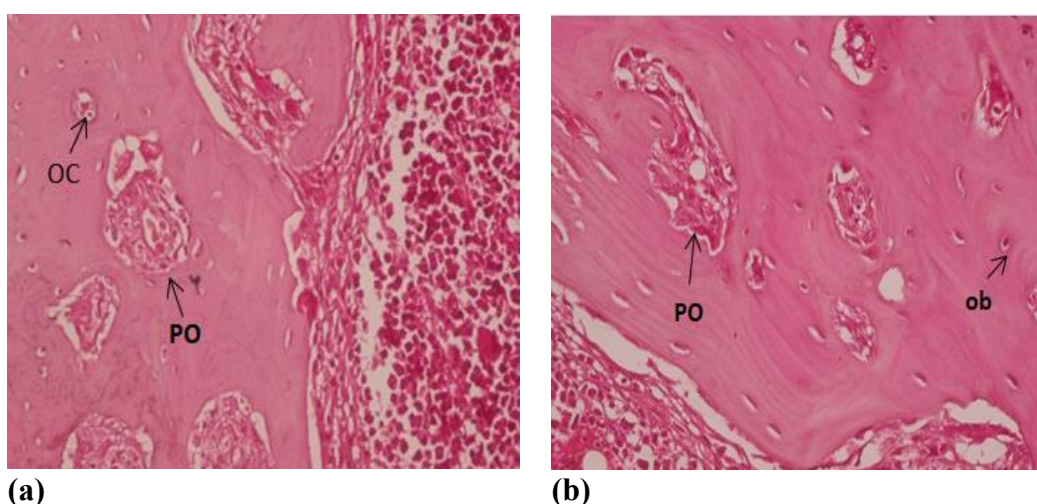


Figure 1: Histological images of randomly selected femur sections from rats treated with Induce Osteoporosis group (glucocorticoid-induced (10 mg/kg body weight)). (a) shows a porosity (PO) and osteoclasts (OC). (b) the osteoblast and porosity (PO) (H&E) 40X. Subsequently, osteoporosis was confirmed to be induced by glucocorticoid administration in the experimental subjects.

These findings validated the successful induction of osteoporosis, which enabled the subsequent evaluation of the plant extract's efficacy in mitigating bone loss in the affected rats compared to other groups.

3.2. Biochemical parameters analysis

Table 2 presents the levels of biochemical parameter, including phosphorus, osteocalcin and catalase in all the studied groups. The results indicated that the levels of Phosphorus, osteocalcin, and catalase significantly decreased in the osteoporotic group compared to the control group. The EGb1, EGb2, and ALN groups considerably regained their values toward normal after 30 days of treatment.

Table 2: Levels of serum phosphorus, osteocalcin and catalase in all the studied groups

Parameters Groups	Phosphorus (mean±SD) mmol/L	Osteocalcin (mean±SD) nmol/L	Catalase (mean±SD) Pg/ml
Control group (G1)	3.846±0.551 ^a	1.154±0.1379 ^a	16.321±1.433 ^a
Osteoporotic group (G2)	2.702±0.496 ^b	0.5696±0.0542 ^d	7.259±0.841 ^d
Alendronate group (70mg/kg body weight) (G3)	3.058±0.484 ^b	0.7165±0.0801 ^c	11.024±1.12 ^c
Ginkgo biloba group (EGb1) 28mg/kg body weight (G4)	3.317±0.525 ^{ab}	0.831±0.0707 ^{bc}	13.645±1.521 ^b
Ginkgo biloba group (EGb2) 56mg/kg body weight (G5)	3.744±0.474 ^a	0.9023±0.069 ^b	13.886±1.373 ^b
P-value	0.010	0.00051	0.00033
LSD	0.552754	0.095297	1.398421

Expresses the significance difference between control group and the treated group

The results showed a significant decrease in Phosphorus levels in osteoporosis when compared with the control group ($p \leq 0.05$). The Phosphorus levels increased significantly ($p \leq 0.05$) in both treated groups when compared to the control group. The results revealed a significant increase in the levels of the studied biochemical factors with aqueous extract of *Ginkgo biloba*, more than ALN in the treated groups. The enhanced effect of *Ginkgo biloba* compared to ALN may be attributed to its multifaceted mechanism of action. Consequently, the increased phosphorus levels in *Ginkgo biloba*-treated rats suggested that they improved bone health and mineralization efficiency compared to ALN alone.

Osteocalcin levels for all studied group were reported in Table 2. The results show a significant variation ($p \leq 0.05$) in the osteocalcin concentration in the control group compared with the osteoporotic group. Additionally, significant difference ($p \leq 0.05$) in the osteoporotic group compared with the groups treated by ALN and *Ginkgo biloba* extract. Furthermore, its level significantly increases in the *Ginkgo biloba* extract groups in comparison to the ALN group, as recorded in Table 2. This is a good indication of the response of infected bone to treatment with *Ginkgo biloba* extract.

The results of CAT for the studied groups were recorded in Table 2. They were shown that there is considerable variation ($p \leq 0.05$) in the CAT level in the treated group compared to the control group, and significant differences in catalase levels in treated groups with ALN and *Ginkgo* compared to the control group. Catalase levels of the treated groups are higher than those in the untreated group. These findings indicate that the treated groups have improved to become similar to the control group. *Ginkgo biloba* extracts contain active components like flavonoids and terpenoids, which possess potent antioxidant and free radical-scavenging properties. Thus, these bioactive compounds can reduce the oxidative damage that occurs in bone cells. Subsequently, increasing CAT levels in osteoporotic rats treated with *Ginkgo biloba* extract indicates the high activity and strong defense mechanisms of *Ginkgo biloba* extract. It demonstrates a superior effect of the extract compared to ALN alendronate. Moreover, *Ginkgo biloba* extract promotes both bone formation and mineralization in infected bones.

3.3. The histopathology of femur bone in rats

1. Control group

Histopathological examination showed normal bone architecture, The Haversian systems and outer, inner, and interstitial bone lamellae comprise the compact bone. Osteocytes are located between lamellae, in their lacunae. In normal bone structure, the periosteum and the compact bone membrane line the bone cortex and exhibit a balance between osteoblasts and osteoclasts. This physiological positioning reflects the normal bone metabolic processes, which are necessary to maintain homeostasis of bone structure, as illustrated in Figure 2. (A and B).

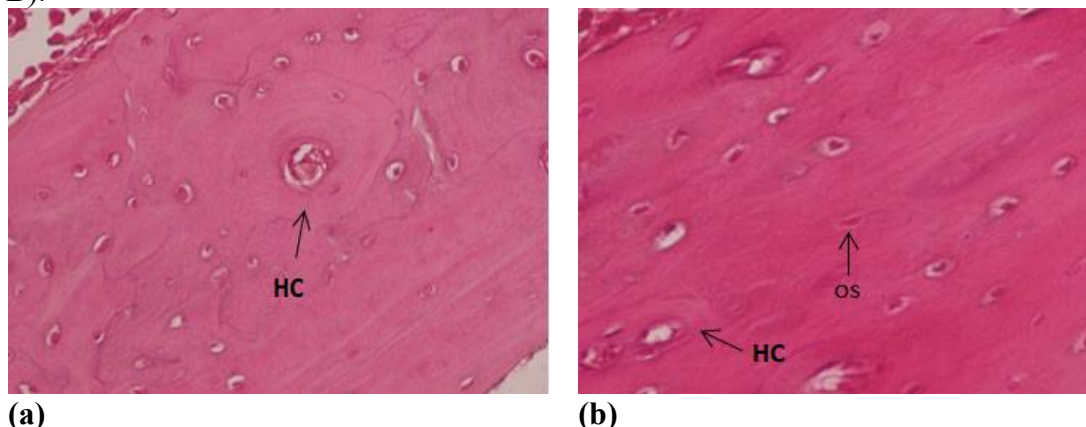


Figure 2: Histological images of randomly selected femur sections from rats treated with control (a) the Haversian canals (HC), (b) the Haversian canals (HC) and osteocytes(OS) (H&E) 40X.

2. Osteoporotic group:

Rats treated with 10 mg/kg body weight of glucocorticoids exhibited abnormal bone histology appearance, with histopathology examination revealing an irregular eroded lining of the endosteum. Enlarged bone marrow gaps were also visible. Compared to the control group, these rats showed an increased number of osteoclasts, along with occasional fissures and pores in the compact bone. The alveolar margin was uneven, the marrow cavities seemed larger, certain osteocyte lacunae appeared broader, while others were empty, and there was a significant decrease in osteoblasts in the osteoporotic group, as shown in Figure 3 (A and B).

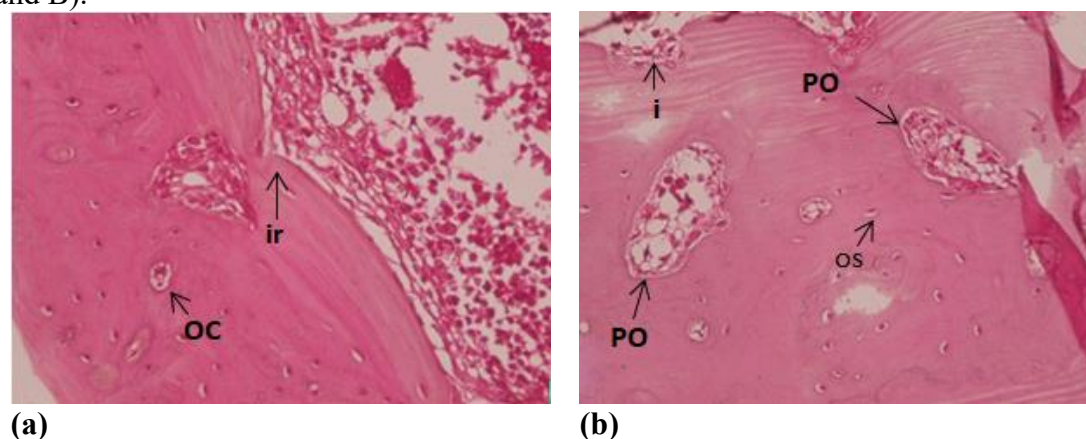


Figure 3: Histological images of randomly selected femur sections from rats treated with glucocorticoid-induced (10 mg/ kg body weight). (a) irregularities, particularly on the inner surface (ir) and osteoclasts (OC). (b) The porosity(PO), osteocytes(OS), and inner surfaces of bone tissue exhibit areas of invasion (i) and osteocytes(OS) (H&E) 40X.

3. Alendronate group:

The results of the alendronate-treated group (70mg/kg body weight) exhibited partial preservation of bone architecture, with a well-maintained histological structure of the compact bone, as shown in Figure 3. Osteogenic cells and osteocytes were abundant within the stroma and lacunae, while osteoclasts were still present at the inner bone margins in contact with the bone marrow. Although alendronate is used in treatment, some lacunae contained residual hemolysis, and the persistence of osteoclasts suggests that bone resorption was not completely inhibited, possibly due to the chronic nature of osteoporosis or an adaptive response to prolonged alendronate exposure. Nevertheless, the bone marrow remained histologically intact, indicating a degree of protection against osteoporotic deterioration, as illustrated in Figure 4 (A and B).

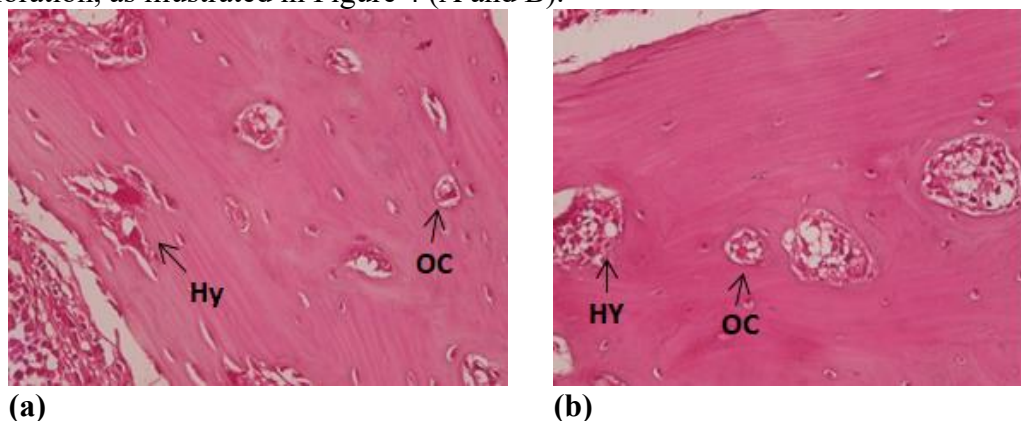


Figure 4: Histological images of randomly selected femur sections from rats treated with alendronate (70mg/kg body weight) (a). increase in the incidence of hemolysis (Hy) within the lacunae and osteoclasts (OC). (b) the osteoclasts (OC), (H&E) 40X.

4. *Ginkgo biloba* group (EGb1):

Figure 5 illustrates the results for the group treated with *Ginkgo biloba* extract at a dose of 28mg/kg body weight. The results revealed reparative changes in the compact bone compared to the osteoporotic group, with an improved histological composition of both the bone tissue and marrow. However, some lacunae contained residual hemolysis, indicating that bone damage was not entirely reversed. A decrease in the number of osteoclasts was evident, suggesting that *Ginkgo biloba* exerted a modulatory effect on bone resorption, though not to the extent of complete inhibition. Additionally, the periosteum appeared well-preserved, implying a potential protective role of *Ginkgo biloba* in maintaining periosteal integrity, as shown in Figure 5 (A and B).

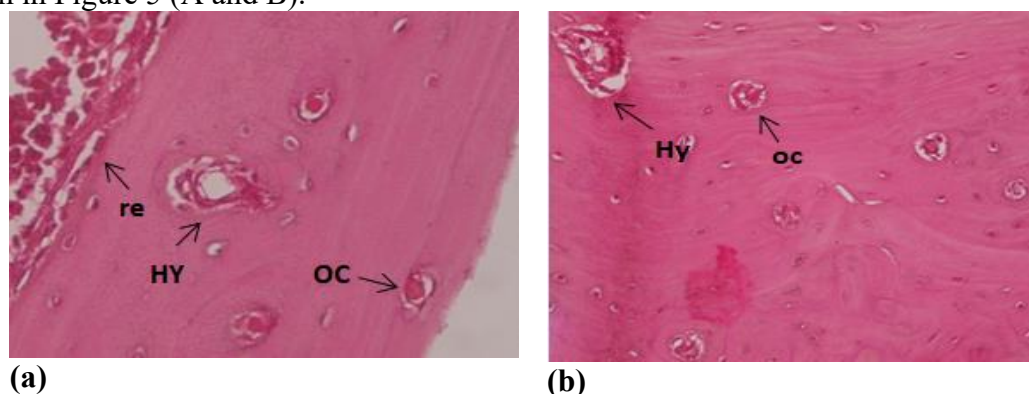


Figure 5: Histological images of randomly selected femur sections from rats treated with *Ginkgo biloba* 28mg/kg body weight) (A) shown repair in the compact bone(re), hemolysis (Hy) and osteoclasts (OC). (B) the osteoclasts (OC) and hemolysis (Hy) within the lacunae, (H&E) 40X.

5. *Ginkgo biloba* group (EGb2):

The histological study of rats' femur bones, exhibiting bone tissue treated with plant extract (*Ginkgo biloba* extract dose 56 mg/kg body weight). The results of this study revealed a balance of osteoblasts and osteoclasts. The exterior periosteum histological makeup was identical to that of the control group. This result indicates that the bone's structural and functional qualities have been completely restored, which contributes to its complete healing. A significant improvement in femoral bone structure was noted. Although there were still a few small gaps, its thickness was nearly the same as that of the control group. These groups demonstrated a greater improvement in bone tissue, as shown in Figure 6 (A and B).

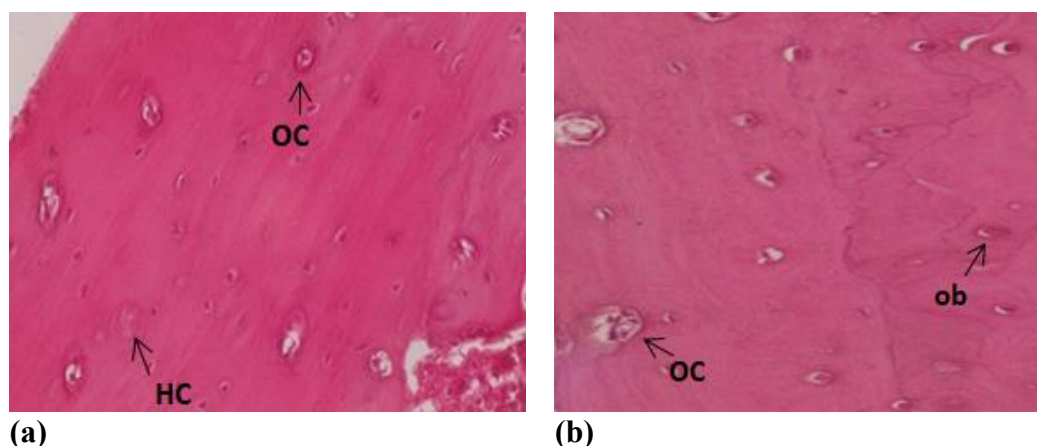


Figure 6: Histological images of randomly selected femur sections from rats treated with *Ginkgo biloba* 56mg/kg body weight) (A). The haversian canals (HC) and osteoclasts (OC). (B) the osteoblaste (Ob) and osteoclasts (OC) ,(H&E) 40X .

4. Discussion

Osteoporosis is a common condition that occurs in the bones, leading to the loss of bone mass[18]. Glucocorticoid-induced osteoporosis is the most prevalent type of secondary osteoporosis, which can result from long-term pharmaceutical use or underlying medical disorders [19], in patients with immunological and rheumatological conditions who need long-term glucocorticoid medication. Glucocorticoid-induced osteoporosis (GIOP) is a significant issue. Prolonged use of glucocorticoids reduces bone mineral density and increases the risk of fractures by encouraging bone resorption and inhibiting bone growth [20]. In the current study, it was found that glucocorticoid leads to a decrease in Phosphorus, osteocalcin, and CAT. The current results are supported by previous studies [21-23]. while glucocorticoids are commonly used to treat a variety of disorders, a major side effects is their detrimental impact on bone, which can lead to glucocorticoid-induced osteoporosis.

Alendronate is an aminobisphosphonate, structurally, it is similar to Isopentenyl diphosphate (IPP), It is the most extensively used medication for preventing and treating postmenopausal osteoporosis, osteoporosis in men, and corticosteroid-induced osteoporosis [24]. However, the administration of ALN results in significant adverse effects, including musculoskeletal discomfort, gastrointestinal irritation, nausea, jaw osteonecrosis, and cardiovascular risks. ALN is also characterized by limited oral absorption and bioavailability [24]. In this study, Phosphorus was found to be increased (ALN) compared with the osteoporotic group. This is consistent with the literature. [25]. Also, current results showed an increase in levels of osteocalcin and catalase. In (ALN) group, these results are in agreement with previous studies [26,27].

The recovery of phosphorus, osteocalcin and CAT levels in the case of *Ginkgo biloba* leaves extract (EGb) therapy was successful; this is consistent with similar studies [28,29].

This may be attributed to the extract's bioactivities, such as anti-inflammatory and anti-oxidative stress, which render it more active in the treatment of bone problems, which are usually linked to inflammation and oxidative stress [30]. One of the most important active ingredients in *Ginkgo biloba* leaves extract is flavonoids, which have anti-inflammatory, antioxidant, and free radical scavenging properties [12].

The histopathological data showed normal bone architecture, and a microscopic examination of bone pieces from the control group indicated a well-organized, undamaged structure.

In the current study, histopathological analysis of glucocorticoid-treated animals showed a reduction in osteoblasts, consistent with findings reported by Gado et al [31]. Because of the development of several intracortical cavities of varying widths, the femoral cortex of osteoporotic rats was thinner and more porous than that of the control group. Long-term glucocorticoid use is linked to a range of side effects, including osteoporosis. This medication has a variety of impacts on bone metabolism, the most significant of which is a decrease in osteoblast number and bone matrix production[10]. It has been demonstrated that glucocorticoids reduce bone mass and thereby increase the risk of fractures [32].

Amongst bisphosphonates, sodium ALN stands out due to its high affinity to bone tissue[33]. Although osteoclasts were still present despite the use of treatment, this differs from other studies that demonstrated that alendronate (ALD) can inhibit bone loss by suppressing osteoclasts activity and decrease level of bone resorption [34], but it agrees with a study conducted by M Sato *et al*[35], who reported that during acidification, alendronate is locally produced and binds to resorption surfaces, the increase in concentration stops resorption and membrane ruffling without harming the osteoclasts. It also showed residual in the incidence of hemolysis within the lacunae of the compact bone, which may be due to the bisphosphate causing gastrointestinal side effects, limiting its therapeutic usefulness [36].

The prevalence of osteoporosis has led to increased interest in drug research related to this disease. The histological test of the femur bone of rats, showing the bone tissue treated with plant extract (*Ginkgo biloba* 56 mg/kg body weight) showed a balance between osteoblast and osteoclast, and well-organized femur bone, which is similar to the control group. Ginkgolide B (GB), a significant ingredient in *Ginkgo biloba*, enhances bone density and inhibits mature osteoclast development *in vitro*[37]. Plant extracts contain bioactive components such as flavonoids, alkaloids, carotenoids, terpenes, and vitamins, which biotic or abiotic nano-catalysts can stimulate. The effect of these plant extracts is due to the presence of such bioactive components with anti-oxidant, anti-inflammatory, and antibacterial properties, which have minimal side effects on general health[38].

The results of the current study clearly and accurately show that *Ginkgo biloba* leaf extract has a significant positive effect on osteoporosis, as measured by biochemical parameters and bone histological studies in the osteoporotic groups treated with standard drug and *Ginkgo biloba* extract and the control group. The results indicated that the effectiveness of the *Ginkgo biloba* extract was very similar to that of the control group, while the standard drug did not provide this level of effectiveness.

Conclusion

The adverse side effects of pharmacological agents have led researchers to explore natural therapeutic compounds that may be both effective and safe for the treatment of osteoporosis with fewer adverse effects. The findings imply that *Ginkgo biloba* extract could be used as an option to treat osteoporosis, especially that caused by long-term corticosteroid use.

6. Acknowledgements

The authors thank and appreciate the Biotechnology Center at Al-Nahrain University, especially the staff of the Chemical Laboratory, for the facilities and great efforts that helped in completing this study.

7. Ethical approval

All institutional and national guidelines for the care and use of laboratory animals were followed. The study was conducted with the approval of the Scientific Committee of the Department (Registration number: 22-5302 on 21 August, 2024) designated for the application of the animal model for practical experimental purposes.

8. Conflict of Interest

The authors declare that they have no conflicts of interest.

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