Research Article

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The Anti-osteoporotic Effect of *Avena sativa* Seeds Ethanolic Extract on Methylprednisolone -induced Osteoporosis in Female Rabbits (*Lepus cuniculus*)

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Abstract

The experiment was conducted to investigate the role of *Avena sativa* L. (oat) seeds ethanolic extract on osteoporosis in female rabbits treated with methylprednisolone, by studying the bone mineral density(BMD), serum Ca and P, Vit. D, PTH, and histopathological alterations of femur bone. Fifty female rabbits were used, were randomly divided into five equal groups, each group contains 10 adult female rabbits. Control negative group(G^{-ve})**,**Control positive group(G^{+ve}) (Meth-Pred) received S/C methylprednisolone of 0.2 mg/kg per day for 30 days then received orally 1ml/kg normal saline per day for two months, Group3 (Meth-Pred+As) received similar dose of Meth-Pred then treated with 600 mg/kg *Avena sativa* seed extract orally per day for two months, Group4(Meth-Pred+Alend) received similar dose of Meth-Pred then treated by alendronate 3.6 mg/kg BW orally weekly for two months, Group5 (Meth-Pred+ Alend + As) received similar doses of Meth-Pred then treated by Alend then treated with *Avena sativa* seed extract. This study persisted for three month and till after one week of the medicine's withdrawal. The results showed that the BMD, serum Ca, phosphorus, Vit. D, a significantly declined ($p \leq 0.05$), as well as a significantly high ($P \leq 0.05$) in serum PTH of the animals after two month of treatment, and after one week of withdrawal of treatment in control(+ve) and alendronate group compared with control(-ve), but when treated rabbits with *Avena sativa* ethanolic extract in third group, showed an improvement in the parameters above return to its normal values and produced excellent results. Histopathological alterations of the femoral bone of control (+ve) group including abnormal histological appearance of the bone, showed widening in the Haversian canal, large cavities, erosion cavity containing osteoclasts, and lacunae appear without osteocytes. Whereas treated with *Avena Sativa* seeds ethanolic extract in third group were effective in arresting histopathological alterations and normal architecture compared with the control (-ve) group. This study concluded that the ethanolic extract of *Avena Sativa* seeds has an effective therapeutic agent for the treatment and prevention of osteoporotic bone loss.

Keywards: ***Avena Sativa*** **seeds, Ethanolic Extract, Femur bone, Rabbits, Osteoporosis**

التأثير المضاد لهشاشة العظام للمستخلص الإيثانولي لبذور الشوفان على هشاشة العظام التي يسببها الميثيل بريدنيزولون في أناث الأرانب

الخلاصة

أجريت التجربة لمعرفة دور المستخلص الإيثانولي لبذور الشوفان على هشاشة العظام الناجم عن العلاج بالميثيل بريدنيزولون في إناث الأرانب من خلال دراسة الكثافة المعدنية للعظام ، قياس تركيز الكالسيوم والفسفور في الدم ، فيتامين د، هرمون الغدة جارالدرقية والتغيرات النسجية المرضية لعظم الفخذ . تم استخدام خمسون انثى ارنب، قسمت الى خمسة مجاميع كل مجموعة تحتوي على عشرة انثى ارنب ، المجموعة الاولى (السيطرة السالبة) ،، المجموعة الثانية (مجموعة الميثيل بريدنيزولون) مجموعة السيطرة الموجبة:

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حقنت دواء الميثيل بريدنيزولون بجرعة 0.2 ملغم/ كغم من وزن الجسم تحت الجلد لمدة شهر واحد ثم جرعت 1مل من المحلول الفسلجي الطبيعي لمدة شهرين، المجموعة الثالثة (مجموعة الميثيل بريدنيزولون + مستخلص بذور الشوفان): حقنت دواء الميثيل بريدنيزولون بنفس الجرعة لمدة شهر يوم ثم جرعت 600 ملغم/ كغم من وزن الجسم من مستخلص بذور الشوفان لمدة شهرين ،المجموعة الرابعة (مجموعة الميثيل بريدنيزولون + دواء الالندرونيت): حقنت دواء الميثيل بريدنيزولون بنفس الجرعة لمدة شهر ثم جرعت دواء الالندرونيت اسبوعيا بجرعة 3.6 ملغم/ كغم من وزن الجسم لمدة شهرين، المجموعة الخامسة (مجموعة الميثيل بريدنيزولون + دواء الالندرونيت + المستخلص الايثانولي لبذور الشوفان): حقنت دواء الميثيل بريدنيزولون لمدة شهر ثم جرعت اسبوعيا دواء الالندرونيت ثم جرعت بمستخلص بذور الشوفان لمدة شهرين بنفس الجرع. واستمرت هذه الدراسة حتى بعد اسبوع واحد من التوقف عن العلاج . اظهرت النتائج ان هنالك انخفاض معنوي في الكثافة المعدنية للعظام ، تركيز الكالسيوم والفسفور في الدم و فيتامين د الى جانب ذلك هنالك زيادة معنوية في هرمون الغدة جار الدرقية في اناث الارانب بعد شهرين من العلاج وبعد اسبوع واحد من سحب الدواء في مجموعة الميثيل بريدنيزولون ومجموعة الميثيل بريدنيزولون ودواء الالندرونيت مقارنة مع مجموعة لسيطرة السالبة، بينما عندما عولجت الحيوانات بالمستخلص الإيثانولي لبذور الشوفان في المجموعة الثالثة أظهرت تحسنًا في المعايير المذكورة أعلاه ، حيث عادت إلى قيمها الطبيعية وحققت نتائج ممتازة حتى بعد أسبوع واحد من التوقف عن العلاج. اظهرت التغيرات النسجية لعظم الفخذ في مجموعة السيطرة الموجبة ان المظهر النسيجي للعظم غير طبيعي وهناك توسع في قناة هافرسيان, وجود تجاويف كبيرة, بالإضافة الى وجود تجاويف متآكلة تحتوي على ناقضات العظم ,الاحياز(الجوبة) كانت خالية من الخلايا العظمية , في حين أن العلاج بالمستخلص الإيثانولي لبذور الشوفان في المجموعة الثالثة كان فعالاً في إيقاف التغيرات النسيجية المرضية وعودة العمارة الطبيعية مقارنة بمجموعة السيطرة. خلصت هذه الدراسة إلى أن المستخلص الإيثانولي لبذور الشوفان له عامل علاجي فعال في علاج هشاشة العظام ومنع فقدان العظام

Introduction

Therapy with corticosteroids (GCs) includes the treatment of allergic and inflammatory conditions as well as the suppression of undesired or improper immune system actions, whereas their massive and prolonged use is continuously associated with multiple complications, such as osteoporosis (1). Reduced bone mass and deterioration of bone microarchitecture are characteristics of osteoporosis (2). Additionally, it contributes to an increase in bone fragility, which result in disability and mortality of the elderly (3,4). Multiple pathways contribute to glucocorticoid-induced osteoporosis (GIO), it is suppress the formation of the bone-forming cells, osteoblasts, and induce their apoptosis (5). Although bisphosphonates are frequently used to treat or prevent osteoporosis (6,7), some side effects, such as gastrointestinal intolerance (8), and osteonecrosis of the jaw have been noted (9,10), there is a need to find new approaches to the current treatments. Because of its advantageous properties and lower complications than chemical medications, medicinal plant-derived pharmaceutical products are employed in medicine. Finding novel, efficient, and safe therapies is therefore necessary(11,12). Oat (*Avena sativa* L.) belongs to the grass family (*Gramineae*) and contains a variety of chemical substances, including carbohydrates, proteins, sterols, lipids, alkaloids, saponins, and flavonoids(13,14). Additionally, it has high antioxidant properties due to phenolic compounds(15), and contains β-glucan(16,17,18), starch, and amylase, also, minerals such as phosphorus iron, calcium, potassium, magnesium, copper, zinc, silicon, selenium(19), and contains several vitamins like Vitamin B1, B2, B6, B12, Niacin, Vitamin C, Vitamin A, Vitamin E. (20).The aim of this study was to investigate the anti-hyperparathyroidism and anti-osteoporotic action of *Avena sativa* seeds ethanolic extract and alendronate in a short treatment period.

Materials and Methods

-Collection of seeds and extraction:

Seeds of *Avena sativa* L.(oat) was obtained from local markets in Basra -Iraq. These seeds were extracted technique according to (21).

Ethics

The experimental design, and procedures used in this study were reviewed and approved in accordance with animal welfare ethical standards by the Scientific Committee of the Department of Physiology, Biochemistry, and pharmacology, College of Veterinary Medicine, University of Baghdad, in its session held on 4/7/ 2021, and the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Acute Toxicity Study:

Acute toxicity study was carried out by using graded doses *Avena sativa* ethanolic extract on female rabbits. The animals were fasted for 12hrs prior to the oral administration of extract. The animals were divided into twelve groups, eleven of them were administered series doses of each extract that have been used in this study, while the twelve group administered distilled water and considered the control. The extract is diluted in distilled water, and administered orally in graded doses (5000, 6000,7000, 8000, 9000, 10000, 11000, 12000, 13000,14000,15000 mg/kg B.W), as one animal at one dose of serial extract, and monitoring the animal for 24 hours for behavioral effects such as nervousness, ataxia, excitement, alertness, dullness, and death, in which the not dosed remaining rabbits let to next step, then used the other diluted extract dose to another rabbit and monitoring to 24hours.

-experimental animals:

The present study was carried out at the College of Veterinary Medicine, University of Basra, during the period extended three month and after one week of treatment withdrawal. Rabbits were left for two weeks in the animal house for the adaptation to the experimental conditions. A total fifty mature female local rabbits (*Lepus cuniculus*). The animals were housed in separate cages, and were fed an unlimited supply of green leaves, fodder, and water *adlibitum*. Weighted 1500- 2000gm, and aged 8 month, were divided into five equal groups, each of which had ten adult female rabbits for the following:

1. group1(G^{-ve}):received orally1ml/kg normal saline daily for (3)months. 2. Group2(G^{+ve}) (methylprednisolone group):received S/C methylprednisolone of (0.2 mg/kg) for (1)month(22), then received orally 1ml/kg normal saline per day for (2) months 3. Group3 (Meth-Pred +As) : received S/C methylprednisolone of (0.2 mg/kg) for (1)month then received 600 mg/kg *Avena sativa* seed extract orally per day for (2)months 4.Group4 (Meth-Pred+Alend):received S/C methylprednisolone of (0.2mg/kg) for (1)month then treated by alendronate 3.6mg/kg.BW weekly orally for (2)months **5.**Group5 (Meth-Pred+ Alend + As): received S/C methylprednisolone of (0.2mg/kg) for (1)month then treated by alendronate 3.6 mg/kg BW orally weekly and 600 mg/kg *Avena sativa* seed extract orally per day for(2)months. After three months of treatment, half of the rabbits in each group were sacrificed, and the other half were done so after one week of treatment withdrawal.

Dual- energy X-ray absorptiometry (DEXA)

Rabbits were anaesthetized by using mixture of I/M ketamine (35mg/kg) xylazine (5mg/kg) (23). All scans were performed using DXA(Hologic QDR-1000 System, Hologic Inc., Waltham, USA) after 1 month, 2 months, 3 months, and 1 week of withdrawal of treatment. The high- resolution scan was performed to estimate the bone mineral density (BMD) at the femur of female rabbits.

Collection of Blood Samples.

The heart puncture method and a disposable ten-cc syringe were used to collect ten ml of blood from each rabbit after the experiment's

three months and one week of treatment withdrawal. Blood was put into a tube without anticoagulant, and the serum was obtained by centrifuging the tube for 10 minutes at a speed of 3000 rpm. After that, this serum was placed into Eppendorf tubes and stored at (-20°C) until it was employed to study parameters, like, Ca and P according to method of (24) by the use of a special kit(bio System, Spain), Vitamin D according to (25), and Parathyroid hormone was measured according to method of (26) by the use of a special kit (Mono bind Inc. lake forest CA 92630, USA).

Histological examination :

The right femur bone was collected, from each group, decalcified in (EDTA) solution in accordance with (27). Then processed till embedding in paraffin, and were stained with H&E(28).

Statistical analysis:

was carried out by one-way covariance (ANOVA) test, by SPSS program V. 21 (29)

Results and Discussion :

Acute Toxicity Study for *Avena sativa* L Seeds Ethanolic Extract .

The results clearly indicated there was no mortality observed - along the acute toxicity experiment of this extract till a dosage rate of 15000 mg/kg B.W. As well as there were no behavioral and any other signs of poisoning observed. Therefore, *Avena sativa* seeds Ethanolic Extract were considered practically not toxic. In accordance with this doses of 600 mg/kg B.W were selected for experimental study.

Effect of *Avena sativa* L Seeds Ethanolic Extract and Alendronate on Femur Bone Mineral Density (BMD) in Methylprednisolone-induced Osteoporosis in Female Rabbits.

There was a significant decrease ($p \leq 0.05$) in BMD in the control positive group compared with the control (-ve) group, also there was non-significant differences ($p \leq 0.05$) between control (+ve) group and alendronate alone group(MP+ Ale), while there was no significant change ($p \leq 0.05$) was showed in BMD between group treated with *Avena sativa* (MP+As) and control(-ve), but the results showed a significant increase ($P \leq 0.05$) in BMD of MP+ Ale +As groups compared with control(+ve) but it was still significantly less than that of the control (-ve) group after one month from treatment. But after two month, a significant decline ($p \leq 0.05$) in BMD in the control(+ve) group compared with the control (-ve) group and other treated groups, whereas there was no significant change ($p \leq 0.05$) was showed in BMD of female rabbits treated with *Avena sativa* (MP+As) group and control(-ve), on the other hand, the results showed no significant change ($p \leq 0.05$) in BMD of MP+ Ale +As and alendronate alone (MP+ Ale) groups, after one week of treatment withdrawal, the results of BMD revealed a significant decrease ($p \leq 0.05$) in the control (+ve) group compared with the control (-ve) group and another treated groups, whereas a significant increase ($p \leq 0.05$) in BMD of the group treated with *Avena sativa* (MP+As) compared with control(+ve), but the results showed a significant decrease ($P \leq 0.05$) in BMD of MP+ Ale +As and alendronate alone (MP+ Ale)group compared with the control (+ve) group (table 1).

Table 1. Effect of *Avena sativa* L Seeds Ethanolic Extract and Alendronate on Femur BMD (g/cm^3) in Methylprednisolone-induced Osteoporosis in Female Rabbits

Groups	BMD (g/cm^3)		
	One Months	Two Months	Withdrawal
C –ve	0.37± 0.01 a	0.41± 0.03 a	0.41± 0.03 b
C +ve	0.22± 0.02 c	0.18± 0.01 d	0.17± 0.01 e
MP+As	0.36± 0.01 a	0.42± 0.01 a	0.44± 0.07 a
MP+ Ale	0.24± 0.02 c	0.31± 0.02 c	0.29± 0.08 d
MP+ Ale +As	0.25± 0.02 b	0.33± 0.01 bc	0.32± 0.01 c
LSD	0.038	0.025	0.033

Values expressed as Mean ±SD
Different small letters refer to significant differences(P≤0.05) between experimental groups

Effect of *Avena sativa* L Seeds Ethanolic Extract and Alendronate on serum Ca and P in Methylprednisolone-induced Osteoporosis in Female Rabbits.

The obtained results in table (2), after two months and after one week of treatment withdrawal, revealed a significant decrease (p≤ 0.05) in serum Ca in the control (+ve) group compared with the control (-ve) group, while there was no significant differences (p≤0.05) was observed in serum Ca between *Avena sativa* (MP+As) group and control (-ve) group, so there were no significant changes(p≤0.05) between control(+ve) group, alendronate alone (MP+ Ale), and MP+ Ale +As groups after two month but, after one week of treatment withdrawal a significant increase (p≤ 0.05) in serum Ca in the MP+ Ale +As group. The results of phosphorus after two month, showed a significant low (p≤ 0.05) in the control(+ve) group compared with the control (-ve) group, but the results of P showed no significant differences (p≤0.05) between control(+ve) group, alendronate alone(MP+ Ale), and MP+ Ale +As group, but the results of P showed a significant increase (P≤0.05) in a *Avena sativa* (MP+As) compared with the control (+ve) group. After one week of treatment withdrawal, there was no significant change (p>0.05) shown in serum P in a *Avena sativa* (MP+As) group and control(-ve) group, also there was no significant change (p≤0.05) between control(+ve) group, alendronate alone (MP+ Ale), but the results showed a significant increase (P≤0.05) of MP+ Ale +As groups compared with control(+ve).

Table 2. Effect of *Avena sativa* L Seeds Ethanolic Extract and Alendronate on serum Ca and P in Methylprednisolone-induced Osteoporosis in Female Rabbits.

Groups	Two Month Ca mg/dl	Withdrawal Ca mg/dl	Two Month P mg/dl	Withdrawal P mg/dl
C –ve	14.28± 0.49 a	14.32±0.47 a	7.95± 1.15 a	7.96± 1.13 a
C +ve	10.18± 0.24 d	9.62±0.11 c	4.33± 0.58 c	4.11± 0.65 c
MP+As	14.42± 1.42 a	14.62±0.76 a	6.64± 0.97 b	8.02± 0.67 a
MP+ Ale	11.53±1.69 cd	9.98±0.59 c	4.70± 1.46 c	4.18± 1.37 c
MP+Ale +As	12.94±0.56 bd	13.20±0.14 b	5.14± 0.36 c	5.16± 0.35 b
LSD	2.29	1.85	2.47	1.89

Values expressed as Mean ±SD
Different small letters refer to significant differences(P≤0.05) between experimental groups

Effect of *Avena sativa* L Seeds Ethanolic Extract and Alendronate on Vit. D and PTH in Methylprednisolone-induced Osteoporosis in Female Rabbits.

In the table (3) methylprednisolone caused a significant decline (p< 0.05) in Vit. D after two months of treatment and after one week of treatment withdrawal in the control(+ve) group compared with the control(-ve) group, also showed no significant differences (p>0.05) between the control(+ve) group, and alendronate alone(MP+ Ale) group, while

showed no significant change (p>0.05) in serum Vit. D in a *Avena sativa* (MP+As) and control (-ve) groups compared with other groups, there was significant increase (p≤0.05) shown in serum Vit. D of MP+ Ale +As group when compared to the control (+ve) group after two month but decline after one week of treatment withdrawal. The results of PTH after two months of treatment, and after one week of treatment withdrawal, showed a significant increase (p≤0.05) in the control (+ve) group compared with the control (-ve) group and another treated groups, but showed no significant differences (p≤0.05) between *Avena sativa* (MP+As) group and control(-ve), so no significant differences (p≤0.05) was shown in serum PTH between alendronate alone(MP+ Ale), and MP+ Ale +As group.

Table 3. Effect of *Avena sativa L* Seeds Ethanolic Extract and Alendronate on Vit. D and PTH in in Methylprednisolone-induced Osteoporosis in Female Rabbits.

Groups	Two Month Vit. D ng/ml	Withdrawal Vit. D ng/ml	Two Month PTH Pg/ml	Withdrawal PTH Pg/ml
C –ve	27.80±0.07 a	28.20±0.21 a	23.40±0.07 c	23.36±0.05 c
C +ve	15.16±0.11 c	15.00±0.12 c	55.18±0.15 a	56.28±1.22 a
MP+As	25.68±2.03 a	26.54±1.75 a	26.24±0.11 c	26.22±0.14 c
MP+ Ale	17.20±0.55 c	16.12±0.22 c	43.91±16.13 b	45.80±16.53 b
MP+ Ale +As	22.04±1.81 b	19.70±3.43 b	38.24±6.32 b	38.00±9.06 b
LSD	9.77	5.29	19.09	19.35

Values expressed as Mean ±SD

Different small letters refer to significant differences(P≤0.05) between experimental groups

Histopathological Changes

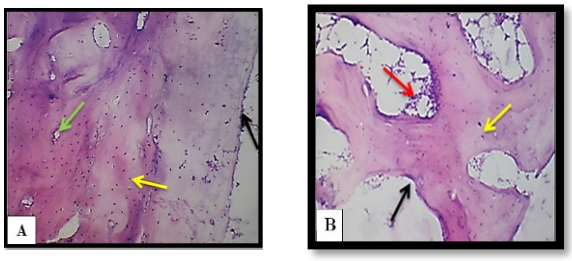

Figure (1)Histological section of femur bone tissue from a control (-ve), showed the normal micro-architecture of the bone, A. showing the cortical bone, normal shape, osteocytes inside their lacunae(yellow arrow), Haversian canals are observed (green arrow), the endosteum is lined by osteoblast cells (black arrows).B: trabeculae bone consist of osteocytes cells inside the lacunae, with large empty cavity between the mature trabecular bone formation

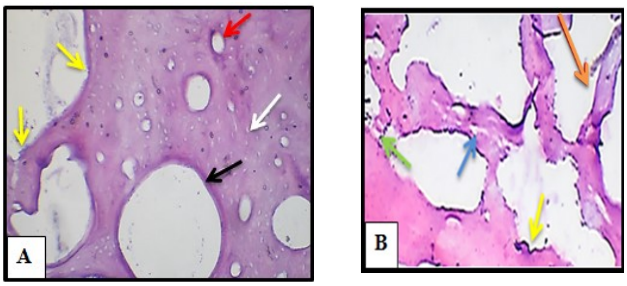

Figure (2) Section of femur bone tissue from a control (+ve), A: showing the cortical bone, widening in the Haversian canal(red arrow), large cavities (black arrow), erosion cavity containing osteoclasts (yellow arrow), and lacunae appear without osteocytes (white arrow) B: trabeculae bone, characterized by thin wall (orange arrow), bone specula's not anastomosis with others, and have bland end (green arrow), and apparent thinned out trabeculae (blue arrow) (H & E,10X).

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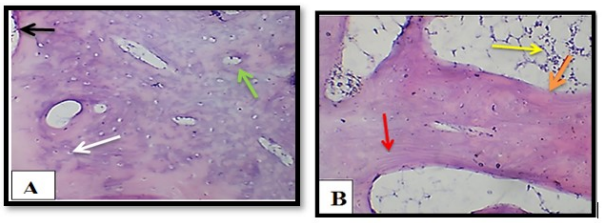

Figure (3) Section of femur bone tissue from a *Avena sativa*
seeds ethanolic extract treated(MP+As)group, A: showing the cortical bone revealed the marked normal Haversian canals (green arrow), numerous mature osteoblasts (black arrow), well differentiated osteocytes in their lacunae (white arrow) B: trabeculae bone, consist lamellae indicates formation of compact bone(red arrow), proliferation of mature osteocytes, formation of red bone marrow (yellow arrow), showing branching and anastomosing trabeculae of normal thickness and shape (orange arrow) (H & E,10X).

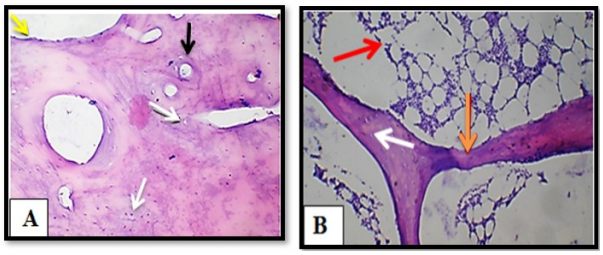

Figure (4) Section of femur bone tissue from a Alendronate treated group(MP+ Ale), A: cortical bone, some osteocytes inside their apparently large lacunae, while other lacunae appear without osteocytes (white arrow), and Haversian canals (black arrow), and non-differentiated osteoblasts (yellow arrow). B: trabeculae bone formation characterized by thin wall (orange arrow), immature osteocytes and large empty space that filled with bone marrow (red arrow) (H & E,10X).

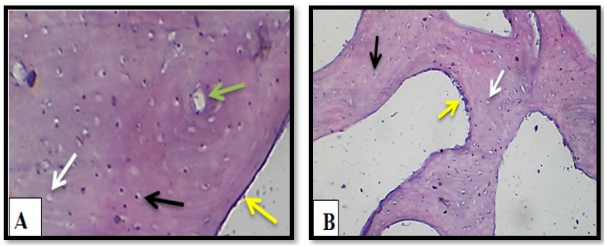

Figure (5) Section of femur bone tissue from a MP+ Ale +As treated group, A: cortical bone showed the some lacunae appear without osteocytes (white arrow), and presence of mature osteocytes (black arrow), remarkable multinucleated osteoclasts (yellow arrow), and large size Haversian canal (green arrow). B: trabeculae bone, increase in trabecular area compared with control (+ve) group (H & E,10X).

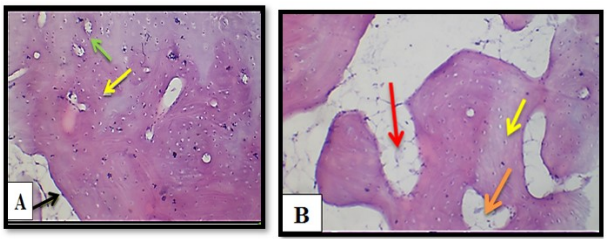

Figure (6) Section of femur bone tissue from a *Avena sativa* seeds ethanolic extract treated (MP+As) group after one week withdrawal, A: cortical bone, showed numerous mature osteocytes (yellow arrow) in their lacunae, normal Haversian canals (green arrow), and showed osteoblasts (black arrow). B: trabeculae bone, consist of osteocytes cells inside the lacunae, with large empty cavity between the mature trabecular bone formation filled with bone marrow(red arrow), many trabeculae bone formation well developed with compromised space (orange arrow) (H & E,10X).

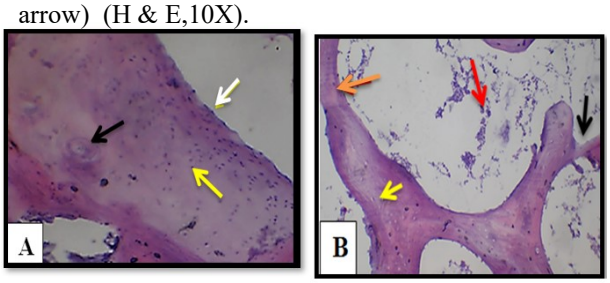

Figure (8) Section of femur bone tissue from a MP+ Ale + As treated group after one week withdrawal, A: cortical bone, massive proliferation of immature osteocytes (yellow arrow), presence of osteoblasts(white arrow) and large size Haversian canal(black arrow). B: trabeculae bone formation characterized by thin wall (orange arrow), bone specula's not anastomosis with others and have bland end (black arrow), with immature osteocytes, and large empty space that filled with bone marrow (red arrow) (H & E,10X)

There is no information available on the ethanolic extract of *Avena sativa* seeds' effects on osteoporosis. This is the first investigation into oat seeds' potential to prevent osteoporosis caused by glucocorticoids. BMD has been demonstrated to be significantly decreased with osteoporosis, making it a valuable clinical indication of changes in bone quality(30). In the current study, femur BMD might be given a more precise image of BMD. Inhibition of bone formation is the main consequence of glucocorticoids in bone (31). This is because of

a decline in the number of osteoblasts, and their function(32). The decline in cell number is secondary to a low in osteoblastic cell replication, and differentiation, and an increase in the apoptosis of mature osteoblasts (33). These results are agreement with Sobhani *et al*.; (34) who showed that the treatment with glucocorticoids induced a decline in the total body bone mineral content, and low in BMD. Our results support previous studies that indicated that loss of bone formation was the major cause of the reduction of bone mineral density (35, 36). The findings of the current study, a benefit that the *Avena sativa* extract may have by inhibiting the development of osteoclasts differentiation, and could modulate osteoclastogenesis by altering the expression of RANKL, and OPG. The β-glucan rich oat seeds were able to inhibit declines in BMD, through the promotion of osteoblast differentiation, bone formation, and the suppression of bone resorption, and osteoclast activity, so it able to preserve bone mass and strength, and to raise the rate of bone formation (37,38). Reduced blood calcium and phosphorus levels in the control positive group in this study showed clear evidence of osteoporotic alterations, bone contains an abundance of calcium, which is essential for maintaining bone mineral density. Glucocorticoid therapy led to a significant decrease in calcium levels. This could be due to the fact that glucocorticoids promote the excretion of calcium through the urine, impede the reabsorption of Ca in the renal tubules, which results in hypocalcemia, and decline the absorption of Ca through the intestines (39,40). The group that received methylprednisolone showed significant changes in serum phosphorus levels as well. Phosphorus levels may have decreased as a result of increased renal excretion and changes to their transport across the brush border membrane (41). Similar findings were described by Hozayen *et al*.; (42) They found that GCs significantly reduced the levels of Ca and P in the serum. Treatment with produced an appreciable raise in the levels of Ca and P. The high calcium and phosphorus content of *avena sativa* seeds may be a contribute the elevated serum Ca and P levels (43). This result demonstrates the ability of oat seeds can improve intestinal Ca absorption. An effect opposing the effect of GCs that rise Ca urinary excretion is by reducing its intestinal absorption and conversion to bone (44). Administration of glucocorticoids may reduce intestinal absorption of calcium and increase its excretion (45), hyperparathyroidism which may cause calcium alteration associated with a compensatory increase in PTH resulting in calcium release from the skeleton thus causing bone loss (46). The high calcium and phosphorous content of oat seeds, which has been found to have beneficial effects on bone and restored the lowered levels of bone Ca and P to normal values, is most likely the cause of the seeds' positive effect on bone density (47, 48). Additionally, it has been shown that the saponin in oat seeds has an anabolic impact on bone metabolism by promoting osteoblast proliferation (49). Glucocorticoids influence vitamin D metabolism, which lowers the production of active vitamin D (1, 25 dihydroxycholecalciferol), and reduces its biological activity in tissues. Glucocorticoids may directly contradict the peripheral effects of vitamin D by reducing intestinal calcium absorption and promoting renal calcium excretion, leading to a negative calcium balance (50,51).The most common source of ω -6 fatty acids is linoleic acid (LA), which is present in high concentrations in oats, that essential fatty acids improve calcium absorption, enhance the effects of vitamin D, decline urinary calcium excretion, rise bone calcium and bone strength (52). An important systemic regulator of the metabolism of calcium and phosphate is parathyroid hormone (53). In order to restore normal plasma calcium levels, high levels of PTH transfer bone calcium into the blood, which contributes to the development of bone

loss. As a result, higher levels of PTH are associated with increased bone resorption rate (54),that is confirmed with what we did find in our study after glucocorticoid administration. It is possible for blood levels of parathyroid hormone to rise as a result of glucocorticoids' ability to reduce gastrointestinal calcium absorption and increase renal calcium excretion (55). One of the main causes of osteoporosis is an excessive level of PTH. Secondary hyperparathyroidism is typically caused by glucocorticoids' negative calcium balance (56). After administering an ethanolic extract of *avena sativa* seeds to rabbits treated with methylprednisolone, the level of PTH was significantly lowered. This may be due to the polyphenol compounds found in oat seeds, which are known to have beneficial effects on bone health and may be able to reduce elevated PTH levels (57). Additionally, oligosaccharides from oat seeds, such as β-glucan, have been shown to suppress PTH and lower the rise in the rate of bone turnover, due to their capacity to diminish osteoclastic activity, which in turn lowers the rate of bone resorption (58).

The histopathological findings of the femoral bone of the control (+ve) group showed damage of architecture, These results were in consistent with earlier result of (59,60). GCs produced osteoporosis results from variation in bone turnover. It increases resorption by prolong the lifespan of osteoclasts and lowers formation by inducing apoptosis in osteoblasts and osteocytes, which explains why some empty lacunae are present (61). When treatment with *Avena sativa* seeds ethanolic extract, prevent of the side effects of methylprednisolone. Oat seeds contain an potent antioxidant known as avenanthramides (Avns), which has been shown to increase the activity of the enzymes superoxide dismutase (SOD) in skeletal muscle, liver, and kidneys, Additionally, glutathione peroxidase activity in the heart and skeletal muscles has been shown to be enhanced(62), reduced the generation of reactive oxygen species(63). Additionally, the ethanolic extract of *Avena sativa* seeds is rich in copper, an essential co-factor of the lysyl oxidase enzyme, which is involved in the cross-linking of the extracellular matrix proteins, collagen, and elastin, and is obviously necessary for maintaining bone integrity. This demonstrates the oat seeds' anabolic effects on bone(57).

Conclusions

Conclude from this study that the ethanolic extract of *Avena Sativa* seeds has an effective therapeutic agent as anti-osteoporosis by increasing bone mineral density and they were superior to alendronate.

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Conflict of interest

The authors declare no conflict of interest.

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