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Therapeutic Effect of *Equisetum arvense* L. on Bone and Scale Biomarkers in Female Rats with Induced Osteoporosis

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Abstract

Equisetum arvense L. (common horsetail) has medicinal characteristics that have been known since ancient times and are used to cure a variety of ailments. The effect of *E. arvense* L. on rats with induced osteoporosis was investigated. Forty female rats were randomized in 5 equal groups (n=8). The first group was working as tricks (SHAM) (negative control), while the other 4 groups were operating with ovariectomized (OVX) and fed on basal diet deficient in calcium and were given orally prednisone acetate (4.5 ml/kg body weight/day twice a week) for two weeks as source of glucocorticoid, to induce osteoporotic models. Three weeks after ovarian removal, Group 2 kept as positive control (OVX) and the other three groups were fed on basal diet supplemented with 5, 10 and 15% of dried *E. arvense* L, respectively for 8 weeks. The results showed that rats fed on dried *E. arvense* L. at all tested levels had significant increase (P<0.05) in serum of calcium (Ca), phosphorus (P), bone alkaline phosphates (b-ALP), free thyroxin (T4), calcitonin (CT) and superoxide dismutase (SOD) while significant reduction (P<0.05) in parathyroid hormone (PTH) and malondialdehyde (MDA)was detected. In addition, significant increase (P<0.05) in bone mineral density (BMD), bone mineral content (BMC), Ca and P contents in Femur ash were recorded in cured groups as compared to the positive control group. Our findings exhibit that *E. arvense* L. had a potent anti-osteoporotic effect in ovariectomized rats due to enhanced bone building, delayed bone loss and normalization of hormones levels that regulate calcium metabolism. Thus *E. arvense* L. might represent as a natural therapy for the avoidance of osteoporosis in women at postmenopausal period.

Key words: Equisetum arvense L.; Osteoporosis; Serum samples; femur bones marks; Thyroxin; Calcitonin; Parathyroid hormone; Ovariectomized; Rats

1. Introduction

Osteoporosis is a worldwide condition that affects both men and women, and it is becoming more prevalent as the world's population becomes older [1]. Osteoporosis is characterized by bone loss and structural degeneration of bone tissue, resulting in increased bone fragility and fracture risk, most commonly in the hip, wrist, and spine. The achievement of highest bone mass will continue until late adolescence or the beginning of adulthood [2], depends on hormone and growth factor interactions, heredity, physical exercise, and nutrition, especially Ca and vitamin D [3]. Age, menopause in women, smoking, Ca and vitamin D deficiency, low weight and muscle mass, anticonvulsants, and corticosteroids are all variables that contribute to bone loss [4]. After menopause, ageing causes a deficit in estrogen hormone, which causes an increase in bone resorption by osteoclast genesis and is the most common cause of osteoporosis in older women [1]. Women have a 30-50 % lifetime risk of osteoporotic fractures, while men have a 15-30% lifetime risk. Preventive interventions often include physical activity, dietary supplements and medication [5].

There is a growing desire for "green medicine," healthier and safer option with less or no side effects and do not result in substantial increase in bone mass as compared to synthetic medications [6]. *Equisetum arvense* L. (common horsetail) has medicinal characteristics that have been known since ancient times and are used to cure a variety of ailments. The powder form aerial part of *Equisetum arvense* L. is

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eaten alone or with milk or other hot beverages [7]. It is an excellent source of biologically active substances and nutrients, such as vitamins (C, E, B1, B2, and B6) and minerals (K, Ca, P, Fe, and Zn) [8]. Some oriental doctors still recommend *Equisetum arvense* L. to help strengthen bones and prevent the onset of osteoporosis [9], attribute this to the plant's silica content, which accounts for 25% of the dry weight [10].

Silica aided in the structure of bone and connective tissue by enabling Ca and other mineral deposition, declining the number of osteoclast cells, motivating osteoblast activity, stimulating collagen synthesis, and facilitating glycosaminoglycan and collagen production [11]. Horsetail has been shown in a few European clinical studies to speed up the healing of shattered bones. When horsetail is introduced to the diet, the risk of osteoporosis decreases significantly, due to its contents of silicon, which stimulates skeletal structure growth and stability [12]. The purpose of this study was to shed light on *Equisetum arvense* L. supplementation on both serum and bone markers of osteoporotic ovariectomized female rats.

2. Materials and methods

The chemical analysis and biological experimental were carried out at the Laboratories of the Regional Center for Food and Feed, Agricultural Research Center, Giza-Cairo.

2.1. Materials:

Equisetum arvense L. (common horsetail) was obtained from the Agriculture Research Centre. Casein, Vitamins, Minerals, L-cystine, starch and Cellulose were obtained from Morgan Company for Chemicals, Cairo, Egypt. Sucrose and oil were bought from the local market. Kits for serum analysis was obtained from Alkan Company for Bio diagnostic Reagents, Dokki, Cairo, Egypt. Forty adult female albino rats (200±5 g b.wt with 10 weeks old) were obtained from Helwan Farm, Ministry of Health and Population, Cairo, Egypt.

2.2. Methods:

Identification for Kingdom Plantae Down to Species *Equisetum arvense* L. was conducted at the Agriculture Research Center.

Rank	**Scientific Name**
Kingdom	***Plantae*** **– Plants**
Subkingdom	***Tracheobionta***
Division	***Equisetophyta***
Class	***Equisetopsida***
Order	***Equisetales***
Family	***Equisetaceae***
Genus	***Equisetum*** **L.**
Species	***Equisetum arvense*** **L.**

2.2.1. Dried *Equisetum arvense* L. preparation:

E. arvense L. was collected in May 2021, thoroughly washed in running water, dried at 40°C for 6 hours, then minced to powder and stored in refrigerated multilayer paper bags.

2.2.2. Analytical Methods:

Dried *E. arvense* L. was analyzed by standard methods for moisture, protein, fat, ash and crude fiber according to [13]. Total carbohydrate was calculated by difference. Gross energy was calculated by using the factors as described by [14] according to the following equation:

Gross energy =

$$4 \times (\text{Protein \%} + \text{Carb. \%}) + 9 \times (\text{Fat \%}).$$

2.2.3. Mineral analysis:

Total phosphorus was determined according to Taussky and Shorr method [15] potassium (K) and Ca were determined using an atomic absorption spectrophotometer (Perkin–Elmer Instrument model 2380) [16].

2.2.4. Silica Estimation:

Silica was measured in the sample's acid insoluble ash (wet ashing) by AOAC method [17]

2.2.5. Determination of Antioxidant content:

The phosphomolybdenum assay was adopted to estimate the antioxidant content [18], and Folin–Ciocalteu assay were adopted to estimate the total phenols content [19].

2.2.6. Procedure for ovariectomy in female rats:

The bilateral ovariectomy in rats was conducted under ether an aesthesia using sharp dissecting scissors to make two dorsolateral incisions. The peritoneal cavity was then reached after cutting the skin and dorsal muscles. The uterine horn was removed, as well as the fatty tissue that around the ovary. A cut was performed under the clamped area to remove the ovary. The connection between the Fallopian tube and the uterine horn was pinched with artery forceps. A single catgut suture was used to seal the skin bilaterally. Both areas of the procedure were treated with a tincture iodine solution (antiseptic) [20]. The ovaries were similarly exposed but not removed in a sham (SHAM) procedure.

2.2.7. Biological study:

Forty adult female rats housed with 12-h light–dark cycles at constant room temperature (24 °C) and 20 % humidity and fed a standard diet ad libitum for one week. The basal diet was consisted of 14% protein (casein), 4% corn oil, 0.25% choline bitartrate, 1% vitamin mixture, 3.5% mineral mixture, 10% sucrose, 5% cellulose, 0.18% L-cystine and the

remainder was starch. The diet prepared according to Reeves et al., method [21]. After this week rats randomly divided into five groups. Frist group (SHAM) (n=8) kept as negative control group and fed on basal diet only, the other rats, (n=32) were ovariectomized and left for 3 weeks post-operation to settle osteoporosis induction as the same time these rats were fed on basal diet deficient in calcium, in addition to given orally prednisone acetate (4.5 ml/kg body weight/day twice a week) for two weeks as source of glucocorticoid, to establish osteoporotic models [22]. One group of them was served as a positive control group, the other three groups were fed on basal diet supplemented with dried *E. arvense* L. at the level of (5, 10 and 15%), respectively.

At the end of the experiment (8 weeks), rats were fasted for 12 hours, and blood samples were obtained from medial canthus of the eyes of rats by means of fine capillary glass tubes in a centrifuge tube without any anticoagulant and centrifuged for 20 minutes at 3000 r.p.m. to obtain serum. The femurs excised and wrapped in gauze moisturized with 0.9% NaCl solution and kept below -20°C until the bone marker determination [23].

2.2.8. Biological Evaluations:

The amounts of feed consumed and/or wasted, were recorded every day and the total feed intake (FI) was calculated. In addition, body weight (BW) of rat's was recorded weekly. Body weight gain percentage (BWG%) and feed efficiency ratio (FER) were calculated [24] by using the next equation:

$$\text{BWG\%} = \frac{\text{Final body weight (FBW)} - \text{Initial body weight(IBW)}}{\text{Initial body weight}} \times 100$$

$$\text{FER} = \frac{\text{Body weight Gain(BWG) (g)/day}}{\text{Feed intake(FI) (g)/day}}$$

2.2.9. Chemical analysis:

Serum levels of Ca and P were determined depending on [25, 26], respectively. Additionally, serum CT and PTH were estimated through enzyme linked immune sorbent assay (ELISA), by [27]. Free thyroxine (T4) concentration was determined using radioimmunoassay (RIA) method of [28]. Oxidative stress markers: SOD and MDA were determined by these methods [29,30] respectively. Serum bone-specific alkaline phosphate [31] was estimated by colorimetric assay using specific enzyme kits (Sigma-Aldrich Chemical Co., USA).

2.2.10. *Bone minerals content (BMC) and bone* minerals density (BMD) measurements:

The BMC and BMD of femur using dual energy X-ray absorptiometry (DEXA; National Research Center - Dokki-Giza) and Lunar PIXImus 2 2.0 software was carried out according to [32]. The femur weighed after removal of the soft tissues around it. To obtain the ash, drying the femur bone overnight at 100°C, followed by incineration at 1000° C in muffle apparatus, was conducted. The remaining ash weighed, solubilized with 0.1Mol/L HCl, transferred into volumetric flask and completed to 100 ml with 0.1Mol/L HCl according to [33]. The final solution used for estimation of Ca [25] and P [34] in the ash using colorimetric methods.

2.2.11. Statistical analysis:

Results were presented as mean± standard Error (SE). Data were analyzed statistically by SPSS program, one-way ANOVA followed by post hoc multiple were used to make a comparison among different groups [35].

3. Results and Discussion

Table 1 : Chemical composition of *E. arvense L.*

Nutrients		*E. arvense* L.
MACRONUTRIENTS		
Moisture		**9.63 ± 0.07**
Protein	**g/ 100g dried**	**15.33 ± 0.04**
Fat		**2.35 ± 0.05**
Ash		**12.17 ± 0.04**
Crude fiber		**12.92 ± 0.03**
Carbohydrates		**47.61 ± 0.01**
Gross energy		**272.91±0.1**
MICRONUTRIENTS		
Silica	**g/ 100g dried**	**8.69±0.54**
K		**2.79 ± 0.12**
P		**0.19 ± 0.01**
Ca		**1.50 ± 0.02**
ANTIOXIDANT CONSTITUENTS		
Total antioxidants (mg/ 100 g ascorbic acid)		**1991.66 ± 12.25**
Total phenols (mg/ 100 g gallic acid)		**192.65± 8.96**

Values are expressed as Means ± SE.
K: Potassium, P: Phosphorus, Ca: Calcium

The proximate gross chemical composition of *E. arvense* L. is shown in Table (1). The major macronutrients component in *E. arvense* L. are carbohydrates followed by protein. Silica and potassium had the highest content of minerals followed by calcium in *E. arvense.* The total antioxidants content of *E. arvense* L. amounted to 1991.66 mg/ 100 g ascorbic acid, where the total phenolic constituents amounted to 192.65 mg/ 100 g gallic acid.

The above results are in accordance with [36]. The plant contains abundant minerals as, Ca, P, iron, manganese and silica [37,38]. In this study silica content in *E. arvense* L. was found to be 8.69%. Comparable results are obtained by [39] who mentioned that *E. arvense* L. contained silicic acid and silicates (5-8%), Ca (1.3%), K (1.8%), sodium,

zinc and magnesium. Serum Ca and P are ordinarily utilized as biochemical markers of bone development and building. Moreover, Ca, vitamin D and PTH are basic controllers of bone reforming [40].

E. arvense L. contains a variety of phenolic compounds [41], that have been connected to constructive outcomes on bone metabolism and preservation of bone health [42, 43]. Moreover, [44] reported that silica enhances the bone mineralization by decreasing excretion of calcium. Apigenin has also been reported to influence bone cell behavior [45]. Luteolin diminishes bone resorption [46]. Oleanolic acid has found to apply an osteoprotective controls in ovariectomy rats by stimulating differentiation of osteoblasts [47].

49]. It turns out also, that obesity may not confer greater protection against fracture in those people [50]. Also, horsetail is a popular weight loss supplement in Latin America, because Horsetail acts as a diuretic and reduces fat in rats as well as increases IL-10, which may boost weight loss [3, 51,52, 53]. Moreover, [54] reported that rats fed on diet supplemented with (5 or 10%) *E. arvense* L. had significant decline (P<0.05) in FBW as compared to both control groups.

Results in Table (3) showed the concentrations of serum Ca, P and b-ALP in the OVX rats (+ve control group) were significantly lowered (P<0.05) as matched to (SHAM) rats by -47.64, -48.61 and -20.13 respectively. Feeding rats on basal diet supplemented

Table 2. Effect of *E. arvense* L. on body weight status of osteoporotic female rats.

Groups / parameters		IBW(g)	FBW(g)	BWG%	FI	FER
Control (-ve) (SHAM)		**200±5.77[a]**	**240±5.77[b]**	**20.03±0.57[b]**	**18±0.56[b]**	**1.11±0.00[a]**
Control (+ve) (OVX)		**202±3.05[a]**	**257±4.04[a]**	**27.23±1.38[a]**	**22±0.57[a]**	**1.23±0.07[a]**
OVX + *E. arvense L.*	**(5%)**	**199±2.08[a]**	**224±1.00[c]**	**12.57±0.71[c]**	**17±1.0[bc]**	**0.74±0.07[b]**
	(10%)	**204±2.51[a]**	**206±1.52[d]**	**0.99±0.86[d]**	**15±0.56[cd]**	**0.07±0.06[c]**
	(15%)	**205±3.60[a]**	**195±2.96[d]**	**-4.54±0.25[e]**	**14±0.58[d]**	**-0.32±0.02[d]**

Values are expressed as Means ± SE.
Values at the same column with different letters are significant at P<0.05.

There were no significant changes in the initial body weight among all rats. It was observed that the positive group had significant (P<0.050 increase in the FBW as compared to the healthy rats. Rats fed on diet supplemented with *E. arvense* L. at different levels had significant weight reduction (P<0.05) as matched to the +ve control group. There was significant variation (P<0.05) in the FBW among the treated groups fed on 5 and 10% of *E. arvense,* while no significant change in FBW between the groups fed on either 10 or 15% of *E. arvense*.

In regarding to BWG% and FER, groups supplemented with *E. arvense* L. at any levels had meaningful decrease (P<0.05) in BWG% and FER compared to both control groups. Moreover, there were significant differences (P<0.05) in BWG% and FER among the groups fed on (5, 10 and 15 %) of *E. arvense*. However, there was no statistical change in FI between the groups fed on 5 and 10% of *E. arvense.* The same trend was observed between the groups fed on 10 and 15% of *E. arvense*. In general, we found that the highest weight reduction was observed at the group fed on 15% of *E. arvense*.

Several studies demonstrated high danger of break and osteoporosis in individuals with high BMI [48,

with *E. arvense* L. at different levels caused a significant growth (P<0.05) in serum Ca, P and b-ALP as compared to the positive control group. Moreover, there was no significant variation in Ca content between the groups fed on 5 and 10% of *E. arvense* L.. Also, between the groups fed on 10 and 15% of *E. arvense*. Regarding, to P and b-ALP content, it was clear that, there was significant difference (P<0.05) in serum P and b-ALP among the three tested levels of *E. arvense*. The mean value of serum Ca was increased by 48.53, 66.71 and 84.28%, additionally, the mean value of P was increased by 29.31, 54.09 and 83.62%, furthermore, the mean value of b-ALP was increased by 5.1, 13.9 and 21.1% for the groups fed on 5, 10, 15 % of *E. arvense,* respectively. The highest increase in serum Ca, P and b-ALP was observed at the group fed on 15% of (*E. arvense*).

The obtained results are harmony with those announced by [27, 55, 56] who's reported that *Equisetum's* silica content supports the absorption and utilization of Ca and synthesis and stabilization of collagen by prolyl hydroxylase enzyme. Feeding rats on *E. arvense* L. caused a significant increase (P<0.05) in serum calcium and phosphorus as assessed to osteoporosis rats [54].

Moreover, another study showed that *E. arvense* L. may prove beneficial in treatment of osteoporosis by boosting bone formation activity [57].

Table 3.: Effect of *E. arvense* L. on some markers of bone development on osteoporotic female rats.

Parameters / Groups		Ca (mg/dl)	% of increase	P (mg/dl)	% of increase	b-ALP (IU/L)	% of increase
Control (-ve) (SHAM)		12.39±0.73[a]	-	9.04±0.36[a]	-	136.60±0.39[a]	-
Control (+ve) (OVX)		6.49±0.37[d]	-47.61	4.64±0.22[d]	-48.67	109.10±1.17[e]	-20.13
OVX + E.. arvense L.	(5%)	9.64±0.34[c]	48.53	6.00±0.06[c]	29.31	114.72±0.99[d]	5.1
	(10%)	10.82±0.27[bc]	66.71	7.15±0.21[b]	54.09	124.28±0.98[c]	13.9
	(15%)	11.96±0.26[ab]	84.28	8.52±0.42[a]	83.62	132.10±0.86[b]	21.1

Ca: calcium, P: phosphorus, b-ALP: Bon specific alkaline phosphatase.
Values are expressed as Means ± SE., Values at the same column with different letters are significant at P<0.05.

It has an important role in normalization of bone mineral state, bone calcification and formation of collagen, thus decreasing risk of bone fractures and increasing bone healing rate [38]. *E. arvense* L. have remarkably high silica content in the whole plant kingdom that helps in the utilization of Ca and stabilization of collagen by prolyl hydroxylase enzyme [10]. Also, silicon from *E. arvense* L. in the diet can rebuild and enhance the activity of these cells, developing any pathological circumstances [37, 58].

Table 4. Effects of *E. arvense* L. on serum CT, T4 and PTH of osteoporotic female rats.

Parameter / Groups		CT (pg/ml)	T4 (µg/dL)	PTH pg/ml
Control (-ve) (SHAM)		10.46±1.33[a]	6.86±0.28[a]	1.51±0.56[e]
Control (+ve) (OVX)		4.30±0.66[e]	2.90±0.05[d]	2.91±0.39[a]
OVX + *E. arvense* L.	5%	6.70±0.72[d]	4.13±0.08[c]	2.60±0.37[b]
	10%	8.23±0.33[c]	4.93±0.20[b]	2.01±0.76[c]
	15%	9.30±0.40[b]	5.53±0.26[b]	1.71±0.86[d]

CT: Calcitonin, T4: free thyroxine and PTH: parathyroid hormone
Values were expressed as Means ± SE., Values at the same column with different letters are significant at P<0.05.

Regarding to the effects of *E. arvense* L. on serum CT, T4 and PTH as presented in Table (4), the obtained results revealed a significant increase (P<0.05) in the concentration of CT and T4 due to *E. arvense* L. supplementation at the three distinct levels. On the other hand, PTH level was significantly (P < 0.05) lowered following *E. arvense* L. treatment at the three tested doses as compared to the +ve control group. Moreover, there was a significant variation in the level of CT and PTH among the three treated groups., while there was no change in the mean value of serum T4 between the groups fed on 10 or 15% of *E. arvense*. The best results of serum CT, T4 and PTH are recorded at the rats treated with 15% of *E. arvense*.

Several studies found a clear relationship between PTH and fat mass fat mass [59, 60]. The current results denoted that supplementation with *E. arvense* L. significantly improved serum T4 and reduced PTH. These results were consistent with this study which found the higher serum PTH levels in individuals with high FBW can have negative effects on cortical bone [61].

Table 5. Effects of *E. arvense* L. on serum MDA and SOD of osteoporotic female rats.

parameters / Groups		MDA µmol/L	%of reduction	SOD (U/ml)	%of increment
Control (-ve) (SHAM)		8.98±0.32[e]	-	85.93±0.16[a]	-
Control (+ve) (OVX)		34.33±0.56[a]	-	47.59±0.72[e]	-
OVX+ *E. arvense* L	5%	28.34±0.33[b]	17.44	55.97±1.00[d]	17.60
	10%	18.85±0.26[c]	45.09	64.26±0.97[c]	35.02
	15%	12.26±0.14[d]	64.28	76.76±1.43[b]	61.29

MDA: Malondialdehyde, SOD: Superoxide Dismutase
Values are expressed as Means ± SE., Values at the same column with different letters are significant at P<0.05.

Results in Table (5) demonstrated that the positive control group had considerable increase (P<0.05) in the mean value of serum MDA but had a significant decline in the level of SOD compared to the -ve control group. The supplement with *E. arvense* L. at 5,10,15% significantly lowered (P<0.05) the mean level of serum MDA by 17.44, 45.09, 64.28% respectively and significantly enhance serum SOD by 17.60, 35.02, 61.29% respectively as compared to the +ve control group. There were significant variations (P<0.05) in serum MDA and SOD among the treated groups with different levels of *E. arvense*. Additionally, the most improvement for the concentrations of MDA and SOD were recorded at the group supplemented with15% *E. arvense* L. by 61.29 and 64.28% respectively.

E. arvense L. has good antioxidant capacity, which can reduce different types of radicals [62]. Also, [63, 64] reported that the level of SOD, CAT, and reduced glutathione were improved, and tissue MDA were dropped significantly in the *L. sativum* (200 and 400 mg/kg) treated groups.

Data in Table (7) regarded that femur ash weight, bone Ca and P content of OVX group (+ve control group) were significantly decreased (P<0.05), compared to the (SHAM) rats. Feeding rats on basal diet supplemented with *E. arvense* L. at 5, 10 and 15% produced a significant increase (P<0.05) in femur ash weight by 21.83, 26.43, 45.97% respectively, bone Ca by 16.11, 30.80, 37.53% respectively and P content by 22.65, 72.26, 116.40 % respectively as compared to the +ve control rats. Moreover, there was no significant diversity in femur ash weight between groups fed on *E. arvense* L. at 5 and 10%, while bone Ca content did not change between the groups fed on 10 and 15% of *E. arvense.* Concerning, to bone P content, it was observed that there was a significant difference (P<0.05) among the three tested levels of *E. arvense.* Feeding *E. arvense* L. at 15% caused the highest increase in bone ca and P content as matched to other treated groups.

Table 6. Effect of *E. arvense* L. on BMC and BMD in femur bone of osteoporotic female rats.

Groups / Parameters		Femur weight (g)	% of increase	(BMC)* (g/cm2)	% of increase	(BMD)* (g/cm2)	% of increase
Control (-ve) (SHAM)		**2.73±0.21ᵃ**	-	**1.32±0.05ᵃ**	-	**0.210±0.01ᵃ**	-
Control (+ve) (OVX)		**1.23±0.07ᵈ**	-	**0.35±0.03ᵉ**	-	**0.053±0.01ᵉ**	-
***OVX* + *E. arvense* L**	**(5%)**	**1.59±0.03ᶜ**	**29.26**	**0.52±0.01ᵈ**	**48.57**	**0.09±0.003ᵈ**	**69.81**
	(10%)	**1.84±0.02ᶜ**	**49.59**	**0.85±0.02ᶜ**	**142.85**	**0.13±0.008ᶜ**	**145.28**
	(15%)	**2.25±0.14ᵇ**	**82.92**	**1.02±0.03ᵇ**	**191.42**	**0.17±0.009ᵇ**	**220.75**

*BMC: bone mineral content, BMD: bone mineral density
Values are expressed as Means ± SE. , Values at the same column with different letters are significant at P<0.05

The positive control group had a significant decrease (P<0.05), in the mean femur weight, BMC and BMD as contrasted to the (SHAM) group as shown in Table (6). The supplementation with dried *E. arvense* L. at 5,10,15% significantly increased (P<0.05) the mean value of femur weight, by 29.26, 49.59, 82.92% respectively, BMC by 48.57, 142.85, 191.42% respectively and BMD by 69.81, 145.28, 220.75% respectively, compared to the +ve control group. There was a significant change (P<0.05) in the weight of the femur between the groups treated with *E. arvense* L. at either 5 or 10% and the level of 15% of *E. arvense.*

Furthermore, there was a significant change (P<0.05) in BMC and BMD among the rats treated with the three tested levels of *E. arvense*. The highest increment of femur weight, BMC and BMD are recorded for rats treated with 15% of *E. arvense* L. by 82.92, 191.42 and 220.75 %, respectively.

This might be due to silica content of *E. arvense* L. that affect the mineral element composition of rat femur and vertebra [44].

The present study also showed that ovariectomy in rats considerably decreased femur weight and bone mineral density in femur ash. These results were partially like those reported by [65, 66, 67].

In this study BMC and BMD increased in rats fed on diet supplemented with *E. arvense*. These results were in harmony with [68] who demonstrated that treatment with *E arvense (*120 mg/kg), has fundamentally improved the BMD and BMD compared to the control group. [69] reported that effect of *E. arvense* L. on OVX rats led to increased bone length as a consequence of decreased chondroblast differentiation. In addition, [54] reported significant rise (P<0.05) in femur BMC and BMD in treated groups fed on (5,10%) *E. arvense*.

These results are concurred with [62] who demonstrated that *E. arvense* L. protected male and female rats against osteoporosis associated with glucocorticoid use, as represented by the elevation of bone minerals (Ca, P). Another study found an increase in the thickness of the cortical and cancellous bones in the group receiving *E. arvense* L. extract, suggesting the bone mineralization ability and action of the plant [3]. The consumption of *E. arvense* L. extract, at 120 mg/kg, raised the mandibular and maxillary BMD. Moreover, [2] demonstrated that the formulation containing lactoferrin, *E. arvense,* soy isoflavone and vit D3 was useful in preventing and treating osteoporosis. Furthermore, another study showed that treatment with *E. arvense* L. extract and Ca supplements were helpful in enhancing bone metabolism in osteoporosis women at postmenopausal time [11].

4. Conclusion

The results of this study denote that administration of diet supplemented with *E. arvense* L. at the tested levels exhibits a potent anti-osteoporotic effect in ovariectomized rats, this effect may be due to enhanced bone building, delayed bone loss and

normalization of serum levels of hormones that regulate calcium metabolism. The study recommends the intake of *E. arvense* L. may be advantageous for the avoidance of osteoporosis in women at postmenopausal period.

Table 7. Effect of *E. arvense* L. on femur ash weight, Ca, and P content of osteoporotic female rats.

Groups	Parameters	femur ash weight (g)	%Of increase	Ca (mg/g ash)	% Of increase	P (mg/g ash)	% Of increase
Control (-ve) (SHAM)		**1.46±0.02[a]**	**-**	**15.70±0.59[a]**	**-**	**5.92±0.03[a]**	**-**
Control (+ve) (OVX)		**0.87±0.03[d]**	**-**	**10.55±0.23[d]**	**-**	**2.56±0.11[e]**	**-**
OVX + *E. arvense* L.	**(5%)**	**1.06±0.02[c]**	**21.83**	**12.25±0.12[c]**	**16.11**	**3.14±0.06[d]**	**22.65**
	(10%)	**1.10±0.01[c]**	**26.43**	**13.80±0.13[b]**	**30.80**	**4.41±0.12[c]**	**72.26**
	(15%)	**1.27±0.01[b]**	**45.97**	**14.51±0.14[b]**	**37.53**	**5.54±0.19[b]**	**116.40**

Ca: Calcium content in bone, P: phosphorus content in bone
Values are expressed as Means ± SE., Values at the same column with different letters are significant at $P<0.05$

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التأثير العلاجي لعشبة ذيل الحصان على المؤشرات الحيوية وقياسات العظام في إناث الفئران المصابة بهشاشة العظام

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الملخص العربي

لعشبة ذيل الحصان .*E. arvense* L خصائص طبية معروفة منذ العصور القديمة وتستخدم لعلاج مجموعة متنوعة من الأمراض. تم دراسة تأثير التدعيم بعشبة ديل الحصان على الفئران المصابة بهشاشة العظام . تم تقسيم عدد أربعون من إناث الفئران بطريقة عشوائيا الي خمس مجموعات متساوية (ن=8). المجموعة الأولى كانت بمثابة مجموعة ضابطة سالبة، بينما أجريت عملية استئصال المبيض لـ الأربع مجموعات الأخرى من الفئران والتي تغذت علي النظام الغذائي الأساسي مع نزع عنصر الكالسيوم من خليط الأملاح المعدنية، كما تم إعطاء تلك الفئران مادة prednisone acetate (4.5 مللي/ كجم من وزن الجسم/مرتين بالأسبوع) عن طريق الفم و لمدة أسبوعين وذلك لإحداث هشاشة العظام. بعد ثلاثة أسابيع من إزالة المبيض، ظلت المجموعة الثانية كمجموعة ضابطة موجبة (OVX) وتغذت المجموعات الثلاث الأخرى على النظام الغذائي الأساسي المدعم ب 5 ، 10، 15% عشبة ديل الحصان المجففة، على التوالي لمدة 8 أسابيع .أظهرت النتائج أن الفئران التي تغذت على عشبة ديل الحصان المجفف عند جميع المستويات التي تم اختبارها كانت لديها زيادة كبيرة (P<0.0.5) في مستوي الكالسيوم، الفوسفور، الفوسفاتيز القلوي بالعظام، هرمون الثيروكسين الحر، والكالسيتونين وSOD وانخفاض معنوي كبير في هرمون البارا ثيرويد والمالونداهيد. بالإضافة إلى ذلك، لوحظ وجود زيادة كبيرة (P<0.05) في كثافة الاملاح في العظام، ومحتوى الاملاح بالعظام، ومحتوي الكالسيوم والفوسفور في رماد عظم الفخذ في المجموعات المدعمة بجميع مستويات من عشبة ديل الحصان مقارنة بالمجموعة الضابطة الموجبة. تشير النتائج التي توصلنا إليها أن عشبة ديل الحصان كان له تأثير علاجي لهشاشة العظام في الفئران التي تم نزع المبيض لها وذلك بسبب تعزيز بناء العظام، وتأخير فقد العظام وضبط مستويات الهرمونات التي تنظم التمثيل الغذائي للكالسيوم . وبالتالي قد يعتبر عشبة ديل الحصان علاج طبيعي لتجنب هشاشة العظام لدى النساء في فترة ما بعد انقطاع الطمث.