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Original Article

Anatomy Section

Phytoestrogenic Effects of *Equisetum arvense* (Horsetail) Extract in an Oestrogen-deficient Rat Model: An Experimental Study

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ABSTRACT

Introduction: Postmenopausal osteoporosis, primarily driven by oestrogen deficiency, is characterised by reduced bone mass, compromised microarchitecture, and increased fracture risk. Hormone Replacement Therapy (HRT) is effective but associated with serious adverse effects such as increased risks of breast cancer and cardiovascular events prompting interest in plant-based phytoestrogens. *Equisetum arvense* (EA) (field horsetail), rich in flavonoids and silica, is traditionally used for bone health, yet its oestrogenic potential in oestrogen deficient models remains underexplored.

Aim: To assess the oestrogen-like activity of EA (horsetail) extract in an oestrogen deficient rat model (ovariectomised rats) by analysing both histological features and biochemical parameters.

Materials and Methods: The present preclinical experimental animal study was conducted at Cape Bio Lab and Research Centre, Marthandam, Tamil Nadu, India, between January and June 2023, 54 female Albino Wistar rats (three-month-old, 200-220 g) were bilaterally ovariectomised to induce oestrogen deficiency. One-month postsurgery, rats were divided into sham operated, Ovariectomised (OVX) control, and treatment groups. The treatment groups received both *E. arvense* extract (60 mg/kg/day) and estradiol for 45 and 90 days. After overnight fasting, blood samples were collected via retro-orbital plexus under anaesthesia. Serum samples were analysed for biochemical parameters, including osteocalcin, Alkaline Phosphatase (ALP), Liver Function Tests (LFTs), and Renal Function Tests (RFTs). Femur bones were harvested for histological examination at the end of treatment.

Results: Phytochemical analysis of EA's ethanolic extract revealed a diverse array of secondary metabolites, including carbohydrates, proteins, flavonoids, saponins, glycosides, tannins, phenolics, and fixed oils. OVX rats displayed notable bone loss and elevated markers of bone resorption, hepatic stress, and renal dysfunction. Treatment with *E. arvense*, especially over 90 days, significantly improved bone architecture ($p<0.01$), lowered Osteocalcin and ALP levels ($p=0.63$) and normalised LFT ($p<0.001$) and RFT markers ($p=0.004$). Protein profiles also returned to near-normal levels ($p<0.05$). The effects were comparable to those observed in estradiol-treated groups, with no statistically significant difference between the two treatment arms. The current study revealed that supplementation with EA notably enhanced bone microarchitecture and normalised critical biochemical markers in OVX rats.

Conclusion: The EA exhibited significant phytoestrogenic, bone-protective, hepatoprotective, and nephroprotective effects in OVX rats. These findings highlight its promise as a natural alternative to Oestrogen replacement therapy for managing postmenopausal symptoms, warranting further clinical evaluation.

Keywords: Bone turnover, Liver function, Nephroprotection, Postmenopausal osteoporosis

INTRODUCTION

Osteoporosis is a metabolic bone disease characterised by low bone mass and microstructure degeneration of bone tissue, which leads to enhanced fragility. As a result, individuals with osteoporosis are at a high-risk of fracture. Women have a higher-risk of osteoporosis than men. In 2005 to 2006, 10% of older women in the United States had osteoporosis at the femur neck, whereas only 2% of men did [1]. In India, the overall prevalence of osteoporosis among postmenopausal women aged 50 years and above is around 29% at both the femoral neck and lumbar spine. Furthermore, in a more general Indian population with an average age of 50 years, the condition affects approximately 6.9% of individuals, with a higher rate in women (11.1%) compared to men (4.2%) [2]. Ovarian dysfunction leading to oestrogen deficiency is recognised as the primary factor responsible for osteoporosis in postmenopausal women [3]. Research demonstrates that oestrogen therapy can mitigate the decline in bone mass after menopause [4,5]. Nevertheless, such therapy is associated with adverse effects, including increased risks of breast cancer, coronary heart disease, stroke, and venous thromboembolism [6,7]. These complications restrict its long-term use, thereby necessitating safer alternatives for osteoporosis management.

In this regard, phytoestrogens- plant-derived, nonsteroidal polyphenolic compounds with oestrogen-like activity have attracted significant attention. They are classified into several groups: isoflavones (genistein, daidzein, biochanin A), lignans (enterolactone, enterodiol), coumestanes (coumestrol), flavonoids (quercetin, kaempferol), and stilbenes (resveratrol) [8,9]. Isoflavones are mainly obtained from soy products, cereals, legumes, and certain meat sources [10], while lignans occur abundantly in flaxseed, oilseeds, cereals, legumes, fruits, and vegetables [11]. Phytoestrogens act primarily through binding to Oestrogen Receptors (ER), producing both oestrogenic and antioestrogenic effects [12,13]. Due to their structural resemblance to oestrogen, numerous studies indicate their beneficial influence on postmenopausal symptoms, cardiovascular health, bone metabolism, and certain cancers [8]. Hence, phytoestrogens present considerable potential in the prevention of postmenopausal bone loss.

EA, a perennial herb belonging to the order Equisetales, has long been used in traditional medicine for its diuretic, anti-inflammatory, and bone-strengthening properties. Additionally, it has been employed to manage bleeding, serve as a topical antiseptic, and treat various ailments including kidney and liver disorders as well as gastric ulcers [14,15]. EA is rich in a variety of phytoconstituents,

including alkaloids, phytosterols, tannins, triterpenoids, and phenolic compounds such as flavonoids, styrylpyrones, and phenolic compounds-indicates potential oestrogenic and antioxidant activity [16,17]. The sterile stems are particularly noted for their high content of silicic acid and silicates (5-8%), along with appreciable amounts of potassium (1.8%), calcium (1.3%), and trace elements such as aluminum, sulfur, magnesium, and manganese [18-20]. However, scientific data supporting its use as a phytoestrogen, especially in models of oestrogen deficiency remain scarce.

This research aimed to investigate the oestrogen-like effects of EA extract using histological and biochemical assessments in ovariectomised rats- a widely accepted animal model for simulating postmenopausal oestrogen deficiency [21]. The findings could offer important insights into the potential of *E. arvense* as a natural therapeutic option for conditions associated with low oestrogen levels.

MATERIALS AND METHODS

The present preclinical experimental animal study was conducted at Cape Bio Lab and Research Centre, Marthandam, Tamil Nadu, India, between January and June 2023. The study received approval from the Institutional Animal Ethics Committee (IAEC), and the approval letter number is (CBLRC/IAEC/15/01-2023).

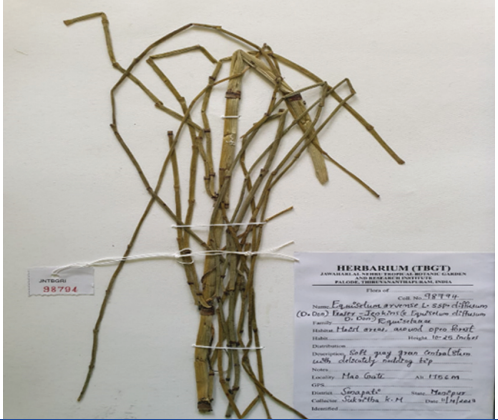
Inclusion and Exclusion criteria: Inclusion criteria included healthy female Albino Wistar rats, aged approximately 3 months and weighing 200-220g that had undergone acclimatisation for one week under controlled conditions (temperature 22±3°C, relative humidity 50-55%, and 12-hour light/dark cycle). Exclusion criteria included any rats showing signs of illness, congenital deformities, or surgical complications following ovariectomy.

A total of 54 female Albino Wistar rats were used in the study. The sample size was determined based on similar experimental models evaluating phytoestrogens in ovariectomised rats, ensuring statistical validity for biochemical and histological analysis across control and test groups.

Study Procedure

Collection of plant material: The Plant EA collected from Senpati District, Manipur. The collected plant materials were shade-dried until complete removal of moisture, ensuring they were suitable for grinding. The dried samples were then finely powdered using a mechanical blender and stored in airtight containers with appropriate labelling for future use.

Plant authentication: The plant materials were taxonomically identified and authenticated by Taxonomist, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Trivandrum, India, Voucher specimen is maintained in the Research Department of Jawaharlal Nehru Tropical Botanical Garden [Table/Fig-1].



[Table/Fig-1]: Herbarium of *Equisetum arvense*.

Preparation of plant extracts: After authentication, the fresh healthy plant of EA dried properly in shade for three weeks, segregated, pulverised by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container, and used for further studies. About 1 kg of air-dried plant of EA was extracted in soxhlet assembly with ethanol. The extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The colour and consistency of the extract were also noted. All the solvents used for this entire work were of analytical reagent grade (Merck, Mumbai).

Phytochemical analysis: The preliminary phytochemical screening was performed to detect the presence of alkaloids, flavonoids, tannins, saponins, glycosides, proteins, amino acids, triterpenoids, steroids, carbohydrates, fixed oils, and phenolic compounds. All reagents used were of analytical grade (Merck, Mumbai).

Animals to be used: Female Albino Wistar Rats (n=54) three-month-old, weighing about 200-220gm obtained from Cape Bio Lab and Research Centre. All animal are allowed to acclimatise in animal housing standard conditions, temperature of (22±3°C) relative humidity (50-55%) and a 12 hour light/dark cycle before being used for the study [22].

Toxicological study: Acute toxicity study was performed according to OECD guidelines 423 (Organisation of Economic Co-operation and Development). The toxicological evaluation of EA extract administered orally at 60 mg/kg/day for 90 days revealed no significant adverse effects in female Wistar rats. The extract demonstrated a high safety margin, with an LD50>5000 mg/kg and no evidence of organ toxicity on both biochemical and histopathological grounds. These findings support the extract's suitability for further pharmacological development as a phytoestrogenic agent [23].

Experimental osteoporosis (Ovariectomy in female rats): After a period of adaptation (one week), the female rats were anesthetised with ketamine hydrochloride and xylazine, and their ovaries were removed bilaterally according to the method described by Lasota and Danowska (2004) [24]. After recovery from surgery, the extract was administered to the rats in an oral dose of 60 mg/kg body weight for 45-90 days [25] and estradiol of 0.2mg/kg body weight administered for 45-90 days [26].

Experimental design and procedures: After one week of recovery from surgery, the OVX rats were randomly divided into four groups. The experimental groups were as follows: group 1 (n=6): Control negative (sham operated), rats received daily saline water orally. Group 2a (n=6): Control positive (OVX-45 days), group 2b (n=6): Control positive (OVX-90 days), both the control positive group rats received the saline water. Group 3a (n=6) rats received EA-OVX-45 days, group 3b (n=6): received EA-OVX-90 days. Group 4a (n=6) OVX rats received estradiol- 45 days, group 4b (n=6) OVX rats received estradiol- 90 days and treatment commenced and continued for 90 days.

One day after the end of treatment, rats from each group were fasted overnight. Blood samples were withdrawn by heparinised capillary tube from the retro-orbital plexus of each the rats were first anesthetised with an intraperitoneal injection of 2 mL ketamine (100 mg/mL) and 1 mL of xylazine (20 mg/mL) [27]. Blood samples were allowed to clot, and then centrifuged at 3000 rpm for 15 minutes to separate serum, which kept at -20°C till biochemical analysis.

Femur bones were collected from all experimental groups under anaesthesia with an intraperitoneal injection of 2 mL ketamine (100 mg/mL) and 1 mL of xylazine (20 mg/mL) [27]. Histological staining is performed on the section of Left femur bone in all groups. Samples were fixed in 10% Neutral Buffered Formalin (NBF). After paraffin embedding, 3-4-µm serial sections were prepared and then stained with Haematoxylin and Eosin (H&E).

Histological analysis was conducted on femur bone. Samples were fixed in 10% NBF, processed and done paraffin embedding,

sectioned at 3-4 µm thickness, and stained with Haematoxylin and Eosin.

Biochemical and histological parameters studied included:

- Bone turnover markers (Osteocalcin and ALP), Liver function markers- Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Renal markers - Creatinine (mg/dL), Urea (mg/dL), Protein profile -total protein (g/dL), albumin (g/dL).
- Bone microarchitecture (histological analysis of femur):

Instrumentation included the Lyca microtome RTS 2125 and reagents such as Merck's Haematoxylin and Eosin staining kits were used throughout the study.

STATISTICAL ANALYSIS

Data were expressed as mean±SEM. One-way Analysis of Variance (ANOVA) detected overall significant differences ($p<0.05$) across all parameters. Bonferroni post-hoc test was applied to identify specific intergroup differences. Statistical analyses were performed using SPSS software (Version 25.0, IBM Corp., Armonk, NY, USA).

RESULTS

Phytochemical analysis: Phytochemical analysis of EA's ethanolic extract revealed a diverse array of secondary metabolites, including carbohydrates, proteins, flavonoids, saponins, glycosides, tannins, phenolics, and fixed oils [Table/Fig-2]. Notably, alkaloids were present, consistent with the plant's known antimicrobial and diuretic properties. However, phytosterols and triterpenoids were absent, possibly due to solvent polarity limitations. These findings highlight species-specific biosynthetic pathways, underscoring the unique therapeutic potential of *E. arvense*.

Phytochemical	*Equisetum arvense* (EA)
Alkaloids	+
Carbohydrates	+
Proteins and amino acids	+
Phytosterols	-
Glycosides	+
Saponins	+
Flavonoids	+
Tannins and phenolics	+
Triterpenoids	-
Fixed oils	+

[Table/Fig-2]: Phytochemical composition of *Equisetum arvense*.

Biochemical analysis: After 45 days, ovariectomised rats showed a significant increase in osteocalcin levels compared to controls ($p<0.001$), reflecting accelerated bone resorption. Administration of *E. arvense* markedly lowered osteocalcin ($p=0.012$), with values closely resembling those of the estradiol group ($p<0.001$ vs OVX). Although ALP activity was elevated following ovariectomy, estradiol treatment produced no significant effect ($p=1.0$), while *E. arvense* supplementation resulted in a significant reduction ($p<0.001$). Overall, these results suggest that *E. arvense* effectively restores bone turnover markers to near-normal levels, similar to estradiol [Table/Fig-3].

Group	Osteocalcin (ng/mL)	*p-value	ALP (IU/L)	*p-value
1 (Control negative)	4.18±0.15		256.5±3.2	
2A (OVX 45 days)	7.43±0.29		280.0±2.7	
3A (OVX+EA 45days)	6.33±0.20	p=0.012	273.7±2.4	p<0.001
4A (OVX+estradiol 45days)	4.23±0.14	p<0.001	270.8±2.6	p=1.0

[Table/Fig-3]: Bone turnover markers (Osteocalcin and ALP)- *Equisetum arvense* 45 days.
*p-value: in comparison with group 2a

After 90 days of ovariectomy, osteocalcin and ALP levels were markedly elevated compared to controls, indicating sustained bone turnover and resorption. Treatment with *E. arvense* for 90 days produced a noticeable reduction in both osteocalcin and ALP values, though these changes were not statistically significant ($p=0.63$ and $p=0.16$, respectively). In contrast, estradiol treatment significantly decreased osteocalcin ($p<0.001$), while its effect on ALP remained non-significant ($p=1.0$). These findings suggest that prolonged *E. arvense* supplementation tends to normalise bone turnover markers, albeit less effectively than estradiol [Table/Fig-4].

Group	SGOT (U/L)	p-value	SGPT (U/L)	p-value
1 (Control negative)	144.2±2.4		56.4±1.8	
2A (OVX 45 days)	166.4±2.8		74.1±2.1	
3A (OVX+EA 45 days)	150.4±2.7	p<0.001	65.3±2.0	p=0.009
4A (OVX+estradiol 45 days)	158.7±2.6	p<0.001	59.9±2.1	p<0.001

[Table/Fig-4]: Bone turnover markers (Osteocalcin and ALP)- *Equisetum arvense* 90 days.
*p-value: in comparison with group 2A

Ovariectomy for 45 days significantly elevated SGOT and SGPT levels compared to the control group, indicating hepatic stress. Treatment with *E. arvense* markedly reduced both SGOT ($p<0.001$) and SGPT ($p=0.009$) levels relative to the OVX group, reflecting partial hepatoprotection. Estradiol supplementation also significantly lowered these enzymes ($p<0.001$), restoring values close to normal [Table/Fig-5].

Group	Osteocalcin (ng/mL)	*p-value	ALP (IU/L)	*p-value
1 (Control negative)	4.18±0.15		256.5±3.2	
2B (OVX 90 days)	7.79±0.33		297.0±2.4	
3B (OVX+EA 90 days)	5.93±0.26	n.s. (p=0.63)	269.9±2.5	n.s. (p=0.16)
4B (OVX+estradiol 90 days)	4.54±0.17	p<0.001	261.5±2.6	n.s. (p=1.0)

[Table/Fig-5]: Liver function markers (SGOT and SGPT)- *Equisetum arvense* 45 days.
*p-value: in comparison with group 2A

At 90 days post-ovariectomy, SGOT and SGPT levels were markedly elevated compared to the control group, indicating progressive hepatic dysfunction. Supplementation with *E. arvense* significantly reduced both SGOT ($p<0.001$) and SGPT ($p<0.001$), restoring values close to baseline and showing strong hepatoprotective effects. Estradiol treatment produced a similar significant reduction in enzyme levels ($p<0.001$), confirming its protective role [Table/Fig-6].

Group	SGOT (U/L)	p-value	SGPT (U/L)	p-value
1 (Control negative)	144.2±2.4		56.4±1.8	
2B (OVX 90days)	180.7±2.7		88.1±2.4	
3B (OVX+EA 90days)	148.5±2.6	p<0.001	57.9±2.0	p<0.001
4B (OVX+estradiol 90days)	148.6±2.5	p<0.001	61.4±2.1	p<0.001

[Table/Fig-6]: Liver Function Markers (SGOT &SGPT)– *Equisetum arvense* 90 days.
*p-value: in comparison with group 2A

Ovariectomy for 45 days caused a rise in serum creatinine and urea levels compared to controls, indicating early renal dysfunction. Treatment with *E. arvense* did not significantly alter creatinine levels ($p=1.0$) but significantly reduced urea concentrations ($p<0.001$) compared to the OVX group. Estradiol treatment showed a similar trend, with no significant effect on creatinine but a moderate reduction in urea ($p=0.028$) [Table/Fig-7].

Group	Creatinine (mg/dL)	p-value	Urea (mg/dL)	p-value
1 (Control negative)	0.62±0.02		28.7±1.1	0.62±0.02
2A (OVX 45days)	0.66±0.02		33.7±1.2	

3A (OVX+EA 45 days)	0.64±0.02	1.0	30.1±1.1	p<0.001
4A (OVX+estradiol 45 days)	0.66±0.02	1.0	31.3±1.2	p=0.028

[Table/Fig-7]: Renal Markers (creatinine and urea) *Equisetum arvense* 45 days.
*p-value: in comparison with group 2A

Ninety days of ovariectomy significantly increased serum creatinine and urea levels compared to controls, indicating progressive renal impairment. Supplementation with *E. arvense* brought creatinine values close to normal (n.s., p=0.059) and significantly reduced urea levels (p=0.004) relative to the OVX group, demonstrating protective effects. Estradiol treatment also lowered creatinine significantly (p<0.001), but its effect on urea was not significant [Table/Fig-8].

Group	Creatinine (mg/dL)	p-value	Urea (mg/dL)	p-value
1 (Control negative)	0.62±0.02		28.7±1.1	
2B (OVX 90days)	0.70±0.02		36.6±1.3	
3B (OVX+EA 90days)	0.63±0.02	0.059	29.9±1.1	p=0.004
4B (OVX+estradiol 90 days)	0.65±0.02	<0.001	32.1±1.2	p=1.0

[Table/Fig-8]: Renal markers (creatinine and urea) *Equisetum arvense* 90 days.
*p-value: in comparison with group 2A

At 45 days, ovariectomy caused a slight reduction in serum protein and albumin compared to controls, suggesting impaired hepatic protein synthesis. *E. arvense* treatment significantly lowered total protein (p=0.001) and albumin (p<0.001) levels compared to the OVX group, while estradiol maintained protein levels without significant change but markedly improved albumin concentrations (p<0.001). These findings indicate that both treatments modulate protein metabolism, with estradiol showing stronger effects on albumin restoration [Table/Fig-9].

Group	Total protein (g/dL)	p-value	Albumin (g/dL)	p-value
1 (Control negative)	6.37±0.07		3.35±0.05	
2A (OVX 45days)	6.35±0.08		3.13±0.07	
3A (OVX+EA 45days)	6.25±0.08	0.001	3.11±0.06	p<0.001
4A (OVX+estradiol 45 days)	6.35±0.06	1.0	3.20±0.06	p<0.001

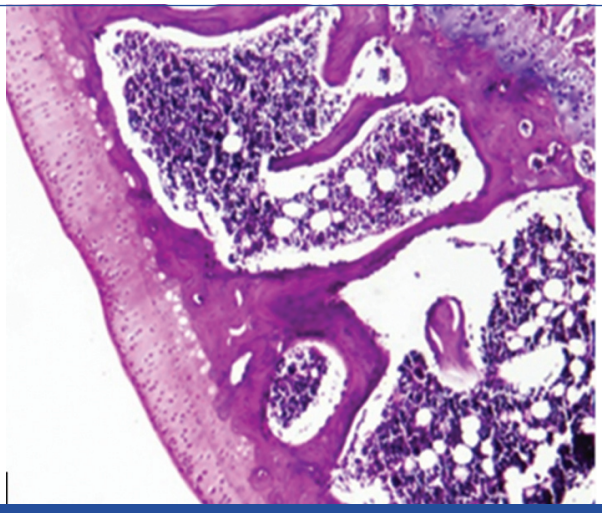
[Table/Fig-9]: Protein profile (total protein and albumin) *Equisetum arvense* 45 days.
*p-value: in comparison with group 2A

At 90 day, supplementation with *E. arvense* significantly restored both total protein (p<0.001) and albumin (p<0.001) to near-control levels. Estradiol treatment produced a similar effect, with significant improvement in albumin concentrations (p<0.001), highlighting the hepatoprotective role of both interventions [Table/Fig-10].

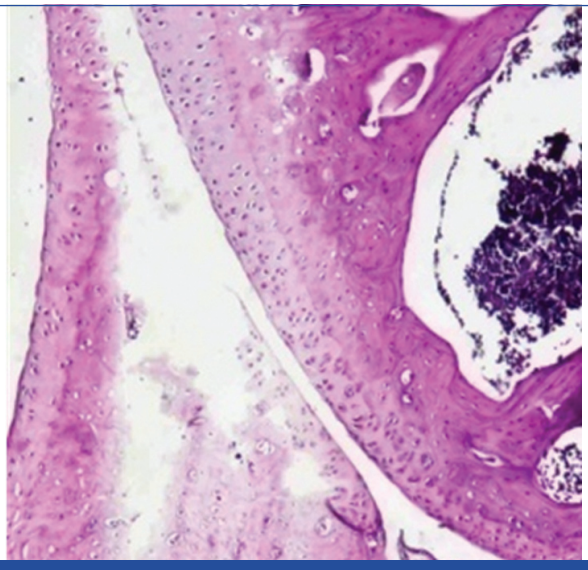
Group	Total protein (g/dL)	p-value	Albumin (g/dL)	p-value
1 (Control negative)	6.37±0.07		3.35±0.05	
2B (OVX 90days)	6.34±0.06		2.86±0.07	
3B (OVX+EA 90days)	6.37±0.07	<0.001	3.35±0.05	p<0.001
4B (OVX+estradiol 90 days)	6.34±0.06	1.0	3.31±0.06	p<0.001

[Table/Fig-10]: Protein profile (total protein and albumin) *Equisetum arvense* 90 days.
*p-value: in comparison with group 2A

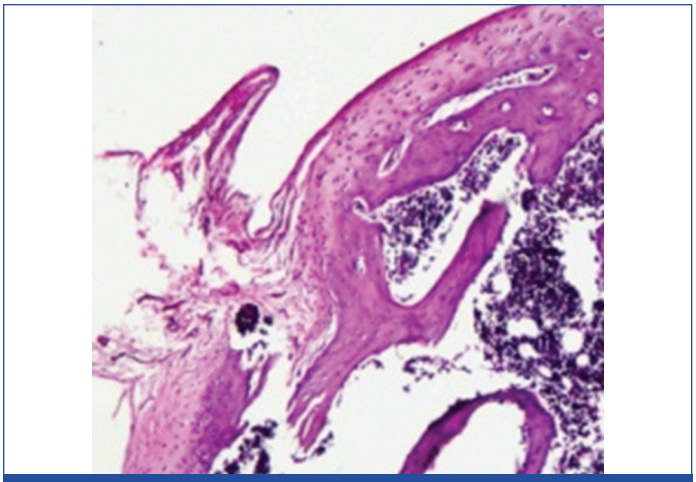
Histological examination of the upper end of the femur bone of albino Wistar rats in the control group showed that the trabeculae were numerous, thick, and densely interconnected, and the intervening marrow spaces showed haematopoietic elements, representing optimal bone mass with mineralised matrix. The cortex was well-preserved and thick [Table/Fig-11]. In the 2a group, OVX control rats observed for 45 days showed faintly stained, short bony trabeculae with free-floating trabeculae and a large proportion of the marrow space and few decalcified osteoid bones [Table/Fig-12]. In the 2b group OVX, control rats kept for 90 days showed faintly stained cortical area, thickness reduced and decalcified osteoid bone are seen. Severely thinned bony trabeculae and floating trabeculae with reduced connectivity Substantial loss of trabecular bone mass [Table/Fig-13]. In the 3a group, OVX rats treated with EA-45 days showed interconnected bony trabeculae. It was shown the potential of protection from osteoporotic changes induced because of ovariectomy was observed. The cortical layer is relatively thick and continuous. Some osteoblast-like cells line the trabeculae, suggesting active bone formation [Table/Fig-14]. In 3b group, OVX rats treated with EA-90 days shows thick bony trabeculae compared to group 2b with only occasional floating trabeculae. The cortical structure was relatively thick and intact, with no clear evidence of resorptive activity, active osteoblast-like cell presence was evident along some trabecular surfaces, indicating ongoing bone formation. It was characterised by negative histochemical reaction (absence of osteoclast) along with a thick cortex, which is a marker of significant improvement compared with 2b group [Table/Fig-15]. In 4a group -OVX rats treated with estradiol-45 days showed minimal surface cartilage irregularities and trabecular bone appears moderately diminished in volume. The few active remodelling is visible [Table/Fig-16]. In 4b group, OVX rats treated with estradiol-90 days compared to other groups, estradiol showed slightly more pronounced trabecular density and marrow quality, reflecting its strong anti-osteoporotic properties. Trabeculae were thick and interconnected. No presence of osteoclastic pits, necrosis, or fibrosis. The findings align with known effects of oestrogen replacement therapy in preventing post-ovariectomy bone loss [Table/Fig-17].



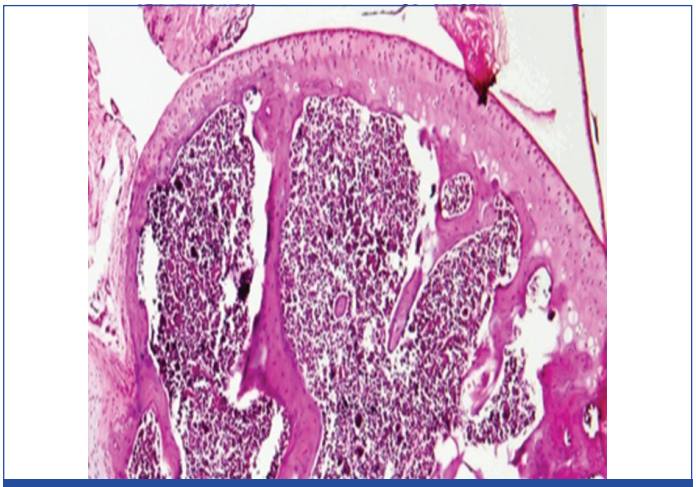
[Table/Fig-11]: Non OVX control (H&E, 40×).



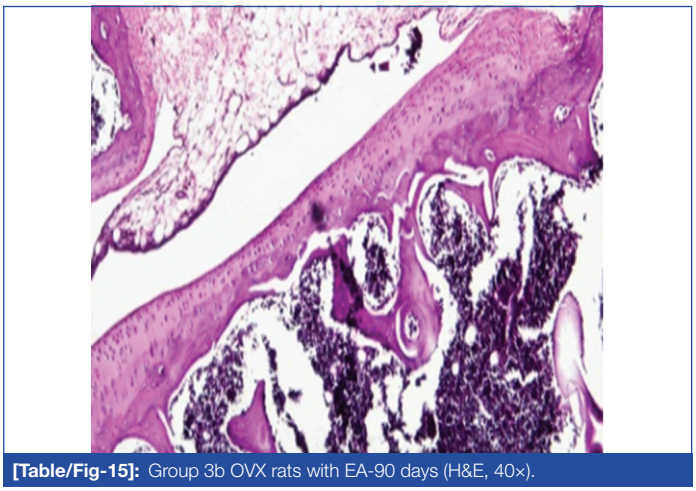
[Table/Fig-12]: Group 2a: OVX control rats-45 days (H&E, 40×).



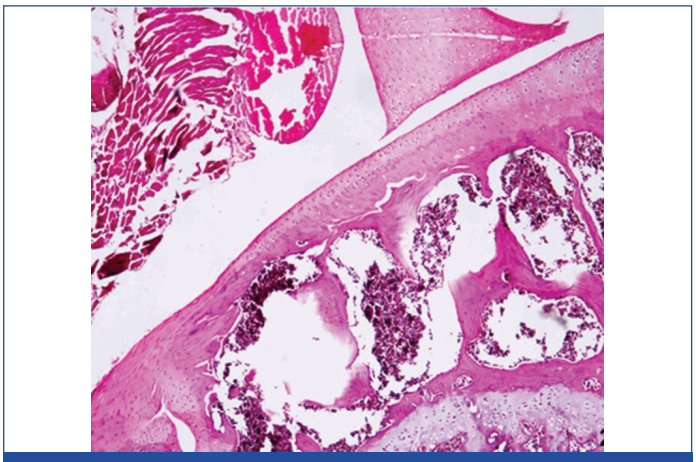
[Table/Fig-13]: Group 2b: OVX control rats -90 days (H&E, 40×).



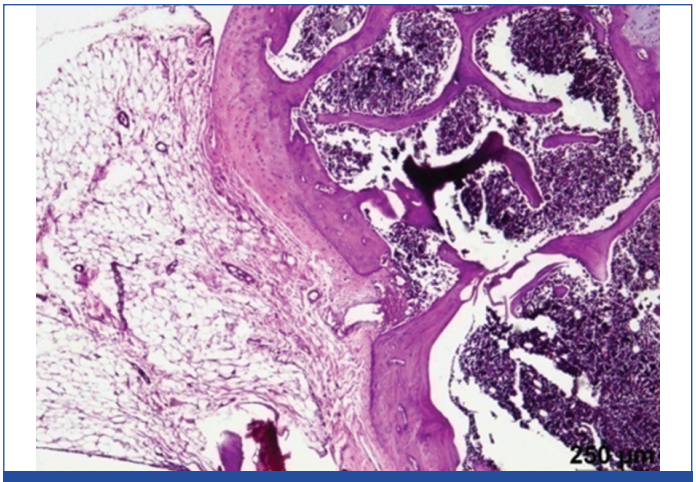
[Table/Fig-14]: Group 3a- OVX rats with EA-45 days (H&E, 40×).



[Table/Fig-15]: Group 3b OVX rats with EA-90 days (H&E, 40×).



[Table/Fig-16]: Group 4a-OVX rats with estradiol-45 days (H&E, 40×).



[Table/Fig-17]: Group 4b-OVX rats with estradiol-90 days (H&E, 40×).

DISCUSSION

Medicinal plants have long been used in the treatment of various diseases. A crucial first step in exploring their pharmacological potential is the evaluation of their toxicological profile [28,29]. EA a perennial herb, has traditionally been used to manage several health conditions. Baracho NCV et al., conducted an acute toxicity study on male Wistar rats, administration of graded doses (30 mg, 50 mg, and 100 mg/kg) of *E. arvense* showed no signs of toxicity [30]. Research by Oh H et al., indicated that the aerial parts of *E. arvense* possess hepatoprotective and antioxidant properties [31]. However, contrasting findings were reported by Semprini M et al., who observed liver damage in Wistar rats following oral administration of the plant [32]. Notably, there is a lack of clinical data regarding its dosage range and effects specifically in female albino Wistar rats.

The current study reveals that supplementation with EA notably enhanced bone microarchitecture and normalised critical biochemical markers in OVX rats. These outcomes support its traditional application in promoting bone health. The most substantial effects were observed in the 90-day treatment group, corroborating earlier research that emphasises the potential of botanicals rich in flavonoids and silica in the management of osteoporosis. Kotwal SD et al., reported anabolic/osteoprotective effects of *E. arvense* in OVX rats when combined with bone-mineralising nutrients, showing improved bone parameters that are consistent with our histological and biochemical improvements [33].

The serum biochemical results further corroborate the histological findings. In OVX rats, levels of bone turnover markers- osteocalcin and ALP- were significantly elevated, indicating heightened bone resorption. Treatment with EA brought these markers closer to baseline values seen in the sham and estradiol-treated groups, suggesting its bone-protective and anti-resorptive properties [34]. Gulcin I et al., Ibrahem ES et al., and Bessa Pereira C et al., suggests that the bone-protective effects of E. arvense may be linked to its bioactive components, including silica and flavonoids, which have been shown to support bone metabolism by enhancing osteoblast function and suppressing osteoclast activity [35-37]. Regarding liver function, OVX rats displayed increased levels of SGOT and SGPT, indicating hepatic stress likely triggered by oestrogen deficiency. EA treatment, particularly over a 90-day period, normalised these enzyme levels, pointing to its hepatoprotective potential. Remarkably, the liver-protective effects of EA were comparable to, or slightly better than, those of estradiol- important given the known hepatotoxic risks of prolonged synthetic oestrogen use [38]. Improvements were also noted in kidney function following EA administration. Elevated creatinine and urea levels in untreated OVX rats were significantly reduced with EA, especially after 90 days. This nephroprotective effect is likely due to the antioxidant properties of phenolic compounds found in *E. arvense*, which help reduce oxidative stress on renal tissues [39].

Histopathological evaluation of femoral bone tissues showed that EA-treated rats retained trabecular thickness, exhibited improved cortical structure, and demonstrated the presence of osteoblast-like cells along trabecular surfaces- indicative of active bone regeneration. In contrast, the OVX control groups (2a and 2b) displayed severe trabecular loss, demineralisation, and disrupted bone connectivity, which are typical of oestrogen deficient osteoporosis [40,41]. Notably, rats in the EA-90 group (3b) showed bone architectural restoration comparable to those in the estradiol-treated group (4b), with fewer signs of osteoclastic activity. These findings suggest a pro-osteogenic role of EA, possibly mediated via ER interaction. This effect may be attributed to its bioactive components-especially flavonoids such as kaempferol and quercetin, along with silica- which are known to stimulate collagen synthesis, enhance osteoblast function, and interact with ERs to influence bone remodeling [42-44].

Beyond skeletal effects, OVX animals demonstrated signs of liver and kidney dysfunction, including elevated levels of SGOT, SGPT, creatinine, and urea. EA-treated groups, particularly those receiving 90 days of treatment, showed marked improvements in these parameters, implying both hepatoprotective and nephroprotective effects. These findings are consistent with earlier studies documenting the antioxidant capacity of *E. arvense*, which mitigates oxidative stress in hepatic and renal tissues by neutralising reactive oxygen species [45,46]. Importantly, EA's effect on liver function- especially normalisation of SGPT- was similar to or exceeded that of estradiol, a notable benefit given the known hepatotoxic risks associated with prolonged synthetic oestrogen use [47,48].

In terms of protein metabolism, the total protein and albumin levels, which were reduced due to oestrogen deficiency, were significantly restored in the EA-90 group. This improvement suggests enhanced hepatic protein synthesis and a possible anti-inflammatory action. While moderate improvements were noted after 45 days of EA treatment, the 90-day protocol resulted in near-complete normalisation, emphasising the value of extended treatment duration

Taken together, these findings highlight EA as a promising botanical alternative to HRT for treating postmenopausal osteoporosis and its associated systemic impairments. Given its broad range of actions including oestrogenic support for bone, and protection of liver and kidney function further molecular-level investigations are warranted. Future research should explore the specific cellular pathways involved, including roles of ER-α and ER-β, and assess long-term efficacy and safety through advanced preclinical and clinical models.

Limitation(s)

The present study has a few limitations. Primarily, the use of an ovariectomised rat model though commonly used to simulate postmenopausal conditions cannot fully capture the complexity of human hormonal and metabolic responses. Additionally, the underlying molecular mechanisms by which EA exerts its effects were not investigated, including its potential binding to ER subtypes (ER-α and ER-β), involvement in signaling pathways, or influence on gene expression. Furthermore, while histological and biochemical parameters were assessed, direct measurements of Bone Mineral Density (BMD) using techniques like DXA or micro-CT were not conducted. Lastly, the study evaluated only one therapeutic dose of EA, leaving its dose-response relationship unexplored.

CONCLUSION(S)

EA supplementation was found to enhance bone microarchitecture and reduce bone turnover markers in oestrogen deficient rats. In addition to its skeletal effects, it safeguarded liver and kidney function, indicating broad systemic benefits. Notably, 90 days of treatment yielded results comparable to estradiol, supporting its role as a promising phytoestrogen with therapeutic potential. This study is important because postmenopausal osteoporosis and oestrogen deficiency not only compromise bone strength but also impact overall systemic health. Although estradiol therapy is effective, its prolonged use is linked to risks such as breast and endometrial cancers and cardiovascular issues. Therefore, discovering a plant-derived option like *E. arvense* provides a safer approach to managing postmenopausal complications, especially for women who are unable or unwilling to undergo HRT. Future research should focus on comprehensive dose-response evaluations, identification of the active phytoconstituents, and exploration of underlying molecular mechanisms. Human clinical trials are essential to confirm its efficacy and safety in clinical settings.

Authors' contributions: SKM was responsible for collecting the plant material, preparing the plant extract, performing the ovariectomy procedure, collecting tissue samples for histopathological analysis, interpreting the results, and analysing the study data; VN and provided guidance in the design and drafting of the research work; JP provided guidance and technical supports; J contributed to the study design, data interpretation and statistical analysis of data.

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REFERENCES

[1] Kanis JA, Johnell O, De Laet C, Johansson H, Oden A, Delmas P, et al. A meta-analysis of previous fracture and subsequent fracture risk. Bone. 2004;35(3):375-82.
[2] Kaushal N, Vohora D, Jalali RK, Jha S. Prevalence of osteoporosis and osteopenia in an apparently healthy Indian population—a cross-sectional retrospective study. Osteoporos Sarcopenia. 2018;4(2):53-60.
[3] National Institutes of Health Osteoporosis Consensus Development Panel. Osteoporosis prevention, diagnosis, and therapy. JAMA. 2001;285(6):785-95.
[4] Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: Scientific review. Jama. 2002;288(7):872-81.
[5] Ettinger B, Ensrud KE, Wallace R, Johnson KC, Cummings SR, Yankov V, et al. One year of estrogen replacement therapy prevents bone loss in older postmenopausal women without increasing bone density above premenopausal levels. Ann Intern Med. 1994;121(10):834-41.
[6] Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-33.
[7] Cushman M, Kuller LH, Prentice R, Rodabough RJ, Psaty BM, Stafford RS, et al. Estrogen plus progestin and risk of venous thrombosis. JAMA. 2004;292(13):1573-80.
[8] Patra S, Gorai S, Pal S, Ghosh K, Pradhan S, Chakrabarti S. A review on phytoestrogens: Current status and future direction. Phytother Res. 2023;37(7):3097-120.
[9] Delgadillo JA, Retana-Márquez S, Hernández H, Flores JA, Muñoz-Gutiérrez M, Duarte G, et al. Effects of phytoestrogens on mammalian reproductive physiology. Tropical and Subtropical Agroecosystems. 2012;15(1):S129-45.
[10] Fu S, Zeng G, Zong S, Zhang Z, Zou B, Fang Y, et al. Systematic review and meta-analysis of the bone protective effect of phytoestrogens on osteoporosis in ovariectomized rats. Nutr Res. 2014;34(6):467-77.
[11] Landete JM. Plant and mammalian lignans: A review of source, intake, metabolism, intestinal bacteria and health. Food Res Int. 2012;45(1):410-29.
[12] Wikipedia contributors. Phytoestrogen \[Internet]. Wikipedia; \[cited 2025 Aug 25]. Available from: https://en.wikipedia.org/wiki/Phytoestrogen.
[13] MedlinePlus. Phytoestrogens \[Internet]. PubMed Health;\[cited 2025 Aug 25]. Available from: https://medlineplus.gov/phytoestrogens.html.
[14] Al-Snafi AE. The pharmacology of Equisetum arvense-A review. IOSR Journal of Pharmacy. 2017;7(2):31-42.
[15] Badole S, Kotwal S. Equisetum arvense: Ethanopharmacological and phytochemical review with reference to osteoporosis. Int J Pharm Sci Health Care. 2014;1(4):01-07.
[16] Pallag A, Jurca T, Pasca B, Sirbu V, Honiges AN, Costuleanu M. Analysis of phenolic compounds composition by HPLC and assessment of antioxidant capacity in Equisetum arvense L. extracts. Revista de Chimie. 2016 Aug 1;67(8):1623-27.
[17] Četojević-Simin DD, Čanadanović-Brunet JM, Bogdanović GM, Djilas SM, Ćetković GS, Tumbas VT, Stojiljković BT. Antioxidative and antiproliferative activities of different horsetail (Equisetum arvense L.) extracts. Journal of medicinal food. 2010 Apr 1;13(2):452-59.
[18] Sandhu NS, Kaur S, Chopra D. Equisetum arvense: Pharmacology and phytochemistry- a review. Asian J Pharm Clin Res. 2010;3(3):146-50.
[19] Al-Snafi AE. The pharmacology of Equisetum arvense-A review. IOSR Journal of Pharmacy. 2017;7(2):31-42.

[20] Holzhueter G, Narayanan K, Gerber T. Structure of silica in Equisetum arvense. Anal Bioanal Chem. 2003;376(4):512-17.
[21] Johnston BD, Ward WE. The ovariectomized rat as a model for studying alveolar bone loss in postmenopausal women. BioMed research international. 2015;2015(1):635023.
[22] Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993;123(11):1939-51.
[23] OECD. OECD guideline for testing of chemicals, Section 4: Health effects. Test No. 423: Acute oral toxicity – acute toxic class method \[Internet]. Paris: Organisation for Economic Co-operation and Development; 2001 \[cited 2025 Aug 25]. Available from: https://www.oecd.org/chemicalsafety/testing/1948370.pdf.
[24] Lasota A, Danowska-Klonowska D. Experimental osteoporosis—different methods of ovariectomy in female white rats. Rocz Akad Med Bialymst. 2004;49(Suppl 1):129-31.
[25] Badole S, Kotwal S. Biochemical, hematological and histological changes in response to graded dose of extract of Equisetum arvense in adult female rats. Int J Pharm Sci Res. 2015;6(8):3321-25.
[26] Cegieła U, Folwarczna J, Pytlik M, Zgórka G. Effects of extracts from Trifolium medium L. and Trifolium pratense L. on development of estrogen deficiency-induced osteoporosis in rats. Evid Based Complement Alternat Med. 2012;2012:921684. Doi: 10.1155/2012/921684.
[27] Veilleux-Lemieux D, Castel A, Carrier D, Beaudry F, Vachon P. Pharmacokinetics of ketamine and xylazine in young and old Sprague-Dawley rats. Journal of the American Association for Laboratory Animal Science. 2013;52(5):567-70.
[28] Ali R, Jaimini A, Nishad DK, Mittal G, Chaurasia OP, Kumar R, et al. Acute and subacute toxicity and efficacy studies of Hippophae rhamnoides based herbal antioxidant supplement. Indian J Pharmacol. 2012;44(3):504-08.
[29] De Smet PAGM. Health risk of herbal remedies. Drug Saf. 1995;13(2):81-93.
[30] Baracho NCV, Vicente BBV, Arruda GDS, Sanches BCF, de Brito J. Study of acute hepatotoxicity of Equisetum arvense L. in rats. Acta Cir Bras. 2009;24(6):449-53.
[31] Oh H, Kim DH, Cho JH, Kim YC. Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from Equisetum arvense. J Ethnopharmacol. 2004;95(2-3):421-24. Doi: 10.1016/j.jep.2004.08.015.
[32] Semprini M, Prado FO, Paz K, Regalo SCH, Lopes RA, Sala M. Hepatotoxicity of medicinal plants: Action of Equisetum arvense infusion in rats. Braz J Pharm Sci. 2003;39(2):217-21.
[33] Kotwal SD, Badole SR. Anabolic therapy with Equisetum arvense along with bone mineralising nutrients in ovariectomized rat model of osteoporosis. Indian J Pharmacol. 2016;48(3):312-15. Doi: 10.4103/0253-7613.182880.
[34] Ilen SR. Bone turnover markers: Clinical update. Endocr Connect. 2015;4(2):R70-R75.
[35] Gulcin I, Bursal E, Şehitoğlu MH, Bilsel G, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of Equisetum arvense (horsetail). 2017;20(2):277-90.
[36] Ibrahem ES, Mohamed GH, El-Gazar AF. Therapeutic effect of Equisetum arvense L. on bone and scale biomarkers in female rats with induced osteoporosis. Egyptian Journal of Chemistry. 2022;65(12):457-66.
[37] Bessa Pereira C, Gomes PS, Costa-Rodrigues J, Almeida Palmas R, Vieira L, Ferraz MP, et al. Equisetum arvense hydromethanolic extracts in bone tissue regeneration: In vitro osteoblastic modulation and antibacterial activity. Cell proliferation. 2012;45(4):386-96.
[38] Baracho NC, Vicente BB, Arruda GD, Sanches BC, Brito JD. Study of acute hepatotoxicity of Equisetum arvense L. in rats. Acta Cirurgica Brasileira. 2009;24:449-53.
[39] Batir-Marin D, Boev M, Cioanca O, Mircea C, Burlec AF, Beppe GJ, et al. Neuroprotective and antioxidant enhancing properties of selective Equisetum extracts. Molecules. 2021;26(9):2565.
[40] Collins FL, Rios-Arce ND, Atkinson S, Bierhalter H, Schoenherr D, Bazil JN, et al. Temporal and regional intestinal changes in permeability, tight junction, and cytokine gene expression following ovariectomy-induced estrogen deficiency. Physiological reports. 2017 May;5(9):e13263.
[41] Kalu DN. The ovariectomized rat model of postmenopausal bone loss. Bone Miner. 1991;15(3):175-91.
[42] Prouillet C, Mazière JC, Mazière C, Wattel A, Brazier M, Kamel S. Stimulatory effect of naturally occurring flavonols quercetin and kaempferol on alkaline phosphatase activity in MG-63 human osteoblasts through ERK and estrogen receptor pathway. Biochem Pharmacol. 2004;67(7):1307-13.
[43] Dormousoglou M, Efthimiou I, Antonopoulou M, Fetzer DL, Hamerski F, Corazza ML, et al. Investigation of the genotoxic, antigenotoxic and antioxidant profile of different extracts from Equisetum arvense L. Antioxidants. 2022;11(7):1393.
[44] Mimica-Dukic N, Simin N, Cvejic J, Jovin E, Orcic D, Bozin B. Phenolic compounds in Equisetum arvense as natural antioxidants. Molecules. 2008;13(7):1455-64.
[45] Carneiro DM, Jardim TV, Araújo YC, Arantes AC, de Sousa AC, Barroso WK, et al. Equisetum arvense: New evidences supports medical use in daily clinic. Pharmacogn. Rev. 2019;13:50-58.
[46] Stajner D, Popović BM, Canadanović-Brunet J, Ćetković G, Tumbas V, Boskovic BM. Exploring Equisetum arvense as a source of natural antioxidants. Phytother Res. 2009;23(4):546-50.
[47] Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, et al. Genistein, a dietary-derived inhibitor of in vitro angiogenesis. Proc Natl Acad Sci USA. 1993;90(7):2690-94.
[48] Bhavnani BR. Estrogens and menopause: Pharmacology of conjugated equine estrogens and their potential role in the treatment of menopausal symptoms and osteoporosis. J Steroid Biochem Mol Biol. 2003;85(2–5):473-82.

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