

***Pterocarpus soyauxii* (Fabaceae) heartwood aqueous extract exhibits anti-osteoporotic activities in a postmenopausal-like model**

Abstract

Previous studies showed that *Pterocarpus soyauxii* (*P. soyauxii*) exhibits estrogenic activities ease menopausal disorders. The objective of this study was to evaluate anti-osteoporotic activities of the aqueous extract of *P. soyauxii* heartwood in ovariectomized (Ovx) Wistar rats. To achieve this, an 84-day postmenopausal osteoporosis model was used. Twenty-five female rats were ovariectomized and 5 others were sham-operated (Sham). After 84 days of hypoestrogenism, Ovx animals were divided into 5 groups including a group receiving distilled water at 10 mL/kg, a group receiving estradiol valerate (E₂V) at 1 mg/kg, and three groups receiving *P. soyauxii* extract at 100, 200, and 300 mg/kg; Sham-operated animals received vehicle (10 mL/kg). After 28 days of treatment, animals were sacrificed. Blood was collected in EDTA tubes for blood count and in dry test tubes. Some femoral and seric biochemical analyses were carried out. The relative mass of both tibia and femur, and femoral density were assessed. As result, oophorectomy significantly increased the level of white blood cells (WBC) (p <0.01), MDA (p <0.01), nitrites (p <0.001), and urinary calcium/urinary creatinine ratio (p <0.01). Ovx animals presented a low femoral calcium and phosphorus levels (p <0.05) likewise ALP activity in both serum and femur compared to sham group. Thus, they also presented numerous resorption lacunae in the tibia and femur and a disorganization of tibia trabecular bone. *P. soyauxii* extract at 300 mg/kg significantly decreased WBC (p <0.05), MDA (p <0.01), and nitrites (p <0.001) compared to vehicle. At the dose of 200 mg/kg, *P. soyauxii* extract significantly increased femoral calcium (p <0.05), seric phosphorus (p <0.01), and ALP activity (p <0, 05) in both femur and serum, as well as relative femoral mass (p <0.05) and density (p <0.001). Furthermore, the plant extract at 200 and 300 mg/kg reduced resorptive lacunae and reconstituted trabecular bone in Ovx animals. Overall, aqueous extract of *P. soyauxii* exhibits anti-osteoporotic activities in a postmenopausal-like model in Wistar rats.

Key words: *P. soyauxii*, anti-osteoporotic, postmenopausal, rat.

1- Introduction

Osteoporosis is a systemic disorder characterized by a reduction in bone mass and microarchitectural deterioration of bone tissue, resulting in skeletal fragility [1]. Known as a multifactorial disease, its

susceptibility is determined by genetic influences, environmental factors, and sex hormone status among others [2]. The ever-increasing aging of the world's population makes osteoporosis a major public health problem [3]. Estrogen deficiency in postmenopausal women, however, is known to be an important factor in the pathogenesis of osteoporosis [4]. Prevention and management of this condition are based primarily on Hormone Replacement Therapy (HRT) [5]. Despite the positive effects associated with HRT for osteoporosis, the Women's Health Initiative (WHI) trial reported a risk of cardiovascular diseases and breast cancer associated with this healing [6]. Around the world and particularly in developing countries, herbs are used as a therapeutic alternative to conventional medicine [7]. Plants commonly used to prevent menopausal disorders have among other estrogenic activities that are linked to their richness in a class of molecules called phytoestrogens. These molecules have been shown to possess anti-osteoporotic effects without the adverse effects associated with estrogens, such as estrogen-dependent cancers, reported in experimental studies [8-9]. Previous studies showed that *P. soyauxii*, the subject of this study exhibits estrogen-like effects in a postmenopausal model [10]. Indeed, this study confirmed the ethnobotanical uses of this plant to manage uro-genital issues in women [11-15]. Actually, the aqueous extract of the plant contains phytoestrogens like pterostilben and linoleic acid, thus it reduced menopausal impairment like vaginal atrophy and metabolic syndrome. Nevertheless, effects on postmenopausal osteoporosis have not yet been evaluated. Thus, this study aimed to evaluate the anti-osteoporotic activities of *P. soyauxii* heartwood aqueous extract in a postmenopausal-like model induced by ovariectomy in rat.

2- Material and methods

2.1- P. soyauxii extraction and Ethics

The aqueous extract of *P. soyauxii* was prepared according to the protocol described by Mengue *et al.* [10]. All experiments were conducted following the principles and procedures of the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Cameroon Institutional National Ethics Committee, Ministry of Scientific Research and Technology Innovation (Reg. number FWA-IRD 0001954).

2.2- Animal material

Wistar strain female rats aged 10-12 weeks and weighing between 130-150 g were used. They were housed in plastic cages of 5 animals per cage with free access to tap water and soy-free chow. The animals were ovariectomized using a dorsal approach [9,15].

2.3- Experimental design

Thirty rats were used. Twenty-five rats were ovariectomized and the others were the sham-operated (Sham). 84 days after oophorectomy, the animals were divided into 6 groups of 5 animals each and were treated daily for 28 days as follows: the sham group received distilled water (10 mL/kg) and an Ovx animals received respectively distilled water (10 mL/kg), E₂V (1 mg/kg), and *P. soyauxii* at 100, 200 and 300 mg/kg. Before sacrifice, fasted animals were individually housed in a metabolic cage for 24 h. A urine sample was collected and acidified with 2 mL of 1 mol/L HCl. Collected urine samples were used for the determination of creatinine, calcium, inorganic phosphorus, magnesium. Thus, some osteolysis indexes (Urinary-calcium/Urinary-creatinine; Urinary-magnesium/Urinary-creatinine) were calculated. Vaginal smears were also carried out. Arteriovenous blood was collected both in EDTA tubes for blood count and in dry tests tubes for centrifugation (3500rpm for 15 min). Calcium, magnesium, creatinine, inorganic phosphorus (IP) levels, and alkaline phosphatase activity (ALP) were assessed in serum. Femur and Tibia were collected and weighed. 0.2 g of the head of femur was homogenized in 2 mL of phosphate buffered saline. The homogenate obtained was centrifuged (3500 rpm for 30 min) and the supernatant was used to determine some biochemical bone markers such as calcium, magnesium, inorganic phosphorus, ALP activity as well as femur oxidative status (MDA, GSH, and Nitrites). At last, histopathological analysis of heads of femur and tibia were carried on paraffin-embedded sections stained with hematoxylin-eosin.

2.4- Determination of the relative mass and femoral density

2.4.1- Determination of the relative mass of the femur and tibia

Femur and tibia relative fresh weight were calculated respectively using the formula bellow according to Akhtar *et al.* [16].

$$\text{Organ weight ratio} = \frac{\text{Femur/Tibia weight (g)}}{\text{Body weight (g)}} \times 100$$

2.4.2- Determination of femoral density

Wet femur volume was measured using a plethysmometer and its density was calculated using the formula as described by Lee *et al.* [17]:

$$\text{Femoral density} = [\text{femur wet weight (kg)} \times 1000 (\text{kg/mm}^3) / \text{volume of femur (mm}^3)]$$

2.5- Biochemical assays

ALP activity like creatinine and IP levels, were assessed using commercial diagnostic kits LABKIT. Calcium and magnesium levels were assessed using commercial diagnostic kits Biolabo and Randox respectively.

2.6- Oxidative stress parameters assays

Malondialdehyde (MDA) and reduced glutathione (GSH) in femur homogenate were determined using methods described by Wilbur *et al.* [18] and Ellman [19] respectively while the nitrites content was determined using the method described by Green *et al.* [20].

2.7- Vaginal smears and cell differentiation

Using a micropipette, 10 μ L of a 0.9% NaCl solution was introduced into the vagina of each rat and then aspirated with a bulb. The collected sample was placed on a slide and fixed in increasing baths of 50%, 70% and 80% alcohol. The slides were stained according to the method described by Papanicolaou [21].

2.8- Histopathological analysis.

After fixation of the femur and tibia in 10% buffered formalin, the organs were streamed in 3 xylene baths (10 min per bath) and then dehydrated in alcohol of croissant gradient (70%, 95%, and 100% (3 baths)). Tissues were then clarified in two xylene baths and embedded in liquid paraffin at 60°C for 4 hours. A 5 μ m section of each organ was cut with a microtome, deparaffinized, and stained with hematoxylin-eosin. The microphotographies were obtained using a light microscope (Leitz wetzlar Germany 513) connected with a celestron 44421 camera linked to a computer.

2.9. Statistical analysis

Data were stated as mean \pm standard error on mean. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test using GraphPad Prism 8.0.1. A value of $p < 0.05$ was considered statistically significant.

3- Results

3.1- Effects of *P. soyauxii* on relative mass of femur and tibia and on total femoral protein level

As shown by figure 1, ovariectomy resulted in a significant decrease in relative femur mass ($p < 0.01$) as well as femoral protein level ($p < 0.001$) compared to Sham control. Administration of the *P. soyauxii* extract at 200 and 300 mg/kg significantly ($p < 0.05$) increased relative femur mass compared to Ovx animals. Moreover, treatment with 200 and 300 mg/kg significantly increased femoral protein levels by $p < 0.01$ and $p < 0.001$, respectively, compared to Ovx animals. Besides, ovariectomy and plant extract have no effects on tibia weight.

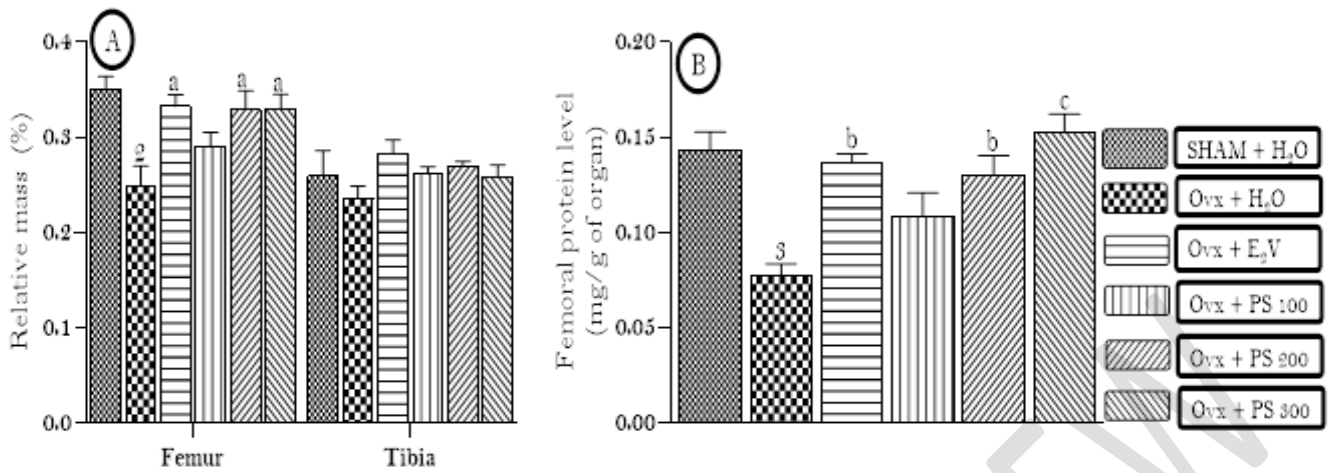


Figure 1: Effects of *Pterocarpus soyauxii* on femur and tibia relative weight (A) and femoral protein level (B). ¹p <0.05; ²p <0.01; ³p <0.001, significant difference compared to Sham control; ^ap <0.05; ^bp <0.01; ^cp <0.001, significant difference compared to Ovx control; PS = *P. soyauxii*.

3.2- Effects of *P. soyauxii* on femur density

The 84-day after Ovariectomy resulted in a significant decrease in femoral density (p < 0.001) compared to Sham control (Figure 2). Administration of *P. soyauxii* significantly increased femoral density at 200 (p < 0.001) and 300 (p < 0.01) mg/kg compared to Ovx animals.

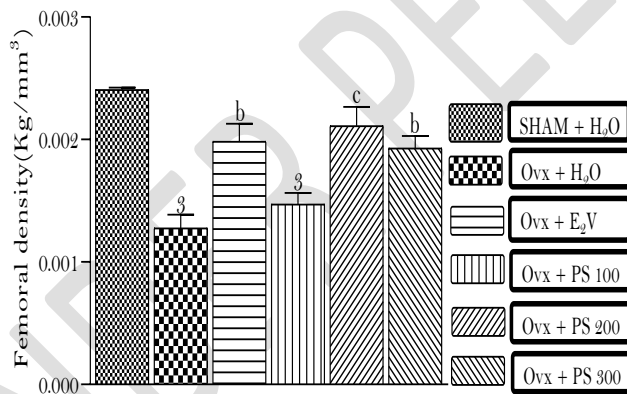


Figure 2: Effects of *P. soyauxii* on femur density.

¹p <0.05; ²p <0.01; ³p <0.001, significant difference compared to Sham control; ^ap <0.05; ^bp <0.01; ^cp <0.001, significant difference compared to Ovx control; PS = *P. soyauxii*.

3.3- Effects of *P. soyauxii* heartwood on some blood elements

The effects of *P. soyauxii* aqueous extract on some blood components are shown in table 1. It shows that ovariectomy resulted in a significant (p < 0.01) increase in white blood cell (WBC) and a non-significant

increase in monocytes and lymphocytes compared to Sham control. The administration *P. soyauxii* extract at

Parameters Groups	WBC ($10^9/L$)	RBC ($10^{12}/L$)	HGB (g/dL)	HCT (%)	LYM ($10^9/L$)	MO ($10^9/L$)	200 mg/kg
SHAM + H ₂ O	6.43 ± 0.73	8.26 ± 0.22	13.95 ± 0.26	45.00 ± 0.77	6.79 ± 1.28	0.18 ± 0.01	reduce
Ovx + H ₂ O	10.14 ± 0.71 ²	8.21 ± 0.22	13.57 ± 0.35	43.62 ± 0.88	8.30 ± 0.76	0.24 ± 0.01	
Ovx + E ₂ V	6.73 ± 0.60 ^a	8.73 ± 0.06	14.12 ± 0.17	46.47 ± 0.33	7.36 ± 1.36	0.18 ± 0.02	d
Ovx + PS 100	8.05 ± 0.70	8.81 ± 0.35	14.07 ± 0.48	47.27 ± 1.73	7.13 ± 0.21	0.20 ± 0.02	
Ovx + PS 200	8.75 ± 0.40	8.83 ± 0.08	14.65 ± 0.16	48.05 ± 0.45	7.91 ± 0.28	0.17 ± 0.02	WBC
Ovx + PS 300	9.45 ± 0.79 ¹	8.77 ± 0.17	14.52 ± 0.33	47.67 ± 1.35	7.60 ± 0.63	0.17 ± 0.02	

by 86.29 % compared to Ovx control.

Table 1: Effects of *P. soyauxii* on some blood constituents

Values represents mean ± SEM (n = 5); ¹p < 0.05; ²p < 0.01, significant difference compared to Sham-operated control; ^ap < 0.05, significant difference compared to Ovx control; **PS** = *P. soyauxii*, **WBC** = White blood cells; **LYM** = Lymphocytes; **RBC** = Red blood cells; **HGB** = Hemoglobin; **HCT** = Hematocrit; **MO** = Monocytes.

3.4- Effects *P. soyauxii* on seric, urinary, and femoral levels of calcium, phosphorus, and magnesium

Table 2 shows that a 112-day of ovariectomy induced a decrease of calcium levels in both seric and femoral homogenate (p < 0.001) in one hand and a significant increase (p < 0.001) in calcium urinary levels in other hand compared to sham control. While the administration of *P. soyauxii* extract at 100 mg/kg increased significantly (p < 0.05) femoral calcium level and decreased likewise the urinary one. The treatment of ovariectomized rats with the aqueous extract of *P. soyauxii* at 200 mg/kg and 300 mg/kg compared to Ovx control, increased significantly (p < 0.05) seric IP, levels while only the extract at 300 mg/kg increased phosphorus levels in the femur.

Table 2: Effects of aqueous extract of *P. soyauxii* heartwood on serum, urine and bone levels of calcium, phosphorus and magnesium

Parameters Groups	Calcium (Mmol/L)			Phosphorus (Mmol/L)		Magnesium (Mmol/L)		
	Serum	Femur	Urine	Serum	Femur	Serum	Femur	Urine
SHAM + H ₂ O	2.23 ± 0.05	3.18 ± 0.33	7.72 ± 0.34	2.73 ± 0.02	10.74 ± 0.25	0.45 ± 0.01	0.32 ± 0.05	2.32 ± 0.10
Ovx + H ₂ O	1.82 ± 0.04 ³	1.88 ± 0.23 ¹	11.07 ± 0.13 ³	2.44 ± 0.05 ²	8.89 ± 0.57 ¹	0.44 ± 0.00	0.20 ± 0.01	2.68 ± 0.23
Ovx + E ₂ V	2.13 ± 0.04 ^a	2.61 ± 0.27	8.33 ± 0.28 ^b	2.69 ± 0.07 ^b	10.54 ± 0.20 ^a	0.45 ± 0.01	0.32 ± 0.05	2.14 ± 0.10
Ovx + PS 100	1.96 ± 0.06 ¹	3.09 ± 0.22 ^a	9.17 ± 0.78 ^a	2.63 ± 0.02	9.73 ± 0.27	0.47 ± 0.00	0.34 ± 0.04	2.25 ± 0.10
Ovx + PS 200	2.09 ± 0.01 ^a	2.94 ± 0.24	8.71 ± 0.23 ^b	2.73 ± 0.01 ^b	10.40 ± 0.49	0.50 ± 0.02	0.25 ± 0.04	2.47 ± 0.17
Ovx + PS 300	1.93 ± 0.04 ¹	2.33 ± 0.24	8.94 ± 0.48 ^a	2.69 ± 0.03 ^b	10.62 ± 0.13 ^a	0.45 ± 0.03	0.26 ± 0.04	2.51 ± 0.49

Values represents mean ± SEM (n = 5); ¹p < 0.05; ²p < 0.01; ³p < 0.001, significant difference compared to Sham-operated control; ^ap < 0.05; ^bp < 0.01; ^cp < 0.001, significant difference compared to Ovx control; **PS** = *P. soyauxii*.

3.5- Effects of *P. soyauxii* on ALP activity, creatinine levels and some osteolysis indices

The table 3 shows the effects of treatment of Ovx rats with aqueous extract of *P. soyauxii* heartwood on femoral and seric ALP activity, seric and urinary creatinine levels and some osteolysis indices. Ovariectomy resulted in a significant ($p < 0.001$) decrease in femoral ALP activity and increase seric ALP activity compared with Sham-operated rats. There was also a significant ($p < 0.01$) increase in the urinary calcium to urinary creatinine ratio in Ovx after 112 days. The administration of *P. soyauxii* at 200 mg/kg significantly reduced seric ALP activity ($p < 0.05$) although it increased significantly ($p < 0.01$) this parameter in femur compared to Ovx control.

Table 3: Effects of *P. soyauxii* on serum and bone PAL activity, urinary creatinine and some osteolysis indices

Parameters Groups	Creatinine (mg/dL)		ALP activity (IU/L)		Uri-Ca ²⁺ /Uri-Crea	Uri-Mg ²⁺ /Uri-Crea
	Serum	Urine	Femur	Serum		
SHAM + H ₂ O	1.02 ± 0.03	15.19 ± 0.19	545.66 ± 8.41	232.05 ± 6.56	0.50 ± 0.02	0.15 ± 0.01
Ovx + H ₂ O	0.99 ± 0.02	16.10 ± 0.54	397.24 ± 15.59 ³	185.15 ± 4.46 ³	0.69 ± 0.02 ²	0.16 ± 0.01
Ovx + E ₂ V	0.99 ± 0.02	15.40 ± 0.24	549.20 ± 16.09 ^{3c}	197.45 ± 2.65 ^c	0.54 ± 0.01 ^a	0.13 ± 0.01
Ovx + PS 100	0.98 ± 0.02	14.83 ± 0.55	398.39 ± 15.73 ³	215.12 ± 4.61 ²	0.62 ± 0.05	0.15 ± 0.03
Ovx + PS 200	0.96 ± 0.01	15.28 ± 0.10	474.71 ± 7.27 ^{1a}	206.55 ± 3.62 ^{1b}	0.57 ± 0.01	0.16 ± 0.01
Ovx + PS 300	0.95 ± 0.01	15.56 ± 0.34	454.33 ± 11.01 ²	211.32 ± 5.06 ^{2a}	0.57 ± 0.02	0.16 ± 0.03

indices

Values represents means ± SEM (n = 5); ¹p < 0.05; ²p < 0.01; ³p < 0.001, significant difference compared to Sham-operated control; ^ap < 0.05; ^bp < 0.01; ^cp < 0.001, significant difference compared to Ovx control; PS = *P. soyauxii*, ALP = Alkaline phosphatase, Uri-Crea = Urinary creatinine, Uri-Ca²⁺ = Urinary calcium, Uri-Mg²⁺ = Urinary Magnesium.

3.6- Effects of *P. soyauxii* on femoral oxidative stress status

Ovariectomy resulted in a significant decrease in GSH levels ($p < 0.01$) and a significant increase in MDA ($p < 0.01$) and nitrite ($p < 0.001$) levels in the femur. *P. soyauxii* extract at 200 and 300 mg/kg doses compared to Ovx animals significantly increased GSH levels by ($p < 0.05$) and ($p < 0.01$) respectively. At all extract doses, there was a significant ($p < 0.001$) decrease in femur nitrite level compared to Ovx animals. MDA level was significantly ($p < 0.01$) decreased following treatment with *P. soyauxii* extract at the dose of 300 mg/kg compared to Ovx female rats (Figure 3).

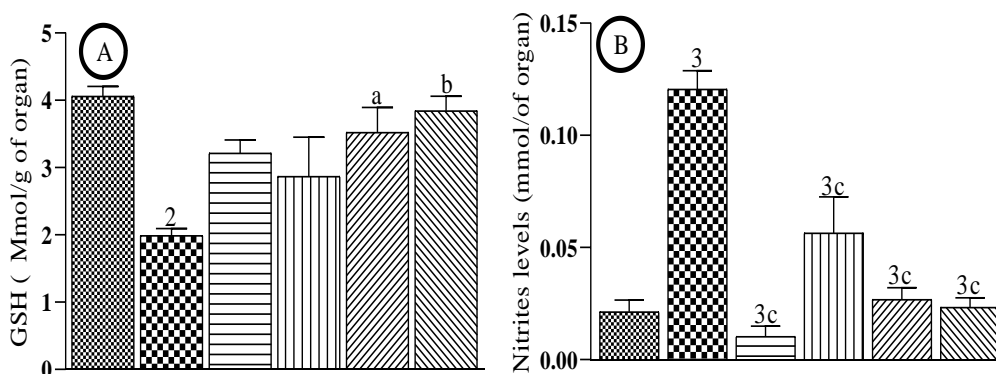
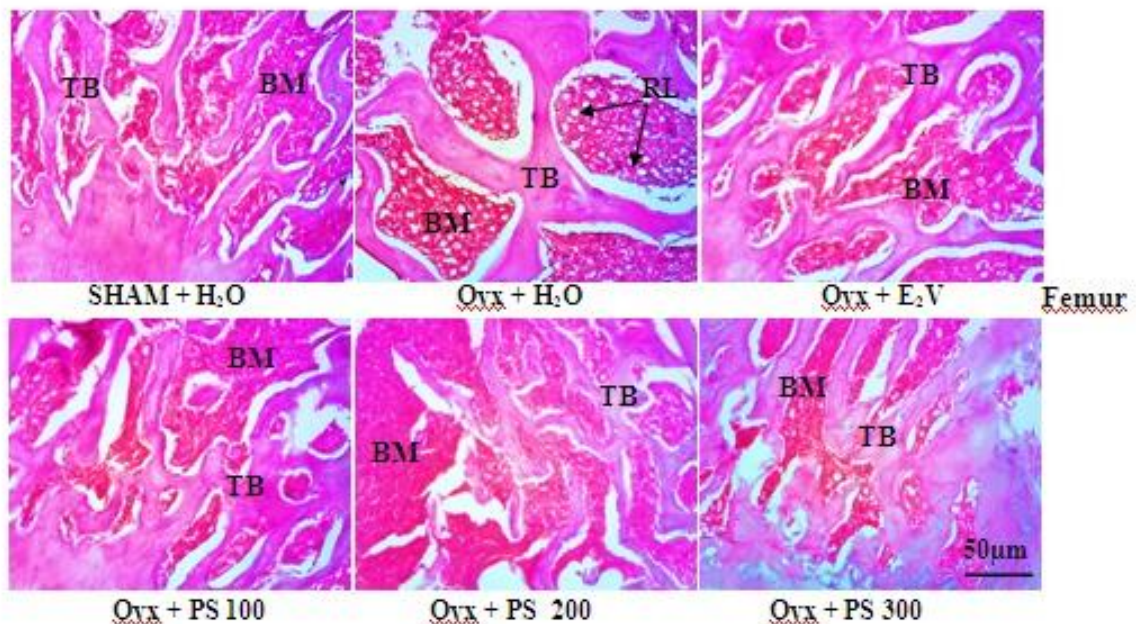


Figure 3: Effects of a 28-day treatment with *P. soyauxii* on GSH (A), nitrites (B) and MDA (C) femur levels. ¹p <0.05; ²p <0.01; ³p <0.001, significant difference compared to Sham-operated control; ^ap <0.05; ^bp <0.01; ^cp <0.001, significant difference compared to Ovx control; **PS** = *P. soyauxii*; **MDA** = Malondialdehyde; **GSH** = Reduced glutathione.

3.7- Effects on the microarchitecture of the femur and tibia

Figure 4 shows the effects of *P. soyauxii* extract on the microarchitecture of the femur and tibia in ovariectomized animals. Ovariectomy increased the number of resorption lacunae in the tibia and femur. Besides, it also induced tibia trabecular disorder. The oral extract corrected these alterations compared to Ovx animals at 200 and 300 mg/kg.



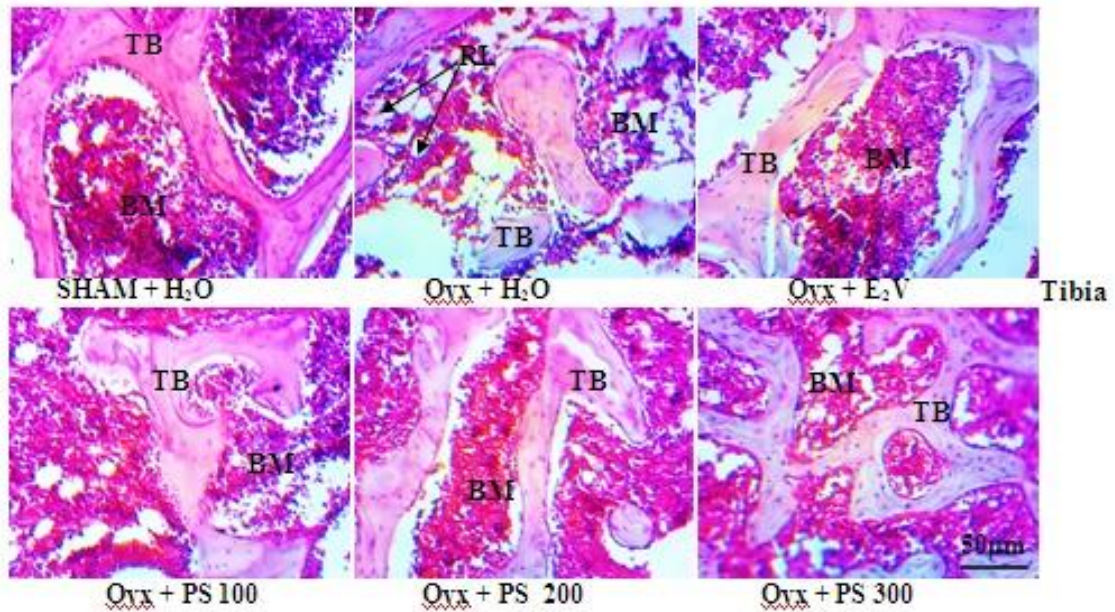


Figure 4: Microphotographs of the femur (100x, H-E) and tibia (200x, HE).
PS = *P. soyauxii*, **RL** = Resorption Lacunae, **BM** = Bone marrow, **TB** = Trabecular bone.

4- Discussion

The potential anti-osteoporotic effects of *P. soyauxii* were evaluated in a model of postmenopausal osteoporosis induced by ovariectomy in Wistar rats. Results revealed that ovariectomy decreased significantly femur relative mass and density compared to Sham-operated animals. Indeed, estrogen is known to induce osteoclast apoptosis and osteoblast proliferation. These actions increase bone matrix synthesis, mass, and density [22-24]. In addition, Braun *et al.* [25] mentioned that high oxidative stress in hypoestrogenism was linked to osteoblast death. Furthermore, Reactive oxygen species (ROS) stimulate osteoclastogenesis [26], thus they promote bone loss. Signaling pathways are consistent in the present work with the increase of ROS like malondialdehyde (MDA) in the femur. This significant increase in MDA level could be considered as an indication of oxidative stress and cell death. Indeed, the increase in MDA level is an indicator of lipid peroxidation and thus of cell death. Some studies had the same results and showed that estrogen deficiency in ovariectomized female rats resulted in decreased relative mass and density of the femur [9, 27]. In the present study, aqueous extract of *P. soyauxii* increased femur density and relative mass in Ovx rats. This result reflects an antiresorptive activity of the plant. Qualitative and quantitative phytochemical analysis revealed flavonoids (formononetin and naringenin) in the extract of the heartwood of *P. soyauxii* [15, 28]. Naringenin is reported to possess anti-osteoporotic activity in ovariectomized rats by stimulating osteoclast apoptosis and osteoblastic proliferation [29]. Besides, formononetin preserves femoral mass and density through its ability to bind to estrogen receptors located on the bone and exert antioxidant

activity to increase bone mineral density [30-32]. In addition, LCMS analysis revealed that *P. soyauxii* aqueous extract possesses linoleic acid [10]; a molecule capable of preventing bone loss by stimulating osteoprogenin (OGP) expression and inhibiting RANKL expression in ovariectomized animals according to Rahman *et al.* [8].

Measurement of bone metabolism markers plays an important role in the diagnosis and treatment of osteoporosis [33]. Bone loss in many studies is evidenced by increased calcium, phosphorus, alkaline phosphatase (ALP) activity in blood and urine, and urinary calcium/creatinine ratio [34]. Other work also explains the development of osteoporosis by a decrease in bone concentrations of calcium, phosphorus, and ALP activity [9,35]. Ovariectomy in the same sense varied seric, femoral and urinary biochemical markers of bone metabolism evaluated in the present work. Indeed, a study reported that ovariectomy modified biochemical markers of bone metabolism [9], by the way, Hewitt *et al.* [36] show that ALP modulates osteoblastic activity and is associated with bone mineralization. Furthermore, hypoestrogenism is related to bone demineralization by Braun *et al.* [25] and the results of the present study corroborate this point. Indeed, 112-day Ovx animals presented a high osteolysis index (urinary calcium/ urinary creatinine). *P. soyauxii* extract, as well as estradiol valerate, reestablished normal values of biochemical markers of bone metabolism compared to Ovx rats. Phytoestrogens are known to possess positive action on bone mineralization and osteoblastic differentiation as well as inhibition of osteoclastic activity [37-38]. Linoleic acid, a phytoestrogen identified in the heartwood aqueous extract [10] is probably responsible for osteoprotective activity by decreasing the concentration of serum biochemical markers of bone resorption in ovariectomized animals [9].

Inflammation may contribute to osteoporosis [39]. The results of the present study show in Ovx animals a significant elevation of bone nitrite; a surrogate for nitrite oxide (NO) in ovariectomized animal. Analysis of hematological parameters also revealed an increase in white blood cells count, in Ovx rats compared to sham-operated rats. Cuzzocrea [40] reported the same results and suggested that the nitrites produced following oophorectomy would come from a macrophagic activity and are a sign of bone inflammation. NO appears to exert biphasic effects by affecting the bone formation and resorption processes in osteoblasts and osteoclasts. Bone formation increased while bone resorption is suppressed at low NO concentration [41]. *P. soyauxii* extract at all doses decreased femoral nitrite level and non-significantly decreased the white blood cell level. Indeed, the work of Saliu *et al.* [42] highlighted the richness of *P. soyauxii* in kaempferol and quercetin. Kaempferol and quercetin potentially protect bone through their anti-inflammatory properties on osteoblastic cells by inhibiting nuclear translocation and NF- κ B activation [43-44]. Linoleic acid a molecule reported in *P. soyauxii* extract [10] is known to inhibit the production of inflammatory cytokines that are messengers for osteoclast recruitment and differentiation [45]; thus, explaining the anti-osteoporotic activity of *P. soyauxii* through the anti-inflammatory pathway.

In addition to inflammatory processes, oxidative stress is an important factor in the pathogenesis of osteoporosis, which is manifested in many studies by microarchitectural alterations of the femur and tibia. In the present work, analysis of oxidative status markers revealed the increase in MDA level and the decrease in GSH level in the femur of Ovx animals compared to sham-operated control; reflecting femoral oxidative stress in Ovx rats. This oxidative stress was correlated with numerous resorption lacunae on histological sections of femur and tibia. This oxidative stress in bones is thought to be related to post-ovariectomy estrogen deficiency. Many studies reported that ovariectomy caused oxidative bone stress in rats marked by an increase in MDA levels and a decrease in GSH levels but also the appearance of resorption lacunae on histological sections of the femur [9]. Indeed, ROS formed under hypo-estrogenic conditions stimulate osteoclast formation and activity, thus they decreased osteoblastic function and osteoblast recruitment, and collagen synthesis [46-47]. The administration of *P. soyauxii* extract reestablished the homeostasis of oxidative parameters in femur and prevented microarchitectural alterations in femur and tibia. Pterostilbenes contain in *P. soyauxii* extract reported to possess antioxidant activity by reducing the expression of NADPH oxidase [10,48]. This activity would explain the antioxidant effects of *P. soyauxii* on bone.

Conclusion

Hypoestrogenism induced by ovariectomy resulted in disorders of bone metabolism and microarchitecture. *P. soyauxii* extract fixed these alterations. The osteoprotective effects of *P. soyauxii* would be related to its antioxidant, estrogenic and probably anti-inflammatory activities. It would be necessary to evaluate *in vitro*, the osteoprotective signaling pathways of *P. soyauxii*.

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