Research Article

Effects of *Combretum micranthum* in Bone Regeneration and Osteoporosis

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ABSTRACT

Osteoporosis is a chronic metabolic bone disease characterized by progressive loss of bone mass and microarchitectural deterioration, resulting in greater fragility and fracture risk. This study explored the potential of *Combretum micranthum* to modulate inflammation, reduce oxidative stress, and enhance bone mineralization, all of which are beneficial for osteoporosis and fracture healing. Forty-five Wistar rats were ovariectomized, fed without treatment for 84 days; 5 rats were sham-operated. Then, twenty ovariectomized rats suffered a tibia fracture, and 5 others were sham-operated. All rats were split into 10 groups of 5 rats each and treated orally during 28 days, with distilled water, 17-β estradiol valerate at 1 mg/kg (E2V), and aqueous extract of *C. micranthum* at 100 mg/kg and 200 mg/kg. At the end of the treatment, animals were sacrificed under anesthesia. Some blood and urinary parameters have been assessed, including lipid-related indicators, oxidative status, and bone biochemical markers. Ovariectomy induced a severe metabolic imbalance, characterized by elevated serum creatinine and serum alkaline phosphatase levels, decreased femoral bone mineral density, and an increased urinary Ca^{2+}/creatinine ratio. It also induced oxidative stress and dyslipidemia, characterized by significantly elevated total cholesterol, triglycerides, and the atherogenic index, and decreased low-density lipoprotein cholesterol. Treatment with E2V and EACM extracts demonstrated efficacy, with near-complete normalization of the parameters, confirming their protective potential against bone loss and metabolic disturbances induced by ovariectomy. *C. micranthum* appears to be a promising alternative to conventional hormonal treatments, offering comprehensive protection against bone, metabolic, and cardiovascular complications induced by estrogen deficiency.

Keywords: Osteoporosis; Combretum micranthum; fracture; ovariectomy; oxidative stress.

INTRODUCTION

Osteoporosis is a chronic metabolic bone disease marked by progressive loss of bone mass and deterioration of bone microarchitecture, ultimately increasing fragility and fracture risk [1]. It is widely recognized as a major public health concern, particularly among postmenopausal women and older adults, but it also affects younger individuals with specific risk factors[2]. The socioeconomic burden of osteoporosis is substantial, given the morbidity, mortality, and healthcare costs associated with osteoporotic fractures [3].

The etiology of osteoporosis is multifactorial[4]. Estrogen deficiency, aging, exposure to chemical agents, and decreased mechanical loading are recognized risk factors [5]. Beyond these classical determinants, recent research has highlighted the pivotal roles of inflammation, oxidative stress, lipid metabolism, and mineralization in the pathogenesis of osteoporosis[6–8]. Chronic low-grade inflammation is increasingly recognized as a central mechanism in osteoporosis. Furthermore, inflammatory signaling pathways, including NF-κB and MAPK, are directly implicated in osteoclastogenesis[9]. Thus, targeting inflammation represents a promising therapeutic approach to mitigate bone loss.

Oxidative stress, defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, plays a critical role in bone metabolism[10]. Excessive ROS induces osteoblast apoptosis, impair osteogenic differentiation, and enhance osteoclast activity, thereby exacerbating bone resorption[11]. Oxidative damage to proteins and lipids within bone cells compromises their function and survival. Antioxidants, particularly those derived from natural sources such as polyphenols and flavonoids, have been shown to counteract oxidative stress and protect bone integrity [12]. Dysregulation of lipid metabolism is another important factor linking systemic metabolic disorders to osteoporosis [8]. Hyperlipidemia and the accumulation of oxidized lipids in bone marrow contribute to inflammation and oxidative stress, creating a vicious cycle that accelerates bone loss [13]. Clinical studies have demonstrated associations between elevated low-density lipoprotein (LDL) cholesterol, reduced high-density lipoprotein (HDL) cholesterol, and decreased bone mineral density. Furthermore, metabolic syndromes such as obesity and type 2 diabetes exacerbate skeletal fragility through lipid-related mechanisms [14]. Therefore, interventions that improve lipid profiles may indirectly benefit bone health.

Bone mineralization is essential for maintaining skeletal strength and resilience. Adequate levels of calcium, phosphorus, magnesium, and trace elements are required

for proper deposition of hydroxyapatite crystals within the bone matrix [10]. Impaired mineralization, whether due to nutritional deficiencies or metabolic disturbances, leads to reduced bone density and increased fracture risk [11]. In addition to supplying minerals, certain bioactive compounds can enhance calcium absorption, stimulate osteoblast differentiation, and promote collagen synthesis, thereby supporting bone formation and mineralization [12, 13].

Despite advances in pharmacological therapies, limitations such as adverse effects, high costs, and poor patient adherence have prompted the search for alternative or complementary strategies, including the use of medicinal plants. Medicinal plants have been used for centuries across diverse cultures to treat bone and joint disorders [14]. Their therapeutic potential lies in their rich composition of bioactive compounds, including polyphenols, flavonoids, saponins, alkaloids, and essential minerals [15]. These constituents exert anti-inflammatory, antioxidant, hypolipidemic, and remineralizing effects, making them particularly relevant for osteoporosis management. Several studies have demonstrated that plant-derived compounds can modulate key signaling pathways involved in bone metabolism [16]. For instance, flavonoids inhibit osteoclastogenesis by suppressing NF-κB activation, whereas polyphenols enhance osteoblast differentiation by upregulating Runx2 and Osterix [17]. Saponins and alkaloids have been reported to improve lipid metabolism, thereby reducing systemic risk factors for osteoporosis [18, 19]. Moreover, mineral-rich plants contribute directly to bone mineralization by supplying essential nutrients [20].

Given the multifactorial nature of osteoporosis, therapeutic strategies that simultaneously target inflammation, oxidative stress, lipid metabolism, and mineralization are highly desirable. Medicinal plants, with their diverse array of bioactive compounds, are uniquely positioned to address these interconnected mechanisms [14]. *Combretum micranthum* has several effects, including anti-inflammatory, antioxidative, and even used in lipid metabolism [21–24]. This plant is used by traditional practitioners in Burkina Faso to fight against osteoporosis and related diseases [25]. The present study aims to evaluate the effects of *Combretum micranthum* on experimental models of osteoporosis. The investigation aims to elucidate how this plant modulates inflammatory responses, mitigates oxidative stress, improves lipid metabolism, and enhances bone mineralization, all of which are beneficial for osteoporosis and fracture healing.

1. MATERIALS AND METHODS

1.1. Chemicals and reagents

All chemicals and reagents utilized in the current study were sourced from Sigma-Aldrich, based in Louis, Missouri, USA. 17-β Estradiol valerate is available under the brand Progynova® in a 2 mg dosage form, and Diazepam (Valium®) is available in a 10 mg dosage form. They were purchased from DELPHRAM, Lille, France, and used as a reference compound to assess the estrogenic effects on bone.

1.2. Plant material and extraction

The leaves of *C. micranthum* were collected at 100 kilometers in the Koulsé region of Burkina Faso in the morning before noon. After being thoroughly washed with water, they were dried in a well-ventilated area, protected from dust and sunlight. A sample was collected, identified, and preserved in the herbarium of Université Joseph KI-ZERBO in Burkina Faso, where it was registered under reference number 7003. Extraction was performed according to the method described by Handa et al. (2008) and adapted by Ouedraogo et al. (2025).

1.3. Animals, modeling techniques, and treatment

All experiments were conducted in accordance with the European Union Animal Care and Use (CEE Council 86/609) guidelines, as adopted by the Cameroon National Welfare Ethics Committee (Reg. No. FWA-IRD00001954). Fifty 3-month-old female Wistar rats (body weight [bw]:183±10 g) were obtained from the University of Yaounde I (Cameroon) for the current investigation. The rats were kept in numerous conventional rat cages (545 x 395 x 200 mm) on a cage rack at 22˚C (air-conditioned) with a 12-hour light/dark cycle. Rats had unlimited access to water and food without estrogen during the treatment. The diet composition was corn (36.6%), bone flour (14.5%), wheat (36.5%), fish flour (4.8%), crushed palm kernel (7.3%), sodium chloride (0.3%), and vitamin complex (Olivitazol, 0,01%). Rats were given a week to get used to their surroundings. Forty-five rats were ovariectomized (OVX), fed without treatment for 84 days; 5 rats were sham-operated (SHAM-U). Then, 5 rats were sham-operated (SHAM-F), and twenty rats suffered a tibia fracture (OVX-F). These rats were randomly divided into 10 groups (5 per group) and treated orally once daily for 28 days. Controls: SHAM-U and SHAM-F (sham-operated, unfractured or fractured), both receiving 10 mL/kg distilled water. OVX + water: OVX-U and OVX-F (ovariectomized, unfractured or fractured) treated with distilled water. OVX + estradiol: E2V-U and E2V-F treated with 17-β estradiol valerate at 1 mg/kg. OVX + extract (100 mg/kg): AECMU-100 (unfractured) and AECMF-100 (fractured). OVX + extract (200 mg/kg): AECMU-200 (unfractured) and AECMF-200 (fractured). The animals were weighed weekly during the treatment period. Twenty-four hours before sacrifice, the animals were placed in metabolic cages to collect their urine and were deprived of food.

1.4. Ovariectomy and fracture model

Rats underwent a bilateral ovariectomy via a back incision [27], general anesthesia, and an intraperitoneal injection of a dose of 10 mg/kg of diazepam (Valium) at a concentration of 5 mg/mL, followed by an injection of 50 mg/kg of ketamine at a concentration of 10 mg/mL with sodium pentobarbital (1.5%, 50 mg/kg). The ovaries were exposed after about 1.5 cm of skin, muscle, and abdominal cavity were removed. A silk thread was used to ligate the oviduct, and a bilateral ovariectomy was carried out. The remaining ten animals had a sham procedure, in which both ovaries

were inspected and put back in their original positions using the same procedure. A unilateral cross-tibial fracture was established in the proximal right tibia 84 days after the ovariectomy, according to Handool et al. (2018)[28], and it was treated under anesthesia using intramedullary nailing (diameter, 1 mm, length, 50 mm).

1.5. Sacrifice and sample collection

At the end of treatment, animals were sacrificed under anesthesia. Blood was taken by cardiac puncture, a part was mixed with an anticoagulant, and agitated for 20 minutes at 3,000 rpm to extract the blood plasma. Before analysis, the collected blood plasma was stored at -15˚C.

Some blood variables (Alkaline phosphatase (ALP) activity, creatinine, inorganic phosphorus, and calcium) were recorded. Two milliliters of 1 mol/L HCl were used to acidify a urine sample. Calcium, phosphorus, creatinine, and osteolysis index (urine-calcium/urine-creatinine) were measured using collected urine samples. The femur and tibia were removed and cleaned of all soft tissues for the histological section. Two milliliters of phosphate-buffered saline were used to homogenize 0.4 g of each organ. After centrifuging the homogenate for 30 minutes at 3500 rpm, the supernatant was used to assess the oxidative status of the femur (malondialdehyde, glutathione, catalase, and superoxide dismutase) and biochemical bone indicators, including calcium, phosphorus, and ALP activity.

1.6. Biochemical analysis and histology

The commercial diagnostic kits LABKIT were used to measure creatinine, calcium, and phosphorus levels, and the BIOLABO kit was used to measure ALP activity. The femur homogenate's levels of malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) were assessed using techniques outlined by Wilbur, Bernhein, and Shapiro (1949) [29], Misra and Fridovich (1972) [30], and Ellman (1959) [31], respectively; the catalase (CAT) content was assessed using the technique outlined by Sinha (1972) [32]. The femurs were fixed in 10% buffered formalin, then treated in three xylene baths for 10 minutes each, and then dehydrated in alcohol gradients (70%, 95%, and 100% (3 baths)). They were then embedded in liquid paraffin at 60°C for four hours after being cleared in two xylene baths. The organ was sliced into a 5 µm piece using a microtome, deparaffinized, and stained with Masson's Trichrome as described by Owona et al. (2021) [33]. A light microscope (Leitz Wetzlar, Germany 513) coupled to a computer-connected Celestron 44421 camera was used to capture the microphotographs.

1.7. Statistical analysis

The statistical analyses were carried out using GraphPad Prism software version 9.5.1. Data are presented as mean ± standard deviation. After testing normality and homogeneity of variance, differences between the groups were evaluated using one-way analysis of variance followed by the Tukey post hoc test. A statistically significant difference was defined as $p < 0.05$.

2. RESULTS

2.1. Effects of *Combretum micranthum* aqueous extract on specific blood parameters in female rats

Ovariectomy (OVX) induced significant changes in blood parameters (Table 1). Treatment with estradiol valerate (E2V) and *Combretum micranthum* extract (AECM) corrected these abnormalities. In OVX animals without fractures (OVX-U) or with fractures (OVX-F), pronounced leukocytosis (15-17 × 10^3/µL) was observed, associated with lymphocytosis (6-8 × 10^3/µL) and monocytosis (≈4 × 10^3/µL), compared to SHAM controls (7-8 × 10^3/µL for WBC; 2-2.5 × 10^3/µL for LYM; 0.5 × 10^3/µL for MON). At the same time, significant decreases ($p < 0.001$) in red blood cell count, hemoglobin, and hematocrit were observed. Treatment with E2V significantly restored all hematological parameters to levels comparable to those of the SHAM control. Similarly, administration of AECM (100 and 200 mg/kg) normalized leukocyte counts and corrected anemia, with sometimes more marked improvements ($p < 0.001$) in hemoglobin and hematocrit (HGB ≈15.8 g/dL with AECM-100). Both treatments reduced hyperlymphocytosis and monocytosis ($p < 0.001$).

Table 1: Effects of *Combretum micranthum* aqueous extract on specific blood parameters in female rats

	WBC 10^3/µL)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	LYM (10^3/µL)	MON (10^3/µL)
SHAM-U	7.16±0.09	8.30±0.07	14.06±0.15	41.23±0.52	2.40±0.14	0.46±0.05
OVX-U	14.81±0.11^{3}	7.35±0.09^{3}	11.76±0.11^{3}	36.04±0.28^{3}	6.22±0.19^{3c}	4.16±0.15^{3}
E2V-U	7.71±0.07^{3c}	9.04±0.06^{3c}	14.26±0.15^{3c}	45.48±0.25^{3c}	2.2±0.10^{c}	0.46±0.05^{c}
AECMU-100	7.42±0.13^{3c}	8.84±0.05^{3c}	15.82±0.19^{3c}	43.6±0.6^{2c}	2.86±0.15^{3c}	0.64±0.05^{3c}
AECMU-200	7.78±0.12^{3c}	8.73±0.08^{3c}	14.62±0.13^{c}	43.24±0.48^{1c}	2.71±0.02^{2c}	0.76±0.05^{3c}
SHAM-F	8.16±0.09	8.30±0.07	15.06±0.15	41.23±0.52	2.40±0.14^{c}	0.46±0.05
OVX-F	16.81±0.11^{3}	7.35±0.09^{3}	11.76±0.11^{3}	36.04±0.28^{3}	8.22±0.19^{3c}	4.16±0.15^{3}
E2V-F	8.71±0.07^{3c}	9.04±0.06^{3c}	14.26±0.15^{3c}	45.48±0.25^{3c}	2.2±0.10^{3c}	0.46±0.05^{c}
AECMF-100	9.14±0.13^{3c}	7.99±0.07^{3c}	14.88±0.13^{c}	40.34±0.23^{3c}	3.54±0.17^{3c}	0.66±0.05^{3c}
AECMF-200	8.06±0.17^{c}	7.65±0.02^{3c}	14.48±0.18^{3c}	44.94±1.10^{3c}	2.36±0.19^{3c}	1.12±0.27^{3c}

Means ± SD; n = 5/group; $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, versus sham-operated + distilled water; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, versus OVX + distilled water; SHAM-U, SHAM-F = unfractured and fractured sham-operated controls receiving distilled water; OVX-U, OVX-F = unfractured and fractured negative controls receiving distilled water, E2V-U, E2V-F = unfractured and fractured positive controls receiving E2V at a dose of 1 mg/kg. AECMU-100,

AECMU-200, AECMF-100, AECMF-200 = unfractured and fractured animals treated with aqueous extract of *C. micranthum* at doses of 100 and 200 mg/kg. WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MO = monocytes, NEU = neutrophils, LYM = lymphocytes.

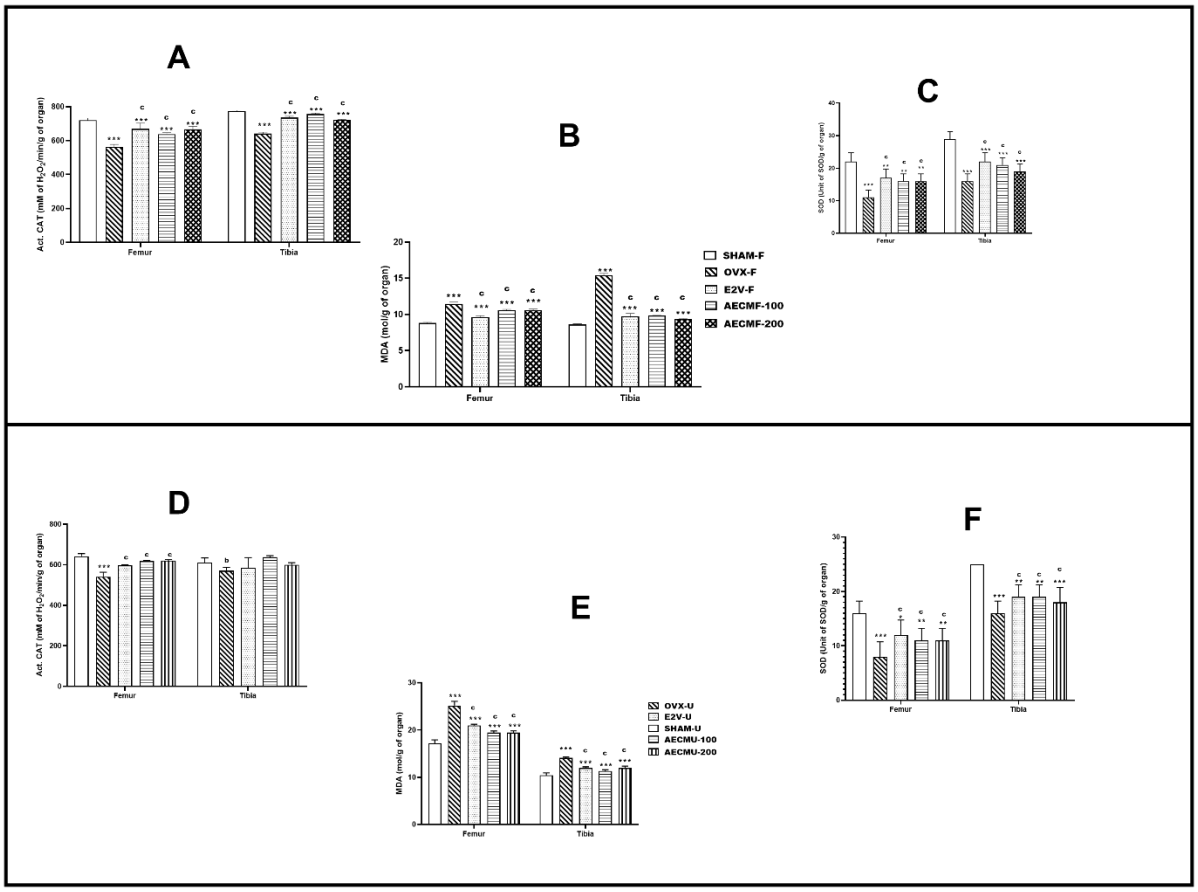

Figure 1: *Combretum micranthum* aqueous extract on oxidative stress parameters in female rats

Means ± SD; n = 5/group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, versus sham-operated + distilled water; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0,001$, versus OVX + distilled water; SHAM-U, SHAM-F = unfractured and fractured sham-operated controls receiving distilled water; OVX-U, OVX-F = unfractured and fractured negative controls receiving distilled water, E2V-U, E2V-F = unfractured and fractured positive controls receiving E2V at a dose of 1 mg/kg. AECMU-100, AECMU-200, AECMF-100, AECMF-200 = unfractured and fractured animals treated with aqueous extract of *C. micranthum* at doses of 100 and 200 mg/kg, CAT = catalase, SOD = superoxide dismutase, MDA = malondialdehyde.

2.2. Effects of *Combretum micranthum* aqueous extract on oxidative stress parameters in female rats

Figure 1 shows the therapeutic effects of treatment with an aqueous extract of *C. micranthum* on CAT levels (A, D); MDA levels (B, E); and SOD levels (C, F) in the femur and tibia of ovariectomized rats, both with and without fractures. The results show that ovariectomy led to a significant increase ($p < 0.001$) in MDA levels in the femur and tibia compared with SHAM-U and SHAM-F animals, and to a significant decrease in the activities of catalase and superoxide dismutase. Treatment with the aqueous extract at various doses significantly reduced ($p < 0.001$) MDA levels compared to OVX-U and OVX-F animals and significantly increased the activity of catalase and superoxide dismutase.

2.3. Effects of *Combretum micranthum* aqueous extract on lipid profile in female rats

In SHAM-U and SHAM-F animals, lipid parameters were within normal physiological ranges, with moderate total cholesterol (TC), low triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and a low atherogenic index (AI) (approximately 2.1) (Table 2). Ovariectomy (OVX) induced dyslipidemia characterized by a significant increase ($p < 0.001$) in TC (53% in OVX-U; 17% in OVX-F), TG (53% in OVX-U; 52% in OVX-F), LDL-C (150% in OVX-U; 89% in OVX-F), and VLDL-C (53% in OVX-U; 52% in OVX-F), associated with a significant decrease ($p < 0.001$) in HDL-C (12% in OVX-U; 29% in OVX-F) (Table 2). Atherogenic index (AI) was significantly increased (75% in OVX-U; 64% in factured animals), reflecting an atherogenic profile. Treatment with E2V partially corrected these abnormalities, reducing TC (29% vs. OVX in the OVX-U group; 7% vs. OVX in OVX-F), TG (23% vs. OVX in both OVX-U and OVX-F groups), LDL-C (51% vs. OVX in OVX-U; 31% in OVX-F), and VLDL-C (23% vs OVX in both cases). HDL-C was restored (+12% vs OVX in OVX-U; +32% in OVX-F), and the AI was significantly lowered ($p < 0.01$) to values close to control (approximately 2.3). AECM extracts (100 and 200 mg) demonstrated dose-dependent efficacy. At 100 mg of extract, TC, TG, LDL-C, and VLDL-C levels were significantly reduced compared to OVX, with an improvement in HDL-C (+7% in OVX-U and +33% in OVX-F). At 200 mg, lipid profiles were even closer to SHAM values,

with normalized TC (approximately 105 mg/dL), reduced TG (approximately 92 mg/dL), restored HDL-C (approximately 45 mg/dL), reduced LDL-C (approximately 40 mg/dL), and an A/I ratio stabilized around 2.3 (Table 2).

2.4. Effects of *Combretum micranthum* aqueous extract on creatinine, alkaline phosphatase, and calcium creatinine ratio in female rats

In the SHAM-U and SHAM-F animals, serum and urinary creatinine concentrations were stable, with elevated PAL activity in serum and femoral tissue, and a moderate urinary Ca^{2+}/creatinine ratio (Table 3). Ovariectomy (OVX) resulted in both groups (OVX-F and OVX-U) in a significant increase ($p < 0.001$) in serum creatinine (84% in OVX-U; 125% in OVX-F) and serum PAL activity (44% in OVX-U; 25% in OVX-F), associated with a decrease ($p < 0.001$) in urinary creatinine (17% in OVX-U; 27% in OVX-F) and femoral PAL activity (41% in OVX-U; 33% in OVX-F). The urinary Ca^{2+}/creatinine ratio nearly doubled (approximately 97%), reflecting increased calcium loss relative to renal function. Treatment with E2V normalized serum creatinine (approximately 0.65-0.69 mg/dL), reduced serum PAL activity (3% of SHAM in OVX-U; +3% vs. SHAM in OVX-F), and improved femoral PAL activity (38% vs. OVX in OVX-U; 33% vs. OVX in F). The urinary Ca^{2+}/creatinine ratio was reduced by 30-31% compared to OVX, indicating a partial improvement in calcium metabolism. AECM extracts (100 and 200 mg) demonstrated efficacy. At 100 mg, serum creatinine was slightly elevated (19 - 49% vs. SHAM), but femoral PAL activity was restored (-7 to +5% vs. SHAM). The urinary Ca^{2+}/creatinine ratio was normalized (-51% vs. OVX in U; -41% vs. OVX in F). At 200 mg, serum creatinine was close to control levels, femoral PAL activity was restored (-6 to +9% vs. SHAM), and the urinary Ca^{2+}/creatinine ratio was reduced from 44% to 42% compared to OVX (Table 3).

Table 2: Effects of *Combretum micranthum* aqueous extract on lipid profile in female rats

	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	AI
SHAM-U	99.25±0.70	82.94±1.25	47.29±0.43	32.10±0.59	16.58±0.25	2.09±0.02
OVX-U	152.03±0.96^{3}	127.09±0.47^{3}	41.49±0.15^{3}	80.13±0.77^{3}	25.41±0.09^{3}	3.66±0.02^{3}
E2V-U	107.53±4.21^{3c}	97.79±0.99^{3c}	46.55±0.35^{c}	39.47±3.24^{3c}	19.55±0.19^{3c}	2.30±0.08^{3c}
AECMU-100	105.36±0.99^{3c}	94.84±0.55^{3c}	44.13±0.15^{3c}	40.15±0.91^{3c}	18.96±0.11^{3c}	2.38±0.02^{3c}
AECMU-200	105.74±0.25^{3c}	91.63±1.05^{3c}	44.59±0.24^{3c}	39.99±0.34^{3c}	18.32±0.21^{3c}	2.37±0.01^{3c}
SHAM-F	102.59±0.77	83.34±1.11	50.45±0.25	31.61±0.70	16.66±0.22	2.03±0.01
OVX-F	119.53±0.38^{3}	126.95±0.29^{3}	35.91±0.35^{3}	59.71±0.57^{3}	25.39±0.05^{3}	3.32±0.03^{3}
E2V-F	110.83±0.52^{3c}	97.92±1.01^{3c}	47.47±0.55^{3c}	41.19±0.58^{3c}	19.58±0.20^{3c}	2.33±0.02^{3c}
AECMF-100	105.46±0.38^{3c}	90.96±1.05^{3c}	47.81±0.25^{3c}	36.55±0.54^{3c}	18.19±0.21^{3c}	2.20±0.01^{3c}
AECMF-200	104.44±0.96^{3c}	91.90±0.76^{3c}	44.08±0.15^{3c}	39.47±0.82^{3c}	18.38±0.15^{3c}	2.36±0.02^{3c}

Means ± SD ; n = 5/group; $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, versus sham-operated + distilled water; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, versus OVX + distilled water; SHAM-U, SHAM-F = unfractured and fractured sham-operated controls receiving distilled water; OVX-U, OVX-F = unfractured and fractured negative controls receiving distilled water, E2V-U, E2V-F = unfractured and fractured positive controls receiving E2V at a dose of 1 mg/kg. AECMU-100, AECMU-200, AECMF-100, AECMF-200 = unfractured and fractured animals treated with aqueous extract of *C. micranthum* at doses of 100 and 200 mg/kg. AI = Atherogenic Index, TC = Total cholesterol, TG = Triglycerides, LDL-C = Low-density lipoprotein cholesterol, HDL-C = High-density lipoprotein cholesterol, VLDL-C = Very low-density lipoprotein cholesterol.

Table 3: Effects of *Combretum micranthum* aqueous extract on creatinine, alkaline phosphatase, and calcium creatinine ratio in female rats

	Creatinine (mg/dL)		PAL (UI/L)		Ca^{2+}_{uri}/Crea $_{uri}$
	Serum	Urinary	Serum	Femur	
SHAM-U	0.63±0.01	1.31±0.02	279.99±5.76	350.74±5.76	60.11±0.41
OVX-U	1.16±0.05^{3}	1.09±0.01^{3}	402.29±8.45^{3}	208.22±2.26^{3}	117.91±0.74^{3}
E2V-U	0.69±0.01^{1c}	1.24±0.02^{1c}	272.91±9.45^{c}	288.07±6.18^{3c}	81.69±2.84^{3c}
AECMU-100	0.75±0.01^{3c}	1.34±0.03^{c}	315.36±9.18^{3c}	327.60±4.16^{3c}	57.68±0.87^{3c}
AECMU-200	0.71±0.01^{3c}	1.30±0.06^{c}	297.17±4.22^{2c}	330.63±2.76^{3c}	66.14±3.67^{3c}
SHAM-F	0.51±0.02	1.24±0.01	317.39±2.26	312.33±4.22	57.87±0.90
OVX-F	1.15±0.01^{3}	0.90±0.02^{3}	397.24±11.07^{3}	209.23±5.76^{3}	113.94±2.09^{3}
E2V-F	0.65±0.01^{3c}	1.25±0.02^{c}	326.48±14.56^{c}	278.98±4.22^{3c}	79.17±3.26^{3c}
AECMF-100	0.76±0.01^{3c}	1.42±0.01^{3c}	305.07±2.65^{c}	326.44±2.77^{3c}	66.77±1.02^{3c}
AECMF-200	0.65±0.02^{3c}	1.42±0.03^{3c}	303.24±3.57^{c}	339.62±10.95^{3c}	65.83±1.73^{3c}

Means ± SD ; n = 5/group; $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, versus sham-operated + distilled water; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, versus OVX + distilled water; SHAM-U, SHAM-F = unfractured and fractured sham-operated controls receiving distilled water; OVX-U, OVX-F = unfractured and fractured negative controls receiving distilled water, E2V-U, E2V-F = unfractured and fractured positive controls receiving E2V at a dose of 1 mg/kg. AECMU-100, AECMU-200, AECMF-100, AECMF-200 = unfractured and fractured animals treated with aqueous extract of *C. micranthum* at doses of 100 and 200 mg/kg.

Table 4: Effects of *Combretum micranthum* aqueous extract on serum, femoral, and urinary calcium and phosphorus in female rats

	Calcium (mmol/L)			Phosphorus (mmol/L)		
	Serum	**Femur**	**Urinary**	**Serum**	**Femur**	**Urinary**
SHAM-U	94.44±0.65	121.60±0.65	79.21±1.21	2.32±0.01	5.27±0.08	4.00±0.04
OVX-U	50.74±0.81^{3}	64.15±0.30^{3}	128.64±0.76^{3}	3.39±0.02^{3}	3.14±0.01^{3}	5.14±0.07^{3}
E2V-U	59.58±0.47^{3c}	91.15±0.92^{3c}	86.58±0.67^{3c}	2.73±0.01^{3c}	4.39±0.01^{3c}	4.01±0.02^{c}
AECMU-100	84.36±0.32^{3c}	99.09±0.61^{3c}	77.36±1.21^{1c}	2.43±0.01^{3c}	5.29±0.09^{c}	4.75±0.15^{3c}
AECMU-200	87.40±0.99^{3c}	103.20±1.10^{3c}	85.92±0.73^{3c}	2.44±0.01^{3c}	5.27±0.07^{c}	4.28±0.01
SHAM-F	90.61±0.47	119.67±1.03	71.81±0.65	2.06±0.03	5.95±0.03	3.88±0.04
OVX-F	54.60±1.12^{3}	81.27±0.65^{3}	103.33±1.13^{3}	4.04±0.02^{3}	3.58±0.01^{3}	6.96±0.04^{3}
E2V-F	58.68±0.67^{3c}	102.92±0.30^{3c}	99.42±1.08^{3c}	2.44±0.01^{3c}	5.97±0.03^{c}	3.98±0.02^{2c}
AECMF-100	91.81±0.30^{c}	111.56±0.77^{3c}	94.75±0.39^{3c}	2.39±0.03^{3c}	5.35±0.05^{3c}	4.50±0.01^{3c}
AECMF-200	97.48±0.62^{3c}	113.62±0.83^{3c}	93.53±1.23^{3c}	2.38±0.03^{3c}	5.44±0.03^{3c}	4.21±0.01^{3c}

Means ± SD; n = 5/group; $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, versus sham-operated + distilled water; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, versus OVX + distilled water; SHAM-U, SHAM-F = unfractured and fractured sham-operated controls receiving distilled water; OVX-U, OVX-F = unfractured and fractured negative controls receiving distilled water, E2V-U, E2V-F = unfractured and fractured positive controls receiving E2V at a dose of 1 mg/kg. AECMU-100, AECMU-200, AECMF-100, AECMF-200 = unfractured and fractured animals treated with aqueous extract of *C. micranthum* at doses of 100 and 200 mg/kg.

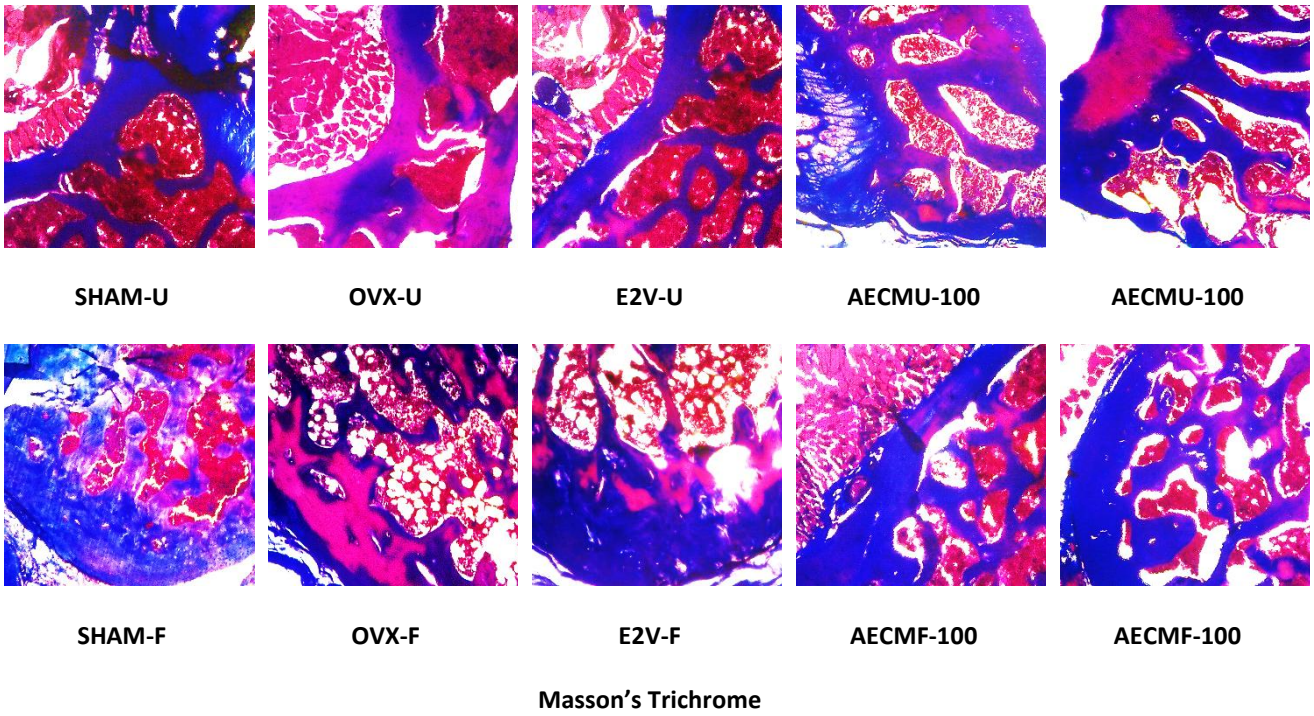

Figure 2: Effect of *Combretum micranthum* aqueous extract on rats' femur structure

SHAM-U, SHAM-F = unfractured and fractured sham-operated controls receiving distilled water; OVX-U, OVX-F = unfractured and fractured negative controls receiving distilled water; E2V-U, E2V-F = unfractured and fractured positive controls receiving E2V at a dose of 1 mg/kg. AECMU-100, AECMU-200, AECMF-100, AECMF-200 = unfractured and fractured animals treated with aqueous extract of *C. micranthum* at doses of 100 and 200 mg/kg.

2.5. Effects of *Combretum micranthum* aqueous extract on serum, femoral and urinary calcium and phosphorus in female rats

In SHAM-U animals, serum and femoral calcium and phosphorus concentrations were elevated, with moderate urinary excretion (Table 4). Ovariectomy (OVX-U) resulted in a significant decrease ($p < 0.001$) in serum calcium (46%) and femoral calcium (47%), as well as a marked increase ($p < 0.001$) in urinary calcium (62%). Serum phosphorus was increased (46%), while femoral phosphorus was reduced (40%). Treatment with E2V-U partially corrected these disturbances, reducing urinary losses and improving bone content. AECMU extracts (100 and 200 mg) effectively restored levels, with the 200 mg dose bringing values close to those of the SHAM-U group. In SHAM-F animals, mineral profiles were comparable, with slightly higher femoral phosphorus. OVX-F induced a decrease ($p < 0.001$) in serum calcium (40%) and femoral calcium (32%), an increase ($p < 0.001$) in urinary calcium (44%), as well as a very marked elevation ($p < 0.001$) in serum (96%) and urinary (79%)

phosphorus. Treatment with E2V-F partially corrected these abnormalities, notably normalizing urinary phosphorus. AECMF extracts (100 and 200 mg) demonstrated plant efficacy, with the 200 mg dose restoring serum calcium to levels above the SHAM-F group (+8%) and femoral phosphorus to near-normal levels (-9%).

2.6. Effect of *Combretum micranthum* on rats' femur structure

The histological analysis of the femur reveals an increase in the number of resorption defects in the OVX-F and OVX-U animals, accompanied by a reduction in collagen density compared to the SHAM-F and SHAM-U animals. Treatment with estradiol and extract at various dosages corrected these abnormalities, revealing distinctly identifiable bone trabeculae, stained blue, reflecting the prevalence of type I collagen in the bone matrix. Osteocytes can be observed within their lacunae, where their dark nuclei are embedded in the matrix. At the periphery of the trabeculae, osteoblasts are positioned at the margins, possessing reddish cytoplasm indicative of bone-forming activity (Figure 2).

DISCUSSION

Osteoporosis is a chronic metabolic bone disease characterized by progressive loss of bone mass and deterioration of bone microarchitecture, ultimately leading to increased fragility and susceptibility to fractures [1]. Osteoporosis is a multifactorial disease [4]. The present study investigated the effects of *Combretum micranthum* extract on osteoporosis, focusing on four interconnected mechanisms: inflammation, oxidative stress, lipid metabolism, and bone mineralization. Our findings provide evidence that *C. micranthum* extract exerts protective effects on bone health through multiple pathways, highlighting its potential as a complementary therapeutic agent. One of the most notable findings was its ability to reduce inflammatory responses by lowering monocyte and lymphocyte levels. Monocytes and lymphocytes play major roles in the inflammatory response [34]. Monocytes secrete tumor necrosis factor (TNF-α) and pro-inflammatory cytokines, including interleukins IL-1, IL-6, and IL-10 [35]. Their reduction suggests that plants can modulate immune signaling pathways implicated in osteoclastogenesis [36]. This aligns with previous reports demonstrating that natural compounds, particularly flavonoids and polyphenols, can suppress NF-κB activation and thereby inhibit osteoclast differentiation [17]. By restoring the balance between bone resorption and formation, plants help preserve bone mass [37]. These findings underscore the importance of targeting inflammation in osteoporosis management, particularly in postmenopausal women, in whom estrogen deficiency amplifies inflammatory signaling [38]. The study also revealed that *C. micranthum* enhances antioxidant defenses, as evidenced by increased activity of enzymes such as superoxide dismutase (SOD), and catalase (CAT). Concurrently, reactive oxygen species (ROS) levels were significantly reduced, along with malondialdehyde (MDA) levels. ROS and MDA have a strong correlation [39]. This dual effect indicates that *C. micranthum* extract not only scavenges free radicals but also strengthens endogenous antioxidant systems [40]. Given that oxidative stress accelerates osteoblast apoptosis and promotes osteoclast activity, the observed antioxidant effects are highly relevant [41]. Our results are consistent with prior studies showing that polyphenol-rich plants can protect against oxidative damage in bone tissue [42]. Previous studies have reported the presence of polyphenols, flavonoids, and triterpenes in the aqueous extract of *C. micranthum* [25]. Importantly, the antioxidant activity may also contribute indirectly to the anti-inflammatory effects, since ROS are known to activate inflammatory pathways [39]. Another noteworthy outcome was the improvement in lipid profiles. *Combretum micranthum* extract reduced serum LDL cholesterol and triglycerides while increasing HDL cholesterol. This hypolipidemic effect is crucial, as dyslipidemia has been linked to reduced bone mineral density and increased fracture risk [43, 44]. Oxidized lipids in bone marrow stimulate inflammation and oxidative stress, creating a vicious cycle that exacerbates bone loss [45]. By improving lipid metabolism, *C. micranthum* extract may break this cycle and indirectly support bone health [46]. These findings resonate with observations that the flavonoid-rich portion of *C. micranthum* has lipid-lowering properties and can have beneficial effects on bone metabolism [46]. However, unlike synthetic drugs, medicinal plants may achieve lipid regulation with fewer side effects, making them attractive for long-term use [47]. *C. micranthum* extract also demonstrated positive effects on bone mineralization. Calcium and phosphate are essential components for bone health [48]. Deficiencies in these nutrients can lead to bone disorders and clinical diseases [49]. In the context of osteoporosis, oxidative stress may contribute to bone demineralization and increased bone fragility [50]. In this study, ovariectomy and fractures reduced bone phosphorus and calcium levels. This decrease has been reported in osteoporosis due to reduced intestinal absorption and increased bone loss [51]. Following a fracture, minerals such as calcium and phosphorus are required to compensate for the demineralization caused by the fracture. Zhang et al. (2024) [52] demonstrated that inflammation induced by fracture and ovariectomy can increase levels of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1β, which are osteoclastogenic. Furthermore, estrogen deficiency increases osteoclast activity, the cells responsible for bone resorption, leading to decreased bone mineral density [53]. Hormonal and metabolic imbalances caused by osteoporosis and estrogen deficiency can lead to increased urinary excretion, including the loss of calcium and phosphorus. Disturbances in calcium homeostasis result in hyperparathyroidism, which increases osteoclast activity. Similarly, alkaline phosphatase (ALP) is an indicator of osteoblastic activity [54]. Its elevation suggests an attempt at bone regeneration in response to demineralization. The osteolysis index measures bone resorption, and its increase indicates greater bone degradation. Studies have shown that isoflavonoids such as genistein and daidzein, as well as flavonoids such as quercetin and kaempferol, may be effective in promoting calcium reabsorption, stimulating

osteoblast activation pathways, and inhibiting osteoclast activity through various mechanisms [55]. The results of histological sections confirm the protective effect of *C. micranthum* extracts against alterations caused by ovariectomy.

CONCLUSION

The study demonstrated that ovariectomy and fracture induced a profound imbalance in bone, renal, and lipid metabolism, reflecting the consequences of estrogen deficiency. Bone mineral loss, impaired renal function, atherogenic dyslipidemia, and oxidative stress were observed, confirming the OVX model's relevance for reproducing the complications of menopause.

Treatment with estradiol (E2V) partially corrected these abnormalities, confirming its known efficacy in preventing osteoporosis and postmenopausal metabolic disorders. However, AECM extracts, particularly at a dose of 200 mg, demonstrated comparable or even superior efficacy, with near-complete normalization of bone, renal, and lipid parameters. These observations suggest that the *C. micranthum* extract exerts osteoprotective, nephroprotective, and lipid-lowering effects, likely linked to bioactive compounds with phytoestrogenic and antioxidant activity. Thus, *C. micranthum* appears to be a promising alternative to conventional hormonal treatments, offering comprehensive protection against bone, metabolic, and cardiovascular complications induced by estrogen deficiency.

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Ethical approval

The committee considered the study that ensured proper adherence to requisite bioethical considerations in experimental studies involving rats at the Cameroon Institutional National Ethics Committee, Ministry of Scientific Research and Technology Innovation (Reg. number FWA-IRD 0001954).

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no competing interests or personal relationships that could have influenced the work reported in this paper.

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