International Journal of Osteoporosis & Metabolic Disorders

ISSN 1994-5442

science alert

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OPEN ACCESS International Journal of Osteoporosis and Metabolic Disorders

ISSN 1994-5442
DOI: 10.3923/ijom.2017.1.9

Research Article
Low Dose *Marantodes pumilum* Leaf and Roots Extracts Preserved Bone Structure in Ovariectomized Rats

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Abstract

Background: *Marantodes pumilum* var. alata, a phytoestrogen-rich herb, has been reported to exert protection on bone of estrogen-deficient animals against osteoporosis. However, comparative osteo-protective activity of its leaf and root extracts has not been fully elucidated. **Objective:** The aim of this study was to investigate and compare the osteo-protective effects of aqueous leaf and root extracts of *Marantodes pumilum* var alata in ovariectomized rat model. **Methodology:** Twenty-seven female rats were divided into nine groups: sham-operated (Sham); ovariectomized control (OVXC); 64.5 μg kg^{-1} day^{-1} estrogen treatment (ERT); 20 mg kg^{-1} day^{-1} (MPv20), 50 mg kg^{-1} day^{-1} (MPv50) and 100 mg kg^{-1} day^{-1} (MPv100) doses leaf extract treatments and; 20 mg kg^{-1} day^{-1} (MPr20), 50 mg kg^{-1} day^{-1} (MPr50) and 100 mg kg^{-1} day^{-1} (MPr100) doses root extract treatment groups. After 8 weeks treatment period, the left femora were excised and investigated using Micro-computed tomography (μ-CT). Results were analysed using one-way ANOVA and Tukey's post hoc test. **Results:** The MPv20, MPv50 and MPr20 groups showed significantly higher ($p<0.05$) bone mineral density on the trabecular bone while all treatment groups recorded significantly higher ($p<0.05$) tissue mineral density on the cortical bone when compared with OVXC group. Trabecular bone number and separation were significantly higher and lower ($p<0.05$), respectively, in both MPv20 and MPr20 groups. Significantly higher ($p<0.05$) cortical bone area fraction and thickness in MPv20 group and medullary area in MPr20 group were observed. **Conclusion:** Lower dose (20 mg kg^{-1} day^{-1}) of both leaf and root extracts of *Marantodes pumilum* var alata preserved bone mineral density and micro-architecture of estrogen-deficient rats better than higher doses.

Key words: *Marantodes pumilum*, micro-computed tomography, bone, ovariectomy, phytoestrogens

Received: September 20, 2016 Accepted: November 18, 2016 Published: December 15, 2016

Citation: Tijjani Rabiu Giaze, Ahmad Nazrun Shuid, Ima Nirwana Soelaiman, Norliza Muhammad and Norazlina Mohamed, 2017. Low dose *Marantodes pumilum* leaf and roots extracts preserved bone structure in ovariectomized rats. Int. J. Osteoporosis Metab. Disorders, 10: 1-9.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to remarkable increase in population of older people, chronic non-communicable diseases such as arthritis, diabetes, dementia, glaucoma and osteoporosis are now the major cause of death in the elderly[1,2]. Osteoporosis is a medical condition characterized by deterioration of bone micro-architectural structure and loss of bone mass causing decreased bone strength and increased risk of fracture[3,4]. It is said to be clinically present when Bone Mineral Density (BMD) falls more than 2.5 Standard Deviation (SD) below the standard reference for maximum bone mineral density of young adult female[5]. In postmenopausal women, it is thought to be due to estrogen deficiency that follows cessation of ovarian function. It was reported to affect approximately 200 million women worldwide and 1 in every 3 women older than 50 years will experience osteoporosis-related fracture[6,7]. Higher incidence of osteoporosis in women than in their male counterpart has been reported to be due to a sharp increase in bone turnover activities (remodeling) following onset of menopause[8,9]. A higher prevalence of osteoporosis, which may be attributed to lower body mass index and height, was reported in Asian population than in Western and African populations[10].

Estrogen Replacement Therapy (ERT), a gold standard treatment for post-menopausal osteoporosis[11,12], was reported to cause improvement in bone mineral density, decrease in bone loss and a consequent decrease in fracture risk[4]. However, following research outcomes of increased risk of breast cancer, pulmonary edema, stroke, colorectal cancer, endometrial cancer, hip fracture and death associated with chronic use of ERT[13-15], experts have argued that its use should be reserved for management of pressing menopausal symptoms such as hot flashes[16]. A number of other drugs such as vitamin D and calcium supplementation, bisphosphonate, calcitonin, Selective Estrogen-Receptor Modulator (SERM), anabolic steroids, parathyroid hormone, phytoestrogens and isoflavonoids have recorded decreased risk of bone fracture and are recommended as alternative to ERT for management of post-menopausal osteoporosis[17,4]. So far, these agents have not been shown to match the clinical outcome of estrogen replacement therapy and are also reported to be associated with debilitating side effects[18]. Consequently, there is an increased demand in alternative and complementary medicine by post-menopausal women[19]. In view of these challenges there is need for further research in pursuant of a safe and effective alternative to ERT.

Marantodes pumilum var. alata [synonyms: *Labisia pumila* (Blume) Fern.-Vill; Labisia *pumila* (Blume) Mez; *Ardisia pumila* Blume] is a herb belonging to family Myrsinaceae[20]. It is popularly known as the queen of herbs (Kacip Fatimah) in Malaysia where it is widely cultivated and used in traditional medicine as medication for women health. It was reported to possess phytoconstituents such as quercetin, myricetin, kaempferol, syringic acid, vanillic acid and gallic acid with estrogen-like biological activities (phytoestrogens)[21-25]. Previous pharmacological studies have reported osteo-protective effects of its root and whole plant extracts on the femur bone of estrogen-deficient rats[26,27]. In view of phytochemical reports of varying nature and possibly amount of phytoestrogen content in the leaf and root parts of *Marantodes pumilum* var. alata current study is designed to further investigate the comparative osteo-protective properties of its leaf and root extracts in post-menopausal osteoporosis rat model using micro-computed tomography (μ-CT) to measure changes in bone morphometric parameters as well as bone and tissue mineral densities (BMD and TMD) in the trabecular and cortical bone, respectively. This study will shed some light on the different effects exerted by the different parts of the plant. This will in future enable optimum use of the plant in alternative therapy of postmenopausal osteoporosis.

MATERIALS AND METHODS

Plant material: The leaves and roots of the *Marantodes pumilum* var. alata variety of the plant were collected from a cultivated site, Delima Jelita Herbs, in Kedah, Malaysia and identified at the herbarium of Department of Biological Sciences and Natural Resources, Universiti Kebangsaan Malaysia where voucher specimens were prepared and deposited (UKM-HF131). Collected leaf and root specimens were garbled, dried, ground and individually extracted by reflux method at 60°C for 2 h with distilled water at a plant-to-water ratio of 1:10 and 1:15 for roots and leaves, respectively. The resultant extracts were then freeze-dried to give a dry extract and then stored (at -20°C) for experimental use.

Animal treatment: Twenty-seven healthy female Sprague-Dawley rats (aged 4-5 months and weighing 200-250 g) were obtained from the Laboratory Animal Unit, Universiti Kebangsaan Malaysia (UKM) and grouped according to the experimental design (n = 3). They were housed in plastic cages (at 25±3°C, natural day-night cycle and humidity) and given free access to standard diet, Gold Coin, Selangor-Malaysia (containing 0.97% calcium, 0.85% phosphorus and 1.05 IU g^{-1} of Vitamin D3) and filtered tap water *ad libitum*. Before commencement of the study, the

animals were allowed to acclimatize to the laboratory environment for 7 days. Appropriate doses of plant extracts (*Marantodes pumilum*) and standards (estrogen-Premarin®) were freshly prepared in deionized water and administered to animals as oral gavages (0.1 mL/100 g) on daily basis for 8 weeks.

Study design: Twenty-seven rats were divided into nine groups (n = 3): sham-operated (Sham), ovariectomized control (OVXC), Estrogen treatment (ERT) that received 64.5 μg kg^{-1} day^{-1} dose of estrogen (Premarin®; leaf extract treatments, MPv20, MPv50 and MPv100, that received 20, 50 and 100 mg kg^{-1} day^{-1} doses plant leaf extract, respectively and root extract treatments, MPr20, MPr50 and MPr100, that received 20, 50 and 100 mg kg^{-1} day^{-1} doses of plant root extract, respectively. All animals, except the sham-operated group, were ovariectomized. Plant extracts and standard drug (estrogen) were administered as oral gavages for a predetermined period of 8 weeks[28]. In the course of treatment, animal body weight changes were monitored using electronic balance (Fisher Scientific, Model No. 51100213). After treatment, animals were sacrificed and left femora were then excised and investigated for changes in bone morphometric parameters and mineral densities using micro-computed tomography (Skyscan 1076). This study was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee, UKMAEC (FP/FAR/2016/ NORAZLINA/28-JAN./720-JAN.-2016-DEC.-2017) and conducted in accordance with the US guidelines on laboratory animal use and care as contained in National Institutes of Health (NIH) publication, 2015.

Ovariectomy: The method described by Kajuria *et al.*[29] with slight modification, was adopted. Animals were anesthetized with 0.1 mL/100 g dose of a mixture of ketamine (80 mg kg^{-1}) and xylazine (10 mg kg^{-1}), intraperitoneally. The abdominal area was shaved with an electronic clipper and cleaned with 70% alcohol. A small peritoneal incision measuring 0.4-0.6 cm was made vertically on the outer skin with surgical scalpel blade no. 11 on the middle part of the abdomen between the 2nd and 3rd nipples. Through the incisional opening, the underlining muscle tissue was incised vertically to create 0.3 cm opening through which surrounding adipose tissue was pulled out to expose underlying uterine tube that branches right and left. The right and left ovaries were then exteriorized by gentle retraction and cut-off. The uterine horns were then returned to the peritoneal cavity and wound was sutured in two layers (muscle and skin) and disinfected with povidone iodine solution. Animals were allowed for a post-operative healing period of two weeks before commencement of treatment.

Bone sample collection: At the end of 8 weeks treatment period, rats were humanely sacrificed using cervical dislocation technique and their left femora were dissected using surgical blade (No. 11) and scissors. Dissected bones were cleansed of all surrounding soft tissues, wrapped with sterile gauze soaked in phosphate buffer solution and stored at -80°C.

Computed tomography analysis: Micro-computed tomography investigation was done on dissected left femora *ex vivo* using micro-computed tomography (μ-CT) machine (Skyscan 1076, serial no. 09G02065). Each bone sample was covered with a paraffin wax sheath and placed in sample holder for scanning using a scanner system (Skyscan 1076 G015619) at a mode of 9 μm voxel size, 82 kVp voltage, 112 μA current, 0.5 mm AL filter, 4000×2672 resolution, 2050 exposure and 0.8° rotation[30]. The scanning Region of Interest (ROI) was set at 1.5 mm beneath the growth plate and extending 2.0 mm towards the proximal direction of the distal femora[31]. Scanned X-ray images were then reconstructed using NRecon Skyscan software. Reconstructed images were then processed and analyzed with 3D Skyscan analyzer software (CTAN) at ROI of 200 slices from an offset of 100 slices from a reference slice to obtain bone mineral densities and quantitative morphometric parameters of both trabecular and cortical bone. Bone Mineral Density (BMD) and Tissue Mineral Density (TMD) of trabecular and cortical bone, respectively, were determined by measuring and comparing attenuation values of the test to a phantom rod containing known density of calcium hydroxyapatite. On the trabecular bone, morphometric parameters: bone volume fraction (BV/TV), trabecular thickness, trabecular separation and trabecular number were determined while on the cortical bone, medullary area, average cortical bone area fraction and average cortical thickness were measured.

Statistical analysis: All results obtained were expressed as Mean±SEM. Analysis was done using SPSS software (version 20). Results were first tested for normality of distribution using Kolmogorov-Smirnov test before analysis with one-way analysis of variance (ANOVA) and Tukey's post hoc test[26]. Only results with difference at $p<0.05$ were considered significant.

RESULTS

Bone and tissue mineral density: Bone Mineral Densities (BMD) were significantly higher ($p<0.05$) in MPv20, MPv50 and MPr20 treatment groups than in OVXC group (Fig. 1). On the cortical bone, sham, ERT and all doses of leaf and root extracts

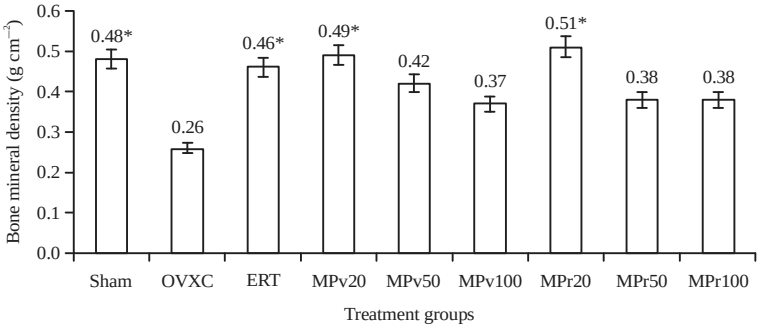

Fig. 1: Effects of aqueous leaf and root extracts of *Marantodes pumilum* var. alata on bone mineral density of femora in ovariectomized rats

Values are expressed as Mean±SEM. *Significant difference from OVXC group ($p<0.05$), Sham: Sham-operated group, OVXC: Ovariectomized control group, ERT: Estrogen treatment group, MPv20: 20 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv50: 50 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv100: 100 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPr20: 20 mg kg^{-1} *Marantodes pumilum* root treatment group, MPr50: 50 mg kg^{-1} *Marantodes pumilum* root treatment group and MPr100: 100 mg kg^{-1} *Marantodes pumilum* root treatment group

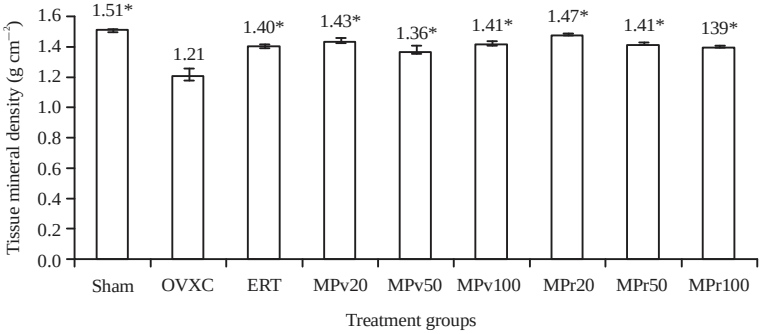

Fig. 2: Effects of aqueous leaf and root extracts of *Marantodes pumilum* var. alata on tissue mineral density of femora in ovariectomized rats

Values expressed as Mean±SEM. *Significant difference from OVXC group ($p<0.05$), Sham: Sham-operated group, OVXC: Ovariectomized control group, ERT: Estrogen treatment group, MPv20: 20 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv50: 50 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv100: 100 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPr20: 20 mg kg^{-1} *Marantodes pumilum* root treatment group, MPr50: 50 mg kg^{-1} *Marantodes pumilum* root treatment group and MPr100: 100 mg kg^{-1} *Marantodes pumilum* root treatment group

Table 1: Effects of *Marantodes pumilum* var. alata aqueous leaf and root extract on morphometric parameters of trabecular bone of Sprague-Dawley rat's femora

	Treatment groups								
Parameters	Sham	OVXC	ERT	MPv20	MPv50	MPv100	MPr20	MPr50	MPr100
Bone volume fraction (%)	14.64±2.01*	9.00±4.26	16.42±1.92*	10.59±4.02	8.21±2.47	7.10±2.78	21.66±7.27*,a	7.35±2.45	6.13±1.36
Trabecular number (1/mm)	2.51±0.37*	0.98±0.18	2.46±0.24*	2.30±0.61*	1.60±0.25	1.40±0.50	2.98±0.85*	1.56±0.70	1.01±0.19
Trabecular thickness (mm)	0.07±0.00	0.06±0.01	0.07±0.00	0.07±0.01	0.00±0.00	0.00±0.00	0.0±0.00	0.06±0.04	0.06±0.00
Trabecular separation (mm)	0.27±0.02*,b	0.87±0.30	0.27±0.05*,b	0.38±0.10*,b	0.71±0.17	0.88±0.28	0.25±0.04*,b	0.50±0.12	0.58±0.09

Values expressed as Mean±SEM. *Significant difference from OVXC group. [a]Significant difference from MPv20, MPv50, MPv100, MPr50 and MPr100 groups. [b]Significant difference from MPv50, MPv100, MPr50 and MPr100 groups ($p<0.05$, ANOVA), Sham: Sham-operated group, OVXC: Ovariectomized control group, ERT: Estrogen treatment group, MPv20: 20 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv50: 50 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv100: 100 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPr20: 20 mg kg^{-1} *Marantodes pumilum* root treatment group, MPr50: 50 mg kg^{-1} *Marantodes pumilum* root treatment group and MPr100: 100 mg kg^{-1} *Marantodes pumilum* root treatment group

showed significantly higher ($p<0.05$) Tissue Mineral Densities (TMD) when compared with the OVXC group (Fig. 2).

Trabecular bone morphometry: To an extent similar to ERT and Sham groups, both MPv20 and MPr20 groups showed significantly higher ($p<0.05$) trabecular number and lower trabecular separation when compared with OVXC group (Table 1). MPr20 group also showed significantly higher Bone volume fraction (BV/TV) when compared with Sham, ERT, OVXC and other treatment groups ($p<0.05$), but no difference

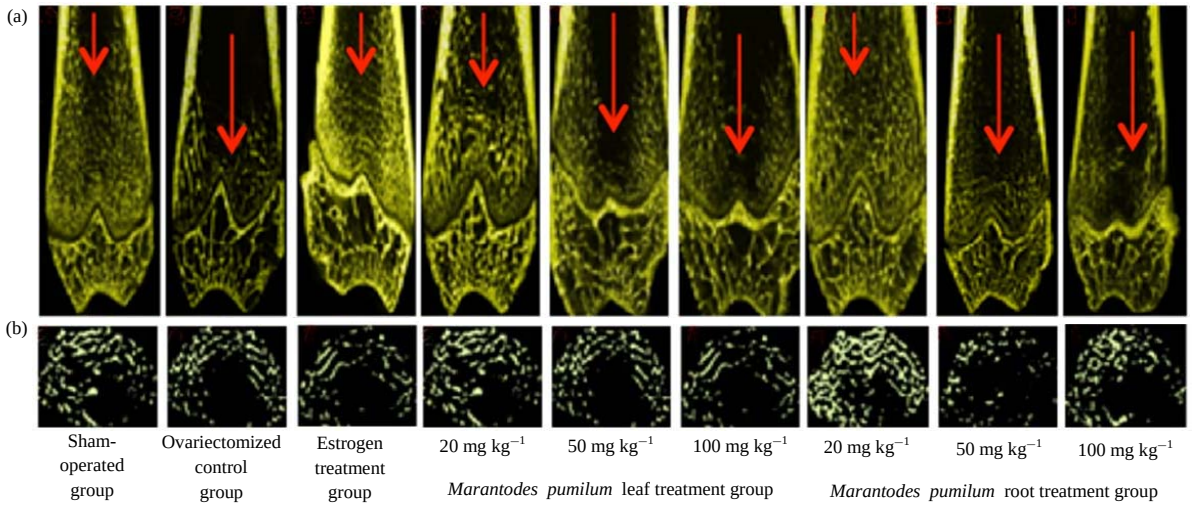

Fig. 3(a-b): Effects of aqueous leaf and root extracts of *Marantodes pumilum* var. alata on trabecular structures of distal femora in ovariectomized rats (a) Corona view and (b) Transverse view

Table 2: Effects of aqueous leaf and root extracts of *Marantodes pumilum* var alata on TMD and morphometric parameters of cortical bone of Sprague-Dawley rat's femora

	Treatment groups								
Parameters	Sham	OVXC	ERT	MPv20	MPv50	MPv100	MPr20	MPr50	MPr100
Medullary area (mm^2)	10.60±0.61*	15.22±1.23	11.50±1.01*	9.97±0.52*	14.20±1.07	18.37±5.636	11.33±1.03*	12.69±1.22	14.56±0.85
Cortical area fraction (%)	42.70±1.76	37.02±0.74	39.60±0.98	45.40±2.17*,a	38.80±0.56	36.12±1.52	42.64±0.53	35.62±1.24	33.38±1.70
Cortical thickness (mm)	0.40±0.01*,a,b	0.35±0.01	0.35±0.01	0.40±0.01*,a,b	0.37±0.01	0.34±0.01	0.37±0.01	0.35±0.01	0.33±0.01

Values expressed as Mean±SEM. * Significant difference from OVXC group, [a]Significant difference from ERT group, [b]Significant difference from MPr50, MPr100 and MPv100 ($p<0.05$ ANOVA), Sham: Sham-operated group, OVXC: Ovariectomized control group, ERT: Estrogen treatment group, MPv20: 20 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv50: 50 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv100: 100 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPr20: 20 mg kg^{-1} *Marantodes pumilum* root treatment group, MPr50: 50 mg kg^{-1} *Marantodes pumilum* root treatment group and MPr100: 100 mg kg^{-1} *Marantodes pumilum* root treatment group

was seen in trabecular thickness across all treatment and control groups. The µ-CT images (Fig. 3) also exhibited clear visual differences in density of trabeculae where MPv20 and MPr20 appeared to possess higher trabeculae than OVXC and other treatment groups.

Cortical bone morphometry: Medullary area values were significantly higher ($p<0.05$) in MPv20 and MPr20 groups when compared with the OVXC group while on the bone area fraction and cortical thickness, only MPv20 group showed significantly higher ($p<0.05$) values than the OVXC (Table 2).

Body weight: Significant weight gain, as seen in OVXC group, was observed in all treatment groups when compared with Sham group (Fig. 4a, b) ($p<0.05$). The MPr20 group further showed significantly higher weight gain ($p<0.05$) when compared with the other groups (Fig. 4b).

DISCUSSION

Results obtained at the end of the study revealed that estrogen treatment as well as all treatment doses of both leaf and root extracts of MPva failed to inhibit the significant ($p<0.05$) weight gain associated with estrogen deficiency induced by ovariectomy as seen in the OVXC (Fig. 4). These findings are contrary to previous study results that reported significant ($p<0.05$) inhibition of weight gain in ovariectomized rats[32]. The discrepancy could be attributed differences in phytoconstituents of extracts used as, in current study, extracts from the root and leaves of the plant were investigated separately whereas, in previous study, extract from the whole plant was used[32].

On the trabecular bone, 20 mg kg^{-1} day^{-1} dose of root extract and 20 and 50 mg kg^{-1} day^{-1} doses of leaf extract significantly preserved BMD when compared to OVXC group ($p<0.05$). When compared with ERT and Sham groups, no significant difference in BMD levels was seen in all treatment

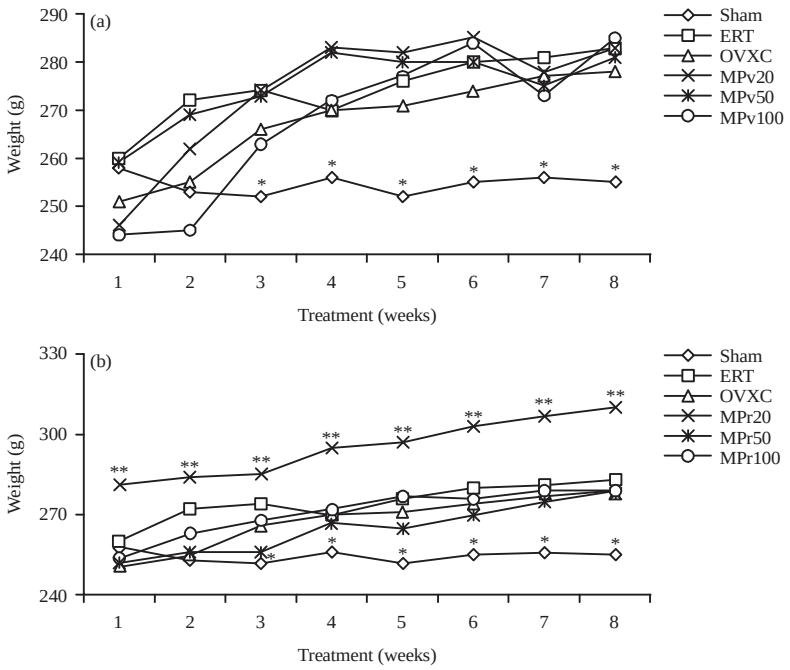

Fig. 4(a-b): Effect of aqueous leaf and root extracts of *Marantodes pumilum* var. alata on body weight of ovariectomized rats, (a) Leaf extracts and (b) Root extracts

Sham: Sham-operated group, ERT: Estrogen treatment group, OVXC: Ovariectomized control group, MPv20: 20 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv50: 50 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPv100: 100 mg kg^{-1} *Marantodes pumilum* leaf treatment group, MPr20: 20 mg kg^{-1} *Marantodes pumilum* root treatment group, MPr50: 50 mg kg^{-1} *Marantodes pumilum* root treatment group and MPr100: 100 mg kg^{-1} *Marantodes pumilum* root treatment group, Values expressed as Mean±SEM, *Significantly different from OVXC and all other treatment groups, **Significantly different from Sham, OVXC and all other treatment groups ($p<0.05$)

groups (Table 1). This result differs from outcome of previous study that revealed MPva root extract failed to prevent the loss of calcium content, a vital bone mineral, when compared to estrogen control group[26]. Observed deviation could be attributed to the fact that slightly higher dose (20 mg kg^{-1} day^{-1}) of plant extract was used in present as compared to lower dose, 17.5 mg kg^{-1} day^{-1}, used in previous study[26]. The choice of doses used in this study was based on suggestion by author of previous study[26] and reports of no-adverse-effect level (NOAEL) following sub-chronic and reproductive toxicity studies at 1000[33] and 800 mg kg^{-1} doses[34], respectively. Moreover, densitometry result from µ-CT processing is usually drawn from x-ray attenuation accrued to the entire mineral constituents of the bone and not due to calcium content only. On the cortical bone, similar to the sham and ERT groups, all leaf and root treatment groups showed significantly higher ($p<0.05$) Tissue Mineral Densities (TMD) values than the OVXC group (Fig. 2).

Morphometry parameters of trabecular bone micro-architecture, trabecular number and trabecular separation, were significantly higher ($p<0.05$) and lower, respectively, in both MPv20 and MPr20 groups when compared with OVXC group (Table 1). When compared with ERT and Sham groups, both MPv20 and MPr20 groups showed similar degree of protection of trabecular number and trabecular separation. The MPr20 group additionally protected the BV/TV better than ERT, Sham, OVXC and other treatment groups ($p<0.05$). Thus lower dose (20 mg kg^{-1} day^{-1}) of both root and leaf extracts, like ERT and Sham, preserved trabecular bone micro-architecture from estrogen deficiency-induced changes better than higher doses of 50 and 100 mg kg^{-1} day^{-1} doses. However, at 20 mg kg^{-1} day^{-1} dose, the root extract showed higher protective potency than the leaf extract. Micro-computed tomographic images obtained also revealed visual changes in density of the trabeculae that appeared to be consistent with the outcomes of quantitative morphometry (Fig. 3). The OVXC group appeared to have the lowest density of trabeculae while, similar to the Sham and ERT groups, MPv20 and MPr20 groups appeared to possess the highest density of trabeculae. Depletion in trabeculae to an extent similar to that of OVXC group was seen in other treatment groups in the order: MPv50<MPv100<MPr50<MPr100. These findings are similar to previous histomorphometric study[27] that revealed that *Marantodes pumilum* var. alata root extract was able to preserved ($p<0.05$) morphometric parameters (bone volume

fraction, trabecular thickness, trabecular number and trabecular separation) of stained femur bone section of estrogen-deficient rats. But, because previous morphometric study[27] utilized a technique (Nikon Eclipse 80i microscope) in which data on underlying bone structures (3 Dimension) are drawn from assumptions of correlation from surface structures (2 Dimension) and in view of consistent plate-to-rod changes in bone during remodeling process, bone SMI and DA couldn't be obtained thus making the results doubtful. Moreover, previous study[27] did not explore extracts from the leaves and roots individually.

Morphometric parameters of the cortical bone micro-architecture, average cortical bone fraction and cortical thickness, were significantly ($p<0.05$) higher in MPv20 when compared with OVXC and other treatment groups to an extent similar to healthy animals (Sham). Medullary area was significantly lower ($p<0.05$) in both MPv20 and MPr20 groups when compared to OVXC group (Table 2). Thus, on the cortical bone, lower dose of 20 mg kg^{-1} day^{-1} of both leaf and root extracts was also more protective than higher doses of 50 and 100 mg kg^{-1} day^{-1}. However, in this case, the leaf extract showed higher potency than the root extract as it preserved the average cortical bone fraction and cortical thickness better.

Complementing bone mineral density, the morphometry of bone micro-architecture also plays important role as a determinant of mechanical bone strength. The predictive value of mineral density and structural parameters are more reliable in prediction of fracture and diagnosis of other bone conditions[35]. Micro-computed tomography has become a gold standard tool for assessing bone morphology and micro-architecture in mice, rats and other small animals. Unlike conventional histomorphometric evaluation, utilized in previous study, CT utilizes X-ray attenuation data to reconstruct a 3D image of the intact bone with high precision in a much shorter time[36]. Because of its high resolution and 3D imaging nature, it is able to distinguish between differential changes in trabecular and cortical bone with excellent reproducibility and accuracy[37]. In addition to quantitative morphometry, MCT can also be used to estimate bone tissue mineralization by comparing X-ray attenuation in bone samples with hydroxyapatite phantom of unknown density as a standard[38]. Results obtained in this study showed that aqueous leaf and root extracts of MPva at lower dose of 20 mg kg^{-1} day^{-1} were able to reverse changes in BMD, TMD as well as morphometric parameters of trabecular and cortical bone induced by estrogen deficiency (ovariectomy) to an extent similar to estrogen and better than higher doses of 50 and 100 mg kg^{-1} day^{-1}. The little or no activity shown at higher doses simply implies that the extract exhibited a dose-dependent decline in its osteo-protective properties that could be due pharmacodynamic and pharmacokinetic factors influenced by biological variations of experimental animals. In comparison, at 20 mg kg^{-1} day^{-1} dose, the root extract showed slightly better osteo-protective effects on trabecular bone as it protected BV/TV significantly higher ($p<0.05$) than both positive controls (Sham and ERT) and other treatment doses of plant extract. Vice versa, the leaf extract was relatively more protective than the root extract on the cortical bone as it showed significantly higher ($p<0.05$) average cortical bone fraction and cortical thickness when compared with ovariectomized control group. Therefore, the root and leaf extract have differential protective effects on the trabecular and cortical bone. Observed differences may be attributed to the variation in nature and possibly concentration of phytochemicals present in the leaf and root extracts of the plant. The mechanism(s) via which *Marantodes pumilum* var. alata elicits its osteo-protective effects is yet to be understood. However, because phytoestrogens such as isoflavone have been reported to cause an improvement in bone density in lumbar spine of postmenopausal women possibly due to their anti-oxidant and anti-inflammatory properties[25,39], osteo-protective action of *Marantodes pumilum* var. alata is being proposed to be due to its phytoestrogen content[40,41].

CONCLUSION

Lower dose of 20 mg kg^{-1} day^{-1} of *Marantodes pumilum* var. alata root and leaf extracts possess better osteo-protective properties in postmenopausal osteoporotic rats than higher doses of 50 and 100 mg kg^{-1} day^{-1} to similar degree as estrogen treatment. Relatively, the root extract protected the trabecular bone structures better than the leaf extract while, on the cortical bone, the leaf extract showed better osteo-protection than the root extract.

SIGNIFICANCE STATEMENTS

This is the first study that looked into the individual osteo-protective effects of the aqueous leaf and root extracts of *Marantodes pumilum* var. alata in post-menopausal rat model using investigative technique, computed tomography, which explores bone structures from a real 3D perspective. Results obtained at the end of this study revealed that lower dose of 20 mg kg^{-1} day^{-1} of both leaf and root extracts of *Marantodes pumilum* var. alata preserved bone mineral density as well as micro-architecture of both trabecular and cortical bone of post-menopausal rat better than higher doses of 50 and 100 mg kg^{-1} day^{-1}. This data provides useful

information on the optimal dose and scope of osteo-protective activity of *Marantodes pumilum* var. alata that may find usefulness in planning future researches.

ACKNOWLEDGMENTS

The authors wish to acknowledge the support of Faculty of Medicine, Universiti Kebangsaan Malaysia and Malaysian Ministry of Agriculture (Grant: NH1014D031). We also wish to acknowledge the technical support from Azlan bin Mohd Arlamsyah and Fadhlullah Zuhair Jafar Sidik of Department of Pharmacology, Faculty of Medicine, UKM; Nor-Ashila Aladdin of Department of Pharmacognosy, Faculty of Pharmacy, UKM; and Fauzi Mohammed Busra of Tissue Engineering Center (TEC), HUKM.

REFERENCES

1. Avendano, M., M.M. Glymour, J. Banks and J.P. Mackenbach, 2009. Health disadvantage in US adults aged 50 to 74 years: A comparison of the health of rich and poor Americans with that of Europeans. Am. J. Public Health, 99: 540-548.
2. Kinsella, K.G. and H. Wan, 2008. An Aging World: 2008. U.S. Department of Commerce, Washington, DC., Pages: 91.
3. Keating, F.S.J., N. Manassiev and J.C. Stevenson, 2000. Estrogens and Osteoporosis. In: Menopause: Biology and Pathology, Rogerio, A.L., K. Jennifer and M. Robert (Eds.). Academic Press, California, pp: 509-514.
4. Anonymous, 1993. Consensus development conference: Diagnosis, prophylaxis and treatment of osteoporosis. Am. J. Med., 94: 646-650.
5. WHO., 2007. WHO scientific group on the assessment of osteoporosis at primary health care level. Summary Meeting Report, World Health Organization, Geneva.
6. International Osteoporosis Foundation, 2009. Facts and statistics about osteoporosis and its impact. J. Bone Min. Res., 4: 113-118.
7. Cooper, C. and L.J. Melton III, 1992. Epidemiology of osteoporosis. Trends Endocrinol. Metab., 3: 224-229.
8. Ismal, N.M., 1997. Postmenopausal osteoporosis: Epidemiology, pathophysiology and treatment. Malays. J. Pathol., 19: 21-25.
9. Lindsay, R., 1995. Estrogen Deficiency. In: Osteoporosis: Etiology, Diagnosis and Management, Riggs, B.L. (Ed.). Raven Press, New York, pp: 133-160.
10. Loh, K.Y. and H.K. Shong, 2007. Osteoporosis: Primary prevention in the community. Med. J. Malays., 62: 355-358.
11. Watts, N.B., J.P. Bilezikian, P.M. Camacho, S.L. Greenspan and S.T. Harris *et al*., 2010. American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the diagnosis and treatment of postmenopausal osteoporosis. Endocr. Pract., 16: 1-37.
12. Al-Azzawi, F., 2008. Prevention of postmenopausal osteoporosis and associated fractures: Clinical evaluation of the choice between estrogen and bisphosphonates. Gynaecol. Endocrinol., 24: 601-609.
13. Furness, S., H. Roberts, J. Marjoribanks and A. Lethaby, 2012. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. Cochrane Database Syst. Rev. 10.1002/14651858.CD000402.pub4.
14. Shumaker, S.A., C. Legault, S.R. Rapp, L. Thal and R.B. Wallace *et al*., 2003. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: The Women's Health Initiative memory study: A randomized controlled trial. J. Am. Med. Assoc., 289: 2651-2662.
15. Writing Group for the Women's Health Initiative Investigators, 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. J. Am. Med. Assoc., 288: 321-323.
16. Stuenkel, C.A., M.L.S. Gass, J.E. Manson, R.A. Lobo, L. Pal, R.W. Rebar and J.E. Hall, 2012. A decade after the Women's health initiative-the experts do agree. Fertil. Sterility, 98: 313-314.
17. Richards, J.B., L. Joseph, K. Schwartzman, N. Kreiger, A. Tenenhouse and D. Goltzman, 2006. The effect of cyclooxygenase-2 inhibitors on bone mineral density: Results from the Canadian multicentre osteoporosis study. Osteoporosis Int., 17: 1410-1419.
18. Rao, L.G. and A.V. Rao, 2013. Oxidative Stress and Antioxidants in the Risk of Osteoporosis-Role of the Antioxidants Lycopene and Polyphenols. In: Topics in Osteoporosis, Valdes-Flores, M. (Ed.). InTech, USA., pp: 117-162.
19. Rees, M., 2011. Management of the menopause: Integrated health-care pathway for the menopausal woman. Menopause Int., 17: 50-54.
20. The Plant List, 2010. Version 1 of the plant list. http://www.theplantlist.org/.
21. Burkill, I.H., 1935. Dictionary of the Economic Products of the Malay Peninsula. Vol. 2, Crown Agents of the Colonies, London, England, Pages: 1290.
22. Chua, L.S., S.Y. Lee, N. Abdullah and M.R. Sarmidi, 2012. Review on *Labisia pumila* (Kacip Fatimah): Bioactive phytochemicals and skin collagen synthesis promoting herb. Fitoterapia, 83: 1322-1335.
23. Karimi, E., H.Z.E. Jaafar and S. Ahmad, 2011. Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of *Labisia pumila* Benth. Molecules, 16: 4438-4450.
24. Ali, Z. and I.A. Khan, 2011. Alkyl phenols and saponins from the roots of *Labisia pumila* (Kacip Fatimah). Phytochemistry, 72: 2075-2080.

25. Jamal, J.A., P.J. Houghton, S.R. Milligan and I. Jantan, 2003. The oestrogen and cytotoxic effects of the extracts of *Labisia pumila* var. alata and *Labisia pumila* var. pumila *in vitro*. J. Sains Kesihatan, 1: 53-60.
26. Shuid, A.N., L.L. Ping, N. Muhammad, N. Mohamed and I.N. Soelaiman, 2011. The effects of *Labisia pumila* var. *alata* on bone markers and bone calcium in a rat model of post-menopausal osteoporosis. J. Ethnopharmacol., 133: 538-542.
27. Fathilah, S.N., A.N. Shuid, N. Mohamed, N. Muhammad, I.N. Soelaiman, 2012. *Labisia pumila* protects the bone of estrogen-deficient rat model: A histomorphometric study. J. Ethnopharmacol., 142: 294-299.
28. Ima-Nirwana, S., M. Norazlina and B.A.K. Khalid, 1998. Pattern of bone mineral density in growing male and female rats after gonadectomy. J. Asean. Fed. Endoc. Soc., 16: 21-36.
29. Khajuria, D.K., R. Razdan and D.R. Mahapatra, 2012. Description of a new method of ovariectomy in female rats. Rev. Bras. Reumatol., 52: 462-470.
30. Effendy, N.M., I.I. Nurul, M. Norazlina and N.S. Ahmad, 2015. An Evidence-Based Review of Micro-CT Assessments of the Postmenopausal Osteoporosis Rat Model. Int. J. Pharmacol., 11: 177-200.
31. Boyd, S.K., P. Davison, R. Muller and J.A. Gasser, 2006. Monitoring individual morphological changes over time in ovariectomized rats by *in vivo* micro-computed tomography. Bone, 39: 854-862.
32. Fazliana, M., W.M.W. Nazaimoon, H.F. Gu and C.G. Ostenson, 2009. *Labisia pumila* extract regulates body weight and adipokines in ovariectomized rats. Maturitas, 62: 91-97.
33. Singh, G.D., M. Ganjoo, M.S. Youssouf, A. Koul and R. Sharma *et al*., 2009. Sub-acute toxicity evaluation of an aqueous extract of *Labisia pumila*, a Malaysian herb. Food Chem. Toxicol., 47: 2661-2665.
34. Ezumi, M.F.W., S.S. Amrah, A.W.M. Suhaimi and S.S.J. Mohsin, 2007. Evaluation of the female reproductive toxicity of aqueous extract of *Labisia pumila* var. alata in rats. Indian J. Pharmacol., 39: 30-32.
35. Ulrich, D., B. van Rietbergen, A. Laib and P. Ruegsegger, 1999. The ability of three-dimensional structural indices to reflect mechanical aspects of trabecular bone. Bone, 25: 55-60.
36. Bouxsein, M.L., S.K. Boyd, B.A. Christiansen, R.E. Guldberg, K.J. Jepsen and R. Muller, 2010. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J. Bone Miner. Res., 25: 1468-1486.
37. Szabo, K.A., C.E. Webber, C. Gordon, J.D. Adachi, R. Tozer and A. Papaioannou, 2011. Reproducibility of peripheral quantitative computed tomography measurements at the radius and tibia in healthy pre-and postmenopausal women. Can. Assoc. Radiologists J., 62: 183-189.
38. Fajardo, R.J., E. Cory, N.D. Patel, A. Nazarian and A. Laib *et al*., 2009. Specimen size and porosity can introduce error into μCT-based tissue mineral density measurements. Bone, 44: 176-184.
39. Gambacciani, M., M. Ciaponi, B. Cappagli, L. Piaggesi and A.R. Genazzani, 1997. Effects of combined low dose of the isoflavone derivative ipriflavone and estrogen replacement on bone mineral density and metabolism in postmenopausal women. Maturitas, 28: 75-81.
40. Effendy, N.M. and A.N. Shuid, 2014. Time and dose-dependent effects of *Labisia pumila* on bone oxidative status of postmenopausal osteoporosis rat model. Nutrients, 6: 3288-3302.
41. Sanusi, R.A.M., N.A. Ab Shukor and M.R. Sulaiman, 2013. Anti-inflammatory effects of *Labisia pumila* (Blume) F. Vill-Naves. Aqueous extract. Sains Malaysiana, 42: 1511-1516.