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Original Research Article (Experimental)

Cinnamomum burmanini Blume increases bone turnover marker and induces tibia's granule formation in ovariectomized rats

Nia Kania [a, *], Wahyu Widowati [b], Firli Rahmah Primula Dewi [c], Antonius Christianto [c], Bambang Setiawan [d], Nicolaas Budhiparama [e], Zairin Noor [f]

[a] *Research Center for Osteoporosis, Department of Pathology, Ulin General Hospital, Medical Faculty, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia*
[b] *Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung, West Java, Indonesia*
[c] *Malang In Silico Club, Malang, East Java, Indonesia*
[d] *Research Center for Toxicology, Cancer, and Regenerative Medicine, Department of Medical Chemistry and Biochemistry, Medical Faculty, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia*
[e] *Budhiparama Institute of Hip and Knee Research and Education Foundation for Arthroplasty, Sports Medicine and Osteoporosis, Jakarta, Indonesia*
[f] *Research Center for Osteoporosis, Department of Orthopedics and Traumatology, Ulin General Hospital, Medical Faculty, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia*

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ABSTRACT

Background: Bone fragility and an increase in susceptibility to fracture osteoporosis is characterized by a reduction in bone mass and the micro-architectural deterioration of bone tissue. There is no previous study regarding the effect of *Cinnamomum burmanini Blume* on osteoporosis.
Objective: This study was aimed to investigate the effect of *C. burmanini Blume* on bone turnover marker, mineral elements, and mesostructure of ovariectomized rats.
Materials and methods: Thirty female Wistar rats were randomly divided into five groups, which included a control group (sham surgery), ovariectomy group (OVX), and ovariectomy groups in the presence of ethanolic extract of *C. burmanini Blume* (EECB) at doses of 12.5; 25; 50 mg/kg body weight (BW). Analysis of serum C-telopeptide collagen type I (CTX) and osteocalcin (OC) were done by enzyme-linked immunosorbent assay (ELISA). Tibia mineral elements and mesostructure were analyzed by X-ray Fluorescence and Scanning Electron Microscopy, respectively. *In silico* study was performed by modeling protein structure using SWISS-MODEL server and Ramachandran plot analysis.
Results: The increase in OC and CTX were significantly attenuated by treatments of EECB. Ovariectomy significantly decreased Cu/Zn ratio compared to sham-operated rats ($p < 0.05$). Mesostructure of ovariectomized rats was significantly different compared with the control group.
Conclusion: Cinnamon was able to normalize bone turnover markers, but, the mesostructure of hydroxyapatite crystal growth was achieved at the highest dose extract. *In silico* study showed that the active compound of EECB could not only support osteoclastogenesis process by decreasing the binding energy between RANKL and RANK, but also by inhibiting the interaction between OPG and RANK.

1. Introduction

Bone fragility and an increase in susceptibility to fracture osteoporosis are characterized by a reduction in bone mass and the micro-architectural deterioration of bone tissue [1]. The loss of ovarian function is the main factor for bone loss in aged females. The pathophysiology of this “ovary-related’ bone loss is not clear and cannot be simply explained by either increased bone resorption or decreased bone formation [2]. The model of bilaterally ovariectomized rats mimics the accelerated bone loss which manifests in postmenopausal women [3]. Intensified bone loss was manifested by a significant decrease in the mineral content/bone mass ratio [4]. Previous study shows that ovariectomized rats showed a significant gradual increase in serum calcium, phosphorus, zinc, and copper levels compared to the sham control [5,6].

* Corresponding author.
E-mail address: kaniazairin@yahoo.com (N. Kania).
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Estrogen replacement therapy (ERT) has been used as drug of choice for prevention of postmenopausal bone loss, but evidence indicates that long-term unopposed ERT may cause an increased risk of ovarian and endometrial cancer [7,8]. Thus, the alternative therapeutic strategy with a proven efficacy and safety should be developed for the prevention and treatment of osteoporosis using medicinal plants with low side effects. Cinnamon is one of the well-known and oldest spices, which has been used for centuries in several cultures [9]. Although there are several origins of Cassia cinnamon, two kinds that are considered as the important are Chinese Cassia (*Cinnamomum cassia Blume, syn. Cinnamomum aromaticum Nees*) and Indonesian Cassia (*Cinnamomum burmanini Blume*). Chinese Cassia is cultivated in southern China, Burma, and Vietnam, whereas Indonesian cassia is mainly from Indonesia [10]. Cinnamon is the bark of *Cinnamomum cassia* and has been used as traditional folk herb to treat inflammation for thousands of years in Asia. It is also used in food industry as an antioxidant and spicy agent [11]. Previous research using cinnamon extract was shown its effect of increasing the estradiol level, LH secretion and in turn affecting the estrogen and progesterone synthesis [12]. Therefore, the aim of the current study was to clarify the effect of cinnamon on bone turnover marker, mineral elements, and mesostructure of ovariectomized rats in an osteoporosis model of rats.

2. Materials and methods

2.1. Preparation and extraction of cinnamon

Cinnamon was obtained from Materia Medica Garden, Batu, East Java, Indonesia. The plant was identified as *Cinnamomum burmaninii* by an officer from Materia Medica Garden, Batu, East Java, Indonesia. The preparation and extraction of cinnamon were performed according to the maceration extraction method [13]. One kilogram of dried cinnamon stem was extracted with distilled in 70% ethanol by a maceration method for five days, filtered and collected until there was colorless ethanol filtrate. The collected ethanol filtrate was evaporated using a rotatory evaporator to produce an ethanol extract of cinnamon. The ethanol extracts of cinnamon were stored at 4 °C.

2.2. High-performance liquid chromatography analysis

Methanol was filtered using membrane millipore with polytetrafluoroethylene (PTFE, Whatman® brand) and water was filtered using cellulose nitrate membrane. Placed in the HPLC eluent reservoir. HPLC tool analysis was balanced for appropriate conditions (optimum conditions): mobile phase of methanol: water = 8: 2, stationary phase was C − 18, flow rate was 0.75 ml/min. When HPLC tool achieved a straight baseline, the standard extracts were injected and measured. The standards, including eugenol 1.7 mg (Sigma Aldrich; Cas E51791), cardamom oil 1.4 mg (Sigma Aldrich, Cas W224111), coumarin 0.6 mg (Chengdu Biopurify Phytochemicals Ltd., Cas No: 91-64-5), *trans*-cinnamic acids 0.5 mg (Sigma Aldrich, Cas W228826) and cinnamon extract 4.1 mg of EECB were filtered using PTFE membrane and homogenized for 5 min. 20 ml of samples were injected into the HPLC column, and the chromatograms were detected using UV at a wavelength 254 nm. The experiment was performed triplicate.

2.3. Animal

Thirty, adult albino female Wistar rats, twelve months old, weighing 150–200 g were used in this study. The animals were acclimatized for 1 week to our laboratory conditions prior to experimental manipulation and were exposed to a 12-h light and 12-h dark cycle at room temperature of 24 °C. They had free access to standard laboratory chow and water *ad libitum*. The animals were randomly assigned into five groups: control group (SHAM) whose animal was sham ovariectomy, ovariectomy group (OVX) where animals received standard ovariectomy, and three ovariectomy groups which receiving the ethanolic extract of *C. burmanini Blume* (EECB) by gavage at dose 12.5 (OVX + $EECB_{12.5}$); 25 (OVX + $EECB_{25}$); and 50 (OVX + $EECB_{50}$) mg/kg body weight (mg/kg BW). The EECB dose was determined based on previous research with some modification [14]. For the OVX rats, it was divided randomly after two weeks recovery from ovariectomy surgery. The animals received EECB by oral gavage for one month. The period of administration was conducted based on a previous experiment using cinnamon extract, in which one-month administration already showed the effect of its treatment [15–18]. All animal procedures were approved by the ethical committee of the Medical Faculty, Lambung Mangkurat University prior the study.

2.4. Ovariectomy surgery procedure

Under ketamine (50 mg/kg) and xylazine (8 mg/kg) anesthesia, twenty four animals from the OVX groups underwent bilateral ovariectomy by ventral incisions while six were sham-operated (control) [19]. The treatment with extract was performed after two weeks recovery from ovariectomy surgery. Wound healing evaluation was conducted based on previous methods with some modification to decide the recovery time and to decide the treatment starting time [20]. The successful osteoporosis modeling animal was determined through identification for osteoblast gene expression using PCR and followed by histochemical staining (data not shown) based on previous research [21]. At the end of the experiment, animals in all groups were sacrificed. Serum and bone tissues were removed.

2.5. Tissue preparation

At the end of the treatment, rats in all groups were anesthetized; their blood was drawn by cardiac puncture. Both tibia and femur were collected, weighed, and later rinsed with physiological saline. All tibia samples were stored at glutaraldehyde until analyzed.

2.6. Analysis of bone turnover markers

The serum bone formation markers osteocalcin was measured using Rat Osteocalcin/Bone Gla Protein OT/BGP ELISA kits from NovaTeinBio, Inc (Cambridge, MA, USA). The serum bone resorption marker C-telopeptide of type I collagen (CTX) kit was purchased from NovaTeinBio, Inc (Cambridge, MA, USA) [22].

2.7. Analysis of bone mineral elements

Bone mineral element analysis was evaluated by X-Ray Fluorescence (XRF). For XRF analysis, the tibia bones were inserted into the bone tube, then put in the proper place in equipment. The processed bones were then analyzed at 10–20 kV accelerating voltages by an XRF (PANalytical MiniPAL 4). All procedures were done at Physic and Central Laboratory, Faculty of Mathematic and Natural Science, University of Malang [22].

2.8. Analysis of the bone cell number

Cellular parameters were obtained from decalcified sections of right femur bones. The bones were decalcified in the EDTA solution for five weeks and then dehydrated in graded concentrations of ethanol before being embedded in paraffin wax. The decalcified

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