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Basic nutritional investigation

Catechin-rich oil palm leaf extract enhances bone calcium content of estrogen-deficient rats

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ABSTRACT

Objective: Postmenopausal estrogen deficiency often causes bone density loss and osteoporosis. This study evaluated the effects of an oral administration of oil palm leaf extract (OPL) on bone calcium content and structure, bone density, ash weights, and serum total alkaline phosphatase (T-ALP) of estrogen-deficient ovariectomized (OVX) rats.

Methods: Female Sprague-Dawley rats were divided into five experimental groups: 1) intact (normal control); 2) ovariectomized (OVX control), and OVX rats supplemented with 3) 2% (w/v) green tea (OVX + GT), 4) OPL 150 mg/kg of body weight, or 5) OPL 300 mg/kg of body weight in the drinking water.

Results: After 3 mo, the OVX control rats had significantly decreased femur and tibia masses (−5% and −3%, respectively), ash (−15% and −10%), calcium content (−0.5% and −2.7%), and bone density and T-ALP concentrations (−40%) compared with intact rats. The catechin-rich OPL dose dependently increased the OVX bone density and structure, femur and tibia masses (by +8% and +12% respectively), ash (by +30% and +20% respectively), calcium (by +3% and +5%), and T-ALP concentrations (by +76%) compared with the OVX rats. The increases by OPL were higher than that in OVX + GT and control intact rats.

Conclusion: The catechin-rich OPL increased the bone mass in estrogen-deficient rats by increasing osteoblast activities to higher levels than in normal rats and those supplemented with GT. This was shown by the modulation of serum T-ALP levels, bone calcium content, total mineral content, and bone histologic structure. The OPL is a potential inexpensive ingredient for protection against osteoporosis and influences bone metabolism by encouraging bone formation.

Introduction

There are few reports on functional food or herbal supplements that can effectively increase bone mineral content or prevent bone mineral loss under estrogen deficiency, which is important for postmenopausal women. Osteoporosis is almost inevitable in the aged, especially for postmenopausal women. Bone ailment treatments cost about US$130 billion worldwide. The increasing osteoporosis incidence indicates a great need for the exploration of more anti-osteoporotic functional food ingredients and preventive or therapeutic agents.

A positive relation exists between fruit and vegetable consumption and bone health, bone mass, and bone metabolism in humans [1]; however, this is obviously insufficient. Green tea (GT) consumption has mitigated bone loss in aged women and decreased the risk of osteoporotic fractures [2]. GT contains catechins or tea polyphenols, mainly (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate, (−)-epicatechin, and (−)-epigallocatechin [2,3]. Oil palm leaf (OPL) is an underused byproduct of the oil palm industry that is abundant in tropical countries of Southeast Asia, Africa, and South America. The OPL contains more biophenolic compounds than GT [4]. High-performance liquid chromatographic analysis of the OPL has shown that a significant amount of the phenolic compounds in OPLs consists of GT catechins, namely epigallocatechin (0.08%), catechin (0.30%), epicatechin (0.01%), EGCG (0.28%), epicatechin gallate (0.05%), and their glycosides [4]. The OPL is a potential economically viable, new source of GT catechins. Traditionally, the palm leaves are woven into bags for boiling rice, and the phenolic compounds in the leaves diffuse into the rice to produce

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a pleasant flavor. The good antioxidant and phytoestrogenic properties of OPLs [5] may help prevent bone loss in postmenopausal women. Palm leaves have been consumed by ruminants for decades without any reported toxicity. This study evaluated the effects of the OPL on femur and tibia bone densities in estrogen-deficient rats compared with intact rats and GT-supplemented ovariectomized rats. The ovariectomized rat is used to induce estrogen deficiency and has been used as an animal model of postmenopausal bone loss [6].

Materials and methods

OPL extract

Oil palm (*Elaeis guineensis*) leaves were collected from the Universiti Putra Malaysia plantation, washed, chopped, and dried in a 40°C oven for 24 h. The dried leaves were milled and extracted with 50% aqueous ethanol under continuous agitation in a turbo extractor at room temperature for 2 h. After filtration, the solution was spray dried under vacuum to a dark green OPL extract powder. The extract was stored at −20°C in tightly sealed packaging until used.

Animal study

Thirty female Sprague-Dawley rats, weighing 100 to 150 g, were purchased from Chenur Supplier (Kajang, Selangor, Malaysia). The rats were housed in clean cages (three rats per cage) under an 11-h light/13-h dark cycle at 25 ± 3°C, with ad libitum access to normal rat chow (Gold Coin, Kuala Lumpur, Malaysia) and water.

After a week of acclimatization, the pubertal rats (about 55 d old) were randomly allocated to five experimental groups ($n = 6$, minimized number as suggested by the animal ethics committee): 1) intact rats (normal control); 2) ovariectomized rats (OVX control); and OVX rats supplemented with 3) 2% GT (OVX + GT), 4) OPL 150 mg/kg of body weight (OVX + OPL-150), or 5) OPL 300 mg/kg of body weight (OVX + OPL-300) in their drinking water. The doses selected are equivalent to the daily dose of regular GT drinkers.

After the acclimatization period, ovariectomy was performed under ketamine and xylazine anesthesia. The surgical sites were sutured, bandaged, and closely monitored for 1 wk to ensure complete wound healing [7]. The procedures used were to minimize pain or discomfort and were in accordance with the animal ethics guidelines of the Faculty of Medicine, Universiti Putra Malaysia.

At the end of the 3-mo study period, the rats were sacrificed by an overdose of ketamine and xylazine. Blood from the 13-h fasted rats was collected by cardiac puncture and the serum was separated and stored at −20°C for biochemical analyses. The right and left femurs and tibias were dissected out, cleared of all soft tissues, and weighed. Serum calcium, serum inorganic phosphorus, and serum total alkaline phosphatase (T-ALP) were analyzed using an automatic analyzer (902 Roche, Hitachi, Tokyo, Japan).

The bones were dried for 24 h at 100°C and their dry masses were recorded. Then, the right bones were heated in a furnace at 550°C to 600°C for 24 h for the ash weights. The ash was then pulverized and hydrolyzed with 6 M HCl, and the bone calcium content was determined using an atomic absorption spectrophotometer (model Z-6100, Hitachi).

The left femurs were fixed in formaldehyde sodium phosphate (Na_2HPO_4) for 24 to 48 h. Longitudinal sections (4 μm thick) were cut using a microtome (Polycut E, Leica, Nussloch, Germany) and stained with Harris hematoxylin and eosin. The left proximal femur, trabecular bone microarchitecture, and static histomorphometric parameters were observed under a light microscope equipped with an image analyzer (U-LH100HL, Olympus, Tokyo, Japan). The selected regions were 1 mm below the growth plate and extended distally for 1.5 mm.

Statistical analyses

Statistical analyses were performed using Minitab 14 (Minitab, Inc., State College, PA, USA). The data were presented as mean ± standard deviation. One-way analysis of variance was used to compare the data among the groups and $P < 0.05$ was considered a statistically significant difference.

Results

Ovariectomy caused significant decreases in femur and tibia ash weights. The OVX rats had significantly lower femur and tibia ($P < 0.05$) ash weights, by −15% and −10%, respectively, compared with the intact rats. The GT-treated OVX rats exhibited increased femur and tibia ash weights, by +20% and +5%, with weights almost equal to those of the intact control rats (Fig. 1).

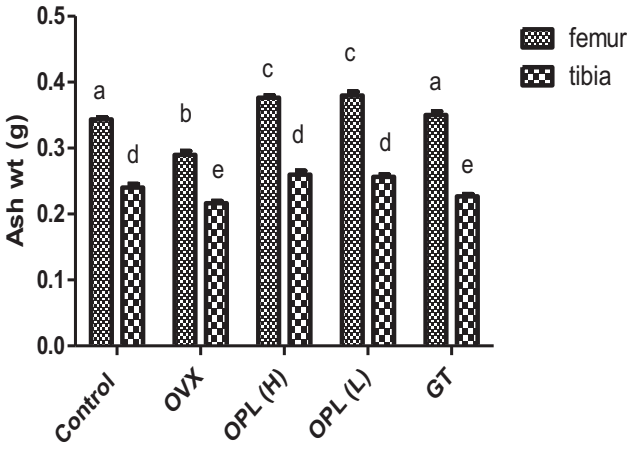

Fig. 1. Ash weights of the femur and tibia. The control, OVX, and GT groups were considered control groups and the OPL (H) and OPL (L) were considered treatment groups. Values are presented as mean ± SD of three replications ($n = 6$). Different letters indicate significant differences ($P < 0.05$) in the bone ash content. Control, intact rats; GT, ovariectomized rats supplemented with 2.0% (w/v) green tea in the drinking water; OPL (H), ovariectomized rats supplemented with oil palm leaf extract 300 mg/kg of body weight in the drinking water; OPL (L), ovariectomized rats supplemented with oil palm leaf extract 150 mg/kg of body weight in the drinking water; OVX, control ovariectomized rats.

The OVX rats treated with OPL-150 and OPL-300 exhibited increased femur and tibia ash weights, by about +30% and +20%, respectively, compared with the OVX rats, with ash weights greater than in the intact control rats.

Ovariectomy caused similar decreases in the fresh and dry masses of the femur and tibia. The OVX rats had lower femur and tibia ($P < 0.05$) masses, by −5% and −3%, respectively, compared with the intact rats. The OPL treatments of OVX rats increased the femur and tibia masses by about +8% and +12%, respectively, compared with the OVX rats, which were greater than those in the intact control rats. The GT + OVX rats had similar femur and tibia masses as the intact control rats (Table 1), but the effects against bone loss in OVX rats were more pronounced in the tibia (+7% increase).

A comparable pattern was observed with the dry masses of the femur and tibia. The OVX + OPL-150 rats had significantly increased femur and tibia dry masses compared with the OVX rats (Table 1), by +3.5 and +9.5%, respectively, whereas the OVX + OPL-300 showed increased weights, by +5% and +7%, respectively. The tibia dry mass of OPL-treated OVX rats was significantly greater that of the intact control rats, whereas the femur dry mass was similar to that of the intact control rats. The OVX + GT tibia and femur dry masses were greater than those in the OVX rats but were similar to those of intact control rats, showing that GT prevented bone mineral loss but did not increase bone mass.

The femur and tibia calcium content of the OVX rats were significantly lower than those of the intact control rats (−0.5% and −2.7%, respectively). The OVX + GT treatment significantly ($P < 0.05$) prevented calcium loss in the tibia but not in the femur (Fig. 2), with increases of only +0.03% for the femur and +3.3% for the tibia. The calcium content of the femur and tibia in the OVX + OPL-150 and OVX + OPL-300 groups were significantly ($P < 0.05$) higher (+2.6% and +5%, respectively) compared with those in the OVX group and higher than those in the intact control rats (Fig. 2).

As presented in Table 2, there were no significant differences in the serum calcium and phosphorus levels among the groups.