Nutrition 31 (2015) 205–213

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Preventive effects of withaferin A isolated from the leaves of an Indian medicinal plant Withania somnifera (L.): Comparisons with 17-β-estradiol and alendronate

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ARTICLE INFO

Article history:
Received 6 December 2013
Accepted 6 May 2014

Keywords:
Premenopausal
Antiresorptive
Osteogenic
Trabecular microarchitecture
Microcomputed tomography

ABSTRACT

Objective: Bone protective effects of withaferin A (WFA) from leaves of Withania somnifera (L.) were evaluated in preventive model of Balb/c mice with 17 β-estradiol (E2) and alendronate (ALD).
Methods: Adult female Balb/c mice, 7 to 9 wk, were bilaterally ovariectomized (OVx) to mimic the state of E2 deficiency. Immediately after surgery mice were administrated WFA at doses of 1, 5, 10 mg/kg/d while other two OVx groups received ALD or E2 for 2 mo. Sham and OVx groups with vehicle and no treatment served as controls.
Results: WFA administration increased new bone formation, as well as improving micro-architecture and biomechanical strength of the bones. It prevented bone loss by reducing expression of osteoclastic genes tartrate resistant acid phosphatase (TRAP) and receptor activator of nuclear factor κ B (RANK). Increase in bone turnover marker, osteocalcin (OCN) and inflammatory cytokine tumor necrosis factor-alpha (TNF-α) because of ovariectomy were reduced with WFA treatment, with effects comparable to E2 administration. Histomorphometric analysis of uterus shows that WFA was not fraught with estrogenic or antiestrogenic effects. At cellular level, WFA promoted differentiation of bone marrow cells (BMCs) and increased mineralization by inducing expression of osteogenic genes. WFA has bone protective potential as its treatment prevents bone loss that is comparable to ALD and E2.
Conclusions: It is surmised that WFA in preclinical setting is effective in preserving bone loss by both inhibition of resorption and stimulation of new bone formation before onset of osteoporosis with no uterine hyperplasia.

Introduction

Withania somnifera (L.) also known as Indian ginseng is used as a dietary supplement throughout United States [1]. In India Withania somnifera (L.) roots and leaf preparation are traditionally used for its various beneficial effects [2–5]. Purification of Withania somnifera (L.) extract by high-performance liquid chromatography shows the presence of alkaloids and withanolides of ergosterol type, and are markers for genus *Withania* [1]. These withanolides enriched extract consist mainly of withaferin A (WFA) (Fig. 1A) and withanolides A and D, that are most studied for their biological activities [6]. A recent study from our laboratory shows that WFA, the most abundant withanolide, is useful in mitigating ovariectomized (OVx) induced bone deterioration by proteasomal inhibition and down regulating E3 Smurf1/2 ligase [7]. Whether, WFA could also be useful

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http://dx.doi.org/10.1016/j.nut.2014.05.010

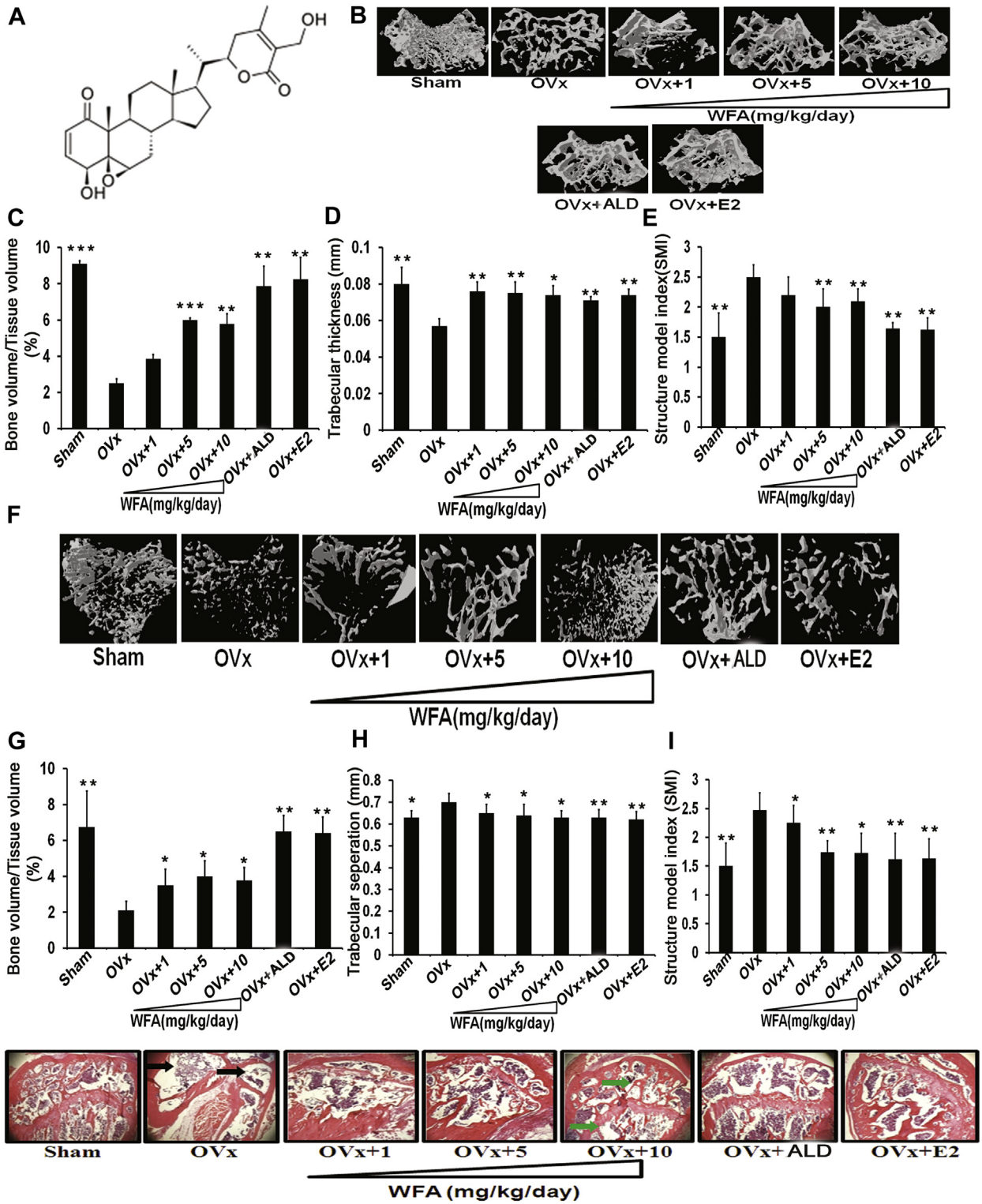

Fig. 1. WFA treatment prevents femoral trabecular microarchitecture deterioration in OVx mice. (A) Chemical structure of withaferin A (WFA). (B) Representative μ-CT images of femoral epiphyseal regions of various experimental groups (upper panel). Quantification of μ-CT data of various trabecular parameters are presented in the lower panels as percentage of Bone volume/Tissue volume (% BV/TV), Trabecular Thickness (Tb.Th) and Structure module index (SMI) (C-E) respectively. Values are expressed as Mean ± SD; n = 10 mice/group. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with the OVx + vehicle group WFA prevented tibial trabecular microarchitecture deterioration in OVx mice. (F) Representative μCT images of tibia metaphysis of various experimental groups (upper panel). Quantification of μCT data on various trabecular parameters are presented in the lower panels percentage of Bone volume/Tissue volume (% BV/TV), Trabecular Separation (Tb.Sp), Structure module index (SMI) (G-I) respectively. (J) Photomicrographs of femur epiphysis trabecular structure at 10 X. Values are expressed as Mean ± SD; n = 10 mice/group. *$P < 0.05$ and **$P < 0.01$ and compared with the OVx + vehicle group.