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ORIGINAL ARTICLE

Comparative study of two types of herbal capsules with different *Epimedium* species for the prevention of ovariectomised-induced osteoporosis in rats

Shi-Hui Chen [a,*,1], Xin-Luan Wang [a,b,1], Li-Zhen Zheng [a], Yi Dai [c], Jia-Yong Zhang [d], Bao-Lin Guo [e], Zhi-Jun Yang [d], Xin-Sheng Yao [c,], Ling Qin [a,b,***]**

[a] *Musculoskeletal Research Laboratory, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, PR China*
[b] *Translational Medicine R&D Centre, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, PR China*
[c] *Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou, PR China*
[d] *School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, PR China*
[e] *Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, PR China*

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Summary *Background/Objective: Epimedii Folium* is the most important osteogenic herb formulated for the traditional Chinese Medicine Xian Ling Gu Bao (XLGB) capsule. The present study compared XLGB capsules containing two different *Epimedium* species, i.e., either *Epimedium pubescens* (XEP) or *Epimedium koreanum* (XEK), with the focus being on the chemical constituents and antiosteoporotic efficacy.
Methods: Ultra performance liquid chromatography was used to demonstrate the different chemical constituents. Biomechanical tests, histological, and cytological evaluation were performed to characterise and compare the bone mineral density, bone strength, microstructure

* Corresponding author. Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Hong Kong SAR, PR China.
** Corresponding author. Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, 601 Huangpu Avenue, Guangzhou, PR China.
*** Corresponding author. Translational Medicine R&D Centre, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, 1068 Xueyuan Avenue, Shenzhen University Town, Shenzhen, PR China.
E-mail addresses: chensh@ort.cuhk.edu.hk (S.-H. Chen), tyaoxs@jnu.edu.cn (X.-S. Yao), lingqin@cuhk.edu.hk (L. Qin).
[1] These authors contributed equally to this paper.

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of bone tissue, and biological activity between XEP and XEK using an established ovariectomised (OVX) rat model.
Results: Six flavonoids with different contents between XEK and XEP were identified. As compared with the OVX group, significantly higher bone mineral density, elastic-modulus, and compressive strength were found in both the XEK group and XEP group ($p < 0.05$ for all, $n = 8$). Histomorphometric data presented significantly higher osteoblast surface ratio and osteoid area accompanied by significantly lower values of erosion surface and adiopocytes area in two treatment groups ($p < 0.05$, $n = 6$). XLGB Fufang with either XEK or XEP all showed significant preventive effects in OVX-induced osteoporosis and deterioration of bone mechanical properties.
Conclusion: The significance of the current preclinical experimental study was that these two *Epimedium* species used for formulating XLGB capsules were equally effective for the prevention of oestrogen-depletion induced osteoporosis.

Introduction

Osteoporosis and osteoporotic fractures occur at metabolically more active and faster deteriorating trabecular regions such as at the spine and hip [1,2]. Prevention and treatment of osteoporosis is essential in avoiding osteoporotic fractures, especially in postmenopausal women. Hormone (oestrogen) replacement therapy is one of the first-line effective countermeasures for relieving menopausal symptoms and for the prevention and treatment of postmenopausal bone loss [3]. However, the Women's Health Initiative trial data caused concerns being raised both in postmenopausal women and their physicians due to the increased risk of breast cancer and cardiovascular events [4].

Herbal Fufang or formula is a popular alternative in traditional Chinese Medicine (TCM) developed for the prevention and treatment of osteoporosis and related bone metabolic diseases [5,6]. Xian Ling Gu Bao Fufang (XLGB) was developed based on the modification of the empirical "Miao minority" medicine, which was traditionally used to tone the "kidney system" and nourish bones in oriental herbal medicine [7]. XLGB capsule was officially approved by the Chinese State Food and Drug Administration and was served as an over-the-counter drug for the treatment of osteoporosis, osteoarthritis, aseptic osteonecrosis, and fractures [5]. As a phytoestrogen-rich TCM [8], preclinical studies showed that XLGB improved bone mineral density (BMD) and mechanical properties in ovariectomised (OVX) rat models [9]. Clinical data demonstrated its positive effects in promoting fracture healing [10] and in the treatment of osteoporosis [10]. Recently, a multicentre, double blind, placebo-controlled, and dose-effect clinical trial further confirmed the safety and efficacy of XLGB in postmenopausal women with osteoporosis [5].

As a herbal Fufang, XLGB consists of six herbs with different percentages in weight as follows: *Herba Epimedii folium* (70%), *Dipsaci Radix* (10%), *Salvia Miltiorrhizae Radix et Rhizoma* (5%), *Anemarrahenae Rhizoma* (5%), *Psoraleae Fructus* (5%), and *Rehmannia Radix* (5%) [5]. Undoubtedly, as the "monarch" herb in XLGB Fufang based on TCM theory, the source of *Herba Epimedii* is of great importance for its long-term sustainable development. In Chinese Pharmacopoeia before 2005, there were five species of *Herba Epimedii*: *Epimedium brevicornum* Maxim., *Epimedium sagittatum* (Sieb.et Zucc.) Maxim., *Epimedium pubescens* Maxim., *Epimedium wushanense* T.S. Ying, and *Epimedium koreanum* Nakai [11]. Icariin was the index component for the quality control of the antiosteoporotic herbal Fufang [11]. Since the 2010 Chinese Pharmacopoeia, *E. wushanense* T.S. Ying has been separated as a new TCM and epimedin-C has been selected as the index component for its quality control [12]. Different species of *Herba Epimedii* have varying flavonoids with different concentrations. Even the same species may have different compositions if they are from different locations, e.g., *E. sagittatum* in the Guizhou province contains more icariin and epimedin-C than that in Henan or Hubei province [13]. It is mandatory to specify the species and locations of herbs for making herbal Fufang in TCM. XLGB contains *E. pubescens* from the Sichuan province in its formulation, where its index component icariin has at least 1.4% of the total flavonoids for quality control [13]. With the increasing demand for XLGB as preventive herbal Fufang against osteoporosis in an aging society in China, it is indispensable to seek other species of *Herba Epimedii* for formulating XLGB herbal medicine for clinical applications. *E. koreanum* in the Chinese Pharmacopoeia became a potential candidate with following three distinguished advantages: (1) large resources available in Liaoning province [12]; (2) almost all of the flavonoids are found in *E. pubescens* although the content of each flavonoid may vary [13]; and (3) the content of icariin, the index component in XLGB for its quality control, in *E. koreanum* is found more than that in *E. pubescens* from Sichuan used for formulating commercial XLGB [13].

In order to evaluate the potential of *E. koreanum* from Liaoning for potential replacement of *E. pubescens* from Sichuan used for formulating XLGB Fufang, we prepared XLGB particles containing *E. koreanum* (XEK) using the same manufacturing process as the commercial ones made of *E. pubescens* (XEP). Ultra performance liquid chromatography (UPLC) was used to demonstrate the different chemical constitutes in XEK and XEP; and then those compounds with different contents were tested for their bioactivity *in vitro*.