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Estrogenic/antiestrogenic activities of a *Epimedium koreanum* extract and its major components: *in vitro* and *in vivo* studies

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ABSTRACT

The estrogenic and antiestrogenic activities of Epimedii Herba, which is a traditional medicinal herb used in Korea and China were investigated in this study. The *in vitro* estrogen receptor (ER) mediated estrogenic/antiestrogenic activities of an Epimedii Herba extract (Epi ext) and its major components were determined using an estrogen responsive element driven reporter gene assay in MCF-7/ERE and HEK293T cells. The Epi ext exhibited ERα- and ERβ-mediated estrogenic activity with an EC_{50} of 5.0 and 17.8 μM in HEK293T cells, respectively. Prenylflavonoid glycosides such as icariin (ICA), epimedin A, B, and C did not show any *in vitro* estrogenic or antiestrogenic activities. Icaritin (ICT) and quercetin exhibited *in vitro* ER mediated estrogenic activity with a more potent interaction with ERβ. *In vivo* estrogenic activities of the Epi ext, ICA and ICT were compared using an uterotrophic assay. Although the potency of *in vitro* estrogenic activity was in the order of ICT > Epi ext > ICA, ICA had the strongest estrogenic activity and next ICT in ovariectomized rats. These results collectively suggest that phytoestrogens possess both estrogenic and antiestrogenic activity, and that the differential expression of these two compounds with opposing activities is dependent on the physiological environment in terms of estrogen level, which may be the case in humans.

1. Introduction

Estrogens play important roles regulating complex cellular events associated with female sexual development and maintaining the health of reproductive organs, bone, and the nervous and cardiovascular systems (Felson et al., 1993; Kohrt and Birge, 1995). Cellular signaling of estrogens is mediated through the ERα and ERβ estrogen receptors. Binding of estrogen or estrogen-like compounds to ERα or ERβ induces a conformational change in the receptor, promotes receptor dimerization, either as homodimers (ERα/ERα or ERβ/ERβ) or heterodimers (ERα/ERβ), translocates the dimers to the nucleus, where of estrogen responsive elements (ERE) on DNA recognize the molecules, and modulate the transcription of target genes in estrogen-responsive tissues. The target genes activated by these events, and, hence, the physiological responses, depend on the dimer pair activated by the ligand (Sotoca et al., 2008; Powell et al., 2012). Although both ERα and ERβ are mediators of the effects of estrogens, they have distinct or even opposing biological effects in certain cells where the action of estrogen ligands depends on a balance between ERα and ERβ. In contrast to ERα-promoted cancer cell growth, ERβ inhibits cancer cell proliferation (Hartman et al., 2006; Powell et al., 2012).

Phytoestrogens are compounds produced naturally in plants that have the ability to interfere with estrogen action either by interacting directly with the ERs or indirectly by modulating endogenous estrogen concentrations (Makela et al., 1999; Park et al., 2012). Many phytoestrogens bind with a higher affinity to ERβ than to ERα, suggesting that they may induce physiological effects through this ER subtype. However, binding affinity to ERα or ERβ alone does not necessarily predict the potency of a phytoestrogen to activate ER subtype signaling (Swedenborg et al., 2009). Accumulating evidence from *in vitro* experiments, animal studies,

Abbreviations: Epi ext, 70% ethanol extract of *Epimedium koreanum*; ERE, estrogen responsive element; ICA, icariin; ICT, icaritin; ER, estrogen receptor; OVX, ovariectomy, ovariectomized; REA, relative estrogenic activity.

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and human clinical trials suggests that in addition to their beneficial effects, phytoestrogens may also increase the risk of several hormone-dependent diseases in humans (Humfrey, 1998). The perceived health beneficial properties of phytoestrogens extend beyond hormone-dependent breast and prostate cancers and osteoporosis to include cognitive function, cardiovascular disease, immunity and inflammation, and reproduction and fertility (Dixon, 2004).

Epimedii Herba (Korean Pharmacopoeia, 2008), the aerial parts of *Epimedium* species (Berberidaceae), has been traditionally used in Korea and China for treating "coldness", gonadal dysfunction, male impotence, to improve female health, strengthen bones and tendons, and to relieve pain in the back and knees (Yap et al., 2007; Xie et al., 2005). Several studies have investigated the ability of an Epimedii Herba extract (Epi ext) to prevent bone loss in relation to the estrogenic effect in animals (Zhang et al., 2006; Xie et al., 2005) and in human clinical trials (Zhang et al., 2007). More than 260 compounds have been isolated; 141 flavonoids, 31 lignins, 12 ionones, nine phenol glycosides, six phenylethanoid glycosides, five sesquiterpenes, and a number of other compounds representing a wide spectrum of secondary metabolite classes have been isolated and identified from the genus *Epimedium*. The most prominent components are the prenylated flavonol glycosides (Ma et al., 2011). Among them, icariin (ICA), epimedin A, B, and C, and hyperin are the main components in Epimedii Herba (Islam et al., 2008; Chen et al., 2008). ICA, the principal *Epimedium* prenylflavonoid, significantly inhibits human phosphodiesterase-5 and induces nitric oxide synthase expression in corpus cavernosum smooth muscle (Liu et al., 2005; Xin et al., 2003). In animal studies, administering ICA and *Epimedium* flavonoids inhibits bone resorption, stimulates bone formation, prevents osteoporosis in ovariectomized (OVX) rats (Zhang et al., 2006, 2007), and improves erectile function in aged male rats (Makarova et al., 2007). Icaritin (ICT), the aglycon of ICA, has estrogenic properties and stimulates estrogen-driven cells (Wang and Lou, 2004).

Phytoestrogens interfere with endogenous estrogen action either by acting as agonists when endogenous estrogen levels are low or by acting as antagonists when endogenous estrogen levels are high. Therefore, OVX rats are frequently used to assess the beneficial effects of phytoestrogens such as their ability to inhibit bone resorption (Zhang et al., 2006; Xie et al., 2005). Although Epimeii Herba possesses estrogenic activity (Wang and Lou, 2004), no studies have been conducted on the *in vitro* and *in vivo* estrogenic and/or antiestrogenic activities of the major compounds purified from Epimedii Herba, particularly the epimedins.

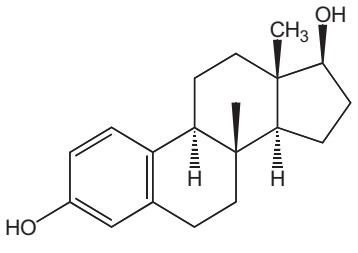
Thus, the aim of the study was to compare the ER α- or β-mediated estrogenic and antiestrogenic effect of a 70% ethanol extract of Epimidii Herba (Epi ext) and its major compounds (ICA, hyperoside, and epimedin A, B, and C) (Fig. 1) and their aglycons (ICT and quercetin) using an *in vitro* reporter gene assay. Additionally, the *in vivo* estrogenic and antiestrogenic actions of the Epi ext, ICA, and ICT were determined using an uterotrophic assay in OVX adult rats after oral administration.

2. Materials and methods

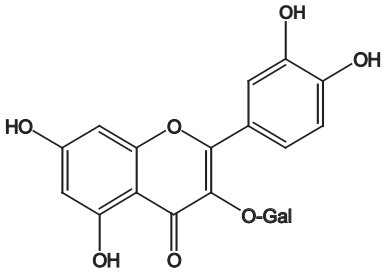
2.1. Preparation and identification of the major compounds from E. koreanum

Epimedii Herba was purchased from a farmer in Chulwon (Kangwondo, Korea) and authenticated by Professor Je-Hyun Lee, College of Oriental Medicine, Dongguk University (Gyeongju, Korea).

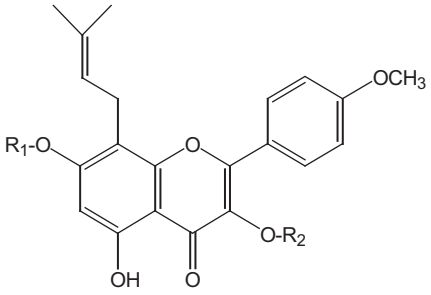
A 500 g portion of Epimedii Herba was extracted with 70% ethanol (3.5 L) by reflux for 3 h at 80 °C. After cooling, the samples were filtered, and the supernatant was collected. The supernatant was concentrated and freeze-dried to give a dried powder. The 70% methanol Epi ext was purified by solvent fractionation and chromatography. Each component was identified using nuclear magnetic resonance (NMR), as well as mass, infrared, and ultraviolet (UV) spectra (manuscript in preparation). The relative composition of the 70% Epi ext was determined using high performance liquid chromatography (HPLC)/UV and liquid chromatography/tandem mass spectrometry (LC/MS/MS), as described previously (Islam et al., 2008).



β-Estradiol



Hyperoside



Name	R_1	
Icariin	Glu	Rha
Epimedin A	Glu	Glc^{2}Rha
Epimedin B	Glu	Rha^{2}Xyl
Epimedin C	Glu	Rha^{2}Rha
Icaritin	H	H

Fig. 1. Chemical structures of 17β-estradiol, hyperoside, and prenylflavonoids from *Epimedium koreanum*.