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In vitro estrogenic activities of Chinese medicinal plants traditionally used for the management of menopausal symptoms

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Abstract

The estrogenic activity of 70% EtOH extracts of 32 traditional Chinese medicinal plants, selected according to their reported efficacy for the treatment of menopausal symptoms, was assessed using a recombinant yeast system with both a human estrogen receptor expression plasmid and a reporter plasmid. Among them, 11 (34%) species proved to be active. *Polygonum cuspidatum* had the highest estrogenic relative potency (RP) (3.28×10^{-3}), followed by *Rheum palmatum* (3.85×10^{-4}), *Cassia obtusifolia* (3.49×10^{-4}), *Polygonum multiflorum* (2.87×10^{-4}), *Epimedium brevicornum* (2.30×10^{-4}), *Psoralea corylifolia* (1.90×10^{-4}), *Cynomorium songaricum* (1.78×10^{-4}), *Belamcanda chinensis* (1.26×10^{-4}), *Scutellaria baicalensis* (8.77×10^{-5}), *Astragalus membranaceus* (8.47×10^{-5}) and *Pueraria lobata* (6.17×10^{-5}). The EC_{50} value of 17β-estradiol used as the positive control was 0.205 ± 0.025 ng/ml (RP = 100). This study gave support to the reported efficacy of Chinese medicines used for hormone replacement therapy.

Keywords: Phytoestrogens; Hormone replacement therapy; Chinese medicine; *Saccharomyces cerevisiae*

1. Introduction

Hormones such as estrogen and progesterone play a very important role in human growth. It is responsible in regulating the complex cellular events associated with differentiation, function and growth of female reproductive tissues. Women in the menopause have always had to suffer bone density reduction, sweating and anxieties because of a lack of hormones (Harlow and Signorello, 2000). Hormone replacement therapy (HRT) was introduced to improve the menopausal symptoms 20 years ago (Nichols et al., 1984), which quickly took effect but increased the risk of breast cancer (Beral et al., 1999). It was found that natural compounds from certain plants called phytoestrogens could be used for management of menopausal symptoms and have few side effects (Thompson, 1993; Glazier and Bowman, 2001).

Traditional Chinese medicine has been used to heal many diseases for thousands of years and is now well known as natural medicine throughout the world. Many herbal medicines such as *Angelica sinensis* (Oliv.) Diels, *Panax notoginseng* (Burk.) F. H. Chen and so on are effective for improving female function according to the oldest traditional Chinese medical book, *Sheng-nong Ben-cao Jing*. It has been proven that some plant extracts have estrogenic components possessing a potential human use in dietary supplements and treatment of menopausal symptoms (Liu et al., 2001).

In vivo and in vitro assays have been developed to test estrogenic substances. Although in vivo assays are widely used,

Abbreviations: DMSO, dimethyl sulfoxide; E_2, 17β-estradiol; EC_{50}, sample concentration at half-maximum β-galactosidase activity; ER, estrogen receptor; ERE, estrogen response elements; hER, human estrogen receptor; HRT, hormone replacement therapy; *lacZEscherichia, coli* β-galactosidase gene; OD_{420}, absorbance at 420nm; OD_{600}, absorbance at 600nm; *o*NPG, *o*-nitrophenol-β-D-pyrogalactoside; RIE, estrogenic relative inductive efficiency; RP, estrogenic relative potency

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they are unsuitable for large-scale screening and their utility is further limited due to the cost and relatively poor sensitivity. In vitro assays, however, are based on well-elucidated mechanisms of action and utilize more definitive end points than in vivo assays (Zacharewski, 1997). Among them, the assay system based on the binding of a ligand to estrogen receptor is the simplest. Yeast cells carrying the human estrogen receptor (hER) gene, estrogen response elements (ERE) and *Escherichia coli* β-galactosidase gene (*lacZ*) are very suitable for large-scale screening and sensitive analysis of estrogenic compounds. It is useful for the assay and discovery of novel estrogenic substances in natural specimens (Breithofer et al., 1998; Routledge and Sumpter, 1996).

In this study, a recombinant yeast with both a human estrogen receptor expression plasmid and a reporter plasmid was employed to search for phytoestrogens in selected Chinese medicinal plants, which have been used for hormone replacement therapy. A total of 32 Chinese medicinal plants (Table 1) were selected according to their reported efficacy for the treatment of menopausal symptoms and the estrogenic activities of their crude 70% EtOH extracts was assessed in order to give support to their reported activity and find crude drugs containing phytoestrogens in high concentration or highly active.

2. Materials and methods

2.1. Chemicals

17β-Estradiol (E_2) and *o*-nitrophenol-β-D-pyrogalactoside (*o*NPG) were purchased from Sigma. Yeast nitrogen base without amino acids was purchased from Fluka. All other reagents used in the study were of analytical grade.

2.2. Plant materials and extraction

A total of 32 Chinese medicinal plants were purchased from Darentang drugstore in Dalian, China, originating from different regions in China. The plants were identified by Dr. H. Sun, College of Pharmacy, Hei Longjiang University. Voucher specimens were preserved in College of Bio and Food Technology, Dalian Institute of Light Industry, China. The voucher numbers are shown in Table 1.

The minced plants (100 g) were extracted with 70% EtOH (800 ml) at room temperature for 24 h with shaking (Chen et al., 1987). The extracts were taken to dryness under reduced pressure at a temperature of 40–50 °C to yield gummy solids. The extraction yields (w/w) ranged from 0.6 to 12.1% (Table 1). The extracts were kept in a refrigerator for further activity assay.

2.3. Yeast strain and growth

For all transactivation assays *Saccharomyces cerevisiae* strain BJ3505 originally developed by Glaxo was used, which was kindly provided by Prof. W.Z. Wu, Institute of Hydrobiology, Chinese Academy of Sciences. This strain carried the hER expression plasmid YEPE10 and the estrogen responsive reporter plasmid YRPE2 (Santiso-Mere et al., 1991). The reporter gene (β-galactosidase) was controlled by ERE. The activity of β-galactosidase resulted in a color reaction, which was measured absorbance at 420 nm. The absorbance at 600 nm was selected to measure cell density and viability. The yeast strain was grown at 30 °C, 180 rpm, in selective medium with 50 μM $CuSO_4$ but without tryptophane and uracil (Wu et al., 2002a).

2.4. Preparation of test samples

The plant extracts were dissolved in dimethylsulfoxide (DMSO) and used as samples for screening tests. 17β-Estradiol was dissolved in DMSO and used as positive control.

2.5. Design of the experiments

The experiments were designed according to Wang et al. (2003) with some modification. For all experiments, overnight cultures were diluted to $OD_{600} = 1.0$ prior to the induction of hER expression and addition of 17β-estradiol (positive control), test samples or DMSO (negative control). All the samples at concentrations of 0.1–1000 μg/ml (dried extract/ml) and 17β-estradiol at concentrations of 0.001–10 ng/ml were prepared in DMSO. The final concentration of DMSO in the assays was less than 1.0%. In this test, 5 μl of samples, 17β-estradiol or DMSO were added to 995 μl of yeast culture containing 50 μM$CuSO_4$, which induced the expression of estrogen receptor (ER). After incubation at 30 °C for 2 h with shaking (150–180 rpm), the yeast cells were used for β-galactosidase assays.

2.6. β-Galactosidase assays

For the β-galactosidase assays, 100 μl cell suspensions were added to the wells of a 96-well microplate. The cells were permeabilized by addition of 100 μl assay buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 2 mg/ml *o*NPG, 38 mM β-mercaptoethanol, 0.01% Triton X-100, 15 U/ml lyticase). The microplate was incubated at 30 °C until the color became yellow, which resulted from β-galactosidase cleavage of *o*NPG. Then, 100 μl of 1 M Na_2CO_3 was added to stop the reaction. In our test system, for 17β-estradiol, the incubation time was 50 min, and for the samples, it was less than 90 min. The resulting absorption was measured at 420 nm with a plate reader (TECAN, Austria). Each test sample and E_2 was assayed in triplicate. E_2 as a positive control and DMSO as a negative control for activity were performed in each test run.