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Estrogen Bioassay of Ginseng Extract and Arginmax®, a Nutritional Supplement for the Enhancement of Female Sexual Function

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ABSTRACT

***Purpose*: To determine whether ArginMax® (The Daily Wellness Co., Sunnyvale, CA) or the *Panax ginseng* extract it contains has any estrogenic activity. ArginMax for Women, a nutritional supplement for optimization of sexual health, contains L-arginine, ginseng, ginkgo, damiana, multivitamins, and minerals.**

***Methods*: A human endometrial adenocarcinoma cell line, Ishikawa, which contains an alkaline phosphatase (AP) enzyme sensitive to estrogen stimulation, was used in a bioassay to determine whether *Panax ginseng* or ArginMax contained estrogenic components.**

***Results*: Neither ArginMax nor *Panax ginseng* stimulated AP at any of the concentrations tested.**

***Conclusions*: No estrogenic activity was evident in the sample of *Panax ginseng* extract tested or in a sample of ArginMax containing this extract in combination with other ingredients.**

INTRODUCTION

RECENT CONTROVERSY FROM THE RESULTS OF THE Women's Health Initiative (WHI) study[1] on the risks of hormone replacement therapy (HRT) has encouraged women to rethink whether they should take HRT or just endure their menopausal symptoms. Many women who stop other forms of HRT attempt to alleviate menopausal symptoms with nutritional supplements, arguing the need to examine the estrogenic potential of such compounds. Decreased libido and sexual dysfunction have been reported to increase after menopause.[2,3] A previous study[4] demonstrated the efficacy of ArginMax® (The Daily Wellness Co., Sunnyvale, CA) for improvement of sexual desire and satisfaction in women ranging in age from 24 to 71 years. Vaginal dryness, frequency of orgasm, and degree of clitoral sensation were also significantly improved by this supplement. The current study was conducted to determine whether this supplement exhibits any estrogenic activity as part of its mode of action.

ArginMax for Women is a proprietary nutritional supplement for female sexual fitness that combines L-arginine, *Panax ginseng*, *Ginkgo biloba*, and damiana leaf (*Turnera aphrodisiaca*) with vitamins A, C, E, B_6, B_{12}, biotin, folate, niacin, pantothenic acid, riboflavin, and thiamin, and the minerals calcium, iron, and zinc. Anecdotal reports

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have suggested that ginseng might have estrogenic activity.[5,6] Although the proposed mechanism of action for ArginMax in female sexual function through the nitric oxide pathway may explain improvements in vaginal dryness,[4] the improvements in frequency of orgasm, degree of clitoral sensation, and sexual desire imply that the brain and a central feedback loop also may be involved. In addition, hormonal influences, especially the effects of phytoestrogens from ginseng or other ingredients, have not been ruled out. Because women are concerned about ingesting any form of estrogen, this study was undertaken to determine whether estrogenic activity is present in ArginMax.

One previous study of potential treatments for menopause symptoms demonstrated no significant estrogen receptor (ER) binding activity by either *Panax ginseng* or American ginseng, although several other herbs (red clover, chasteberry, and hops) did bind to the ER.[7] A study on breast cancer cells found that American ginseng and estradiol equivalently induced RNA expression of an estrogen-regulated gene (pS2).[8] However, the American ginseng preparation caused a dose-dependent decrease in cell proliferation, in contrast to estradiol, which significantly enhanced cell proliferation. Concurrent use of American ginseng and breast cancer therapeutic agents caused significant suppression of cell growth for most drugs evaluated. An *in vitro* study of ginseng extracts binding to steroid receptors demonstrated that *Panax* and Siberian ginseng had demonstrable affinity for progestin and mineralcorticoid and glucocorticoid receptors, but only the Siberian ginseng bound to ERs.[9]

The possibility of estrogenic activity in a nutritional supplement containing *P. ginseng* is of interest because estrogen has been linked to tumor development in both uterine endometrium and breast tissue.[10–13] In addition, estrogen is widely used in oral contraceptives and HRT, and there could be a possibility of interference with these therapies with concomitant use of ArginMax or *P. ginseng*. With a potentially large number of women seeking remedies for loss or lack of libido,[14] the presence of estrogen in this supplement could present a safety issue. This study was designed to determine if ArginMax or the ginseng extract it contains has estrogenic activity using the Ishikawa cell bioassay.

MATERIALS AND METHODS

ArginMax, 18.5 mg/ml, and *P. ginseng* (standardized 30% ginsenosides), 2.5 mg/ml, in tissue culture medium were stirred for 3 hours. Each homogeneous suspension was serially diluted 10-fold for a total of eight separate log dilutions. The highest concentration at which the material ap-

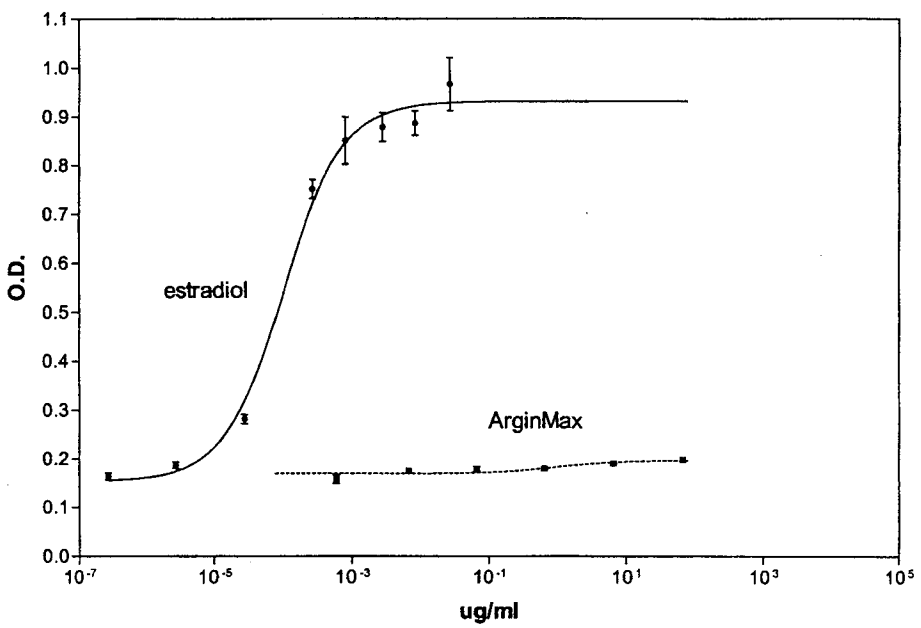

FIG. 1. Effect of estradiol and ArginMax on the induction of alkaline phosphatase in Ishikawa cells.

peared to be soluble was 185 μg/ml ArginMax and 25 μg/ml *P. ginseng*. Each solution was diluted 3 fold in the assay, decreasing the final concentration in the assay by two thirds, thus making the maximum tested concentration of ArginMax 61.6 μg/ml and that of *P. ginseng* 8.3 μg/ml.

The log dilutions of ArginMax, *P. ginseng*, and estradiol (for comparison) dissolved in tissue culture medium were tested for estrogenic activity by stimulating alkaline phosphatase (AP) in Ishikawa cells, a method developed by Littlefield et al.[15] This bioassay has been shown to provide a sensitive and accurate measurement of estrogenic activity. To perform the assay, a solution containing 50 μl of each test compound in quadruplicate was added to a 96-well plate containing 25,000 Ishikawa cells and 100 μl estrogen-free tissue culture medium (a 1:1 mixture of phenol red-free Ham's F-12 and Dulbecco's modified Eagle's medium [DMEM] and 5% charcoal stripped calf serum [GIBCO, Grand Island, NY]). The charcoal treatment removed the endogenous estrogens from the serum. The cells were grown at 37°C in a humidified atmosphere containing 5% CO_2. After 3 days, the cells were washed, frozen, thawed, and assayed for AP activity using the chromogenic substrate *p*-nitrophenyl phosphate.

RESULTS

As can be seen in Figures 1 and 2, estradiol induced the formation of AP at very low concentrations, with an EC_{50} of $\sim 4 \times 10^{-10}$ M (0.1 ng/ml). Significant stimulation was seen at 3×10^{-11} M estradiol, with maximal stimulation at about 10^{-9} M. Neither ArginMax nor *P. ginseng* stimulated AP at any of the concentrations tested.

Estradiol maximally stimulates the Ishikawa cells at a concentration of about 0.1–1 ng/ml. The estrogen produced a minimally detectable response at approximately 30 pg/ml. As 17β- estradiol is the most potent of the naturally occurring estrogens, phytoestrogens would be expected to be less potent. In these experiments, ArginMax and *P. ginseng* were tested at maximal concentrations of 185 and 25 μg/ml, respectively (amounts that exceeded the minimally effective dose of estradiol by a factor of 1,000,000). Even at such high concentrations, ArginMax and *P. ginseng* showed no signs of estrogenic activity.

DISCUSSION

Following information from the WHI on the slightly increased risk of heart attack and breast cancer ascribed to one form of HRT, many wo-

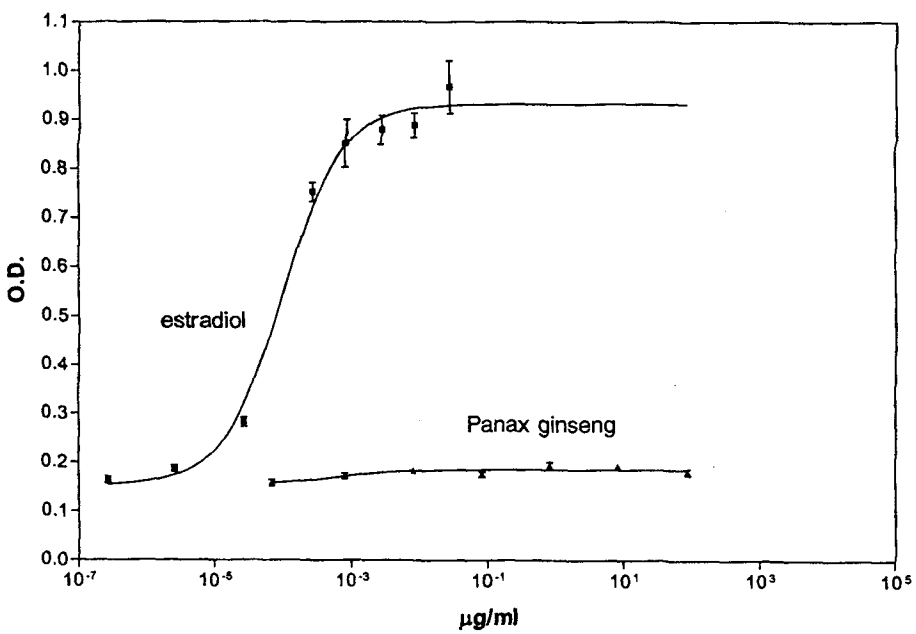

FIG. 2. Effect of estradiol and *P. ginseng* on the induction of alkaline phosphatase in Ishikawa cells.

men have become fearful of all forms of HRT. Many are turning to nutritional supplements and homeopathic remedies. Rarely do such preparations have clinical evidence of efficacy or safety. Information on estrogen-mediated activity and the potential side effects in supplements used to treat menopausal symptoms is essential for women seeking alternatives to HRT. Experimental evidence demonstrating a lack of estrogenic activity in ArginMax, as well as in other nutritional supplements, is critical before these compounds can be recommended for the relief of specific menopausal symptoms.

CONCLUSIONS

This study demonstrates the absence of estrogenic AP activity in both *P. ginseng*, a component of ArginMax, and in the complete preparation. The lack of AP activity is reasonable reassurance that potential estrogen-mediated side effects are not a concern with ArginMax, a supplement that has been shown to improve sexual desire and satisfaction in women.[4]

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