

Commercialized Ginseng Products have Estrogen Receptor β Binding Properties with possible correlation to Zearalenone Contamination

by

Benjamin Schaeffer Koch

Dr. Sandra L. Gray

Clemson University Endocrinology Physiology Laboratory

Abstract

All over the world herbal products are used as therapies for sicknesses and general well being. Ginseng is an herb that gained popularity for medicinal purposes in the Far East with references to its use in ancient times. Traditionally, ginseng is used for physical, mental, and sexual rejuvenation. Many commercialized ginseng products use these claims to promote sales, and as a result, ginseng is one of the top five herbal products sold in the United States. Ginseng products are touted as being capable of relieving menopausal symptoms as well. This study investigated the possibility that commercially available ginseng products had estrogen receptor beta binding properties and that zearalenone, a mycotoxin formed by *Fusarium* fungi, was a possible source of estrogen receptor binding.

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Corresponding Author:

Koch@musc.edu

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Introduction

Alternative medicine is defined as various herbal and other miscellaneous treatments that have not been accepted by the mainstream, or Western, medical establishment (11). The use of alternative medicine in the United States is very popular with 425 million visits to alternative practitioners compared to 388 million visits to primary care practitioners in 1990. It was estimated that in 1996 the sale of herbal products would reach around \$2 billion and that approximately 63% of the population would use herbal medicine in their health care (5).

Ginseng has been used as a medicinal herb for over 2000 years. It has been shown to

interact with the CNS, cardiovascular, endocrine, and immune systems (2). Commercial products may use one or more of ginseng's different species. Some of the most common forms of ginseng used are *Panax ginseng* (Asian ginseng), *Panax quinquefolius* (American ginseng), and *Eleutherococcus senticosus* (Siberian ginseng). *Panax ginseng* and *P. quinquefolius* are the most frequently used forms in herbal products. In the past, *P. ginseng* grew in China, Korea, and parts for Siberia, but presently the wild plant is rare, and most of the ginseng root found in the market is grown commercially. *Panax quinquefolius* was harvested at one time exclusively from native populations in North

America but is now grown commercially for domestic use and as well as export (14). Although Siberian ginseng is a member of the same plant family, Araliaceae, it is not a true ginseng (21).

Ginseng products are available in a variety of forms such as capsules, powders, tinctures, teas, and whole roots. Many other products like toothpaste, beverages, power bars, and baby foods include ginseng in their ingredients. Advertisements of commercial products suggest ginseng use provides benefits such as improvement of stamina and concentration, facilitation of healing, increased resistance to stress, and improvement in work efficiency. (21).

Many ginseng products are marketed specifically for postmenopausal women and this has led to studies investigating how such effects might occur. One study found that both *Siberian ginseng* extract and *Panax ginseng* extract have an affinity for progesterin, glucocorticoid, and mineralcorticoid receptors. The same study stated that *Siberian ginseng* binds to estrogen receptors (3). Another study showed that estradiol and *P. quinquefolius* extracts induced RNA expression of presenelin-2 equally well in estrogen receptor (ER) positive cells (6). *Panax quinquefolius* extract was responsible for diminishing the proliferation of the ER positive breast cancer cell line, MCF-7 (2). A different study showed that *P. quinquefolius* increased cancer growth (1). There are also case reports of mastalgia where in one case a 70-yr-old woman presented with swollen, tender breasts along with dispersed nodularity after use of a *P. ginseng* powder. Another case study showed a 62-yr-old woman who had undergone a complete hysterectomy 14 years previously, with marked estrogenic effects after using a ginseng product. Estrone, estradiol, and estriol levels were

basically unchanged over the time period of usage (3).

The reports of estrogenic effects of ginseng products requires further study in order to determine what bio-active compounds are causing such effects and to help determine whether or not such compounds should be regulated in commercialized products. In order to understand what substances might take part in estrogenic responses, a logical step would be to determine the competitive activity of a compound to hormone receptors like the estrogen receptors (8).

Zearalenone (ZEA) is a potent estrogenic and anabolic compound that causes reproductive problems in farm animals (16). Zearalenone is a naturally occurring mycotoxin that is produced by the fungal species *Fusarium* which can grow on agricultural products in the field or in storage. It is known to be a health concern even at the parts-per-billion level (18). Zearalenone is quickly transformed within the liver to α - and β -zearalenol by 3 α -hydroxysteroid dehydrogenase. These products are comparatively much more estrogenic than ZEA (9,15). Zearalenone shows distinct estrogenic and anabolic properties in several animal species which is due to its agonistic effect on the estrogen receptor, resulting in serious effects on the reproductive system (20). This mycotoxin is known to affect the reproductive capability, cause vaginal swelling, enlargement of the mammary glands, and testicular atrophy in farm animals (17). The U.S. Food and Drug Administration requires that low levels of this toxin be measured in grains and other agricultural commodities (18).

Herbal products, however, are under no such scrutiny. Most herbal products such as ginseng extracts are never screened for mycotoxins. There is a distinct possibility that during the farming and storage of such products, *Fusarium* growth could occur, leading to the possibility of contamination

with ZEA (8). In this study, it is believed that the detection of estrogen receptor- β binding in commercialized ginseng products can be correlated with ZEA concentration in the products. The objective of this study was to test commercialized ginseng products using a competitive binding assay for interaction with the ER- β and to expose the source of binding in the products using immunoassay.

Materials and Methods

Commercialized ginseng products were acquired through local merchants who were associated with larger store chains.

Chemicals and Reagents

All chemicals were from VWR (Atlanta, GA), Fisher (Atlanta, GA), or Sigma (St. Louis, MO) unless noted otherwise. Estradiol, [2,4,6,7- ^3H (N); 71 Ci/mmol] (^3H -E₂) was purchased from NEN Life Sciences Products (Boston, MA). Human recombinant ER β was obtained from PanVera (Madison, WI).

Extraction

The samples were extracted based on the recommended dosage for the ginseng product. Samples in a solid form were extracted using 15 ml 80% methanol. Liquid samples were extracted with 1ml of 80 % methanol. Samples were mixed with the solvent on a lateral shaker for approximately 8 hours at room temperature. The samples were centrifuged at 1800 x g for 15 min, and the supernatant was removed into a clean tube. The pellet was extracted again with 80 % methanol. Methanol extracts were combined, and placed in a Thermolyne Dri-BathTM (VWR, Atlanta, GA) at 40° C and evaporated to dryness using filtered air. Samples were reconstituted at 1ml/g original material and filtered using 0.45 μm PVDF Acrodisc® (V45W500; Pall Gelman; Ann Arbor, MI).

Competitive Binding Assay

Estrogen receptor β binding studies were performed using the procedure of Obourn with modifications (8, 10). The assay binding buffer consisted of 10 mM Tris, pH 7.5, 10% glycerol, 2 mM dithiothreitol, and 0.1% bovine serum albumin. Various concentrations of test samples were evaporated to dryness and reconstituted in 50 μl binding buffer. Competition assay reactions included test samples evaporated to dryness and reconstituted in 50 μl binding buffer, 50 μl of recombinant receptor beta (5nM) and 100 μl ^3H -E₂ (400 nM) for a total incubation volume of 200 μl . Each extraction replicate was assayed in triplicate. Each assay also included a standard curve using E₂ concentrations at 0.0, 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, and 10.0 ng and non-specific binding (NSB) of ^3H -E₂. Non-specific binding counts were subtracted from total binding to determine specific binding. Standard concentrations (diluted in 100% ethanol) and product extracts were added to assay tubes, evaporated to dryness under filtered air and reconstituted in 50 μl assay buffer. Incubations were conducted for 2 h at room temperature followed by 16 h at 4° C. Separation of bound and free steroid was performed by the addition of 1 ml activated charcoal suspension (3%) in binding buffer. Samples were incubated for 5 min in ice water bath after charcoal addition and centrifuged for 10 min at 1800 x g. Supernatant was decanted into 7 ml scintillation vials, 4 ml Ultima GoldTM (Packard, Cat. No. 6013329; Meriden, CT) scintillation fluid added and samples counted using a Beckman LS 1800 liquid scintillation counter (Irvine, CA). A 4-parameter logistic standard curve was constructed using StatLIA Analysis software (Brendan Scientific; Carlsbad, CA). Concentration of test samples displacing approximately 50% ^3H -E₂ binding from each receptor (IC₅₀) was determined from

the standard curve. The data represent the average \pm SE of six determinations.

Affinities of receptors and estrogenic ligands can be determined by measuring the equilibrium binding of the radioligand in the presence of different concentrations of the unlabeled ligand. These assays are conducted by determining the binding affinity of a test compound for the ER relative to a radiolabeled competitor with a known binding affinity. The plant tissue crude extracts may contain compounds that displace E_2 from its receptor, but concentrations of these compounds may be unknown. A convenient way of expressing the estrogenicity of such extracts is by calculation of estrogen receptor binding equivalents (EBE) in a 1-gram sample. Different extract dilutions incubated with the radiolabeled E_2 for receptor binding, and EBE are based on the extract dilution that displaces approximately 50% of the radioligand. Thus, EBE represent a relative measure of the ER binding potential by compounds from a 1-gram plant tissue sample.

ELISA Determination of Zearalenone

Ginseng root samples were extracted and assayed using the Veratox® Quantitative Zearalenone Test CD-ELISA (Neogen Corporation of Lansing, MI), a kit used commercially to detect food and feed possibly containing ZEA. Each of the ER β binding samples were dried down and reconstituted with 100% ethanol. The samples were then filtered with 0.45 μ m PVDF Membrane Acrodisc® LC 13 mm Syringe Filters (Pall Gelman; Ann Arbor, MI). Aliquots of 100 μ l from each sample were added to the micro-assay plates. Free ZEA in the samples and controls competed for the antibody binding sites with enzyme-labeled ZEA. Micro-assay plates were washed and substrate was added, which binds with the conjugate to produce blue color. Plates were read in a micro-well

reader using a 650 nm filter. Concentrations were read on a log-logit curve generated by the reader from a set of standards provided in the kit. Range of quantification was 50-600 ppb. Root samples were extracted and assayed in two separate experiments.

Results

Affinity of Ginseng Commercial Products for ER β

Extracts of only one of fourteen different products did not have ER β binding properties (Table1). The samples that did have binding properties ranged in estrogen binding equivalents from 10.9 to 306.4 ng/g. Product samples that were in liquid form tended to have less EBE than the products that were sold in a powder form.

Zearalenone Concentrations in Ginseng Commercial Product Extracts

Using ELISA

Raw extracts of commercialized ginseng products from eight sources were screened for traces of ZEA content using CD-ELISA. One of the samples tested had no binding in the ER assay and was used as a negative control. The results of five other samples showed positive evidence that ZEA was in the product samples (Table 2). Four of the five samples that tested positive were from the crude product and the other one was already in an encapsulated form.

Pearson correlation coefficient was 0.89 indicating a significant correlation between the ER β binding and ZEA concentration (Figure 1).

Discussion

There is a growing trend for patients to use alternative therapies for treatment of health symptoms. The days when a physician needed little knowledge about alternative treatments used by the patient are rapidly coming to an end. Ginseng is an herbal product that is used for treatments of numerous symptoms. Women looking for

treatment of menopausal symptoms sometimes turn to ginseng as a form of relief. Ginseng has a long history of use and is generally considered to be safe and have few negative side effects (2). Nonetheless, there are cases of ginseng product use which have resulted in estrogen-like effects.

Several studies have investigated sources of the estrogen-like effect from ginseng root (12, 8). At present herbal products are not under strict regulations and their quality and content are at the discretion of the producer. This has become a major concern due to the risk of harmful substances possibly contaminating the herbal products. A study investigating bust-enhancing creams discussed the possible role of the estrogenic mycotoxin, ZEA, as contributing to estrogenic action of the creams (7, 16). This mycotoxin has been shown to be a health hazardous at the parts per billion level (ref). The FDA tests ZEA and other mycotoxin concentrations in food and agricultural products (18).

Human exposure to ZEA has not been thoroughly investigated, but in rats, mice, swine, and monkeys the mycotoxin is known to cause hyperestrogenicity and severe reproductive and infertility problems. There are also tumor promoting properties similar to estrogen, which may be associated with carcinogenesis, found in ZEA (4, 13, 19). In China, "endemic breast enlargement disease," was linked to *Fusarium*-contaminated grain (7). A recent study showed that the binding capabilities of ginseng extracts were 3-4 times higher for ER β than ER α , hence the study's focus on the ER β (8). This study illustrates the importance of testing commercial ginseng products for ER β binding capabilities and concentrations of ZEA in an effort to increase awareness of the dangerous substances. ZEA and other mycotoxins pose potential health risks to the public, especially those where estrogenic side

effects or exposure are of particular concern. This study's discoveries promote the testing of all herbal products for mycotoxins. The presence of ER binding and ZEA in herbal products can be logically detected using the methods contained within the study.

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Table 1. Estrogen Receptor Beta Binding Equivalents (EBE) from Methanol Extracts of Ginseng Products Using a Competitive Binding Assay

Ginseng Product	Mean EBE (ng/g)	Std. Error	Product Description
A	80.0	13.8	Compacted pill
B	154.8	17.4	Capsule
C	0.0	0.0	Liquid drops
D	225.8	41.8	Powder -precapsule form
E	94.0	10.6	Powder -precapsule form
F	25.5	2.6	Drink
G	306.4	35.3	Powder -precapsule form
H	260.0	19.0	Powder -precapsule form
I	123.3	6.1	Powder -precapsule form
J	74.9	14.7	Powder -precapsule form
K	10.9	1.7	Liquid drops
L	29.6	1.2	Drink
M	123.1	31.2	Powder -precapsule form

Table 2. Zearalenone (ZEA) Concentrations in Methanol Extracts of Ginseng Products

Ginseng Product	EBE (ng/g)	ZEA (ng/g)
A	80.0	0.0
B	154.0	209.5
C	0.0	0.0
D	225.8	197.2
E	94.0	139.7
F	25.5	0.0
G	306.0	248.3
H	260.0	370.8

Note. ZEA concentration was determined using CD-ELISA.

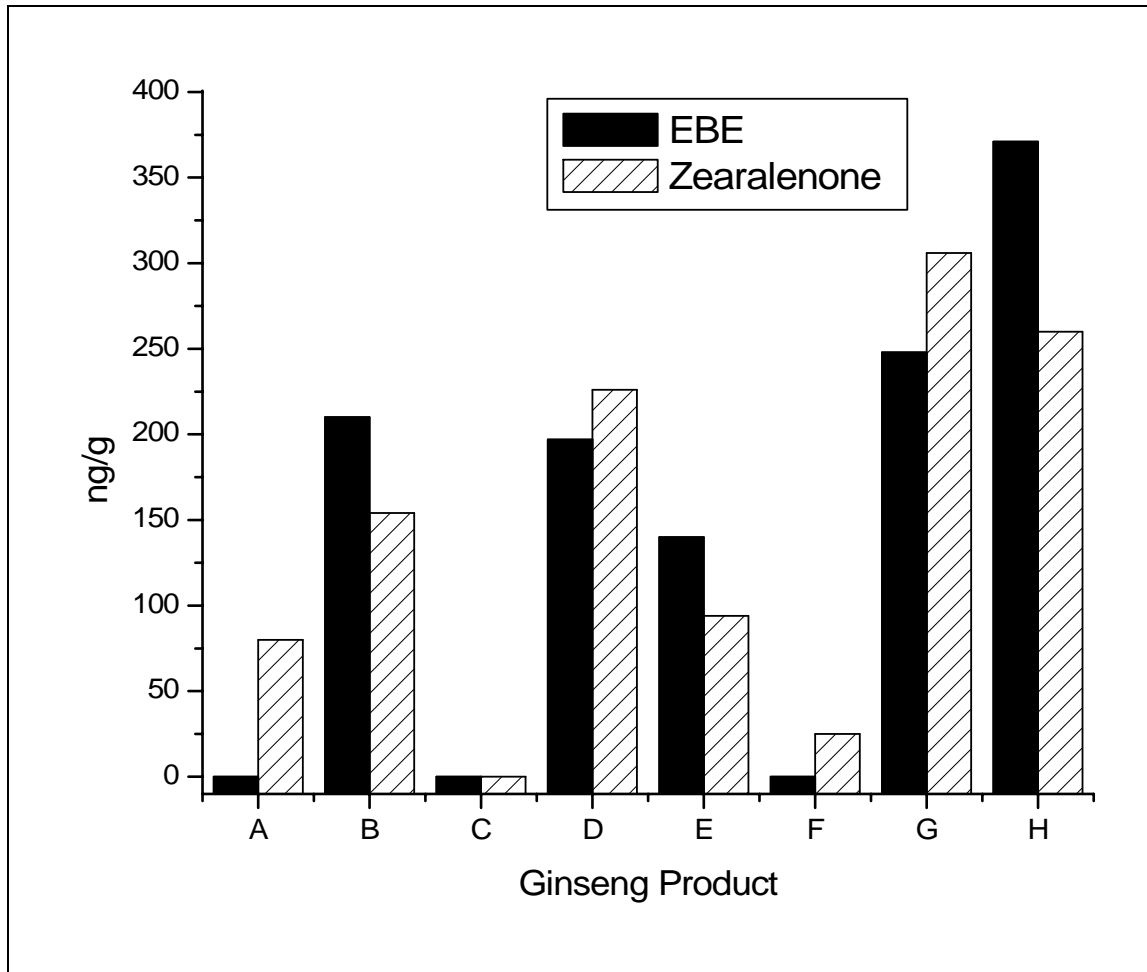


Figure 1. Comparison of ER β estrogen receptor binding equivalents (EBE) with zearalenone concentration from extracted commercial ginseng products (A-H).

Note. Estrogen binding equivalents and zearalenone concentrations in samples were determined using competitive binding assay and CD-ELISA, respectively. Pearson's correlation coefficient (r^2) = 0.89.