Research highlights from the UIC/NIH Center for Botanical Dietary Supplements Research for Women’s Health: Black cohosh from the field to the clinic

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Abstract
In 1999, the Department of Medicinal Chemistry and Pharmacognosy at the College of Pharmacy, University of Illinois (UIC) at Chicago was funded to establish a Botanical Dietary Supplements Research Center from the National Institutes of Health (NIH). The emphasis of the UIC/NIH Center for Botanical Dietary Supplements Research (CBDSR) is botanical dietary supplements (BDS) for women’s health. The Center’s research has focused on BDS that may improve women’s health and quality of life, specifically in the areas of menopause, premenstrual syndrome, and persistent urinary tract infections. Center investigators have overcome many challenges associated with botanical dietary supplements research, including acquiring and identifying plant species for investigation, isolating and identifying active constituents, elucidating the mechanisms of action of these botanicals, and conducting Phase I and Phase II clinical studies. Black cohosh [Actaea racemosa L. (Ranunculaceae)] has been used as a model to illustrate the steps involved in taking a botanical dietary supplement from the field, all the way to clinical trials. Bioassays are described that were necessary to elucidate the pertinent biological studies of plant extracts and their mechanisms of action. The Center has used an innovative multidisciplinary approach to this type of research, and thus has been very successful in fulfilling its specific aims.

Keywords: Actaea racemosa; black cohosh; botanical dietary supplements; estrogen; menopause; multidisciplinary research; serotonin

Introduction
Botanical dietary supplements are widely used by many Americans despite a lack of safety and evidence for most of these products. In 2002, the National Health Interview Survey conducted by the Centers for Disease Control and Prevention indicated that approximately 38.2 million (19%) American adults use natural products, primarily botanical supplements (NCCAM, 2009). Recognition of these statistics led the US Congress to the formation of the Office of Dietary Supplements (ODS) and the National Center for Complementary and Alternative Medicine (NCCAM, 2009), which later established the botanical centers program. The Centers for Botanical Dietary Supplements Research (CBDSR) identify and characterize botanicals, assess bioavailability and bioactivity, explore mechanisms of action, conduct preclinical and clinical evaluations, help select botanicals to be tested in clinical trials, and provide a rich environment for training and career development. The goals of the Centers are to advance the scientific base of knowledge about botanicals, including issues of their safety, efficacy, and biological action. The University of Illinois at Chicago/National Institutes of Health
(UIC/NIH) Center for Botanical Dietary Supplements Research was established in 1999 during the first round of funding from a grant from the NIH via the Office of Dietary Supplements (ODS), the National Center for Complementary and Alternative Medicine (NCCAM), the National Institute of General Medicine Sciences, and the Office of Research in Women’s Health. The center focuses on three areas of concern to women: menopause, premenstrual symptoms, and urinary tract infections.

The UIC/NIH CBDSR uses a multidisciplinary approach to its research, and had from its inception the specific aim of taking a plant from the field to the clinic, ensuring the processing of a safe and effective botanical dietary supplement for human consumption. The Center research team includes faculty from the College of Pharmacy (Departments of Medicinal Chemistry and Pharmacognosy, Biopharmaceutical Sciences, and Pharmacy Practice), the College of Medicine (Departments of OB/GYN and Psychiatry), and the College of Liberal Arts and Sciences (Department of Math and Statistics). The collaboration also involved Northwestern University (Obstetrics and Gynecology) in conjunction with two industrial partners, Pharmavite LLC and Naturex, Inc. The Center faculty and staff selected 12 botanical dietary supplements that were widely used by women in the United States to treat the three specific areas of concern. These botanicals include *Angelica sinensis* (Oliv.) Diels (Apiaceae; dong quai), *Trifolium pratense* L. (Fabaceae; red clover), and *Actaea racemosa* L. (syn: *Cimicifuga racemosa* (L.) Nutt.; Ranunculaceae; black cohosh), which were all used for the management of menopausal symptoms. *Viburnum prunifolium* L. (Caprifoliaceae; black haw) and *Vitex agnus-castus* L. (Verbenaceae; chasteberry) were commonly used to treat premenstrual syndrome (PMS), dysmenorrhea, and cyclic mastalgia. *Ginkgo biloba* L. (Ginkgoaceae; ginkgo), *Glycyrrhiza glabra* L. (Fabaceae; licorice), *Humulus lupulus* L. (Cannabaceae; hops), *Panax ginseng* C.A. Meyer (Araliaceae; Asian ginseng), and *Panax quinquefolius* L. (Araliaceae; American ginseng) were all reported to have estrogenic effects and were thought to be of potential use for the treatment of menopause or PMS. *Vaccinium macrocarpon* Ait. (Ericaceae; cranberry) was commonly used for the prevention or treatment of urinary tract infections, and *Valeriana officinalis* L. (Valerianaceae; valerian) was often used by menopausal women to improve sleep (Farnsworth, 2009). As each of these botanicals was investigated, the results determined whether the plants were appropriate for further studies or, where inactive, they were to be removed from the list.

**Center organization**

The UIC/NIH CBDSR is organized into five research components consisting of three project areas and two clinical evaluation teams (Figure 1). All research projects are supported by an administrative core (Core A) and a technology utilization core (Core B), which supplies expertise in mass spectrometry–liquid chromatography–mass spectrometry. Work flows naturally from the most basic science research in botanicals, which includes selecting the plant species, collection (primarily wild crafting), and isolation and elucidation of bioactive compounds, through established and novel methods of phytochemistry (Project 1) to the biological evaluation and mechanism of action studies (Project 2); to the characterization of metabolism, bioavailability, safety, and pharmacokinetics of active species contained in these botanicals (Project 3); and finally to safety and efficacy determined by Phase I and Phase II clinical trials (Clinical Evaluation Group). A continuous feedback mechanism is in place between projects and cores. The Center by this structural scheme promotes continuous interactivity between basic science researchers and the clinicians administering the Center’s clinical trials, thus following the philosophical construct of translational science.

**Chemical and biological standardization**

The taxonomic identities of plants to be used in the Center research are verified with voucher materials that are stored for future reference. If the plant is collected
in the wild, the process for acquiring the plant, including necessary permits, follows good collection (wild crafting) practices. If the plants are cultivated, good agricultural standards are followed according to guidelines (NCCAM, 2009). Processors and final formulation producers must follow good manufacturing practices to ensure that heavy metals, toxic materials, and chemical and microbial contaminants are not present in the product. All these steps are necessary to ensure traceability, should questions arise during the manufacturing and supply chain process, and are supported in documents published by NCCAM (2009). For human use, botanical dietary supplements should also undergo both chemical and biological standardization. Chemical standardization is common with supplements. The compound to which the supplement is standardized is the predominant chemical but it may not be the biologically active constituent. For active constituents, the Center employs bioassay-directed fractionation using several separation methods, including countercurrent chromatography, which resulted in the isolation and structure characterization using ultraviolet, infrared, proton magnetic resonance, carbon magnetic resonance, mass spectrum, optical rotatory dispersion, and X-ray analyses of >150 pure entities, including such diverse chemical groups as triterpenes, flavonoids, chalcones, phenolic acids, iridoids, alkaloids, depsides, phthalides, diterpenes, lactones, and sterols (Booth et al., 2006; Burdette et al., 2002a, 2002b; Chadwick et al., 2004; Chen et al., 2002a, 2002b, 2002c; Deng et al., 2006; Dietz et al., 2005a; Fabricant et al., 2005; He et al., 2006; Li et al., 2000, 2003; Liu et al., 2001, 2004, 2005; Nikolic et al., 2005; Piersen et al., 2004; Sheng-Hong et al., 2002; Turner et al., 2005, 2007).

In contrast to chemical standardization, biological standardization reflects the biological activity of the plant (or extract) in vitro or in vivo with the use of receptor- and cellular-based or animal assays that identify the biological activities, and may identify and filter out potentially toxic substances. The Center researchers anticipated that the alleviation of hot flashes as well as symptoms associated with premenstrual syndrome are partially mediated by activity at estrogen receptors. Estrogenicity seemed to be common to many of the 12 botanicals, with the exception of cranberry and valerian. Numerous bioassays were developed as data accumulated, which indicated that not all of the plants acted through estrogen mechanisms. Bioassays used in our studies included assays for estrogen receptors (Burdette et al., 2002b, 2003; Deng et al., 2006; Overk et al., 2005; Turner et al., 2005), serotonin receptors (Deng et al., 2006; Dietz et al., 2005b), opioid receptors (Rhyu et al., 2006; Webster et al., 2006; Xu et al., 2002), bacterial antiadherence (Turner et al., 2005, 2007), and antioxidants (Burdette et al., 2002a; Liu et al., 2005). Additionally, the Center developed and conducted studies of metabolism (Nikolic et al., 2005). Three of the plants were not active in our estrogen receptor (ER)-α and -β in vitro screening assays. Because we were unable to verify an estrogen-binding effect of ginkgo, licorice, and the ginsengs, we dropped them from additional work. Black cohosh, while not estrogenic, was retained because of its extensive history of use in women’s health, primarily on the basis of several German clinical trials that showed presumptive evidence of the alleviation of hot flashes in menopause.

### Biological activities of black cohosh

To determine whether any plant extract should be tested in clinical trials, a safety and efficacy profile must be established. For black cohosh, our first step was to perform a literature search using databases such as PUBMED (National Library of Medicine, Bethesda, MD) and the UIC-based NAPRALERT database (Farnsworth, 2009), a searchable database of scientific and ethnomedical botanical and folkloric literature dating from the early 19th century and exclusively focused on natural products and their derivatives. Our literature searches determined that there were sufficient data indicating that black cohosh extracts had significant potential for the treatment of menopausal hot flashes. However, the process of taking an extract of black cohosh into a clinical trial presented many challenges. Scientific and medical literature searches provided many examples of well-characterized studies of black cohosh and were correlated with substantial ethnomedical use of the plant for menopause, especially hot flashes. At the time of initiation of our work, published clinical studies showed no significant adverse effects associated with the ingestion of black cohosh preparations, and no adverse events were observed in any of our black cohosh extract studies to date.

The quality control process of preparing any botanical dietary supplement for an in vivo study must begin with properly collected and identified raw materials. Before any kind of biological or chemical studies are begun on a botanical, quality must be assured. To commence our Phase I study of safety and determination of the appropriate dose, researchers from the Center were sent to work closely with botanist G. W. Ramsey, expert in the North American genus of black cohosh, to collect raw material in the Blue Ridge Parkway and Great Smokey Mountains National Park of North Carolina, Pennsylvania, Tennessee, and Virginia using good field collection practices. Voucher specimens were then housed off-campus in two private herbaria—the Searle Herbarium at the Field Museum of Natural History (Chicago) and the Ramsey-Freer Herbarium at Lynchburg College (Lynchburg, VA). Roots were subjected to macroscopic, microscopic, and DNA
techniques to identify and validate the plant materials. The plant materials were dried and extracted (Liu et al., 2001) and tested in numerous bioassays (Table 1). Since the early mechanism of action for black cohosh presumed that these extracts acted as estrogen receptor agonists, the first step was to clarify the estrogen receptor binding and determine if indeed black cohosh extracts were estrogenic in vivo. No estrogen binding of the extract was found in either ERα or ERβ (Burdette et al., 2003). Center researchers confirmed that a 40% isopropanol and 75% ethanol extract of black cohosh were not estrogenic in an animal study. The extract was administered to ovariectomized rats at doses of 4, 40, and 400 mg/kg body weight (b.w.) per day with or without estradiol at 50 µg/kg b.w. per day. No estrogenic, antiestrogenic, or additive effects were found (Burdette et al., 2003). Having confirmed the lack of estrogenicity of the black cohosh extract, the Center researchers used a series of bioassays for the serotonin (5-HT) receptors 5-HT7, 5-HT1A, and 5-HT1D (Burdette et al., 2003). We confirmed that the 75% ethanol extract bound to the serotonin receptors, and acted as a partial agonist of 5-HT7 (Burdette et al., 2003). Center investigators also showed that black cohosh acts as a mixed competitive ligand and partial agonist of the human mu opioid receptor (Rhyu et al., 2006). Confirming this activity was significant as there is an association of the locus of temperature control in the central nervous system and the endogenous opiate system. Thus, the Center research has shown that black cohosh could potentially modulate the vasomotor symptoms associated with menopause through these two mechanisms. The black cohosh extract was standardized based on the chemical and biological data for use in the clinical trials.

The Center then conducted a Phase I clinical trial of its black cohosh and red clover extracts. The Phase I trial was used to assess preliminary safety of the black cohosh extract, determine the highest nontoxic dose for each botanical, and obtain pharmacokinetic and metabolism data. With these data, we proceeded to perform a Phase II study. The Phase II study was a four-arm trial including black cohosh, red clover, the hormone therapy Prempro (Wyeth Pharmaceuticals, Inc., Philadelphia, PA) as a positive control, and placebo. More than 1500 female volunteers were interviewed for this study, and 88 women were randomized into the 12-month clinical trial. Subjects in the red clover arm were treated with a single oral daily dose of 120 mg of a standardized extract, and the black clover arm were treated with a single oral daily dose of 128 mg of a standardized extract (5.5% triterpenes, IC50 13 µg/mL in the 5-HT7 assay). The Phase II trial experienced recruiting impediments due to the adverse publicity resulting from findings of the Women’s Health Initiative; women interested in participating were reluctant to be in the Prempro arm and opted out during initial screening. The phase II trial has since been completed and is currently undergoing data analysis for the results. This study will provide preliminary data indicating whether black cohosh and red clover are safe and effective for treating hot flashes associated with menopause.

The UIC/NIH CBDSR was established in 1999, and was refunded in 2005. During this time we have identified several promising botanicals used in dietary supplements for women’s health using a multidisciplinary collaborative approach (Figure 2). All of the steps have been defined and implemented and require botanical, chemical, and biological assessment as well as mechanism-of-action studies. Multidisciplinary research on botanical dietary supplements is highly collaborative and necessary for success, as the challenges to this type of work are significant. However, we have been able to overcome these challenges through collaboration and the use of novel chemical, biological, and recruiting techniques. The significant amount of research, training, and publications that have resulted from the UIC/NIH Center shows that this approach to botanical research is very effective.

### Table 1. Biological standardization and mechanism of action.

- ERα and ERβ
- Ishikawa assay (cell-based estrogenicity test)
- Antioxidant
- Mutagenicity
- 5-HT7, 5-HT1A, 5-HT1D
- In vivo estrogenic rat model
- Opioid binding
- COX-2
- Dopamine binding
- GABA_A
- Progestagenic
- Antiadherence

![Figure 2. Multidisciplinary approach to research.](image-url)
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