Published in final edited form as:
*Am J Clin Nutr*. 2008 February ; 87(2): 504S–508S.

# The University of Illinois at Chicago/National Institutes of Health Center for Botanical Dietary Supplements Research for Women's Health: from plant to clinical use1,,2, ,3, ,4

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## Abstract

The University of Illinois at Chicago/National Institutes of Health Center for Botanical Dietary Supplements Research began in 1999 with an emphasis on botanical dietary supplements for women's health. We have concentrated on plants that may improve women's health, especially to reduce hot flashes in menopausal women, alleviate the symptoms of premenstrual syndrome, and reduce persistent urinary tract infections. The primary focus of this article is to describe the operation of our center, from acquiring and identifying botanicals to isolating and identifying active constituents, to elucidating their mechanisms of action, and to conducting phase I and phase II clinical studies. Black cohosh (*Actaea racemosa*; syn *Cimicifuga racemosa*) has been used as a model to illustrate the steps involved in taking this plant from the field to clinical trials. Bioassays are described that were necessary to elucidate the pertinent biological studies of plant extracts and their mechanisms of action. We conclude that this type of research can only be successful with the use of a multidisciplinary approach.

### Keywords

Black cohosh; botanical dietary supplements; menopause; multidisciplinary research

## INTRODUCTION

The University of Illinois at Chicago/National Institutes of Health (UIC/NIH) Center for Botanical Dietary Supplements Research was established in 1999 with 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 US Congress recognized the increased

[2]Presented at the workshop "The Science of Botanical Supplements for Human Health," held at Experimental Biology 2007, Washington, DC, 28 April 2007.

[3]Supported by a research grant from NIH (P50 AT000155) jointly funded by the Office of Dietary Supplements, the National Center for Complementary and Alternative Medicine (NCCAM), the Office for Research on Women's Health, and the National Institute for General Medical Sciences. The black cohosh clinical trial product was developed in collaboration with Naturex, Inc (South Hackensack, NJ), and Pharmavite LLC (Northridge, CA). A secondary clinical study, a spinoff from the center's phase II clinical trial, is supported by grants (R21AT001868 and K01AT002321) from NIH/NC-CAM. The contents are the responsibility of the authors and do not necessarily represent the views of the funding agencies.

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consumer use of alternative and complementary medicine, which prompted the formation of the ODS and the NCCAM, after which the botanical centers were established. The center focuses on three areas of concern to women: menopause, premenstrual symptoms, and urinary tract infections. The center had from its inception the goal of taking a plant from the field to the clinic, ensuring the processing of a safe and effective botanical dietary supplement for human consumption.

Center faculty and staff began by investigating 12 botanicals widely used by women in the United States. These botanicals include Angelica sinensis (Oliv.) Diels (dong quai), Actaea racemosa L. (formerly Cimicifuga racemosa [L.] Nutt.; black cohosh), Ginkgo biloba L. (ginkgo), Glycyrrhiza glabra L. (licorice), Humulus lupulus L. (hops), Panax ginseng C.A. Meyer (oriental ginseng), Panax quinquefolius L. (American ginseng), Trifolium pratense L. (red clover), Vaccinium macrocarpon Ait. (cranberry), Viburnum prunifolium L. (black haw), Valeriana officinalis L. (valerian), and Vitex agnus-castus L. (chasteberry). As each botanical was investigated and determined to be appropriate for further studies or seen to be inactive, this list has been modified.

## ORGANIZATION OF THE CENTER

The center's research components consist of three project areas and two clinical evaluation teams. All 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. Since 1999 our center has been operating as a multidisciplinary unit involving 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). Collaboration also involves Northwestern University (Obstetrics and Gynecology) in conjunction with two industrial partners, Pharmavite, LLC, and Naturex Inc.

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.

## CENTER PHILOSOPHY

The identities of plants to be used are verified with voucher materials that are stored for future reference. If the plant is found in the wild, the process for acquiring the plant—including any copies of necessary permits—must be readily available, and good collection (wildcrafting) practices should be followed. If the plant is grown as a crop, good agricultural standards should be followed. 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 (1).

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. In contrast, 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 effects and may identify and filter out potentially toxic substances. Finally, studies of the mechanism of action, metabolism, bioavailability, and safety in various in vitro and animal models should be performed.

The process that the center uses to work up a plant extract to the level at which its safety and efficacy profile can be determined in phase I and phase II clinical trials is listed in Table 1. The first step of our process, focusing on black cohosh (currently in a phase II clinical trial), was to perform a literature search using databases such as PUBMED (National Library of Medicine, Bethesda, MD) and the UIC-based NAPRALERT database (2), 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 identified the 12 botanicals noted previously, each with many citations for use in women's health. In addition to having a well-established history of use, these botanicals continue to be readily accessible in the marketplace, thus giving added rationale for their selection as obvious targets for research. Since this initial survey for useful plants, we have identified additional plants for study.

## CHEMISTRY

Bioassay-directed fractionation using several separation methods, most recently countercurrent chromatography, 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 (3-22). Most of these were studied for appropriate biological activities.

## SELECTING THE APPROPRIATE BIOASSAYS

We anticipated that the alleviation of hot flashes as well as of symptoms associated with premenstrual syndrome are usually partially mediated by activity at estrogen receptors. Estrogenicity seemed to be common to five (cranberry and valerian being the exceptions) of the 12 botanicals. A broad array of bioassays was 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 (14, 19, 23-26), serotonin receptors (27, 28), opioid receptors (29, 30), bacterial antiadherence (15, 22), and antioxidants (8,17,19). Additionally, we developed and conducted studies of metabolism (11, 27, 28, 31-36).

Three of the plants were not active in our estrogen receptor-a and -f in vitro screening assays (Table 2). Because we were unable to verify an estrogen-binding effect of ginkgo, licorice, and the ginsengs, we dropped them from additional work. Black cohosh, one of the three, 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. At the time, it was believed that black cohosh most likely was working through estrogenic mechanisms and thus we proceeded with black cohosh and the 5 remaining botanicals: dong quai, hops, red clover, chasteberry, and viburnum. Cranberry was chosen for study because of its historical use in alleviating urinary tract infections, and different assays were developed for its study (15, 22). Valerian was also selected for its traditional use in promoting sleep.

## BLACK COHOSH—FROM THE PLANT TO THE CLINIC

### The literature: historical use

The process of taking black cohosh from crude extract through to the clinical trial presented challenges. Our literature search provided many examples of well-characterized studies of black cohosh correlated with substantial ethno-medicinal symptoms of menopause, especially hot flashes. At the time of initiation of our work, clinical studies showed no significant adverse effects from the use of black cohosh preparations. We have not found adverse events associated with our black cohosh study extract to date.

## COLLECTION AND DISPOSITION OF RAW MATERIAL

Center investigators have consistently opined that a quality botanical dietary supplement must originate with quality 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 appropriate dose, we sent a team from the center to work closely with botanist GW 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 2 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 material.

### Bioassay screening and identification of active compounds (biomarkers)

The mechanism of action for black cohosh was not well understood when we began our investigation. Early presumption that black cohosh was an estrogen receptor agonist were countered by other reports indicating no such activity. Our first step was to clarify the estrogen receptor binding question. Initially, we administered a 40% isopropanol extract of black cohosh at doses of 4, 40, and 400 mg/kg per day with or without estradiol at 50μg/kg per day to ovariectomized rats. We found no estrogenic, antiestrogenic, or additive effects either in vitro or in vivo (23). Having confirmed the lack of estrogenic activation, we used a panoply of bioassays, including 5-$HT_7$, 5-$HT_{1A}$, and 5-$HT_{1D}$ receptors (27; Table 3). It is significant that the phenolic acids are more active than the triterpenes and that the phenolic acids do not pass through Caco-2 cells (a model for intestinal absorption), whereas the weakly active triterpenes pass through the monolayer (unpublished data, RB van Breemen, 2007). We also showed that black cohosh acts as a mixed competitive ligand and partial agonist of the human mu opiate receptor (29). Confirming this site is significant for the association of the locus of temperature control in the central nervous system and its activation directly or indirectly with the endogenous opiate system.

### Clinical trials

The center conducted a phase I clinical trial of its black cohosh and red clover extracts and is now conducting a phase II study of these botanicals in a 4-arm trial including black cohosh, red clover, the hormone therapy Prempro (Wyeth Pharmaceuticals, Inc, Philadelphia, PA) as a positive control, and placebo. The phase I trial was needed to assess the safety and determine the highest nontoxic dose for each botanical and to collect preliminary pharmacokinetic and metabolism data. More than 1500 volunteers were interviewed, and 88 were selected to participate in the 12-month study. The red clover arm uses a single daily dose of 120 mg of a standardized extract, and the black cohosh arm uses a single daily dose of 128 mg of a standardized extract (5.5% triterpenes, IC50 13 μg/mL in the 5-$HT_7$ assay). By December 2007 the last volunteer will have completed the study. 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. Postcard notices to selected populations was the most important recruitment tool. The phase II trial will provide preliminary data indicating whether black cohosh and red clover are safe and effective for treating hot flashes associated with menopause. A separate clinical study—Effects of Botanicals on Cognition in Midlife Women—is directly tied to the phase II clinical trial as it is conducted with a subset of the same population; it is examining the association of black cohosh and red clover with cognitive functioning during menopause.

## SUMMARY AND FUTURE DIRECTIONS

Since 1999 we have identified several promising botanicals used in dietary supplements for women's health. All of the steps have been defined and implemented and require botanical, chemical, and biological assessment as well as mechanism-of-action studies. Interdisciplinary research on botanical dietary supplements is highly collaborative and necessary. We plan to continue work on other promising botanicals—isolate active constituents, standardize them chemically and biologically, and initiate phase I and phase II clinical studies—and to study mechanisms of action at the molecular level.

The contributions of the authors were as follows—NRF: was responsible for the overall direction of the project and some of the chemistry; JLB: was responsible for the biological assays described; RBvB: was responsible for the LC/MS work and metabolism; GFP: was responsible for the chemistry; JGG: was responsible for the botany and manuscript drafting; and ECK: was responsible for records maintenance and drafting parts of the manuscript. NRF is a consultant to Pharmavite LLC (Northridge, CA). JLB, GFP, RVvB, ECK, and JGG had no financial interests in any company or organization sponsoring this research, including advisory board affiliations.

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**TABLE 1**

The University of Illinois at Chicago/National Institutes of Health Center for Botanical Dietary Supplements Research—steps to a clinical trial: identifying a safe and effective botanical dietary supplement

| |
|---|
| *1)* Search scientific and ethnomedical botanical literature |
| *2)* Acquire plant material using Good Agricultural Standards (GAS) and Good Collection Practices (GCP) |
| *3)* Determine appropriate bioassays to assess the mechanism of action |
| *4)* Perform bioassay-guided isolation of the active compounds |
| *5)* Establish safety of extract and active biomarkers |
| *6)* Chemically characterize active biomarker compounds |
| *7)* Establish bioavailability of marker compounds |
| *8)* Standardize extract for preclinical and clinical studies |
| *9)* Develop chemically and biologically standardized formulation using Good |
| *10)* Conduct phase I and phase II clinical trials |

**TABLE 2**

Competitive binding of extracts to estrogen receptors (ERs) and their estrogenic activity in Ishikawa cells[1]

| Methanol Extract | 200μg/mL | | 20μg/mL | |
|---|---|---|---|---|
| | ERα | ERβ | Estrogenic | Anti-estrogenic |
| | % | | % | |
| ***Angelica sinensis*** **(Dong Quai)** | 27 | 30 | – | – |
| ***Cimicifuga racemosa*** **(black cohosh)** | 19 | 16 | – | – |
| ***Glycyrrhiza glabra*** **(licorice)** | 33 | 29 | – | – |
| ***Humulus Lupulus*** **(hops)** | 70 | 79 | 25 | 42 |
| ***Panax ginseng*** **(oriental ginseng)** | inactive | | – | – |
| ***Panax quinquefolius*** **(American ginseng)** | inactive | | – | – |
| ***Trifolium pretense*** **(red clover)** | 73 | 74 | 56 | – |
| ***Vitex agnus-castus*** **(chasteberry)** | 57 | 67 | 40 | – |

[1]Preliminary data from a single dose

## TABLE 3

Black Cohosh: $5HT_7$ activity of selected isolated compounds[1]

| ***Compound*** | ***5-HT inhibition: ($IC_{50}$, μg/mL)*** | ***$5HT_7$ percentage inhibition at 250μg/mL*** |
|---|---|---|
| | μmol | % |
| Cimicifugic acid A | 54.0 | – |
| Cimicifugic acid B | 1.2 | – |
| Actein (R,S) | – | 50 |
| 23-Epi-26-deoxyactein | – | 31 |
| 26-Deoxyactein | – | 55 |
| 25-Anhydrocimigenol-3-*O*-β-D-xyloside | – | 55 |
| 25-*O*-Acetylcimigenol-3-*O*-β-D-xyloside | – | 46 |
| 2-*O*-Acetylactein | – | 47 |
| Cimiracemoside I | – | 69 |
| Cimiracemoside M | – | 48 |
| Cimiracemoside O | – | 50 |

[1]Preliminary data, NR Farnsworth, 2007