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Computational analysis for hepatic safety signals of constituents present in botanical extracts widely used by women in the United States for treatment of menopausal symptoms ☆

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ABSTRACT

Black cohosh, red clover, hops, and chasteberry are botanicals commonly used to alleviate menopausal symptoms in the US, and are examined in this study as part of a FDA Office of Women's Health research collaboration to expand knowledge on the safety of these botanical products. Computational approaches using classic (quantitative) structure–activity relationships ((Q)SAR), probabilistic reasoning, machine learning methods, and human expert rule-based systems were employed to deliver human hepatobiliary adverse effect predictions. The objective is to profile and analyze constituents that are alerting for the human hepatobiliary adverse effects. Computational analysis of positively predicted constituents showed that common structural features contributing to the hepatobiliary adverse effect predictions contain phenolic, flavone, isoflavone, glucoside conjugated flavone and isoflavone, and 4-hydroxyacetophenone structures. Specifically, protocatechuic acid from black cohosh, benzofuran and 4-vinylphenol from chasteberry, and xanthohumol I from hops were botanical constituents predicted positive for liver toxicity endpoints and were also confirmed with literature findings. However, comparison between the estimated human exposure to these botanical constituents and the LOAEL and NOAEL in published animal liver toxicology studies for these constituents demonstrated varying margins of safety. This study will serve as regulatory decision support information for regulators at the FDA to help with the process of prioritizing chemicals for testing.

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1. Introduction

According to the North American Menopause Society, Hormone Therapy Statistics for 2009, more than 50 million American women use menopausal hormone therapy (MHT) to relieve menopausal symptoms (NAMS, 2010). It has been estimated that 80 percent of women 40–65 years of age use botanical dietary supplements to reduce menopausal symptoms (Mahady, 2010). A recent report by the University of Illinois at Chicago/Center for Botanical Dietary Supplements Research on Women's Health (UIC/NIH Botanical Center) and others have identified four botanicals that are most frequently used by women in Western countries for MHT (Kurzer and Xu, 1997; Lieberman, 1998; Liu et al., 2001; Tham et al., 1998). The four botanicals with parts of the plant are: the root and rhizome of black cohosh (*Actaea racemosa L.*), the fruit of chasteberry (*Vitex Agnus-castus L.*), the dried female flowering part of hops (*Humulus lupulus L.*), and the flowering tops of red clover (*Trifolium pretense L.*).

The US Pharmacopeia Council of Experts on dietary supplements reviewed safety information for black cohosh products which found possible causality to liver injury and recommended black cohosh products be labeled to include a cautionary statement regarding potential liver adverse effects (Mahady et al., 2008). The US National Institutes of Health (NIH) Office of Dietary Supplements recently published a report on considerations of the safety of black cohosh, including the body of evidence in the open literature regarding hepatotoxicity in association with use of products containing black cohosh, and recommended monitoring liver

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function as mandatory before, during, and at follow-up of any clinical trial, as well as identifying the potential causal agents in the botanical and/or adulterating species (Betz, 2009). The NIH, National Center for Complimentary and Alternative Medicine's (NCCAM) monograph on black cohosh also notes several case reports of hepatitis and liver failure in women who were taking black cohosh, and thus raises concern about possible effects of black cohosh on the liver even though there is no definitive evidence to establish causality (NCCAM, 2010). The NIH led review of post-market adverse event reporting data and two meetings on the current state of knowledge of black cohosh: a 2004 meeting entitled "Workshop on the Safety of Black Cohosh in Clinical Studies" (NCCAM, 2004) and a 2007 meeting focused on understanding the clinical reports of hepatotoxicity, have resulted in regulatory and research recommendations to protect the safety of patients in clinical trials (Betz et al., 2009; Borrelli and Ernst, 2008). In this research, one important area identified is the examination of the chemical constituents in the botanicals that may possibly contribute to the liver injuries observed in humans (Betz et al., 2009; Borrelli and Ernst, 2008). Equally important are the public's concerns regarding the risks of MHT and the prevailing interest in botanical products as alternatives, which provides further impetus for new research focused on the safety of such botanicals.

Given these concerns, the objectives of this investigation are to identify chemical constituents known to be present in botanicals commonly used by American women as an alternative to MHT and to computationally (*in silico*) screen these chemicals for liver toxicity signals. As specified previously by scientific names and parts of plant, the botanicals examined in this investigation are commonly known as, black cohosh, red clover, hops, and chasteberry. The *in silico* screening will entail using specialized software programs that employ computational models based upon (quantitative) structure–activity relationships ((Q)SAR) and chemoinformatic analyses of structural features of positively predicted chemicals for liver toxicity. The results of this study are aimed to assist regulatory decision making on product safety as hepatotoxicity endpoints are directly relevant to the FDA regulatory review of the safety for products which contain these botanicals (*e.g.,* dietary supplements), or for products (*e.g.,* botanical drugs) that may propose to contain them and must be evaluated through formal agency review and approval processes (FDA/CDER, 2004). The acquired knowledge of the composition and scientific data generated from this study will be disseminated to the FDA Office of Women's Health (OWH), Center for Drug Evaluation and Research (CDER) Botanical Drug Review Team, and the Center for Food Safety and Applied Nutrition (CFSAN) Division of Dietary Supplement Programs. Thus, this study aims to build evidence-based knowledge and predictive scientific data for use by these FDA regulatory groups by increasing the quality of safety information available on these botanicals relevant to women's health due to their use for alleviation of menopausal symptoms.

2. Material and methods

2.1. Botanical chemical constituents

Chemical constituents present in published botanical extracts of black cohosh (*Actaea racemosa* L., syn. *Cimicifuga racemosa* (L.) Nutt.), red clover (*T. pratense* L.), hops (*H. lupulus* L.), and chasteberry (*V. agnus-castus* L.) were identified from the Natural Products Alert (NAPRALERT^{sm}) database (<http://napralert.org>). NAPRALERT covers literature comprehensive from at least 1975 through 2003, including natural product clinical study, metabolism data, and ethnomedical information on more than 20,000 species of plants collected through the UIC Health Science Library, public journals, as well as foreign journals published in non-English languages. The chemical composition data found in the NAPRALERT database for each of the four botanicals was used to create unique datasets that represent the known composition of the botanicals based on publically available data. Composition data was extracted only from the specific parts of the plant that are used for the relief of menopausal symptoms.

2.2. Computational toxicology software and models

Specialized computational software programs using (Q)SAR models and chemoinformatics analyses were employed to conduct *in silico* screening of the chemical constituents in the botanicals for various hepatotoxicity endpoints. Five software programs were utilized because of their complementary *in silico* predictive approaches, increasing chances that a chemical would be covered by the chemical domain space of the model. Listed alphabetically, the software used in this study were: (1) BioEpisteme® (version 4.1) provided by Prous Institute for Biomedical Research, S.A., (2) Derek for Windows (version 11) provided by Lhasa Ltd., (3) Leadscope Model Applier (LMA) (version 1.2.1–3) provided by Leadscope Inc., (4) MetaDrug™ (version 6) provided by GeneGo Inc., (5) MC4PC (version 2.1.0.11) provided by MultiCASE, Inc. A description of each software program is summarized in Table 1. The computational software programs were provided to FDA either through an agency approved Cooperative Research and Development Agreement (CRADA), Research Collaboration Agreement, Material Transfer Agreement, or site license with the software vendor. The computer hardware used for the computational toxicology studies in this report was a desktop PC with Microsoft Windows XP Professional version 2002.

The human hepatobiliary adverse effect QSAR models in LMA, MC4PC, and BioEpisteme® were developed and validated as described previously (Matthews et al., 2009; Ursem et al., 2009). Briefly, each of these QSAR models were constructed using a large training dataset that comprises about 1050–1600 pharmaceutical compounds collected from the FDA human post-market surveillance databases (spontaneous reporting system (SRS), adverse event reporting system (AERS)), as well as credible published literature sources (Ursem et al., 2009). Description of the modeling approaches for the Derek for Windows (Dfw), BioEpisteme®, MC4PC, MetaDrug™ and Leadscope Model Applier have been previously described (Ekins et al., 2006; Marchant et al., 2008; Matthews et al., 2008; Saiakhov and Klopman, 2008; Valerio et al.,2010). Predicted positive chemicals were further analyzed with the software, Leadscope Enterprise (version 2.4.15–6) and Leadscope Model Applier (v. 1.3.2–7) provided by Leadscope Inc.

Multiple (Q)SAR models built into the five different computational toxicology software previously mentioned were used. Electronic structure data files (SDFs) containing all chemical constituents of each botanical were used in the *in silico* hepatotoxicity screening. The QSAR modeled endpoints in LMA, MC4PC, and BioEpisteme® software programs were gall bladder disorder, cholestasis and jaundice, cytotoxic injury (liver damage), bile duct disorder, and liver enzyme release; the QSAR endpoints in MetaDrug™ include liver cholestasis, liver lipid accumulation, liver necrosis, liver weight gain, and general hepatotoxicity; and the SAR endpoint in Dfw was hepatotoxicity (see Table 2).

2.3. Consensus predictions for liver injury

Botanical chemical constituents showing predictive results for liver toxicity (i.e. structural alerts, or quantitative probability values) by more than one software programs were considered to signal hepatotoxic potential and were classified as "positive" based on the following criteria in each software programs: