Black Cohosh and Climacteric Symptoms: Growing Knowledge about the Efficacy and Safety

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This paper is dedicated to Professor Tom J. Mabry for his 75th birthday.

Hormone therapy of perimenopausal and postmenopausal disorders includes, in many cases, treatment with estrogens but many recent studies have raised the question as to whether it brings more dangers than benefits for patients. This has led to an increased use of alternatives, mainly plant derived extracts. Among the botanical supplements and herbal medicinal products, extracts of the rhizome and roots of black cohosh are used worldwide for these purposes. This plant has a long-standing history of being used to treat climateric complaints and its clinical efficacy has been proven in several double-blind placebo controlled studies. In terms of safety, minor and transient adverse effects such as nausea, vomiting, headaches and dizzness have been observed in clinical trials. A few cases of hepatotoxicity have been reported, but a direct association with the use of black cohosh has not been demonstrated. Black cohosh was first thought to be estrogenic in nature, but recent studies have proposed it as selective estrogen receptor modulator (SERM) and serotoninergic, dopaminergic and cholinergic mechanisms have been described. Black cohosh shows great promise for relief of menopausal symptoms, primarly of vasomotor and possibly mood symptoms, with an overall positive safety profile of at least 6 months and likely longer. However, data from longer and in some cases more rigorous clinical trials are necessary to assess high efficacy and to substantiate safety.

Keywords: Black cohosh, *Cimicifuga racemosa*, *Actaea racemosa*, Ranunculaceae, climateric symptoms, efficacy, safety.

Hormone replacement therapy has been routinely employed to relieve the symptoms associated with the decline in endogenous estrogens that is characteristic of menopause. However, recent studies of the Women's Health Initiative evidenced that hormone replacement therapy can increase breast cancer, coronary heart disease, stroke and pulmonary embolism [1]. For these reasons many women are now reluctant to use exogenous hormone therapy and are turning to botanical supplements and herbal medicinal products for relief. Among these herbal drugs, black cohosh (*Actaea racemosa* L., *Cimicifuga racemosa (*L.) Nutt., Ranunculaceae) represents one of the best known alternative therapies to alleviate perimenopausal and postmenopausal symptoms. Black cohosh is a graceful perennial woodland plant bearing spikes of white flowers (Figure 1), native to New England and eastern Canada [2].

Figure 1: Black cohosh

Native Americans and early colonists occasionally used the herb to treat many problems and, in the late 1800's it became the key ingredient in "Lydia Pinkham's Vegetable Compound", a widely popular over-the-counter medicine for menstrual problems, infertility, and unpleasant symptoms of menopause, sold until the latter half of the twentieth century [3-5].

Over the past 40 years, extracts of the plant have been used as an herbal medicine in North America and Europe primarily for the treatment of symptoms related to menopause [6]. There is a range of products currently available on the market, including combination products and single-herb products in different formulations: tinctures, tablets or capsules; solid dosage forms contain powdered root or dried extracts. Black cohosh products have been increasing steadily since the late 1990s, peaking in 2003. Retail sales of black cohosh herbal products ranked ninth in 2002 in terms of the U.S. botanical products' market [7]. Clinical research reported on this plant is quite considerable, including placebo and/or treatment controlled clinical trials [8-11], but none of these trials has been conducted for more than six months [12]. This review reports the growing knowledge about the efficacy and safety of black cohosh in the treatment of climateric symptoms.

Historical overview

According to Lloyd and Lloyd [13] the first description of the plant appears in the *Amaltheum Botanicum* (1705), the final of six volumes of Leonard Plukenet's "Phytographia". Black cohosh was presented under the name "Christopheriana facie, Herba spicata, ex Provincia Floridana". The first modern botanical name is *Actaea racemosa* L., published in Species Plantarum (1753). Later, Linnaeus separated the genus *Cimicifuga* from *Actaea*. But the first botanist to call it *Cimicifuga racemosa* was Thomas Nuttall in his 1818 work "The Genera of North American Plants". The genus name *Cimicifuga* is derived from the Latin *cimex*, the generic name for the bedbug (*Cimex lectularius* L., Cimicidae) and the Latin *fugare*, meaning to drive away. This refers to the fact that some species, including the European *C. europaea* Schipcz., the Asian *C. foetida* L., and the North American *C. elata* Nutt., among others, have herbage with a strong, unpleasant fragrance, hence they were used as insect repellents [14].

Black cohosh has a long history of use among indigenous North American people: some tribes used black cohosh together with elecampane (*Inula helenium* L., Asteraceae) and stoneroot (presumably *Collinsonia canadensis* L., Lamiaceae) as a "tonic" [15]. The Iroquois used root decoctions to promote the flow of milk and to treat rheumatism, a decoction of the root was used as a foot bath (for washing the affected parts) [16]. The Cherokee used alcoholic spirits of the root for the treatment of rheumatism and as a tonic, diuretic, anodyne, emmenagogue, and for its astringent activity. Root tea was used to treat colds, cough, consumption, constipation, fatigue, hives, rheumatism, backache, and as infant sleep medication [17]. Among Algonquians black cohosh root was used as a common remedy for kidney trouble [18]. The plant was known with several vernacular names such as black snakeroot, black root, bugbane, rattle root, rattle top, rattle squawroot, snake root and rattle weed [19].

The first mention of the drug by the profession was made by Benjamin Smith Barton in his *Collections for an Essay towards a Materia Medica of the United States* (1798), reported for its astringent properties [3]. In 1828 Rafinesque [4] expanded the list of applications adding these indications: diuretic, sudorific, anodyne, repellent, emmenagogue and subtonic. In 1832 the eclectic physician John King made a large use of black cohosh, a tincture which he called "Macrotyn", as a primary remedy in both acute and chronic cases of rheumatism and in inflammatory conditions associated with endometriosis, pulmonary afflictions, chorea and neuralgic affections. According to King, Macrotyn was also an important remedy for many afflictions of reproductive organs in females [14,19,20]. *Cimicifuga racemosa* and various preparations, including a fluid extract, were listed in the United States National Formulary for over 100 years, from 1840 until 1946 [19].

Chemistry

The constituents (Figure 2) of the rhizome and roots of black cohosh are not completely known. The triterpene glycosides are considered the main characteristic constituents followed by phenolic compounds [21]. Research on the triterpenoids has resulted in the isolation and identification of more than 40 glycosides mostly belonging to the 9,19-cycloartanol and cyclolanostanol series and typically glycosidated at the C-3 positions with D-xylose or L-arabinose.
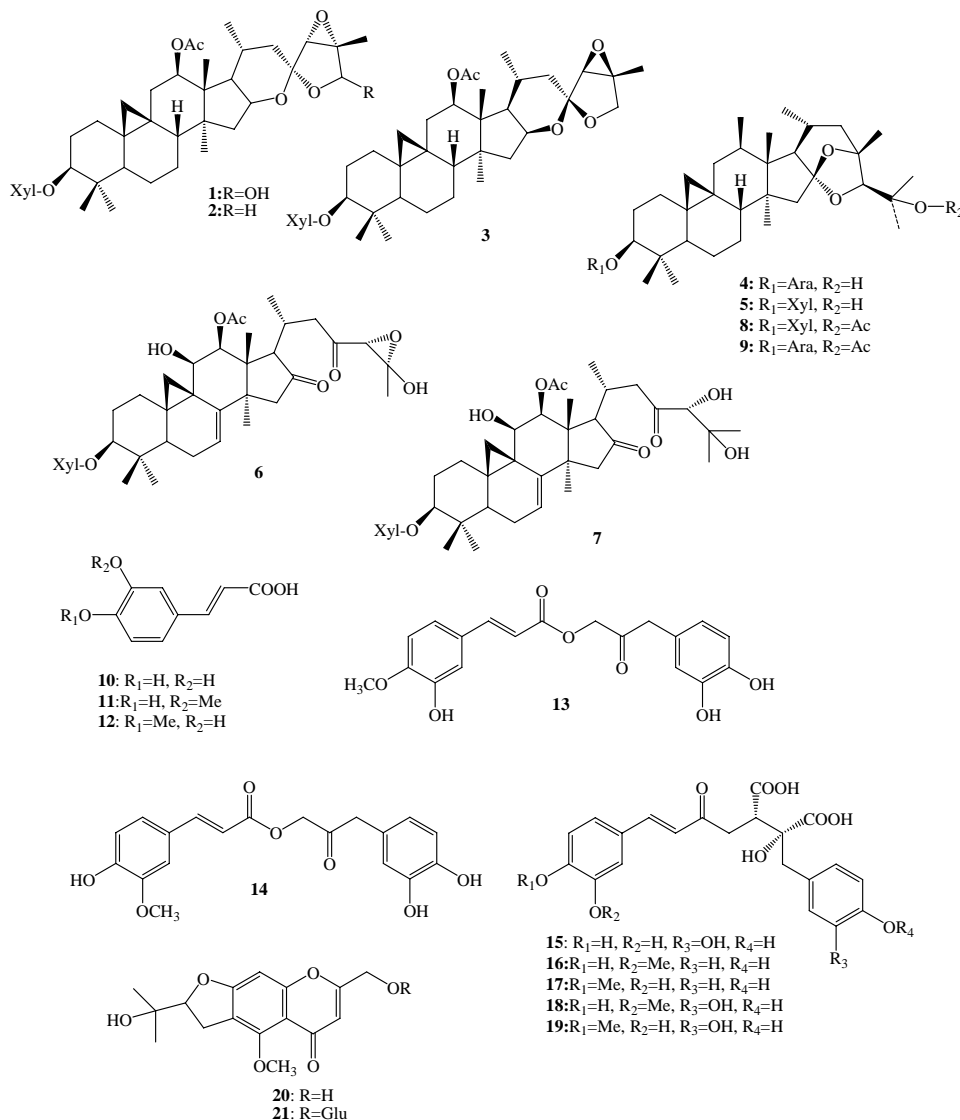
Figure 2: Characteristic constituents of black cohosh rhizome and roots.

Main saponins are actein (**1**, acetylacteol-3-*O*-β-D-xyloside), 26-deoxyactein (**2**), 23-epi-26-deoxyactein (**3**), cimigenol-3-*O*-α-L-arabinoside (**4**), cimigenol-3-*O*-β-D-xyloside (**5**, syn. cimicifugoside or cimigoside), cimicifugoside H-1 (**6**) and H-2 (**7**), 25-*O*-cimigenol-acetylcimigenol-3-*O*-β-D-xyloside (**8**), 25-*O*-acetylcimigenol-3-*O*-α-L-arabinoside (**9**) [22-31]. Numerous polyphenolics have also been isolated [32-34]. They are represented by the ubiquitous cinnamic acid derivatives (caffeic acid **10**, ferulic acid **11**, isoferulic acid **12**), phenylpropanoid ester dimers such as cimiracemate A (**13**) and B (**14**), piscidic and fukiic acids esters, especially fukinolic (2-*E*-caffeoylfukiic)acid (**15**), 2-feruloyl piscidic acid (**16**), 2-isoferuloylpiscidic acid (**17**), cimicifugic acid A (**18**), B (**19**), and the chromones cimifugin (**20**) and its β-D-glucoside (**21**) [35-38]. These two derivatives

were only found in the species *C. racemosa* and are considered, together with cimigenol-3-*O*-β-L-arabinoside (**4**), as suitable specific markers for the distinction of *C. racemosa* from the other *Cimicifuga* species [35]. A few studies reported the presence of the flavonol kaempferol and the isoflavone formononetin [37,39], but this is controversial and some recent works could not confirm its presence in black cohosh rizhome and roots or the commercial extracts [40,41].

Biochemical mechanisms

Most of the *in vitro* studies have been focused on the evaluation of estrogen receptor affinity and the effects on human estrogen receptor-positive breast cancer cell lines using defined or undefined crude extracts of roots and rhizome, commercial extracts,

fractions and pure constituents. Two studies evaluating estrogen receptor competitive binding assay [42-44] demonstrated the endocrine activity of black cohosh using the estrogen receptor-binding assay. A lipophilic extract of the plant was demonstrated as able to bind to estrogen receptors of rat uteri. After fractionation of the extract, three different endocrine active compounds were found: those having no binding for the estrogen receptor but suppressing LH release after chronic treatment (perhaps acting on CNS such as an α-2 agonist), those binding to the estrogen receptor and also suppressing LH release and compounds which are ligands for the estrogen receptor but without an effect on LH release. One of these compounds was identified as the isoflavone formononetin, which has been shown to be a competitor in the estrogen receptor assay, but failed to reduce the serum levels of luteinizing hormone in ovariectomized rats. However, formononetin has not be detected in black cohosh in more recent studies [40-41,45-46]. Similarly, Harnischfeger and Cillien (1996) reported that a butanol-derived and a chloroform-derived subfraction of an alcoholic extract of black cohosh were able to bind to estrogen receptors [47]. Other investigations, including more recent studies, [48-50] contradicted these data. The first study [48] showed no estradiol binding of an undefined extract using cytosolic estrogen receptors from livers of ovariectomized rats, and the second study [49] tested a 50% ethanol/water extract on T47D human breast cancer cell lines. The most recent investigation [50] used a methanolic extract and human recombinant diluted estrogen receptors. A study using 58% ethanol/water extract demonstrated a dopaminegic activity of black cohosh using recombinant human dopamine D2 receptor protein and this action may contribute to the pharmacological profile of the extract [51]. A dopaminergic activity of the extract was also interpreted from the findings obtained assaying a black cohosh extract in primary cell cultures of the rat pituitary gland [52].

Recent data have also demonstrated that black cohosh acts on serotonin receptors which may be the mechanism for relief from hot flashes and improvement in mood [53].

Conflicting results have also been obtained from the studies regarding the influence of black cohosh on estrogen receptor-positive breast cancer cells. Some investigations [54-56] assessed the inhibition of cell proliferation of human breast cancer cell lines T- 47D

and MCF-7. This effect was reduced by estradiol and increased by estrogen antagonists, such as raponticin [54] or tamoxifen [56]. On the other hand, another study [49] observed that a butanolic subfraction of an alcoholic extract stimulated the proliferation of carcinoma AN3 (an estrogen sensitive cell line). Estrogen and genistein used as positive control increased the AN3 proliferation. Estrogen and the butanolic fraction had no effects on the growth of FTDE and MX1 cells (not sensitive estrogen cell lines). Other studies have demonstrated inhibition or at least a lack of proliferative effects as well as proliferative effects: the reason for these conflicting results is not clear [54,57-60]. Two more recent investigations reported the effects of black cohosh extracts on human breast cancer cell lines determined by gene expression profiling. Gaube and coworkers [61] found that black cohosh can inhibit cell proliferation of human breast cancer cell line MFC-7 with an IC$_{50}$ of 15μg/mL. Some 500 genes appeared to be significantly regulated. Grouping the genes according to function showed that mRNAs coding for gene products involved with cell cycle progression and DNA replication were decreased, while genes coding for inhibitory products were unregulated.

A regulation of gene expression in a pro-apoptotic manner was also observed, indicating that the extract may sensitize the cells for apoptotic events. Expression of several enzymes with oxidoreductase activity was induced. Some of these genes are known to be regulated via the aryl hydrocarbon receptor (AhR), suggesting that at least a part of the effects of the black cohosh extract could be via the AhR. The other study by Einbond and coworkers [62] concerned gene expression profiles, yielding insights into the mechanisms by which black cohosh MeOH extract inhibits MDA-MB-453 human breast cancer cell line growth. The investigation was performed using 40μg/mL black cohosh extract and collecting RNA at 6 and 24 h for gene expression analysis.

The microarray results were confirmed with real-time RT-PCR for 18 genes. At 6 h after treatment there was a significant increase in expression of ER stress (GRP78), apoptotic (GDF15), lipid biosynthetic (INSIG1 and HSD17B7) and Phase 1 (CYP1A1) genes and, at 24 h, a decrease in expression of cell cycle (HELLS and PLK4) genes. It was concluded that since the MeOH extract activated genes that enhance apoptosis and repressed cell cycle genes, it may be useful in the prevention and therapy of breast cancer.

Early animal studies on an undefined extract of black cohosh postulated its capacity to possess estrogen-like activity as evidenced by an increase in uterine weight and an induction of estrus [63-64]. Further studies [39,43-44,65] suggested that black cohosh is able to reduce serum LH levels and bind to estrogen receptors (so as to increase the amount of estrogen in the blood which decreases menopausal symptoms). Subsequently, an investigation [48] demonstrated a decrease of the LH levels in ovariectomized rats. An estrogenic action of a commercial extract was confirmed [43] comparing this effect with that of estradiol on serum levels of LH and other parameters regulated by estrogen receptors. In contrast, recently, no estrogen agonistic effects on plasma hormone levels were found (prolactin, FSH, LH) in ovariectomized rats treated with a commercial extract of black cohosh [66]. Also the studies concerning the effects of black cohosh on uterine weight are in conflict. A study [67] using immature mice and ovariectomized rats revealed no signs of uterotrophic or vaginotrophic effects. Similar results were obtained with an extract of black cohosh administered to immature female mice for 14 days: it did not increase uterine or ovarian weight although it significantly prolonged the days of estrus [68] and treatment of ovariectomized mice with black cohosh did not increase uterine weight [59].

On the contrary, it was found that a black cohosh extract administered to ovariectomized rats for three weeks increased uterine growth [54,65,69]. In other studies no modifications in the uterine weight of ovariectomized rats were observed [43,66]. In addition, no increase in the expression of IgF1 and C3 in the uterus of the animals treated with black cohosh was observed, suggesting no estrogenic action of the extract on the uterine tissue [43]. The same author also reported that black cohosh possessed an estrogen agonist effect on bone (femur), increasing the expression of collagen osteocalcin and I. Similarly, it was reported that an isopropanolic extract of black cohosh significantly reduced some urinary parameters of bone metabolism, as well as the bone loss observed in control animals (however not significantly). The effects induced by the extract were similar to those induced by the estrogen raloxifene [70].

A significant reduction in loss of bone mineral density was found in a recent investigation [71]. The findings reported in this study suggest that the mode of action of black coholosh may be described as a Selective Estrogen Receptor Modulator (SERM). It is now known that there are at least two estrogen receptors, ERα and ERβ, which are differently expressed in various organs and may explain observations of both estrogenic and anti-estrogenic effects of black cohosh [60,71-73]. Pharmacological studies in humans at the end of the 1980s were, again, in contrast: vaginal cytological parameters seemed to be influenced in an estrogenic manner [9,25] while no significant changes in serum LH and FSH were observed [10]. An estrogenic mode of action was also found by Duker *et al*. in 1991 [44], while more recent double-blind, randomized studies with women having climateric symptoms showed no significant changes in serum levels of estradiol, LH, FSH, prolactin or sexual hormone binding globulin. Vaginal cytological parameters also remained unchanged [74]. Black cohosh had no effects on endometrial thickness in one study [75] but it increased by more than 1 mm in another [72].

Clinical trials

Much of the clinical research on black cohosh has been conducted in Germany since the 1940s, mostly using commercial standardised 60% ethanolic and 40% isopropanolic extracts of the rizhome and roots as tablets, capsules or drops. Daily doses ranged from 40 up to 160 mg herbal drug, most of those tested being 40 or 80 mg herbal drug or equivalent preparations corresponding to 1-2 mg triterpenes calculated as 26-deoxyactein (**2**) [76].

From 1957 to 1964 a number of case reports and uncontrolled studies were published describing the successful treatment with black cohosh preparations of numerous women presenting climateric symptoms or menstrual disorders [64,74,77-85]. In 1998 a review of clinical and human pharmacological studies [86] which assessed the efficacy of black cohosh for menopausal symptoms concluded that it may be a safe and effective alternative to estrogen replacement therapy in patients for whom estrogen replacement therapy is contraindicated or refused. In a more recent systematic review, [87] four randomized, controlled clinical studies [8-11] were analyzed. The first clinical study examined was conducted in 1985 by Warnecke. Sixty women in menopause were divided into three groups: they received 80 mg daily of black cohosh, estrogens (0.625 mg/day) or diazepam (2 mg/day) for four months. The authors measured the modified Kuppermann Menopausal Index, self-evaluation

depression scale, Hamilton Anxiety Scale and vaginal epithelium status. Black cohosh and conjugated estrogens produced a similar decrease in climacteric complaints and estrogen-like stimulation of the vaginal epithelium status. In regard to this latter, cytological examination revealed a similar percentage of eosinophilic epithelial cells with black cohosh and conjugated estrogens. The women treated with black cohosh had a greater percentage of pycnotic nuclei than those on conjugated estrogens [8]. Another study included in the review, by Stoll in 1987 [9], was quite similar. Eighty postmenopausal women were randomly assigned to a commercial preparation of black cohosh (80 mg/day) versus conjugated estrogen (0.625 mg per day) versus placebo for 12 weeks. Women receiving black cohosh were reported to have a significantly improved Kupperman Menopausal Index and Hamilton Anxiety Scale compared with women receiving conjugated estrogen or placebo. No difference was detected in vaginal proliferation among the three groups [9]. Lehmann-Willenbrock and Riedel reported a study on 60 hysterectomized women with at least one intact ovary. One group was administered twice daily with black cohosh (80 mg/day) for six months. The control group was administered with estriol (1 mg/day) and conjugated estrogens (1.25 mg/day). In both groups a decrease of the modified Kupperman index was observed within four weeks after the beginning of therapy. *C. racemosa* showed no effects on serum concentration of LH and FSH [10].

The study by Jacobson *et al*. [11] was also analyzed in the review. The author tested black cohosh extract versus placebo on 26 female breast cancer survivors. Each woman took one commercial tablet of black cohosh (40 mg) or a placebo twice daily for two months. Menopausal symptoms improved in both groups. No statistically significant differences in blood levels of FSH and LH were noted. There was no significant beneficial clinical effect of black cohosh compared with placebo, except for a reduced number of sweating episodes [11]. The authors of the systematic review [87] concluded that, in spite of plausible mechanisms of actions, the clinical efficacy of black cohosh for the treatment of menopausal symptoms has not yet been convincingly demonstrated; further rigorous studies are warranted [87].

No other systematic reviews have been reported in the literature, but starting from the late 1990s numerous clinical trials related to vasomotor symptoms of black cohosh have been conducted

[88-101]. Among them, three studies in 2005 [88-90], four in 2006 [91-92,96-97], and two in 2007 [98-99], which represent the most recent trials together with clinical trials involving breast cancer patients [11,95,101-101], are briefly summarized in the present contribution.

Two of the studies reported in 2005 [88-89] were double-blind placebo-controlled trials and black cohosh was found significantly better than a placebo in improving the menopause rating scale when used at dosages corresponding to 40 mg herbal drug/day or placebo for three months. The first trial involved 304 women and those in early climacteric phase benefited more than those in the late phase. No relevant group differences in adverse events, laboratory findings, or tolerability were found [88]. The second trial was a multicenter, randomized, double-blind, placebo-controlled clinical trial including 122 menopausal women with ≥3 hot flashes a day [89]. Two main efficacy measures - weekly weighted score of hot flashes and Kupperman Index - and secondary efficacy variables (e.g. menopause rating scale) were defined. Routine safety laboratory parameters and adverse events were documented. The primary efficacy analysis showed no superiority of the tested black cohosh extract compared to a placebo. However, in the subgroup of patients with a Kupperman Index > or = 20 a significant superiority regarding this index was demonstrated (P<0.018). A decrease of 47% and 21% was observed in the black cohosh and placebo group, respectively. The weekly weighted scores of hot flashes (P<0.052) and the menopause rating scale (P<0.009) showed similar results. The results indicate a superiority of black cohosh compared to a placebo in patients with menopausal disorders of at least moderate intensity according to a Kupperman Index > or = 20, but not in the intention-to-treat population as a whole There were no differences between the *Cimicifuga racemosa* group and the placebo group regarding adverse events or other safety assessments. Frequency – 17/83 patients (20%) in black cohosh group and 10/44 patients (23%) in the placebo group – and intensity of adverse events were comparable in the two groups [89].

In 2005 a third study was conducted on 64 postmenopausal women for the same period. Patients were divided into two groups: the first received 40 mg/day black cohosh, the second received low-dose trans-dermal estradiol, i.e. 25 microg every 7 days

plus dihydrogesterone 10 mg/day for the last 12 days of the three-month estradiol treatment. Hot flashes and other climacteric symptoms (vasomotor and urogenital symptoms), as well as anxiety and depression, were evaluated at baseline and after three months. No significant differences were found between the black cohosh and transdermal estrogen; neither black cohosh nor transdermal estrogen had any effect on urogenital symptoms, endometrial thickness, FSH and LH [90].

In 2006 Wuttke and coworkers reported two double-blind, randomized, placebo- and conjugated estrogens-controlled studies. Patients were treated with a commercial black cohosh preparation (daily dose corresponded to 40 mg of herbal drug), conjugated estrogens (0.6 mg/day), or a placebo over a three-month period [91-92]. The first trial involved 62 postmenopausal women and was performed to evaluate markers of bone turnover (bone-specific alkaline phosphatase, CrossLaps), estradiol, follicle-stimulating hormone, luteinizing hormone, SHBG, triglycerides, total cholesterol, high-density cholesterol, low-density cholesterol, and routine clinical chemistry parameters from blood samples. The vaginal "maturity index" was determined from vaginal smears. Black cohosh showed only a weak estrogen-like activity and showed beneficial effects on bone metabolism because it stimulated osteoblast activity, whereas conjugated estrogens inhibited osteoclast activity. No significant effects were seen on coagulation markers and liver enzymes in the blood [91]. The second study in 2006 [92] was carried out to assess efficacy and tolerability of black cohosh in postmenopausal women suffering from estrogen deficiency symptoms. Ninety-five women were involved and the change from baseline of the menopause rating scale (MRS) total score after 12 weeks was defined as the primary efficacy endpoint. Changes from baseline were also analyzed for secondary endpoints including three MRS subscores (major climacteric complaints, somatic complaints, mental score), sweating episodes, and sleeping behavior. Thirty-three women had to be excluded from the intention-to-treat analysis due to protocol violations. Both black cohosh and conjugated estrogens reduced all estrogen deficiency symptoms and improved sleep quality equipotentially. Due to the small sample size the trial was considered as a pilot study but demonstrated that black cohosh is able to reduce estrogen deficiency symptoms to the same degree as conjugated estrogens and with good tolerability [92]. The third study in 2006 was a one-year double-blind, randomized, placebo-controlled trial of black cohosh in the management of hot flashes [96].

Participants were 351 women aged 45 to 55 years with two or more vasomotor symptoms per day; 52% of the women were in menopausal transition and 48% were postmenopausal. Patients were divided into five groups: black cohosh, 160 mg daily; multibotanical with black cohosh, 200 mg daily, and nine other ingredients; multibotanical plus dietary soy counseling; conjugated equine estrogen, 0.625 mg daily, with or without medroxyprogesterone acetate, 2.5 mg daily; or a placebo. Vasomotor symptoms per day, symptom intensity, and Wiklund Vasomotor Symptom Subscale score did not differ between the herbal interventions and placebo at 3, 6, or 12 months or for the average of all the follow-up time points ($P > 0.05$ for all comparisons) with one exception. At 12 months, symptom intensity was significantly worse with the multibotanical plus soy intervention than with placebo ($P = 0.016$). The difference in vasomotor symptoms per day between placebo and any of the herbal treatments at any time point was less than one symptom per day; for the average of all the follow-up time points, the difference was less than 0.55 symptoms per day. The difference for hormone therapy versus placebo was −4.06 vasomotor symptoms per day for the average of all the follow-up time points (95% CI, −5.93 to −2.19 symptoms per day; $P < 0.001$). It was concluded by the researchers that black cohosh used alone, or as part of a multibotanical regimen, showed little potential as an important therapy for relief of vasomotor symptoms [96].

Radowicki and coworkers in 2006 reported a trial using 40 mg black cohosh a day for six months involving 20 women with mean age 52.4 +/- 4.9 years with climacteric syndrome [97]. Kupperman's Index, biochemical parameters and hormonal profile were estimated before and after three and six months of the therapy. Mean values of Kuppermen's Index were decreased from 30.2 +/- 5.7 points before the therapy to 8.5 +/- 6.3 points after three months and to 2.6 +/- 2.1 points after six months of therapy (p < 0.05). No statistical differences in biochemical parameters' concentrations and hormonal profile were observed. Researchers concluded that black cohosh was an effective and safe therapy for climacteric women with contraindications to hormonal replacement therapy [97].

At the beginning of 2007 two trials appeared in the literature [98-99]. The first was carried out to determine the efficacy and safety of the herbal formula (containing black cohosh, dong quai, milk thistle, red clover, American ginseng, chaste-tree berry) marketed for the relief of menopausal symptoms. It was a randomized, double-blind, placebo-controlled trial in 50 healthy pre- and postmenopausal women, aged 44-65 years, to whom oral formulation or matched placebo was prescribed twice daily for three months. A structured questionnaire on the frequency and intensity of menopausal symptoms was completed weekly starting one week before treatment and throughout the three-month treatment period, followed by biochemical tests, breast check, and transvaginal ultrasonography. The women receiving herbal drugs reported a significantly superior mean reduction in menopausal symptoms than the placebo group. The effect of treatment improvements in menopausal symptoms increased over time; by three months there was a 73% decrease in hot flashes and a 69% reduction of night sweats, accompanied by a decrease in their intensity and a significant benefit in terms of sleep quality. Hot flashes ceased completely in 47% of women in the study group, compared with only 19% in the placebo group. There were no changes in findings from vaginal ultrasonography or levels of relevant hormones (estradiol, follicle-stimulating hormone), liver enzymes or thyroid-stimulating hormone in either group. The authors reported that the herbal drug formulation was safe and effective for the relief of hot flashes and sleep disturbances in pre- and postmenopausal women, at least with three months' use [98].

An additional trial was conducted by Chinese researchers [99]. The aim of the study was to investigate the efficacy-safety balance of a commercial preparation of black cohosh in comparison with tibolone in Chinese women with climacteric complaints. This randomized, double-blind, controlled three-month study in five centers in three cities in China enrolled 244 menopausal patients aged 40-60 years with a Kupperman Menopause Index (KMI)$\geq$15. The participants were assigned to either black cohosh, corresponding to 40 mg crude drug/day (N=122), or tibolone 2.5 mg/day (N=122) orally. The primary endpoint was the combination of the Mann-Whitney values (MWV) of the KMI and the frequency of adverse events (benefit-risk balance) at the end of treatment (MWV>0.5 shows superiority; MWV>0.36 shows

non-inferiority). It was found that KMI decreased from 24.7+/-6.1 to 11.2+/-6.2 and 7.7+/-5.8 (black cohosh) and to 11.2+/-7.2 and 7.5+/-6.8 (tibolone) at four and 12 weeks. This remarkable and clinically relevant improvement was similar in both treatment groups (MWV=0.47; 95% CI=0.39-0.54; p(non-inferiority)=0.002) showing statistically significant non-inferiority of black cohosh to tibolone. The KMI-responder rate was similar in both groups (84% and 85%). Safety evaluation showed for both groups good safety and tolerability profiles, however there was a significantly lower incidence of adverse events (p<0.0001) in favor of the herbal treatment. None of the postmenopausal black cohosh patients experienced vaginal bleeding; with tibolone 17 cases were reported. Breast and abdominal pain, as well as leucorrhea, was mostly observed in the tibolone group (p = 0.015, p = 0.008, p = 0.002). No serious adverse events were observed in the black cohosh group, however two occurred in the tibolone group. The benefit-risk balance for black cohosh was significantly (p = 0.01) superior to tibolone (MWV = 0.56; 95% confidence interval [0.51-0.62]). It was concluded that the efficacy of black cohosh is as good as tibolone for the treatment of climacteric complaints, even for moderate to severe symptoms, whereby black cohosh is clearly superior regarding the safety profile [99].

Clinical trials involving breast cancer patients: Hot flashes are a common manifestation of diminished ovarian function as a result of natural menopause but they can also be related to breast cancer therapy and for such patients hormone replacement therapy is strictly contraindicated. Thus, black cohosh was tested in a few trials with patients in concomitant therapy with tamoxifen, raloxifene, or an aromatase inhibitor to evaluate efficacy in the management of hot flashes [11,95,100,101].

The first study by Jacobson and coworkers [11] on 26 breast cancer survivors, as discussed above in the clinical studies paragraph, resulted in no significant beneficial clinical effect of black cohosh compared with placebo, except for a reduced number of sweating episodes [11]. The study by Munoz and Pluchino in 2003 [100] included 136 young premenopausal breast cancer survivor women aged 35-52 years with hot flashes due to tamoxifen administration. Patients who had undergone segmental or total mastectomy, radiation therapy and adjuvant chemotherapy, were randomly assigned to receive tamoxifen 20 mg per day orally (usual-care

group; n = 46) or tamoxifen (same dose and posology) plus a commercial extract of black cohosh (corresponding to 20 mg of herbal drug; intervention group n = 90). Duration of treatment was five years for tamoxifen, according to international standards for adjuvant therapies, and 12 months for the commercial extract [101]. Follow-up included clinical assessment every two months; the primary endpoint was the number and intensity of hot flashes. Comparing patients assigned to the usual-care group with those assigned to the intervention group, the number and severity of hot flashes were reduced after intervention. Almost half of the patients in the intervention group were free of hot flashes, while severe hot flashes were reported by 24.4% of patients in the intervention group and 73.9% in the usual-care group (P<0.01) [100].

Two other correlated studies were conducted by Pockaj and coworkers in 2004 and 2006 [95,101]. The first study [95] was, a pilot, open-label, not-randomized and not-blinded trial involved 21 women having a mean age of 56 years (range, 38-80). Thirteen patients had a history of breast cancer. Six patients were taking tamoxifen or raloxifene. Patients reported an average of 8.3 hot flashes per day during the baseline week. The reduction in mean daily hot flash frequency was 50% (95% CI, 34%-65%), while weekly hot flash scores were reduced 56% (95% CI, 40%-71%) at completion of the study. Overall, patients reported less trouble with sleeping, less fatigue, and less abnormal sweating. No patients stopped therapy because of adverse effects. Black cohosh appeared to reduce hot flashes and had a low toxicity [95]. The second study was a double-blind, randomized, placebo-controlled, cross-over clinical trial with two four-week periods for the treatment of hot flashes in 132 women with a history of breast cancer [101]. Concomitant therapy with tamoxifen, raloxifene, or an aromatase inhibitor was allowed as long as the patient had been on the therapy for one month and was not planning to alter this therapy during the study. Patients receiving black cohosh (a dose corresponding to 1 mg triterpenes) reported a mean decrease in hot flash score of 20% (comparing the fourth treatment week to the baseline week) compared with a 27% decrease for patients on placebo (P = 0.53). Mean hot flash frequency was reduced 17% with black cohosh and 26% with a placebo (P = 0.36). The trial failed to provide any evidence that black cohosh reduced hot flashes more than the placebo [101].

Safety

Adverse events: Safety data from post-marketing surveillance studies found very few serious adverse events [75]. Forty menopausal women were involved and had high doses of balck cohosh preparation (136 mg/day) administered for three months. Twenty-eight women completed the study, with twelve withdrawing for unknown reasons. Administration of black cohosh did not significantly change endometrial status, vaginal cytology, or levels of reproductive hormones from baseline [75].

In the same year (1999) the first review of clinical safety data on black cohosh was published [14] and the authors concluded that clinical data and case reports had demonstrated good tolerance of both ethanolic and isopropanolic extract preparations based on black cohosh [14].

Another clinical evaluation of the safety of black cohosh [5] concluded in 2003 that human clinical trials, postmarketing surveillance studies and other reports involving over 2,800 patients demonstrated a low incidence of adverse events (5.4%). Of the reported adverse events, 97% were considered minor and did not result in the discontinuation of therapy. Black cohosh was considered safe without severe adverse events [5]. Similar findings were obtained in another review in the same year [102] concerning clinical trials and spontaneous reports from the WHO and national regulatory bodies. It was concluded that adverse events arising from the use of black cohosh are rare and generally mild and reversible, as they are in general gastrointestinal upsets and rashes. It was concluded that even if definitive evidence is not available, black cohosh seems to be safe [102].

The most recent clinical evaluation was reported in 2005 by Mahady [103]. Eleven clinical trials since 1982 were included in the study and split into two groups: randomized controlled or comparative trials (six), and uncontrolled studies (five). In terms of safety, transient adverse events such as nausea, vomiting, headaches, dizziness, mastalgia, and weight gain were observed in clinical trials, even if the author evidenced the relatively short treatment period of all clinical studies [103].

The most recent clinical trials (2005-2007) reported in the clinical paragraph of this paper give little additional information with regards to safety as the papers are mainly focused on efficacy. Among the

nine trials, four [90-92,96] gave no information about safety. Only one trial [90] reported that after three months' treatment with 40 mg/day black cohosh or trasdermal estradiol any effect on urogenital symptoms, endometrial thickness, FSH and LH were found [90].

Other trials [88-89, 97-98] reported only a generic statement: no relevant group differences in adverse events, laboratory findings, or tolerability were found but no specific information was given. The study by Frei-Kleiner and coworkers [89] stated that frequency (17/83 patients (20%) in black cohosh group and 10/44 patients (23%) in the placebo group) and intensity of adverse events were comparable in the two groups and in particular, the karyopyknotic index showed no proliferative effects on vaginal epithelium [89]. Rotem and coworkers [98] evidenced no changes in vaginal ultrasonography findings or levels of relevant hormones (estradiol, follicle-stimulating hormone), liver enzymes or thyroid-stimulating hormone in either group [98]. The last paper by Bai and coworkers gave more precise data [99]. The participants (244 menopausal patients aged 40-60 years) were assigned to either black cohosh, corresponding to 40 mg crude drug/day (N = 122), or tibolone 2.5mg/day (N = 122) orally. Three hundred ninety-two adverse events were found in 154/243 subjects. Of those, 139 adverse events occurred in 64 (52.9%) subjects in the black cohosh group and 253 occurred in 90 (73.8%) subjects in the tibolone group. There was a significant difference between the two groups regarding the rate of patients with any adverse event ($p = 0.001$) in favor of black cohosh.

Of the 243 subjects, 130 patients (53.5%) experienced 281 possibly drug-related adverse events: 86 in 49 patients (40.5%) in the black cohosh group, and 195 in 81 patients (66.4%) in the tibolone group. The incidence of these adverse events was much lower in the herbal group compared to the tibolone group ($p < 0.0005$). All adverse events were either known climacteric symptoms or listed adverse drug reactions. Adverse events that might have been interpreted as a sign of a liver dysfunction did not occur.

Gynecological adverse events were of particular interest. The black cohosh therapy demonstrated lower incidences in comparison with tibolone with respect to vaginal bleeding or spotting, breast pain, abdominal pain, leucorrhea. Most of the subjects experiencing bleeding were perimenopausal as they

had been amenorrhoeic for less than 12 months. Average thickness of the uterine intima increased slightly from 2.8±1.3mm at baseline to 3.3±2.0mm at week 12 when analyzed together for peri- and postmenopausal patients in the black cohosh group ($p = 0.012$), 2.9±1.2mm at baseline to 3.4±2.0mm at week 12 in the tibolone group ($p = 0.005$). While such a small increase is without clinical relevance, a subgroup analysis showed that in the black cohosh group this increase was found only in perimenopausal but not in postmenopausal patients (from 2.7±1.1 to 2.9±1.4 mm; $p = 0.148$). In contrast, the mean endometrial thickness significantly increased also in postmenopausal tibolone patients (from 2.8±1.1 to 3.4±2.0 mm; $p = 0.01$).

Intra-group comparison of mean body weight demonstrated a clinically irrelevant increase at week 12 as compared to baseline and week 4 ($p < 0.01$). Inter-group comparison showed that body weight in the tibolone group at week 12 was significantly higher than that in the black cohosh group ($p = 0.027$), while body weight at baseline and week 4 was similar in the two groups. In this trial two serious adverse events occurred in the tibolone group, one related to tibolone (spotting for 28 days with an endometrial thickness of 9.1 mm; the endometrial biopsy indicated endometrial polyps and complex hyperplasia) and the other without any causal relationship to the study medication (traffic accident).

No clinically significant findings were detected from the results of hematology, blood chemistry or urinalysis during treatment of both groups. In particular, neither AST, ALT, gamma-GT nor AP revealed any suspicion of liver dysfunction. Only C-reactive protein increased slightly, but only in the tibolone group ($p = 0.06$), in contrast to the black cohosh-group. Regarding the Clinical Global Impression (Item 3.2) for side effects, the women given herbal treatment responded significantly better than those given tibolone (week 4: $p = 0.002$; week 12: $p = 0.033$). However, the difference in the patient's global assessment of tolerability between the two groups in favor of black cohosh was not statistically significant ($p = 0.13$) [99].

A recent review reported a study concerning safety and efficacy of black cohosh during pregnancy and lactation [104]. Black cohosh, alone or in combination with other medicinal herbs such as "mother's cordial", or "partus preparatus," has a long traditional use and is frequently used by midwives as

a uterine stimulant and labor-inducing aid. The authors concluded that black cohosh should be used with caution during pregnancy, particularly during the first trimester where the labor-inducing properties of black cohosh could be of greatest harm to the fetus. Despite no reports of malformations in the scientific literature, black cohosh should be used with caution in the third trimester and at delivery when used as a labor-inducing aid until further clinical research is conducted. The level of evidence for using black cohosh during lactation is also poor. Black cohosh should be used with caution as *in vitro* evidence suggests estrogenic/anti-estrogenic properties. Clearly more rigorous and well-controlled research is needed in this area [104].

Finally, in the literature there are two case reports concerning adverse events. The first in 2004 involved a 54-year-old woman with severe asthenia and very high blood levels of creatine phosphokinase and lactate dehydrogenase taking a dietary supplement derived from black cohosh (20 mg/day. After suspension of therapy, the patient showed a progressive normalization of biochemical parameters and improvement of clinical symptoms. The authors considered black cohosh to have a causative role because of the temporal relationship between use of the herbal product and myopathy, and for the absence of other identified causative factors [105]. The second paper (2007) [106] concerned a 56-year-old woman who presented localized asymptomatic erythematous plaques on her arms and legs. Histologically her condition was diagnosed as pseudolymphoma. The patient had been taking black cohosh root extract for a year. The first skin lesions appeared six months after initial administration of the herbal drug. Withdrawal of black cohosh resulted in regression and complete remission of the lesions within 12 weeks. As in the previous case, the temporal relationship between cutaneous lesions and use of black cohosh and the lack of recurrence after six months of follow-up suggest the diagnosis of drug-induced pseudolymphoma [106].

Effects on liver: Suspected adverse liver reactions associated with the use of black cohosh were first brought to the attention of the Medicine and Healthcare products Regulatory Agency (MHRA, UK) in 2002 through cases in the literature. In October 2003, media activity surrounded the publication of a case in the USA in which a woman developed liver failure and required a liver transplant after using an herbal remedy that contained black cohosh. The fact attracted great public interest and in February 2004 this matter was discussed at the Committee on Safety of Medicines Sub Committee on Pharmacovigilance (CSM SCOP, a panel of independent advisory experts). At that time, the MHRA had received four spontaneous case reports of liver reactions associated with the use of this drug in the UK. This committee concluded that there was insufficient information to support regulatory action, but recommended that healthcare professionals ask about the use of herbal medicines in patients presenting symptoms and signs of hepatotoxicity and to report eventual cases [107]. A similar conclusion was reported in the same year by the US National Institute of Health [108].

At the beginning of 2005 the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMEA) adopted an assessment report of cases of hepatotoxicity associated with the use of black cohosh [109]. The documents from the committee were forwarded to the CHMP Pharmacovigilance Working Party for consideration.

As of March 31, 2006, there were 21 reports of adverse liver reactions associated with black cohosh in the UK received through the Yellow Card Scheme, and a further 19 cases worldwide were included in a report by the German Federal Institute for Drugs and Medical Devices. These cases included seven from Germany, three from the USA, and six from Sweden. Furthermore, in February 2006, the Australian Therapeutic Goods Agency made reference to nine cases and this agency announced in Australia that warning labels will be introduced on all black cohosh products over a 12-month period: "Black cohosh may harm the liver in some individuals. Use under the supervision of a healthcare professional".

In the same time period the HMPC made a public statement concerning the assessment of 32 cases, including 29 from the EU and three from the USA, and the nine cases published in the literature according to the RUCAM score. As a conclusion, it was reported that there is probably a connection between the use of black cohosh and hepatotoxicity, at least in a few cases [110]. In July 2006 the EMEA issued a public statement to advise both patients and healthcare professionals about suspected hepatic injury caused by black cohosh [111]. According to RUCAM Score [112-113] all the cases were assessed. Among the 31 EU cases collected from National Competent authorities, seven were classified

as "unrelated", five as "excluded" (global score ≤0), seven as "unlikely" (global score 1-2), one possible (global score 3-5), and 11 not assessable because of insufficient information. Among the three cases of non-EU cases received from European National Competent Authorities, two were considered unrelated and one not assessable. Finally, the cases published in the literature were also analyzed. The first striking case was reported in 2002 by Withing and coworkers [114]. A patient required urgent liver transplantation for fulminant hepatic failure after brief use of herbal combination products including black cohosh. The case was considered improbable by HMPC because of the short time between intake of the drug and transplantation (one week) [111]. In 2003 Lontos and coworkers [115] reported the case of a liver failure resulting in transplantation and associated it with the use, for three months, of an herbal preparation containing several ingredients including black cohosh root and *Glechoma hederacea*. However, because of the known hepatotoxic potential of *Glechoma hederacea* (pulegone is one of the active ingredients), and the lack of detailed information, it was not possible to conclude that black cohosh was the relevant cause of liver failure [111].

In 2004 Cohen and coworkers [116] reported a case of autoimmune hepatitis likely triggered by the use of black cohosh. HPMC classified this case as probable, but suggested that the autoimmune hepatitis could have been a manifestation of a multisystem autoimmune disease as well [111]. Levitisky and coworkers [117] reported in 2005 one case of fulminant liver failure after taking 500 mg daily of black cohosh root for five months. HPMC classified it as a probable case because the time frame of onset of reaction is related to the drug exposure. However, 500 mg drug/day is 12 fold the recommended dose of the commission E monograph [111]. Lynch and coworkers [118] published in 2006 an article reporting the case of a 54-year-old woman who had been taking 1000 mg of black cohosh daily for several months. She had a history of hypothyroidism, fibromyalgia, osteoarthritis and depression. The patient was on fluoxetin, propoxyphene and paracetamol concomitantly, moreover, she admitted drinking one or two glasses of wine a day. The patient developed a fulminant hepatic failure and died during liver transplantation in the operating room due to uncontrollable hemorrhage. For this case HPMC classified the connection between black cohosh and hepatic toxicity as probable, but affirm that since

there is no further information on the herbal preparation, the case report is not of great relevance in the assessment of cimicifuga-related hepatotoxicity [111].

EMEA suggested discontinuing use of this herbal drug or its preparations [111] and the MHRA suggested adding warnings to the label of the products [119]. A document was also published by the WHO to disseminate this information and immediately afterwards a few EU countries, but not Canada or the USA, discontinued the marketing of preparations based on black cohosh [120].

After July 2006, the HMPC revised the documents concerning the assessment of case reports in January, March and May 2007. The final document was also revised, including the adverse drug reactions from clinical studies evidencing that overall, in the 15 clinical studies, no cases of significant liver dysfunction were observed [121]. The HMPC suggested advising patients on the label to stop taking black cohosh and consult a medical doctor if they develop signs and symptoms suggesting liver injury [121]. This advice was considered very important by many countries, even by some of those who discontinued products based on black cohosh, leading to readmission of the products on the market.

Hormonal effects: A review of safety related to the hormonal effects of black cohosh was reported by Mahady in 2005 [103]. The author discussed six studies, an old clinical trial [44], three *in vitro* studies [50, 57, 122] and two investigations with rats [123-124] and concluded that black cohosh seems to be not estrogenic and does not stimulate the proliferation of breast or endometrial cancer cells [103].

Two very important papers concerning hormonal effects appeared in 2006 and 2007. The first study [125] was carried out to investigate endometrial safety by assessment of endometrial biopsy samples and the tolerability and efficacy of black cohosh commercial extract. Four hundred postmenopausal women with symptoms related to estrogen deficiency were enrolled in a prospective, open-label, multinational, multicenter study. Treatment duration (daily dose corresponding to 40 mg of herbal drug) was 52 weeks. To determine the probability of endometrial hyperplasia and more serious adverse endometrial outcome, the point estimator and upper limit of 95% CI were calculated. Descriptive statistics were used to assess the secondary

endpoints. Endometrial safety was proven because no case of hyperplasia or more serious adverse endometrial outcome occurred (point estimate: 0.0; upper limit of 95% CI: 0.011). Endometrial thickness, which was measured by endovaginal ultrasonography, did not show an increase. The number and intensity of hot flashes were markedly decreased. The dropout rate was less than 10%, and overall tolerability was good. The lack of endometrial proliferation was proved for the first time after a treatment period of one year [125].

The other study [126] was carried out to determine the effects of black cohosh on mammographic breast density and breast epithelial proliferation in healthy, naturally postmenopausal women with climacteric symptoms. This was a prospective, open, uncontrolled drug safety study in which baseline status was compared with status after six months of treatment by blinded observers. A total of 74 women were treated with 40 mg black cohosh daily, and 65 women completed the study. Mammograms were performed, and breast cells were collected by percutaneous fine needle aspiration biopsies at baseline and after six months. Mammographic density was quantified according to the Wolfe classification or a percentage scale. Breast cell proliferation was assessed using the Ki-67/MIB-1 monoclonal antibody. Safety was monitored by adverse event reporting, laboratory assessments, and measurement of the endometrium by vaginal ultrasound. None of the women showed any increase in mammographic breast density. Furthermore, there was no increase in breast cell proliferation. The mean change +/- SD in proportion of Ki-67-positive cells was -0.5% +/- 2.4% (median, 0.0; 95% CI = -1.32 to 0.34) for paired samples. The mean change in endometrial thickness +/- SD was 0.0 +/- 0.9 mm (median, 0.0). A modest number of adverse events were possibly related to treatment, but none of these were serious. Laboratory findings and vital signs were normal. The findings suggested, again, that black cohosh does not cause adverse effects on breast tissue [126].

Some studies are also related to safety of black cohosh in women with breast cancer who are on tamoxifen. A presentation at the American Association for Cancer Research meeting raised some concerns about increased metastases, but not incidence, of breast cancer in mice using black cohosh [127]. However, no peer-reviewed paper has been published or plausible mechanism of action

presented and the investigators themselves have noted that the histology component of the research is not complete. In fact, previous studies on both *in vitro* investigations with breast cancer cells and *in vivo* data show no stimulation of estrogen-dependent mammary gland tumors with black cohosh [5,50,57-58,93,123].

A very important recent study in 2007 [128] investigated the influence of black cohosh on recurrence-free survival after breast cancer, including estrogen-dependent tumors. It was a pharmacoepidemiologic observational retrospective cohort study that examined breast cancer patients treated at general, gynecological and internal facilities linked to a medical database in Germany. The main endpoint was disease-free survival following a diagnosis of breast cancer. Of the 18,861 patients, a total of 1,102 received black cohosh therapy. The mean overall observation time was 3.6 years. Results showed that black cohosh was not associated with an increase in the risk of recurrence but associated with prolonged disease-free survival. Two years after initial diagnosis, 14% of the control group had developed a recurrence, while the black cohosh group reached this proportion after 6.5 years. In conclusion, an increase in the risk of breast cancer recurrence for women having had black cohosh treatment, compared to women not treated with black cohosh, was considered unlikely [128].

Drug interactions: Finally, there have been no documented cases of drug interactions [129]. In a study on mouse breast cancer cell lines, the interaction of black cohosh extract with four drugs used in cancer therapy and with radiation was evaluated. The results showed that black cohosh extract increases the cytotoxicity of doxorubicin and docetaxel and decreased the cytotoxicity of cisplatin, but does not alter the effects of radiation or 4-hydroperoxycyclophosphamide (4-HC) [130].

Two recent papers deal with the effects of black cohosh or single constituents on several human cytochrome P450 phenotypes [131-132]. The first paper [131] demonstrated black cohosh had CYP3A4 inhibition (IC_{50}=0.027 mg/mL) and bioassay-guided isolation afforded six moderately active constituents, which were identified as cycloartanoid triterpene glycosides (IC_{50} ranged from 0.10 to 7.78 mM). The IC50 value indicates that 40 mg of black cohosh could be estimated to inhibit the metabolism of roughly half a dose of nifedipine for one day [131].

The second study [132] was related to the *in vivo* effects of several herbal drugs, including black cohosh on CYP1A2, CYP2D6, CYP2E1, or CYP3A4/5 activity. Twelve healthy volunteers (six females) were randomly assigned to receive the herbal drug for 28 days. For each subject, a 30-day washout period was interposed between each supplementation phase. Probe drug cocktails of midazolam and caffeine, followed 24 hours later by chlorzoxazone and debrisoquine, were administered before (baseline) and at the end of supplementation. Pre- and post-supplementation phenotypic trait measurements were determined for CYP3A4/5, CYP1A2, CYP2E1, and CYP2D6 using 1-hydroxymidazolam/ midazolam serum ratios (1-hour sample), paraxanthine/caffeine serum ratios (6-hour sample), 6-hydroxychlorzoxazone/chlorzoxazone serum ratios (2-hour sample), and debrisoquine urinary recovery ratios (8-hour collection), respectively. Black cohosh exhibited statistically significant inhibition of CYP2D6 (difference = −0.046; 95% CI = −0.085 to −0.007), but the magnitude of the effect (~7%) did not appear clinically relevant [132].

Another study by the same authors [133] assessed the effects of milk thistle and black cohosh supplementation on CYP3A activity and compared them to a clinically recognized inducer, rifampin, and inhibitor, clarithromycin. Nineteen healthy volunteers (nine females) were randomly assigned to receive milk thistle (corresponding to 440 mg silymarin) or black cohosh (corresponding to 3 mg triterpene glycosides) supplement for 14 days. Subjects also received rifampin (600 mg) and clarithromycin (1000 mg) for seven days as positive controls for CYP3A induction and inhibition, respectively. Midazolam was administered orally before and after each supplementation and control period. The effects of milk thistle, black cohosh, rifampin, and clarithromycin on midazolam pharmacokinetics were determined using noncompartmental techniques. Unlike those observed for rifampin and clarithromycin, midazolam pharmacokinetics were unaffected by milk thistle or black cohosh. Milk thistle and black cohosh appear to have no clinically relevant effect on CYP3A activity *in vivo* [133]. The same group of researchers [134] also reported an investigation on the effects of black cohosh and milk thistle on digoxin pharmacokinetics in humans. Sixteen healthy volunteers were randomly assigned to receive milk thistle (corresponding to 440 mg silymarin) or black cohosh (corresponding to 3 mg

triterpene glycosides) for 14 days, followed by a 30-day washout period. Subjects were also randomized to receive rifampin (600 mg daily, 7 days) and clarithromycin (1000 mg daily, 7 days) as positive controls for P-gp induction and inhibition, respectively. Digoxin (Lanoxicaps, 0.4 mg) was administered orally before and at the end of each supplementation and control period. Serial digoxin serum concentrations were obtained over 24 h and analyzed by chemiluminescent immunoassay. Comparisons of area under the serum concentration time curves from 0 to 3 h (AUC(0–3)), AUC(0–24), Cmax, apparent oral clearance of digoxin (CL/F), and elimination half-life were used to assess the effects of milk thistle, black cohosh, rifampin, and clarithromycin on digoxin pharmacokinetics. No statistically significant effects on digoxin pharmacokinetics were observed following supplementation with black cohosh [134].

Concluding remarks and future directions

Standardized isopropanolic and ethanolic extracts of black cohosh roots and rizhoma are currently available on the European market both as food supplements and as Herbal Medicinal Products after German health authorities endorsed its use for premenstrual discomfort, dysmenorrhea, and menopause [135]. Similarly, the World Health Organization (WHO) [19] and European Scientific Cooperative on Phytoterapy (ESCOP) [76] have recognized its use for "treatment of climacteric symptoms such as hot flashes, profuse sweating, sleeping disorders and nervous irritability." Food supplements containing black cohosh extracts, the herbal drug or herbal combinations are also marketed in the USA, Australia and other regions of the world. The American Herbal Pharmacopoeia has recognized the clinical research on black cohosh, mainly conducted on the Herbal Medicinal products [136] and the North American Menopause Society [137] recommends black cohosh, in conjunction with lifestyle approaches, as a treatment option for women with mild menopause-related symptoms.

Constituents responsible for the activity are not yet completely known, and probably a synergistic effect of different constituents of triterpene glycosides (actein, 27-deoxyactein, cimicifugoside) and cinnamic acid esters could be related to the activity. Also the exact mechanism of action is still unclear even if hundreds of pharmacological studies and clinical trials have been performed. For decades it has been postulated to have estrogenic activity and is

classified as a phytoestrogen, however recent studies show no effect on serum hormone levels (e.g., luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, sex hormone binding globulin (SHBG), or estradiol.

Recent studies have evidenced that it devoids estrogenic effects in the uterus and mammalian gland, it slightly inhibits serum LH levels in ovariectomized rats, and it has a selective estrogen receptor modulator (SERM) activity. The investigators found that black cohosh had an equivalent effect to conjugated estrogens in significantly improving both menopausal symptoms and bone markers compared to placebo. For this reason it can reduce climacteric complaints, have antiosteoporotic effects and reduce VLDLs and LDLs but increase HDLs, thereby reducing the "bad" cholesterol stores and possibly also atherosclerotic events. Central dopaminergic, serotoninergic, and cholinergic mechanisms have also been described.

Recently, the endometrial safety of *Cimcifiuga racemosa* extract has been proven in a 12-month trial in postmenopausal patients with menopausal complaints, demonstrating no cases of hyperplasia or other serious adverse endometrial outcome.

Clinical efficacy of the available black cohosh products to reduce climacteric complaints has been proven in more than 11,000 patients, and up to 2007, more than 1,500 women have been included in several randomized controlled trials comparing the therapeutic suitability of *C. racemosa* for climacteric complaints with a placebo or an active medication, including estrogens and tibolone. A significant superiority of black cohosh to placebo was found if the treatment duration was at least 12 weeks in naturally climacteric women and the black cohosh medication was a Herbal Medicinal Product with certified pharmaceutical quality as approved by medical regulatory authorities. Thus, from a recent analytical investigation on seven US dietary supplements [36] it was found that these products did not meet the label claim for triterpene glycosides, sometimes with one-tenth of the medicinal content or even five times more the medicinal dose per serving. Concomitant use of black cohosh and tamoxifen or analogs in patients with a history of breast cancer showed no negative influences on cancer recurrence or cancer spread during short-term (three months) observation.

Safety studies have demonstrated good tolerability, however, starting in 2002, a few cases of hepatotoxicity have been reported and alerted the relevant international agencies, but a direct association with the use of black cohosh has not been demonstrated. It is important to underline that in none of the four cases considered as "probable" or "possible" did the patients use an Herbal Medicinal Product. On the contrary, on the world market there are numerous brands of black cohosh, and some of them include herbs that can have potential hepatotoxicity, especially the herb blue cohosh (*Caulophyllum thalictroides*) that is also used for gynaecologic disorders but has a much greater potential for toxicity [138]. Based on the evidence available it cannot be concluded that black cohosh, in particular those products of pharmaceutical quality is a cause of liver toxicity when used for up to six months; however, in Germany, many women use this herbal remedy for longer periods of time with physician's supervision [5, 139-140].

In summary, black cohosh shows great promise for relief of menopausal symptoms, primarily of vasomotor symptoms and perhaps mood with an overall positive safety profile for at least six months and likely longer. However, data from longer and in some cases more rigorous clinical trials are necessary to assess high efficacy and to substantiate safety. Finally, there has been almost no research on black cohosh to study health conditions associated with aging such as heart disease, osteoporosis and fracture, although one study compared the effects of black cohosh, conjugated estrogens, and placebos on menopausal symptoms as well as bone markers.

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