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An isopropanolic extract of black cohosh does not increase mammographic breast density or breast cell proliferation in postmenopausal women

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ABSTRACT

Objective: The aim of this study was to determine the effects of the isopropanolic extract of black cohosh (Remifemin) on mammographic breast density and breast epithelial proliferation in healthy, naturally postmenopausal women with climacteric symptoms.

Design: This was a prospective, open, uncontrolled drug safety study in which baseline status was compared with status after 6 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 6 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.

Results: 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.

Conclusions: The findings suggest that the isopropanolic extract of black cohosh does not cause adverse effects on breast tissue. Furthermore, our data do not indicate to any endometrial or general safety concerns during 6 months of treatment.

Key Words: Black cohosh – Mammographic breast density – Breast cell proliferation – Menopause.

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During the last few years, serious concerns have been raised about the long-term safety of combined estrogen-progestogen hormone therapy (HT) and, in particular, about effects on the breast. A number of clinical and observational studies have indicated an increase in breast cancer risk during such treatment.[1,2] There is a need to define alternative treatment regimens for postmenopausal women that have minimal effects on

the breast but are still effective in the treatment of climacteric symptoms.

Several clinical studies, including a recent randomized, double-blind trial, have shown that an isopropanolic extract of the black cohosh rootstock (iCR) is effective in relieving vasomotor symptoms.[3] iCR, which represents a well-characterized pharmaceutical, has long been considered an alternative to synthetic hormones in the treatment of menopausal complaints.[4] Cell cultures and animal experiments suggest that iCR may not stimulate breast cell proliferation but rather may stimulate apoptosis.[5-7] Also, iCR did not stimulate breast tumor growth in the dimethylbenzoic acid rat model.[8] So far there have been no data on the effects of iCR on the breast when treatment is given to postmenopausal women.

Mammographic breast density and breast cell proliferation could be regarded as surrogate markers for the risk of breast cancer.[9-11] Epidemiological studies have repeatedly shown increased breast density to be a strong independent risk factor for breast cancer. Density reveals the effect of the intrinsic hormonal environment and its background genetics on the breast.[10] The basis of risk associated with hormonal therapies may lie in the regulation of cell proliferation. Within populations of cells in vitro and in vivo, a higher rate of cell proliferation may increase the risk of transformation to the neoplastic phenotype. In animal models, as well as in women, we have shown breast cell proliferation to increase in short-term studies on HT.[12,13] An increase in mammographic density has also been reported to occur in a significant number of postmenopausal women during conventional estrogen-progestogen HT.[14-16]

Here we report the effects on mammographic breast density and breast cell proliferation in a group of postmenopausal women after 6 months treatment with iCR.

METHODS

Participants

Postmenopausal women aged 50 to 70 years with a body mass index of 20 to 30 kg/m^2 were recruited for the study. Inclusion criteria were last menstrual bleeding 12 or more months before enrollment in the study or follicle stimulating hormone levels greater than 40 IU/L and estradiol levels less than 20 pg/mL. Women who had used HT in the previous 3 months were excluded from the study, as were those with hypertension (systolic blood pressure >170 mm Hg or diastolic blood pressure >105 mm Hg), hyperlipidemia (total cholesterol >8.0 mmol/L or triglycerides >4.0 mmol/L), or type I or II diabetes mellitus. All study procedures were conducted at the Women's Health Research Unit within the Department of Obstetrics and Gynecology at the Karolinska University Hospital in Stockholm, Sweden. The study was approved by the independent ethics committee (IRB-02-404) and the Swedish Medical Products Agency (151:2002/71141). All women gave their informed consent before participation in the study.

Interventions and treatment

The screening and baseline assessments were performed within 3 weeks before the start of treatment. Mammography and fine needle aspiration (FNA) biopsies were performed within 14 days before the participant started intake of Remifemin (batch no. 229690), one tablet twice daily, and after 6 months of treatment (24-26 weeks). Each tablet contains 0.018 to 0.026 mL liquid extract of black cohosh rootstock (0.78-1.14:1), corresponding to 20 mg herbal drug (ie, 2.5 mg dry extract, extraction agent isopropanol 40% [vol/vol]).

Each woman eligible for inclusion at the end of the screening period received study medication for the first 2 months. During the treatment period, the participant's daily intake of medication and the occurrence of any adverse events (AEs) were recorded in a diary. At two interim visits after 2 and 4 months, study medication for the next period was dispensed, information regarding compliance was obtained, and AEs and concomitant medication were documented. The time window for the two interim visits was a maximum of 80 days after the start of treatment and the preceding interim visit, respectively.

The last visit occurred at the end of treatment (weeks 24-26). Within 14 days after the end of treatment, a follow-up phone call to the participant was made by the midwife. The woman was informed about all results available and asked about the occurrence of any AEs since the end of treatment.

Evaluation of the primary safety variables was performed by blinded assessors when all participants had completed all study-related procedures, including the follow-up call.

Mammographic breast density

Mammography examinations were performed in accordance with the quality control regulations stipulated by the Swedish National Board for Health and Welfare and the Swedish National Radiation Protection Institute. Mammograms were obtained at baseline and at 6 months to determine breast density and to evaluate any abnormalities. Mammography examinations comprised mediolateral oblique and craniocaudal views of both

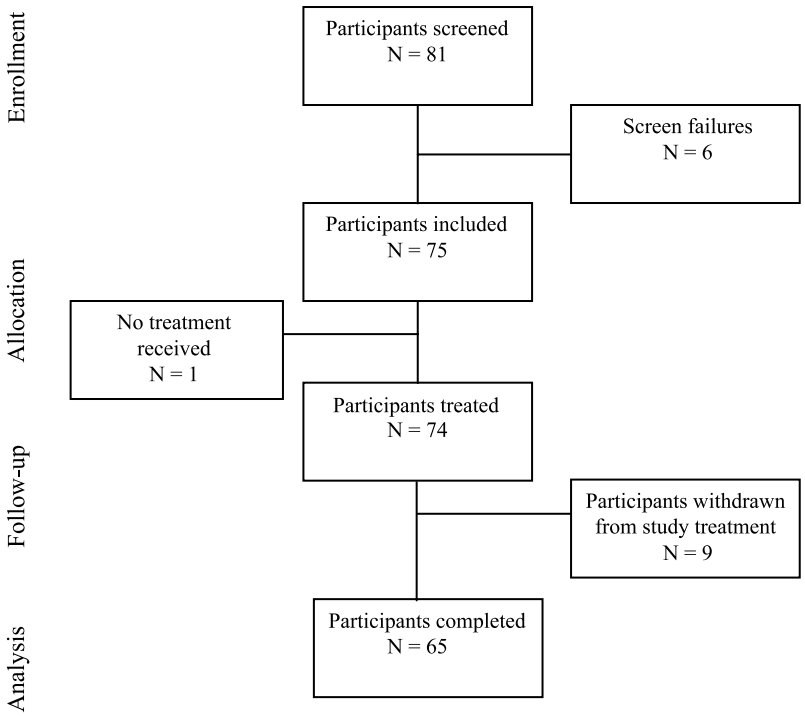

FIG. 1. Flow diagram of the study. A total of 81 women were screened. Six screened women were not included because of various exclusion criteria. One included woman withdrew consent to participate before taking the first dose of study medication. Nine of the participants, included and treated, discontinued participation after the start of study treatment but before study end.

breasts. Only the mediolateral oblique views of each breast were used to assess breast density. All mammograms were assessed at the Karolinska University Hospital by two independent radiologists (G.S. and E.A.), who were blinded to the order of mammograms in each individual. Any difference of opinion in the classification of a mammogram was resolved with a consensus result.

Mammographic density of all coded films was classified according to Wolfe[17] in four categories: N1, essentially normal breast with parenchyma composed primarily of fat and with, at most, a few fibrous connective tissue strands; P1, prominent ductal pattern in up to one fourth of breast volume; P2, prominent ductal pattern in more than one fourth of breast volume; and DY, extremely dense parenchyma, which usually denotes connective tissue hyperplasia. In addition to the Wolfe classification, for each individual woman, all coded films were classified according to a percentage scale with five categories of the amount of dense breast parenchyma in relation to the whole breast area. The five categories were 0 to 20%, 21% to 40%, 41% to 60%, 61% to 80%, and 81% to 100%.

TABLE 1. *Demographic characteristics and medical histories in 65 postmenopausal women*

Age, y	56.8 ± 5.1 (50-70)
Weight, kg	66.7 ± 8.1 (51-84)
Body mass index, kg/m^2	24.2 ± 2.7 (19-30)
Time since last natural menstruation, mo	82 ± 66 (10-276)
Age at menarche, y	13.2 ± 1.4 (9-17)
No. of pregnancies	2.7 ± 2.0 (0-10)
Age at birth of first child, y	26.8 ± 6.0 (17-41)

Values are mean ± SD (range).

FNA biopsy

Percutaneous FNA biopsies of the upper outer quadrant of the left breast were performed at baseline and after 6 months of treatment. FNA biopsy was performed without anesthesia using a needle with an outer diameter of 0.6 mm as described by Franzén and Zajicek.[18] To produce several identical slides, the aspirated cells were mixed with 0.5 mL of 4%

TABLE 2. *Shift table of mammographic status (Wolfe classification of breast parenchyma density), comparing baseline data with data obtained after 6 months of iCR treatment in 65 postmenopausal women*

	End of therapy			
Baseline	N1	P1	P2	DY
N1	—	—	—	—
P1	—	29	—	—
P2	—	—	36	—
DY	—	—	—	—

iCR, isopropanolic extract of black cohosh rootstock. See "Mammographic breast density" for descriptions of Wolfe categories.

TABLE 3. *Shift table of mammographic status (percentage scale classification of breast parenchyma density), comparing baseline data with data obtained after 6 months of iCR treatment in 65 postmenopausal women*

	End of therapy				
Baseline	0%-20%	21%-40%	41%-60%	61%-80%	81%-100%
0%-20%	21	—	—	—	—
21%-40%	—	26	—	—	—
41%-60%	—	1	16	—	—
61%-80%	—	—	—	1	—
81%-100%	—	—	—	—	—

iCR, isopropanolic extract of black cohosh rootstock.

buffered (pH 7.4) formalin in the same syringe as the procured cells. Volumes of 110 μL were cytocentrifuged at 700 rpm for 3 minutes, and the cells were sedimented onto pretreated glass slides.

Immunocytochemical analysis

Slides were stained for the nuclear antigen Ki-67. The Ki-67/MIB-1 monoclonal antibody reacts with a human nuclear antigen, which is present in proliferating cells but absent in quiescent cells. Cell cycle analysis shows that the antigen is expressed in the phases of G1, S, G2, and mitosis.[19] The MIB-1 analysis was performed using reagents supplied by Immunotech SA (Marseilles, France). The staining procedure uses an avidin-biotin peroxidase system, modified for the cytospin technique.

We considered samples obtained by FNA to be assessable only if they contained intact cells and no free-lying nuclei. Because of limited material, cells were not stained for both proliferation and epithelial markers. All slides were examined by an experienced cytopathologist (L.S.), and the vast majority of cells were judged to be of epithelial origin. On average 150 to 200 cells were counted per slide, and only samples with a minimum of 40 cells were included in the analysis. All samples were assessed by an observer blinded to the order of biopsy samples.

Serum analyses

Venous blood samples were drawn at baseline and after 6 months. Serum concentrations of sex hormone–binding globulin (SHBG) were determined by direct chemiluminescence enzyme immunoassay using a commercial kit (Immulite) obtained from Diagnostic Products Corporation (Los Angeles, CA). Insulin-like growth factor I (IGF-I) was determined by chemiluminescence enzyme immunoassay using a commercial kit obtained from Nichols Products Corporation (Advantage, San Juan Capistrano, CA). The detection limits and within- and between-assay coefficients of variation for SHBG were 0.05 nmol/L and 4% and 8%, and for IGF-I were 6 μg/L and 4.8% and 6.7%.

The serum levels of total cholesterol and triglycerides (Ortho Diagnostics, Rochester, NY) were determined by routine clinical methods in the Department of Clinical Chemistry, Karolinska University Hospital. The detection limit and overall coefficient of variation were 1.29 mmol/L and 1.8% for total cholesterol and 0.11 mmol/L and 1.6% for triglycerides.

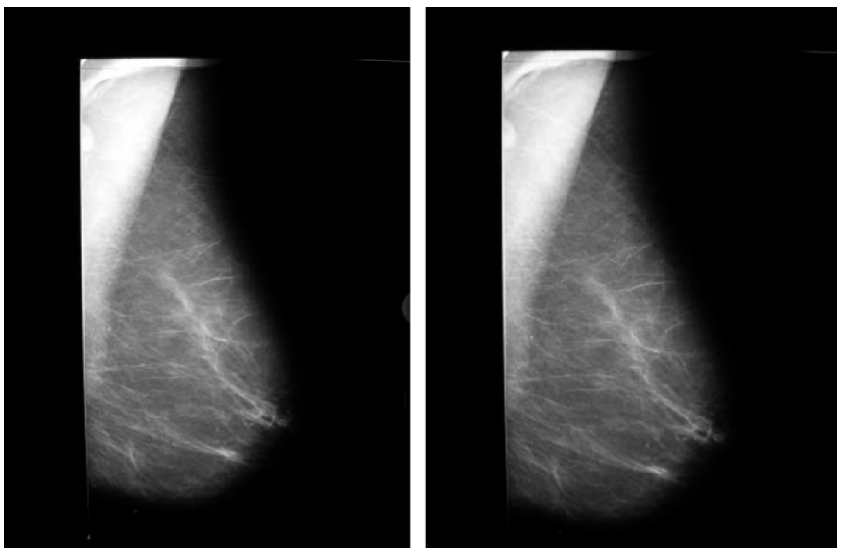

FIG. 2. Mammograms showing density in one individual woman at baseline (**left**) and after 6 months of therapy with an isopropanolic extract of black cohosh rootstock (**right**).

TABLE 4. *Percentage of Ki-67–positive cells in FNA biopsy samples*[a]

	Baseline	End of therapy	*P* (95% CI)
All assessable samples			
N	49	43	
Mean ± SD	0.8 ± 2.0	0.4 ± 0.6	
Median	0.0	0.0	
Range	0-11	0-3	
Paired samples			
N	35	35	
Mean ± SD	0.9 ± 2.2	0.4 ± 0.6	0.947 (−1.32 to 0.34)
Median	0.0	0.0	
Range	0-11	0-3	

[a]All individuals in which any fine-needle aspiration (FNA) sample revealed valid results are shown, as well as all individuals in which both FNA samples revealed valid results (ie, 35 cases). The *P* value is based on the Wilcoxon signed-rank test and the 95% CI upon *t* distribution.

Other assessments

Physical and gynecologic examinations including vital signs (weight, blood pressure at rest, and heart rate) were carried out at baseline and after 6 months. A transvaginal ultrasound examination for measurement of endometrial thickness was also performed. The women were questioned about any untoward medical events at the baseline and at the 2-, 4-, and 6-month visits.

Statistical analyses

The proportion of women whose breast density increased from baseline to the end of therapy, including the exact binomial 95% CI for both classifications, was calculated. Regarding breast density, the proportion of Ki-67–positive cells and numeric variables and change from baseline to the end of therapy was tested by use of the Wilcoxon signed-rank test. *P* values less than 0.05 are considered significant.

RESULTS

A total of 81 women were assessed for eligibility, of which 6 did not fulfill the inclusion criteria for various reasons. Of the 75 women included, 10 women were withdrawn from the study treatment for various reasons and could not be evaluated. Thus, a total of 65 women completed the study (Fig. 1). Demographic and reproductive data are shown in Table 1.

Mammographic breast density

According to the Wolfe classification at baseline, the mammograms from 29 (44.6%) of the women were judged as P1 and 36 (55.4%) were P2. This distribution was the same after 6 months of treatment, and in none of the women was any change, increase or decrease, recorded (Table 2). Also, according to the percentage scale, in none of the women was there an increase in breast density during treatment. Here the baseline distribution of densities was 21 women (32%) in the 0% to 20% category, 26 women (40.0%) in the 21% to 40% category, 17 women (26.2%) in the 41% to 60% category, and 1 woman in the 61% to 80% category. After 6 months, figures were the same except for one woman classified with a decrease from the 41% to 60% to the 21% to 40% category (Table 3). Figure 2 shows mammographic breast density in one individual woman before and after 6 months of treatment.

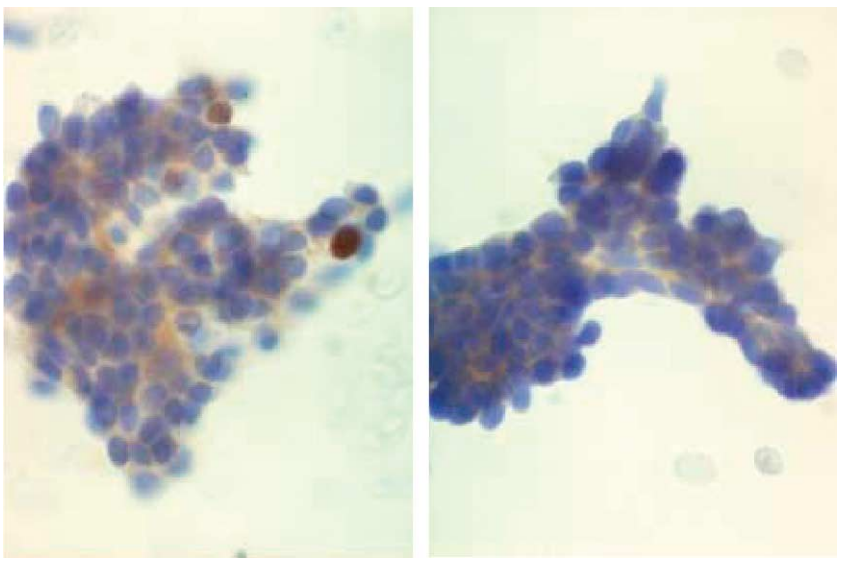

FIG. 3. Ki-67–positive cells in fine-needle aspiration biopsies in one individual woman at baseline (**left**) and after 6 months of therapy with an isopropanolic extract of black cohosh rootstock (**right**).

FNA biopsies and Ki-67 cytology

From the 65 women a total of 130 FNA biopsies were obtained. Ninety-two of these aspirates (71%) were evaluable for MIB-1 content. Thirty-eight biopsy samples (29%) were nonevaluable because of too few cells in the aspirate. As illustrated in Table 4, mean values for the percentage of MIB-1–positive cells showed no change between baseline and 6 months. From 35 of the women (54%), assessable samples were obtained both at baseline and after 6 months. Also in this group, there was no change recorded during therapy. Ki-67–positive breast cells before and after 6 months of treatment with iCR from one individual woman are shown in Figure 3.

Endometrial thickness and other safety parameters

No differences in endometrial thickness were observed before and after treatment. Mean values ± SD for the 65 women were 2.1 ± 0.9 and 2.1 ± 0.9 mm, respectively. Likewise, the biochemical safety parameters did not reveal any significant treatment-induced alterations. Mean values ± SD before and after 6 months for the 65 women for total cholesterol were 5.9 ± 0.8 and 5.8 ± 0.9 μg/L, for triglycerides were 1.4 ± 0.9 and 1.3 ± 0.9 μg/L, for SHBG were 52.1 ± 17.9 and 50.4 ± 16.6 μg/L, and for IGF-I were 136.9 ± 50.1 μg/L and 136.5 ± 45.0 μg/L.

Adverse events

Twelve women (16%) reported AEs possibly related to study medication, and none of these was serious. In detail, we observed seven women with mild gastrointestinal disorders, four with vaginal bleeding/spotting, three with mild/moderate headache, one with vaginal candidiasis, one with a mild increase in breast size, and one with moderately swollen feet and hands for 5 days.

DISCUSSION

iCR has emerged as an effective nonhormonal alternative for the treatment of climacteric symptoms.[3,4] Its mechanism of action is incompletely understood, but preclinical and clinical data show that black cohosh has no systemic estrogenic effects. However, this compound may act as a natural selective estrogen receptor modulator and may have a specific activity within the central nervous system.[20-24] In the present study, none of the 65 postmenopausal women had any sign of increased mammographic breast density or enhanced breast cell proliferation after 6 months of treatment with iCR. According to a power analysis (α error: 0.05; β error: 0.20), the clinical material was sufficient to exclude an increase in mean breast cell proliferation of more than 1.5% (eg, a change from a baseline value of 1% to 2.5% after 6 months). We also should have been able to detect an upgrading of mammographic density in more than 20% of the women.

The design of this study was open and uncontrolled, which should be considered when one interprets the results. However, the investigators were blinded to the order of breast cell samples and mammograms, and the inclusion and exclusion criteria used here were identical to those of our previous placebo-controlled, randomized studies and resulted in similar patient characteristics.[14]

Mammographic breast density is considered to be a strong and independent risk marker for breast cancer.[10] Postmenopausal estrogen-progestogen therapy has been associated with an increased risk of breast cancer, and an increase in mammographic breast density has been found to occur in a significant proportion of women during such treatment.[14-16] The increase in mammographic density seems to be an early event and to occur during the first months of therapy. The change also seems to remain stable during long-term treatment in women using the same regimen.[25] There are several methods, including different visual classification scales and digitized computer-assisted programs, to assess mammographic breast density. Consequently, reports about the degree of mammographic change and effects of different regimens show considerable variation.[26,27]

In previous studies in which the same visual classification scales were used and the evaluation was carried out by the same investigators, we found that 30% to 50% of the women reacted with a marked increase in density to fulfill the criteria for an upgrading of at least one class by the Wolfe and percentage classification scales.[14,15] This stimulatory effect was apparent after 6 months and was quite similar for combinations of estradiol 2 mg and norethisterone acetate 1 mg, estradiol valerate 2 mg and dienogest 2 mg, and conjugated estrogens 0.625 mg and medroxyprogestorone acetate 5 mg.[15] In comparison, treatment with tibolone, a compound with estrogenic, progestogenic, and slightly androgenic properties, resulted in increased breast density in only 2% to 6%.[14] Thus, the results for breast density with iCR were considerably better than those with conventional HT and at least as good as those for tibolone.

The FNA biopsy is an established technique for preoperative diagnosis of palpable lesions in the breast.[28] Numerous reports have shown a high correlation and reproducibility between the cytological

results from FNA biopsies and histological results follow-up from open biopsies or surgical specimens.[29] FNA biopsies cause a minimum of inconvenience for the participant and can be performed repeatedly during different forms of HT. As in previous studies, FNA biopsies were obtained from the upper outer quadrant of the left breast. This area was chosen because on average a higher amount of breast epithelium is found in this location.[30] In a previous analysis of 10 different locations in the macaque breast, there were no significant differences with respect to proliferative activity and receptor expression.[31] We have previously shown that estrogen-progestogen causes a 3- to 5-fold increase in breast cell proliferation during 6 months of treatment.[12,13] In comparison, for tibolone there was no significant effect and no difference compared with placebo.[13] We also showed that women in whom insufficient material was obtained were on average older, had more years since menopause and had a higher body mass index and lower SHBG and IGF-I values at baseline compared with those with assessable samples. Here the cell yield from FNA biopsies and the percentage of assessable samples were quite similar to those in previous studies, but we observed no change in proliferative activity during treatment with iCR. Thus, according to the FNA biopsy analyses, iCR clearly has no effects on breast cell proliferation, in contrast to standard HT regimens.

The results of our study correspond with those of in vitro studies on the effects of black cohosh on human estrogen receptor–positive breast cancer cell lines, which have demonstrated an inhibition of proliferation or at least a lack of proliferative effects.[5-7,32-34] In vivo experiments in the rat showed no apparent influence of iCR on estrogen receptor–positive breast tumors.[8] Also, clinical studies on black cohosh performed in women with breast cancer did not give rise to any safety concerns for the treatment of menopausal symptoms.[35-37]

During 6 months of treatment with iCR we found no change in endometrial thickness. Also, blood lipids, SHBG, and IGF-I did not differ from baseline. A modest number of AEs that were possibly related to treatment were noted, none of which was serious. Apart from the known AEs associated with Remifemin, the profile of the other events comprised well-known symptoms of climacteric transition and was highly similar to events observed in untreated postmenopausal women.

CONCLUSIONS

In conclusion, the findings of this study suggest that 6 months of treatment with isopropanolic extract of black cohosh does not cause adverse effects on human breast tissue. Furthermore, our data do not indicate any endometrial or general safety concerns with iCR treatment of postmenopausal women at the recommended daily dose (extract of 40 mg crude drug) for 6 months.

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