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Safety and efficacy of black cohosh and red clover for the management of vasomotor symptoms: a randomized controlled trial

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Abstract

Objective: The aim of this study was to evaluate the safety and efficacy of black cohosh and red clover compared with placebo for the relief of menopausal vasomotor symptoms.

Methods: This study was a randomized, four-arm, double-blind clinical trial of standardized black cohosh, red clover, placebo, and 0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate (CEE/MPA; n = 89). Primary outcome measures were reduction in vasomotor symptoms (hot flashes and night sweats) by black cohosh and red clover compared with placebo; secondary outcomes included safety evaluation, reduction of somatic symptoms, relief of sexual dysfunction, and overall improvement in quality of life.

Results: Reductions in number of vasomotor symptoms after a 12-month intervention were as follows: black cohosh (34%), red clover (57%), placebo (63%), and CEE/MPA (94%), with only CEE/MPA differing significantly from placebo. Black cohosh and red clover did not significantly reduce the frequency of vasomotor symptoms as compared with placebo. Secondary measures indicated that both botanicals were safe as administered. In general, there were no improvements in other menopausal symptoms.

Conclusions: Compared with placebo, black cohosh and red clover did not reduce the number of vasomotor symptoms. Safety monitoring indicated that chemically and biologically standardized extracts of black cohosh and red clover were safe during daily administration for 12 months.

Key Words: Botanicals – Hot flashes.

Hormone therapy (HT) remains the standard treatment for women experiencing vasomotor symptoms related to menopause.[1] However, when the Women's Health Initiative demonstrated an increased risk of stroke and breast cancer, as well as an increased risk of heart disease in older women using a combination of estrogen and progestin, use of HT decreased signficantly.[2-4] Furthermore, HT has shown less promise than expected in alleviating other symptoms related to the menopausal transition and aging.[3,5,6] These concerns have caused a reduction in the use of HT and increased interest in alternative therapies for the relief of menopausal symptoms such as botanical dietary supplements.[7]

The botanicals black cohosh (*Cimicifuga racemosa* (L.) Nutt.) and red clover (*Trifolium pratense* L.) are popular among women seeking alternative therapies for the management of menopausal symptoms, especially vasomotor symptoms. The roots/rhizomes of black cohosh have been used traditionally by Native Americans for a variety of complaints, and black cohosh has also been used as a treatment of menopausal disorders in Germany for more than 50 years.[8,9] Although not estrogenic, the mechanism of action of black cohosh might involve serotonergic activity.[10,11] Multiple clinical investigations have suggested that black cohosh extracts are effective in reducing the frequency and intensity of hot flashes among perimenopausal and postmenopausal women,[12-15] whereas other randomized controlled trials have reported no vasomotor symptom benefits.[16,17]

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Because the aerial parts of red clover are rich in estrogenic isoflavones, women have also been using red clover products for the management of vasomotor symptoms related to menopause.[18-22] The predominant isoflavones in red clover, biochanin A and formononetin, are the O-methylated precursors of the soy isoflavones genistein and daidzen. They are demethylated in the intestinal tract to genistein and daidzen.[23] Compared with black cohosh, fewer clinical investigations of red clover safety[24] or efficacy[25] for relieving vasomotor symptoms have been reported, but most studies report at best a modest impact.[26]

The primary objective of this four-arm, randomized, double-blind, 12-month phase II clinical trial of black cohosh and red clover compared with placebo was to assess the safety and efficacy of the two botanical products for the management of menopausal symptoms, primarily vasomotor symptoms. Black cohosh and red clover were selected for study because of the high consumer demand for these dietary supplements.[27] The HT arm, the conventional and recognized approach for the relief of vasomotor symptoms in perimenopausal and postmenopausal women, was included as a positive control. In an effort to ensure that well-characterized and reproducible botanical products were evaluated, center investigators oversaw the development of botanical interventions from raw material to chemical and biological standardization and to dosing formulations for the clinical trial.

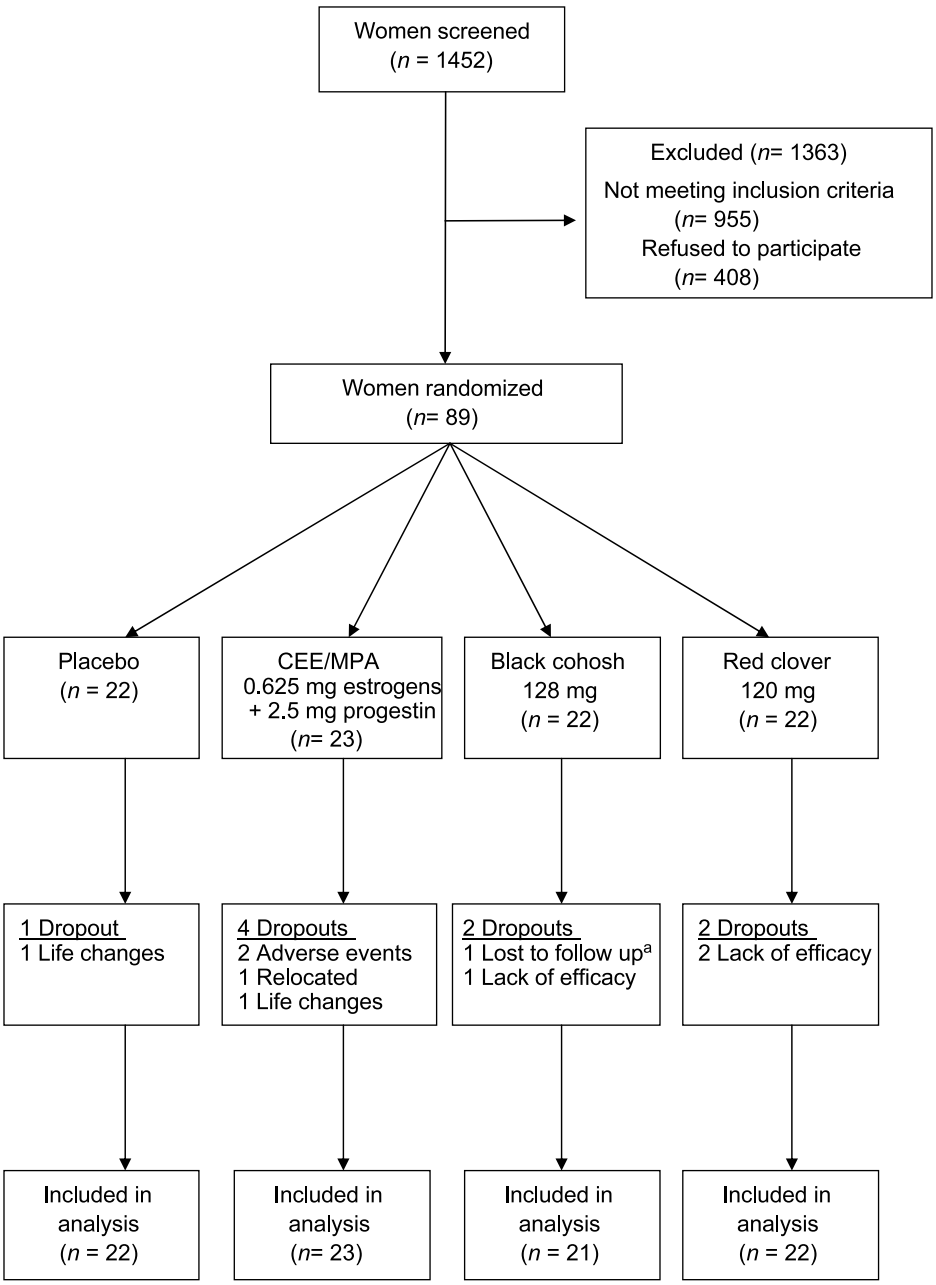

FIG. 1. Study design and profile. CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate. [a]Of the nine women who dropped out of the study early, eight were included in the intent-to-treat analysis. One woman was lost to follow-up.

TABLE 1. *Description of intervention materials according to CONSORT guidelines*

Criteria	Black cohosh	Red clover	CEE + MPA
Nomenclature			
Latin binomial name	*Cimicifuga racemosa* (L.) Nutt. (syn. *Actaea racemosa* L.)	*Trifolium pratense* L.	—
Family name	Ranunculaceae	Fabaceae	—
Common name(s)	Black cohosh, snake root, squaw root, bugbane	Red clover, cow clover, meadow clover, wild clover	CEE + MPA
Proprietary product name(s)	None[a]	None[a]	Prempro (NDC 0046-0875-05)
Is product licensed or registered in the United States?	No[a]	No[a]	Yes (W Ayerst Laboratories, Inc., Philadelphia, PA)
Characteristics of herbal(s)			
Parts of plant used to produce the extract	Below ground parts	Above ground parts	—
Type of product used	Dry extract	Dry extract	—
Type and concentration of extraction solvent used and ratio of herbal drug to extract	75% ethanol 20:1	75% ethanol 10:1	—
Method of authentication of raw material	Botanical: Voucher Specimen confirmed by UIC botanist D.D. Soejarto; Chemical: HPLC	Botanical: Voucher Specimen confirmed by UIC botanist D.D. Soejarto; Chemical: HPLC	—
Lot number of raw material	9-2677	02F-1525	—
Voucher specimen retained; where deposited and reference number	Field Museum of Natural History Herbarium, Chicago, IL (voucher #BC 192)	Field Museum of Natural History Herbarium, Chicago (voucher #BC 189)	—
Dose regimen and quantitative description			
Dose (mg) and duration (length of trial)	128 mg (2 capsules)/d × 12 mo	378 mg (2 capsules)/d × 12 mo	0.625 mg CEE + 2.5 mg MPA (2 capsules)/d × 12 mo
Single capsule content; active component and excipient in an olive green hard shell #2 capsule. [Placebo: 325.3 mg rice powder + 4.7 mg highly dispersed SiO_2]	Black cohosh: 64 mg; excipients: rice powder (320 mg) and highly dispersed SiO_2 (3.8 mg)	Red clover: 189 mg; excipients: rice powder (182 mg) and highly dispersed SiO_2 (4 mg)	Prempro Powder: 180 mg; excipients: rice powder (200 mg) and highly dispersed SiO_2 (4 mg)
Chemical standardization (contents per capsule)	3.64 mg cycloartane spirochetal triterpene glycosides (1.42 mg 23-*epi*,26-deoxyactein, 0.36 mg deoxyactein, 1.38 mg 26*S*-actein, 0.47 mg 26*R*-actein)	116.6 mg isoflavones (57.5 mg biochanin A, 56.6 mg formononetin, 1.6 mg genistein, 0.9 mg daidzen)	—
Biological standardization[b]	Serotonin (5-HT_7-receptor binding) in vitro assay[10,29]: IC_{50}, 18 ± 6 μg/mL	Estrogenic potency in vitro[30,31]: Ishikawa endometrial cells: EC_{50}, 2.0 ± 0.1 μg/mL; estrogen-binding affinity IC_{50}, 18.4 ± 4.9 (ERα) and 1.9 ± 0.8 (ERβ) μg/mL	—
Qualitative testing[b]			
Chemical methods used	ELSD-HPLC[29,32]	DAAD-HPLC[23,29,32,33]	—
Chemical analysis performed by	UIC/NIH Center for Botanical Dietary Supplements Research	UIC/NIH Center for Botanical Dietary Supplements Research	UIC/NIH Center for Botanical Dietary Supplements Research
Retention sample deposited at	UIC/NIH Center for Botanical Dietary Supplements Research	UIC/NIH Center for Botanical Dietary Supplements Research	UIC/NIH Center for Botanical Dietary Supplements Research
Purity testing[c]			
Heavy metals	USP Heavy Metal Method[34,35]	USP Heavy Metal Method[34,35]	—
Cadmium	Cadmium <0.02 ppm[36]	Cadmium <0.02 ppm[36]	—
Lead	Lead <100 ppb[7]	Lead <196 ppb[37]	—
Pesticides	USP Pesticide Screen[38,39]	USP Pesticide Screen[38,39]	—

CONSORT, Consolited Standards of Reporting Trials; CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; UIC/NIH, University of Illinois at Chicago/National Institutes of Health; HPLC, high-performance liquid chromatography; 5-HT_7, 5-hydroxytryptamine receptor 7; EC_{50}, half-maximal effective concentration; IC_{50}, half-maximal inhibitory concentration; ER, estrogen receptor; ELSD, evaporative light scattering detector; DAAD, diode array absorption detection.

[a]Both botanical extracts (black cohosh and red clover) were developed by the UIC/NIH Center for Botanical Dietary Supplements Research. Products have not been licensed nor have proprietary names been adapted.

[b]Standardization and fingerprint methodologies have been described previously.[10,23,29-33]

[c]Purity testing including heavy metals and pesticide contamination was conducted by Covance Laboratories, Inc., Madison, WI.[34-39]

METHODS

Design

This study was a phase II randomized, double-blinded, placebo-controlled safety and efficacy trial of two botanicals, black cohosh and red clover, for the management of vasomotor symptoms in healthy perimenopausal and postmenopausal women (study design: Fig. 1). Women were recruited and randomized into one of four arms: placebo, 0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate (CEE/MPA; Wyeth Pharmaceuticals, Philadelphia, PA), an ethanolic extract of black cohosh below-ground parts (128 mg/d standardized to 7.27 mg triterpene glycosides), or an ethanolic extract of the aerial parts of red clover (398 mg/d standardized to 120 mg isoflavones). The doses of black cohosh and red clover are similar to those used in other clinical trials.[16,17,28,29] The CEE/MPA arm was included as a positive control based on conventional therapy for treatment of vasomotor symptoms in perimenopausal and postmenopausal women. All study materials were identical in appearance, taste, and smell. Stability testing was conducted yearly on all preparations. Further details of the preparation and botanical and chemical standardization of these extracts are listed in Table 1.

The sample size calculation for the primary outcome (reduction in vasomotor symptoms) was based on prior research and powered with the following assumptions. Botanical treatments would reduce vasomotor symptoms by approximately 60%, for example, from 35 hot flashes to 13 hot flashes per week,[30] with a probability of at least 0.80, SD of 10,[31] and an anticipated placebo effect of 35%.[28,29] The null hypothesis to be tested was the equality of reduction in the number of hot flashes between placebo and the botanical groups. This was a two-sided test with an α error rate of 5% and a 5% dropout rate anticipated during the 12-month intervention period. The optimal sample size (n) for the primary outcome was calculated to be 22 per arm, for a total number of 88 women across all four arms of the study. This phase II study was powered only to compare each botanical with placebo.

Setting and participants

The study was carried out through the University of Illinois at Chicago/National Institutes of Health Center for Botanical Dietary Supplements Research in outpatient care facilities at the University of Illinois Medical Center from February 2003 to December 2007 and at the Northwestern University Feinberg School of Medicine from January 2004 to December 2007. The trial began shortly after the results from the Women's Health Initiative were released. The willingness of women to enroll in the study was negatively impacted because of the possibility of being randomized to the HT arm. Therefore, recruitment for this study took longer than initially anticipated. There were no significant differences in the management and recruitment of participants at the two sites because the core study team was the same at both institutions. The institutional review boards of both universities approved the study, and all participants provided written informed consent before study initiation.

Women experiencing at least 35 vasomotor symptoms per week (hot flashes and night sweats) in the 2 weeks before study enrollment, indicating moderate to severe symptoms,[25] and who met all inclusion and exclusion criteria outlined in Table 2 were eligible for enrollment. A total of 1,452 women were screened for enrollment into the study, and 94% were ineligible due to strict eligibility criteria (Fig. 1). Women taking oral HT underwent a washout period of 2 months; women using transdermal hormone preparations or oral botanical supplements required a 1-month washout period. Women who were vegans were excluded due to greater than average consumption of dietary phytoestrogens, and consumption of soy products was prohibited during the trial.

TABLE 2. *Study inclusion and exclusion criteria*

Inclusion criteria	Exclusion criteria
Perimenopausal or Postmenopausal woman with intact uterus	Previous hysterectomy
Must be experiencing at least 35 vasomotor symptoms (HF + NS) per wk	Fewer than 35 vasomotor symptoms (HF + NS) per wk
Amenorrhea >6 mo, <10-y duration	Last menstrual period, >10-y duration
FSH, >40 mIU/mL[a]	Positive pregnancy test or breast-feeding
HT not contraindicated	Obesity, BMI >38 kg/m^2
Able to give informed consent	Previous history of endometrial hyperplasia/neoplasia
	Previous history of cancers of the breast or reproductive tract
	History or presence of myocardial infarction or stroke
	History of severe recurrent depression or severe psychiatric disturbance
	History or presence of cerebrovascular accident, severe varicose veins, sickle cell anemia
	History of alcohol or drug abuse
	Abnormal vaginal bleeding of undetermined cause
	Untreated or uncontrolled hypertension defined as systolic blood pressure >165 mm Hg or diastolic blood pressure >95 mm Hg
	Concurrent administration of medication containing estrogen, progestin, SERM, St. John's wort, bisphosphonates, or dietary phytoestrogens
	History of migraine associated with hormone use
	History or presence of deep vein thrombosis, thrombophlebitis, or thromboembolic disorder
	Current participation in any other clinical trial within 30 d of enrollment
	>5 alcoholic drinks per wk
	Smoker
	Diabetes
	Abnormal transvaginal ultrasound defined as >7-mm thickness
	Abnormal endometrial biopsy or mammogram
	Vegans (vegetarians who tend to consume greater than average doses of phytoestrogens)

HF, hot flash; NS, night sweats; FSH, follicle-stimulating hormone; HT, hormone therapy; BMI, body mass index; SERM, selective estrogen receptor modulator.
[a]FSH was measured only if the last menstrual period was between 6 and 12 months.

TABLE 3. *Baseline demographics and medical history*

	Placebo (n = 22)	CEE/MPA (n = 23)	Black cohosh (n = 21)	Red clover (n = 22)	All (n = 88)	*P*
Mean age (SD), y	52.0 (4.2)	53.3 (4.0)	54.4 (3.9)	52.4 (4.6)	53 (4.2)	0.24
Mean BMI (SD), kg/m^2	30.1 (4.9)	26.0 (3.9)	28.3 (4.5)	30.5 (4.3)	28.7 (4.7)	0.004[a]
Race, n (%)						0.005[a,b]
African American	16 (72.7)	7 (30.4)	8 (38.1)	13 (59.1)	44 (50.0)	
White	5 (22.7)	16 (69.6)	13 (61.9)	5 (22.7)	39 (44.3)	
Hispanic	1 (4.6)	—	—	3 (13.6)	4 (4.5)	
Pacific Islander	—	—	—	1 (4.6)	1 (1.1)	
Past hormone use, n (%)	10 (45.5)	10 (43.5)	9 (40.9)	10 (45.5)	39 (43.8)	0.99
Previous tobacco use, n (%)	8 (36.4)	15 (65.2)	13 (59.1)	9 (40.9)	46 (51.7)	0.13
Alcohol use[c]	1.0 (1.6)	1.5 (1.9)	1.7 (1.9)	1.1 (1.3)	1.3 (1.7)	0.48
Last menstrual period, number of years (SD)	2.8 (2.9)	3.6 (2.9)	3.4 (2.6)	4.1 (2.8)	3.5 (2.8)	0.52
Gravida, n (SD)	2.7 (1.6)	2.1 (1.9)	2.4 (2.0)	3.0 (2.9)	2.6 (2.1)	0.53
No. deliveries (SD)	1.6 (1.0)	1.4 (1.3)	1.5 (1.3)	2.4 (2.4)	1.7 (1.6)	0.12
Endometrial thickness (SD)	4.6 (2.1)	3.8 (2.3)	3.7 (1.3)	3.9 (2.0)	3.8 (1.8)	0.66
Total cholesterol (SD)	200.1 (30.4)	217.8 (37.0)	221.8 (36.8)	209.2 (44.3)	213.2 (39.3)	0.10
Estradiol (SD)	29.7 (19.2)	24.3 (10.5)	26.8 (13.0)	27.9 (13.2)	27.1 (14.2)	0.45
FSH (SD)	79.1 (36.3)	99.3 (38.7)	86.0 (26.8)	70.1 (27.6)	84.8 (33.7)	0.08

CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; BMI, body mass index; FSH, follicle-stimulating hormone.
[a]Statistically significant difference between groups.
[b]*P* value for race from χ^2 test for overall homogeneity of race.
[c]Reported as number of drinks per week (SD).

Randomization and intervention

Prestudy screening was carried out in the clinics a minimum of 14 days before intervention to qualify each woman. Then, a random, computer-generated code assigned two women in each cluster to each of four treatment arms. There were 11 clusters with eight women in each cluster. Thus, from the first set of eight participants, two each were assigned to black cohosh, red clover, placebo, and the CEE/MPA arms. This same process was repeated for all women enrolled in the study. The randomization procedure was the same at both sites. During the intervention, each participant consumed two capsules each evening containing black cohosh, red clover, CEE/MPA, or placebo. The study capsules were identical in appearance, and there was no detectable odor for any of the preparations.

Primary outcome measure

The primary endpoint was determination of the efficacy of black cohosh and red clover for the relief of vasomotor symptoms as compared with placebo and was measured as the reduction of vasomotor symptoms (hot flashes and night sweats), hot flashes alone, and intensity of hot flashes as compared with placebo. Women were asked to maintain diaries for a minimum of 2 weeks before randomization and throughout the 12 months of the study, in which they recorded the number of vasomotor symptoms per day as well as the intensity. Hot flash intensity was scored as 1 indicating "mild" (slight episodes of heat), 2 indicating "moderate" (a few episodes of heat with discrete sweating), and 3 indicating "severe" (more sudden episodes of heat accompanied by sweating, sleep disturbances, and or feelings of irritation or anxiety). Any night sweat that interrupted sleep received an automatic intensity score of 3. The number of hot flashes used to calculate the reduction of vasomotor symptoms included all hot flashes regardless of intensity.

Secondary outcome measures

Secondary endpoints included safety assessment and measurement of other efficacy indicators such as relief of somatic symptoms (eg, insomnia, joint pain, sleep, and fatigue), mood changes (eg, depression and anxiety), sexual dysfunction (eg, vaginal dryness, dyspareunia, libido, and difficulty in achieving orgasm), and health-related quality of life. Previously validated instruments were used to measure secondary endpoints of efficacy: the Greene Climacteric Scale to assess somatic symptoms as well as health-related quality of life,[32,33] a modified version of the Pittsburgh Sleep Quality Index to assess sleep,[34,35] and the Positive and Negative Affect Schedule[36,37] to assess both positive and negative moods. The Kupperman Index was also used to measure somatic complaints.[38,39]

The prerandomization screening visit and exit visit included a medical history, physical examination, gynecologic examination (including transvaginal ultrasound, Pap smear, and endometrial biopsy), and mammogram. Complete blood count, urinalysis, comprehensive serum chemistry, and lipid analysis were carried out at screening, randomization, and months 1, 6, 9, and 12 or early termination. Serum hormone levels (including estradiol, follicle-stimulating hormone, luteinizing hormone, estrone, testosterone, thyroid-stimulating hormone, and sex hormone–binding globulin), body weight, and height were measured at screening, randomization, and 6 (blinded) and 12 months or early termination. Bone density was assessed using dual-energy x-ray absorptiometry at randomization and at 12 months or early termination. During monthly clinic visits, blood samples were obtained for pharmacokinetic analysis, participant diaries were collected, interviews related to quality of life questionnaires were conducted (months 3, 6, 9, and 12), adverse events and concomitant medications were discussed, and study capsules were dispensed.

Monitoring and adverse events

In addition to oversight by two institutional review boards, an independent Data Safety Monitoring Board reviewed the clinical data throughout the study. All participants were examined in the clinic monthly for adverse events and side effects. Safety monitoring including liver function tests to detect any potential serious adverse effects such as hepatotoxicity was carried out at months 1, 6, 9, and 12. The prothrombin time-international normalized ratio was determined to screen for clotting disorders related to potential coumarin-like substances in the botanicals at randomization and at the 1-month safety visit.

Statistical analysis

For the primary endpoint, the number of vasomotor symptoms was computed by the summation of individual daily counts of hot flashes and night sweats during a 1-week period. Hot flash intensity was computed by summing daily individual intensity scores. Changes from baseline for both primary and secondary efficacy parameters were calculated for each treatment at follow-up time points (months 3, 6, 9, and 12). All changes from baseline were compared between placebo and black cohosh, placebo and red clover, and placebo and CEE/MPA at follow-up time points. For each treatment, the baseline data were subtracted from the data at months 3, 6, 9, and 12 to arrive at a reduction of vasomotor symptoms, hot flashes only, and intensity of hot flashes for each treatment as compared with placebo. All women were followed for 12 months or until their withdrawal from the study. Although the study was powered only for the primary outcomes, a number of secondary efficacy and safety parameters were also monitored.

All primary and secondary efficacy parameters were assessed with an intent-to-treat analysis, consisting of all randomized women who had been in the study for at least 3 months. A one-way analysis of variance was used for the analyses of primary and secondary data. The Fisher's least significant difference procedure was used for pairwise comparison of the treatment groups. The primary and secondary data were analyzed post hoc, adjusting for baseline covariates, but this exploratory analysis yielded no clinically meaningful or statistically significant findings. Missing data were imputed using the last-observation-carried-forward method. Statistical analysis was carried out using SAS 9.1 (SAS, Cary, NC). All data are summarized as mean (SD), and P values of less than 0.05 are considered statistically significant.

RESULTS

Participants and follow-up

In total, 89 women were randomized to the study; 80 women completed 12 months, 8 women terminated early but were included in the intent-to-treat analysis, and 1 woman was lost to follow-up, for a retention rate of 98.8% and a lost to follow-up rate of 1% (Fig. 1). The overall adherence rate across groups was 88.6% (percent of women who took more than 80% of their study medications) with no significant differences in compliance between the groups. At baseline, study participants experienced, on average, 52 (placebo) to 71 (CEE/MPA) vasomotor symptoms per week.

The demographics and baseline clinical parameters of the four study groups are shown in Table 3. The average age of the study participants was 53 years, with a last menstrual period averaging 4.3 years earlier. Almost 55% of women in this study were from underrepresented minorities (50% African American and 4.5% Hispanic). Overall, the four groups were similar across most sociodemographic and

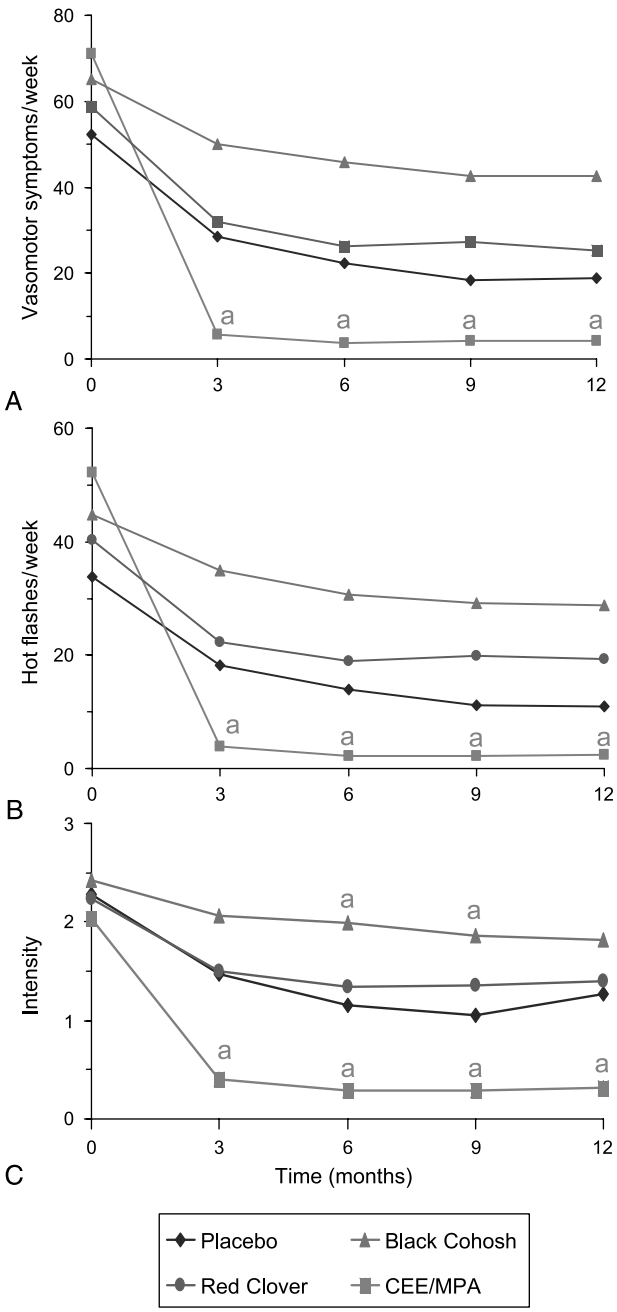

FIG. 2. Change in vasomotor symptoms, by study group: average number of vasomotor symptoms, hot flashes plus night sweats (**A**); average number of hot flashes (**B**); and average intensity of hot flashes (**C**). CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate. [a]Statistically significant difference.

TABLE 4. *Reduction of vasomotor symptoms compared with placebo*

	Placebo vs black cohosh		Placebo vs red clover		Placebo vs CEE/MPA	
	Difference in mean reduction (SD)	*P*	Difference in mean reduction (SD)	*P*	Difference in mean reduction (SD)	*P*
Vasomotor symptoms per week						
3 mo	−8.58 (9.42)	0.37	2.90 (9.31)	0.76	41.60 (9.21)	<0.0001[a]
6 mo	−10.51 (9.27)	0.26	2.58 (0.78)	0.78	37.41 (9.06)	<0.0001[a]
9 mo	−11.20 (9.26)	0.23	−2.45 (9.15)	0.79	33.11 (9.05)	0.0004[a]
12 mo	−10.77 (9.35)	0.25	−0.07 (9.24)	0.99	33.46 (9.14)	0.0004[a]
Vasomotor intensity						
3 mo	−0.46 (0.25)	0.07	−0.08 (0.25)	0.75	0.83 (0.24)	0.001[a]
6 mo	−0.71 (0.26)	0.008[a]	−0.24 (0.26)	0.34	0.61 (0.25)	0.02[a]
9 mo	−0.66 (0.27)	0.02[a]	−0.35 (0.26)	0.19	0.52 (0.26)	0.05[a]
12 mo	−0.42 (0.27)	0.13	−0.19 (0.27)	0.49	0.70 (0.27)	0.01[a]
Hot flashes per week						
3 mo	−5.62 (6.93)	0.42	2.46 (6.84)	0.72	32.63 (6.77)	<0.0001[a]
6 mo	−5.53 (6.65)	0.41	1.58 (6.57)	0.81	30.13 (6.50)	<0.0001[a]
9 mo	−6.78 (6.71)	0.32	−2.05 (6.63)	0.76	27.39 (6.55)	<0.0001[a]
12 mo	−6.81 (6.89)	0.33	−1.94 (6.81)	0.78	26.93 (6.74)	0.0001[a]

CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate.
[a]Statistically significant difference.

clinical variables, although there were significant differences between the groups by race ($P = 0.005$) and body mass index (BMI; $P = 0.004$) (Table 3). The placebo and the red clover groups had a larger percentage of African American women (72.7% and 59.1%, respectively) compared with the black cohosh (38.1%) and CEE/MPA (30.4%) groups. Similarly, there were higher proportions of white women in the CEE/MPA (69.6%) and black cohosh (61.9%) groups. The red clover and placebo groups had the highest mean BMI, and the CEE/MPA group had the lowest mean BMI. All analyses controlled for race and BMI, but neither presented a significant covariate in any analysis.

The primary outcomes of the study are shown in Figure 2 and Table 4. The average number of vasomotor symptoms

TABLE 5. *Selected secondary outcomes compared with placebo*

	Placebo vs black cohosh		Placebo vs red clover		Placebo vs CEE/MPA	
	Difference in mean reduction (SD)	*P*	Difference in mean reduction (SD)	*P*	Difference in mean reduction (SD)	*P*
Total Kupperman score						
3 mo	−4.59 (2.21)	0.04[a]	−2.39 (2.19)	0.28	2.07 (2.16)	0.34
12 mo	−3.54 (2.07)	0.09	−5.00 (2.05)	0.81	2.31 (2.02)	0.26
Vasomotor instability (Kupperman Index)						
3 mo	−0.67 (0.28)	0.02[a]	−0.27 (0.28)	0.33	0.53 (0.28)	0.06
12 mo	−0.47 (0.29)	0.11	−0.09 (0.29)	0.75	0.47 (0.28)	0.09
Total Greene Score						
3 mo	−3.46 (2.00)	0.09	−0.41 (1.98)	0.84	2.13 (1.96)	0.28
12 mo	−0.41 (2.26)	0.86	1.59 (2.24)	0.48	2.33 (2.21)	0.29
Greene Anxiety Score						
3 mo	−0.20 (0.74)	0.78	1.14 (0.73)	0.12	1.01 (0.72)	0.16
12 mo	−0.47 (0.81)	0.56	1.64 (0.80)	0.04[a]	0.83 (0.79)	0.29
Green Vasomotor Score						
3 mo	−1.06 (0.48)	0.03[a]	−0.41 (0.48)	0.39	1.23 (0.47)	0.01[a]
12 mo	−0.21 (0.51)	0.68	0.04 (0.50)	0.93	1.37 (0.49)	0.007[a]
PANAS positive score						
3 mo	0.30 (1.90)	0.87	0.45 (1.88)	0.81	1.16 (1.86)	0.53
12 mo	−3.00 (1.88)	0.11	0.91 (1.86)	0.63	0.83 (1.84)	0.65
PANAS negative score						
3 mo	−0.24 (1.65)	0.41	−0.45 (1.67)	0.78	−0.24 (1.65)	0.88
12 mo	−0.75 (1.60)	0.64	2.41 (1.59)	0.13	0.68 (1.57)	0.66
Impact of hot flashes on sleep (Pittsburgh Sleep Questionnaire)						
3 mo	−0.006 (0.40)	0.98	−0.09 (0.39)	0.82	1.74 (0.39)	<0.0001[a]
12 mo	0.03 (0.47)	0.94	0.45 (0.46)	0.33	1.66 (0.46)	0.0005[a]
Overall quality of sleep (Pittsburgh Sleep Questionnaire)						
3 mo	−0.31 (0.30)	0.32	−0.50 (0.30)	0.10	0.28 (0.29)	0.35
12 mo	−0.06 (0.3)	0.85	−0.27 (0.34)	0.42	0.40 (0.33)	0.23

CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; PANAS, Positive and Negative Affect Schedule.
[a]Statistically significant difference.

per week decreased over time across all groups (black cohosh, 34% reduction; red clover, 57%; placebo, 63%; and CEE/MPA, 94%). There were no statistically significant differences in the number of vasomotor symptoms, hot flashes alone, or intensity of hot flashes between the botanical groups and placebo at 3, 6, 9, or 12 months with one exception. At 6 and 9 months of intervention, there was a significant difference between black cohosh and placebo for vasomotor intensity, with the black cohosh group showing higher symptom intensity compared with placebo. CEE/MPA, as expected, showed a statistically significant decrease in vasomotor symptoms and intensity relative to placebo at all time points (Fig. 2; Table 4).

Secondary outcomes (excluding safety measurements) are shown in Table 5. For most of the secondary outcomes, no significant differences were observed between any of the treatment groups and placebo at any of the time points measured. One positive exception for the botanicals was that red clover showed a significant improvement over time on the Greene anxiety score for reduction in anxiety ($P = 0.04$). Black cohosh showed a significant increase in vasomotor symptoms compared with placebo at 3 months on both the overall Kupperman score ($P = 0.04$) and vasomotor instability ($P = 0.02$) as well as the Greene vasomotor score ($P = 0.03$), but this difference was not observed over time.

The CEE/MPA group showed significant reduction of vasomotor symptoms over time on the Greene Climacteric Scale ($P = 0.007$) as well as an improvement related to the impact of hot flashes on sleep ($P = 0.0005$). However, the women on HT reported no improvement in overall quality of sleep as measured by the Pittsburgh Sleep Quality Index, or for any of the other secondary outcomes.

Although all women in the botanical and placebo groups lost bone mass during the study, the women in the CEE/MPA arm showed a small increase in lumbar spine ($P < 0.001$) and femoral neck ($P = 0.02$) but no positive change at the hip. The CEE/MPA group also had a small but significant improvement in total cholesterol compared with placebo with a reduction of 22 points ($P = 0.05$). The CEE/MPA group showed a significant reduction in one of the liver function parameters (alkaline phosphatase), although this was not clinically meaningful. Hormone levels were unchanged across all groups except the CEE/MPA arm (Table 6).

An important outcome of this study was that there were no significant differences between the botanical treatments and placebo for any of the safety parameters including breast and endometrial safety, liver enzymes, complete blood count, or lipid profiles. In particular, there was no evidence for hepatotoxicity of black cohosh during the 12-month intervention. Furthermore, prothrombin time was unchanged in any arm of the study, indicating that red clover, in particular, did not interfere with blood coagulation (Table 6).

There were no serious adverse events related to either red clover or black cohosh. In the CEE/MPA group, several women reported breakthrough bleeding, and one woman exited the study after 3 months due to recurrent headaches.

TABLE 6. *Safety data, differences at 12 months*

	Placebo vs black cohosh		Placebo vs red clover		Placebo vs CEE/MPA	
	Difference in mean reduction (SD)	*P*	Difference in mean reduction (SD)	*P*	Difference in mean reduction (SD)	*P*
DXA						
Lumbar spine	−0.083 (0.13)	0.52	−0.081 (0.126)	0.52	−0.504 (0.124)	<0.001[a]
Total hip	−0.004 (0.12)	0.98	0.079 (0.121)	0.51	−0.201 (0.119)	0.10
Femoral neck	−0.181 (0.163)	0.27	0.033 (0.161)	0.84	−0.366 (0.159)	0.02[a]
Endometrial thickness	−0.291 (0.576)	0.62	−0.005 (0.569)	0.99	−0.452 (0.563)	0.42
Hormones						
Estrone	8.32 (15.48)	0.59	6.48 (15.30)	0.67	−105.85 (15.13)	<0.001[a]
Estradiol	−7.59 (6.87)	0.27	0.04 (6.78)	0.99	−29.01 (6.71)	<0.001[a]
Testosterone	0.16 (2.83)	0.95	−1.27 (2.79)	0.65	−2.71 (2.76)	0.32
SHBG	1.64 (7.44)	0.82	1.43 (7.35)	0.85	−38.65 (7.27)	<0.001[a]
FSH	5.14 (7.84)	0.51	1.63 (7.75)	0.83	53.58 (7.77)	<0.001[a]
LH	−0.49 (3.43)	0.88	−1.55 (3.37)	0.64	13.53 (3.34)	0.001[a]
TSH	0.03 (0.49)	0.95	0.64 (0.48)	0.19	−0.18 (0.48)	0.69
Lipids						
Total cholesterol	8.21 (11.60)	0.48	5.54 (11.47)	0.63	22.55 (11.34)	0.05[a]
HDL	1.04 (2.88)	0.72	1.91 (2.84)	0.50	−0.729 (2.81)	0.80
LDL	1.55 (9.38)	0.87	−2.09 (9.27)	0.82	10.28 (9.17)	0.26
LFTs						
ALP	5.25 (5.90)	0.38	3.68 (5.83)	0.53	22.75 (5.76)	0.002[a]
ALT	−0.07 (2.79)	0.98	−0.92 (2.76)	0.74	1.19 (2.73)	0.48
AST	5.29 (4.02)	0.20	5.136 (4.059)	0.21	8.08 (4.01)	0.47
PT[b]	−0.19 (0.17)	0.27	−0.001 (0.16)	0.99	0.13 (0.16)	0.43

CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; DXA, dual-energy x-ray absorptiometry; SHBG, sex hormone–binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LFTs, liver function tests; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PT, prothrombin time.

[a]Statistically significant difference.

[b]PT compared between baseline and 1 month.

There were no significant differences between groups reported for any other side effects including gastrointestinal symptoms, nausea, vomiting, or fatigue.

DISCUSSION

This 12-month randomized clinical trial of 89 perimenopausal and postmenopausal women with moderate to severe hot flashes found that neither black cohosh nor red clover had a significant effect on reduction of vasomotor symptoms as compared with placebo. As expected, HT significantly reduced vasomotor symptoms compared with placebo.

There were significant reductions of all vasomotor symptoms from baseline to 12 months within all four groups. The women in the CEE/MPA group had a 94% reduction in vasomotor symptoms (71 vasomotor symptoms per week at baseline to 5 at follow-up), with the placebo group showing the next greatest reduction in vasomotor symptoms (63%; from 52 to 19). The red clover group had a 57% reduction in hot flashes (from 58 to 25), and the black cohosh group had the smallest reduction in vasomotor symptoms, 34% (from 65 to 43).

Previous clinical trials of the efficacy of red clover for the relief of hot flashes have shown either no efficacy or a small beneficial effect.[18,25,40] Although this trial found a 57% reduction in vasomotor symptoms with red clover, the effect was no better than that observed in women using placebo. Despite earlier studies showing evidence to the contrary,[22] red clover use in this study did not improve lipid profiles or bone density. However, red clover reduced anxiety during the course of the study when compared with placebo. The outcome that red clover administration reduced anxiety is consistent with estrogen-receptor (ER) β agonist activity.[41] The red clover extract used in this trial has greater affinity for ER-β than ER-α,[42] and agonists to ER-β have been shown to reduce anxiety in animal models, whereas transgenic female mice lacking ER-β exhibit increased anxiety.[43,44] Although this beneficial effect is a novel observation for red clover, there is precedent in the clinical literature because an extract of rhubarb roots (*Rheum rhaponticum*) that was ER-β positive was found to reduce anxiety in perimenopausal women compared with that in placebo-treated women.[45]

The efficacy of black cohosh for reducing vasomotor symptoms has been evaluated in several other clinical trials, although most have been short term (≤3 mo), and many were sponsored by the manufacturers of their respective formulations. Of nine clinical trials examining the efficacy of black cohosh for relief of menopausal symptoms, six have shown significant reduction in vasomotor symptoms.[12-17,46-48] However, five of these studies reported improvement in menopause rating scales rather than frequency and intensity of vasomotor symptoms, and two of these studies were not placebo controlled. In a recent 12-month randomized, placebo-controlled study by Newton et al,[17] black cohosh did not reduce hot flashes when compared with placebo.

In the current study, the black cohosh group exhibited a 35% reduction in vasomotor symptoms, a response that had been expected from placebo. If only entry and exit assessments had been made without comparison to placebo, considerable improvement in vasomotor symptoms would have been reported for the black cohosh group. The rigorous approach taken in the design of the present clinical evaluation of black cohosh revealed that black cohosh was actually less effective than was placebo (35% vs 63%) in reducing vasomotor symptoms. Although a natural decline in vasomotor symptoms over time is to be expected, the 63% reduction among placebo users was greater and more sustained than anticipated.

In a Cochrane Review of HT trials, women in placebo groups experienced a 58% average reduction (range, 41%-61%) in hot flash frequency during the course of these trials.[1] The black cohosh study by Newton et al[17] (personal communication, Katherine M. Newton, PhD, Center for Health Studies, Group Health Cooperative, 2008) showed a placebo effect of 32% at 6 months and 26% at 12 months. Other botanical clinical trials that report frequency of hot flashes have placebo effects ranging from only 10% to 36%.[15,40] The findings of the current study suggest that future clinical trials of menopausal therapies should be designed for the possibility that the placebo effect may exceed 60%.

Given that many women may continue to use botanicals, another significant outcome of this clinical trial has been the demonstrated safety of black cohosh and red clover. Neither of the botanical products differed from placebo in the incidence of adverse events, and both were found to be safe on breast tissue and endometrial thickness.[14,16,49,50] In the case of red clover, the concern had been raised that it might contain anticoagulant coumarins.[51,52] In response, Booth et al[53] reported that red clover contained no coumarins known to prevent coagulation. As a precaution, the current study measured prothrombin time in all participants at randomization and at the 1-month safety visit. The current study confirms these findings by showing no increase in prothrombin time in women in the red clover group. Based on a few case reports, concerns have been raised that black cohosh might be hepatotoxic in women.[54-56] However, black cohosh did not produce any clinical symptoms of liver damage nor did it increase liver enzymes in this investigation. These results are consistent with the National Institutes of Health report that, "there is no known mechanism with biological plausibility that explains any hepatotoxic activity of black cohosh" and that "millions of women have taken black cohosh with very few adverse events."[57] Most published reports proposing a direct cause and effect link cannot be substantiated because the putative botanicals have not been verified, and in many cases, concomitant drug and/or alcohol use was not reported.[54,55,58-65]

CONCLUSIONS

In conclusion, women taking red clover or black cohosh extracts did not have reduced vasomotor symptoms compared with the placebo group. The placebo group exhibited a higher than expected decrease. Red clover significantly reduced

anxiety over time. Both botanicals were found to be safe during the 12-month period. In particular, red clover showed no anticoagulant activity, and black cohosh was not hepatotoxic. These positive safety outcomes are important because women may be expected to continue to use black cohosh and red clover, regardless of scientific clinical findings. Finally, regarding the manufacture of safe botanical dietary supplements, it is important to emphasize the benefits of using botanicals that have been authenticated and both chemically and biologically standardized.

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