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First-time proof of endometrial safety of the special black cohosh extract (*Actaea* or *Cimicifuga racemosa* extract) CR BNO 1055

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ABSTRACT

Objective: To investigate endometrial safety by assessment of endometrial biopsy samples and the tolerability and efficacy of the special *Actaea* or *Cimicifuga racemosa* extract (CR BNO 1055).

Design: Four hundred postmenopausal women with symptoms related to estrogen deficiency were enrolled into a prospective, open-label, multinational, multicenter study. Treatment duration (daily dose corresponds 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.

Results: Endometrial safety has been 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 flushes were markedly decreased. The dropout rate was less than 10%. The overall tolerability was good.

Conclusions: The lack of endometrial proliferation and improvement of climacteric complaints as well as only a few gynecologic organ–related adverse events are reported for the first time after a treatment period of 1 year. Due to the improved benefit:risk ratio, it must be assumed that the *Cimicifuga racemosa* special extract BNO 1055 is a safe alternative for treatment of climacteric complaints.

Key Words: Black cohosh – Safety study – CR BNO 1055 – Uterus – Liver.

In the peri- to postmenopausal phase of life, more than two thirds of women are suffering from climacteric complaints. Severe vasomotor symptoms including hot flushes occur in 70% to 75% of women. In about half of these women, the symptoms persist for 5 years or longer. Twenty percent of all women are reported to find these symptoms intolerable.[1] Therefore, they are in most cases the decisive factor for starting treatment.

Vasomotor symptoms (hot flushes) occur due to an overactivity of the hypothalamic gonadotropin-releasing hormone pulse generator, which then influences temperature regulatory neurons.[2] Hot flushes are known to correlate with changes of pulsatile luteinizing hormone (LH) secretion,[3] and complex neuroendocrine pathways are involved in the process of temperature regulation, which include epinephrine and serotonin.[4,5]

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Postmenopausal hormone therapy (HT), ie, estrogens with or without progestins, has been the standard therapy for many years because it targets the majority of menopause-associated complaints and diseases such as osteoporosis. However, the recent results of the Heart and Estrogen/Progestin Replacement Study, Women's Health Initiative, and the Million Women Study showed HT-associated risks regarding breast cancer, venous thrombembolism, stroke, and possibly heart disease.[6-8] Therefore, alternative nonestrogen therapies have been looked at with increasing interest to evaluate their potential risks and benefits.

Alternative treatments are herbal extracts such as soy, red clover, and black cohosh (*Actaea* or *Cimicifuga racemosa* [CR]). Due to the isoflavones daidzein and genistein, contained in soy extracts, potential adverse estrogenic effects have to be considered. When taken in high doses over a 5-year period, endometrial hyperplasia was demonstrated to occur in 3.37% of women in the treatment group versus 0% in the placebo group.[9]

Among the herbal medicines, CR extracts may be a promising alternative because CR was able to reduce climacteric complaints and had beneficial effects on bone turnover.[10-14] Despite its assumed organ-selective activity, none of the known phytoestrogens have been detected in the CR extracts.[15-19]

As there are many preparations of CR on the market, each being extracted in different ways, this may be an explanation why some studies claimed the CR extract to act independently of the estrogen receptor and other studies assumed estrogen-like or even antiestrogenic activity.

On the other hand, any estrogenic effect of CR extracts in the uterus of ovariectomized rats could not be demonstrated in animal experimental studies even when administered at high concentrations.[15,20]

Recently it was shown that CR extracts contain substances that bind to serotonin receptors, and this particular mode of action was suggested to be the mechanism by which hot flushes are alleviated.[21] A dopaminergic effect has also been demonstrated,[22] and it is known that estrogens increase dopamine in the brain of postmenopausal women.[23] However, the precise mechanism of effect of the extracts of CR on alleviation of estrogen-deficit–related symptoms remains unknown.

One of the major disadvantages of HT is the fact that estrogen treatment in uterus-intact women needs to be accompanied by progestin treatment as estrogens alone increase the risk of the development of endometrial carcinomas. Because the CR preparations are considered to be alternative treatment, the question is raised whether this herbal alternative is devoid of any potential of inducing hyperplasia in the endometrium. Therefore, the objectives of the present study were to provide a sufficiently precise estimate of the rate of endometrial hyperplasia and/or more serious adverse endometrial outcome, to prove the overall safety/tolerability, and to assess the efficacy of a 12-month treatment with the black cohosh extract CR BNO 1055 (*Actaea* or CR extract). As for studies on HT, the European Agency for the Evaluation of Medicinal Products guidelines were followed.[24]

METHODS

Study design

The open-label, noncomparative, prospective, multicenter, and multinational study was conducted in 44 gynecological practices and outpatient clinics in the Czech Republic and in Poland between 2002 and 2004 (approval numbers: Czech State Institute for Drug Control 1779/02; Polish Central Register of Clinical Trials 196/02). The conduct of the study, monitoring, data management, and statistical analyses were done in accordance with the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. Ethical approval of the study protocol was obtained from nine ethics committees from both countries before the study start.

After receiving written and verbal information about the type, purpose, risks, benefits, and duration of the study and about alternative therapies, the women signed a written informed consent before any study-related measures.[25,26]

The sample size calculation was done according to the European Agency for the Evaluation of Medicinal Products guidelines.[24] For a minimum treatment period of 12 months, at least 300 assessable subjects were necessary to obtain the required precision (upper limit of the 95% CI should not exceed the point estimate by more than 2%). To show endometrial safety comparable to that of HT, the incidence of hyperplasia and/or more serious adverse endometrial outcomes should not exceed 2% as defined by the European Agency for the Evaluation of Medicinal Products guidelines.[24] Therefore, no more than six women with an adverse endometrial outcome of the calculated 300 subjects would have been tolerable. Because a dropout rate of up to 20% was assumed, a total of 400 women had to be enrolled in the study.

Data were collected at visit 1 (weeks –4 to –1), visit 2 (day 0 = baseline), visit 3 (4 weeks after visit 2), visit 4 (13 weeks after visit 2), visit 5 (26 weeks after visit 2), visit 6 (39 weeks after visit 2), and visit 7 (52 weeks after visit 2).

Inclusion/exclusion criteria

Patients fulfilling the following inclusion criteria were enrolled: outpatient; written informed consent obtained; age between 50 and 75 years; postmenopausal with symptoms related to estrogen deficiency; at least seven moderate or severe hot flushes in the weeks before screening visit 1 and visit 2; intact uterus; body mass index 28 or less (kg/m^2); last spontaneous menstrual period at least 2 years ago; follicle-stimulating hormone (FSH) 35 mIU/mL or more; 17β-estradiol less than 40 pg/mL; endometrial thickness 5 mm or less; nonpathological endometrium, ie, the histological slide of the biopsy sample contained sufficient endometrial tissue for diagnosis according to the Committee for Proprietary Medical Products (CPMP) classification[24] modified according to Kurman and Mazur[27] and the endometrium was classified as being of category 2-0 (atrophic and/or inactive endometrium), 3-1 (secretory endometrium, cyclic type), 3-2 (secretory endometrium, progestational type), or 4-0 (proliferative endometrium), *or* insufficient endometrial tissue (endometrium classified as category 1-0, insufficient for diagnosis), and endometrial thickness of 5 mm or less.

Patients presenting with any of the listed exclusion criteria (Table 1) were not included in the study.

TABLE 1. *Exclusion criteria*

Withdrawal of informed consent
Endometrium classified in categories 0-0 and 5 to 8 according to Kurman and Mazur [27]
Suspicious findings on endovaginal sonography
Cervical smear with epithelial cell abnormalities; nonepithelial malignant neoplasms
Estrogen monotherapy, Tibolone, selective estrogen-receptor modulators, antiestrogens during the 6 months before visit 1
Estrogen/progestogen combinations during the month before visit 1
Vaginal application of estriol during the 2 weeks before visit 1
Injectable sex hormones/hormonal implants during the 12/36 months before visit 1
Food/supplements with phytoestrogens during the 6 weeks before visit 1
Acute phlebitis, pancreatitis
Chronic disease with effects on drug absorption, metabolism, excretion
Acute/chronic hepatic diseases or history thereof
Uncontrolled diabetes mellitus
Unstable nontreated hypertension
Combined diagnosis of diabetes mellitus, hypertension, and obesity
Known coagulation disorders
Acute or history of thromboembolic event
Known sickle cell anemia
Refractory hypertriglyceridemia
Vaginal bleeding of unknown etiology during past 2 years
Any malignancy, especially E_2-dependent neoplasms
Herpes gestationis during a previous pregnancy
Migraine accompagnee
Known hypersensitivity to any of the components of the test preparation

Study drug

The study medication, dried aqueous/ethanolic (58% vol/vol) extract CR BNO 1055 of the rhizome of *Actaea* or CR (black cohosh) (Klimadynon/Menofem, Bionorica AG, Neumarkt, Germany), which was manufactured according to Good Manufacturing Practice, was provided as film-coated tablets. The daily dose consisted of two tablets given orally. Each tablet (batch no. 2471502) contained 1.66 to 2.86 mg of native extract corresponding to 20 mg of herbal drug.

Appropriately packed and labeled containers with 200 tablets each were distributed at visits 2, 4, 5, and 6.

Investigated parameters for safety and efficacy

Endometrial biopsies were performed under standardized conditions[9] by means of Pipelle-induced endometrial suction at visit 1, in cases of premature discontinuation, when metrorrhagia occurred, and after 52 weeks (visit 7).[28] A second biopsy was performed in case of classification 0-0 (no endometrial tissue obtained) at visit 1 or 0-0 or 1-0 (endometrial tissue insufficient for diagnosis) at visit 7/premature discontinuation. The samples were read by two independent pathologists according to the predefined CPMP classifications[24] modified according to Kurman and Mazur.[27] In case of differences between the two pathologists, a third independent pathologist assessed those slides to adjudicate the difference. If a unanimous decision among the three pathologists on discussion of the samples in dispute could not be achieved, the most unfavorable of the three competing diagnoses was used for data analyses.[24] The pathologists were neither aware of the study design nor the name or type of the study drug.

The thickness of endometrium was measured including both layers of the endometrium in the sagittal view at the place of maximum thickness in the fundus region by means of endovaginal sonography (frequency of ≥5 MHz) at visits 1, 5, and 7 and in case of premature discontinuation. An endometrial thickness of more than 5 mm under treatment was documented as an adverse event. These women

had to undergo curettage or hysteroscopy after the biopsy was obtained by Pipelle sampling.

The occurrence and intensity (classified according to a five-grade rating: none, spotting, mild, moderate, strong) of bleeding episodes were recorded by means of a patient diary. Bleeding episodes accompanied by additional complaints were documented as adverse events.

A physical examination of the breasts and measurements of blood pressure, heart rate, and body weight were performed at visits 1, 5, and 7 and in case of premature discontinuation.

If there were abnormal findings during the breast examination, mammography had to be performed before enrollment (mammograms up to 12 months before enrollment were accepted), at the end of the trial, and at any time of study termination provided the interval from the last mammogram was longer than 12 months. Ultrasonography of the breast had to be performed upon premature study termination, provided the patient's last mammogram was not older than 12 months and a physical examination of the breast was normal.

Retrospectively, the mammograms were centrally assessed to detect any changes in the breast density patterns when compared to the last mammogram before the start of treatment. The assessment of breast density was carried out according to Wolfe[29] and BI-RADS.[30]

At visits 1, 4, 5, and 7, blood samples were collected for determination of 17β-estradiol, LH, FSH, and markers of osteoblast (osteocalcin) and osteoclast (β-CrossLaps = metabolic breakdown products of collagen 1 α 1) activity, which were determined with the ELECSYS 2010 System (Roche-Diagnostics, Mannheim, Germany). For the explorative evaluation of bone turnover, women were assigned to three subgroups according to their baseline values of β-CrossLaps (high baseline levels: ≥0.5 ng/mL; medium baseline levels: >0.217 to <0.5 ng/mL; low baseline levels: <0.217 ng/mL).

Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides were analyzed by enzymatic color tests.

Creatinine, uric acid, total protein, sodium, potassium, total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma-glutamyl transferase (γ-GT), alkaline phosphatase, serum glucose, a complete blood workup, hemoglobin, and the status of hemostasis (international normalized ratio) were analyzed at visits 1, 5, and 7 by a central laboratory.

The occurrence of (serious) adverse events (type, severity, duration, the causal relationship) was recorded and assessed.

The validated Menopause Rating Scale II (MRS II) was completed by the patient herself at visits 2 to 7 to assess changes of climacteric complaints (Table 2).[31] The frequency (number/day) and severity of hot flushes (mild = flushes without perspiration or limitation of normal daily function, moderate = flushes with or without perspiration that limited but did not prevent daily function, severe = flushes with perspiration that prevented normal daily function) were registered daily in a patient diary.

Statistical analyses

Primary outcome of the study was the occurrence of endometrial hyperplasia or more serious adverse endometrial outcome (carcinoma) after continuous application of the trial medication for 52 weeks. Before further analysis, data sets were classified as positive if endometrial hyperplasia and/or a more serious outcome was found and negative if an adverse outcome was not found. The point estimator for the probability of endometrial hyperplasia or more serious outcome under the trial drug and its upper limit of 95% CI were calculated.[32]

Statistical analyses for the secondary endpoints were based on the 375 women who completed the treatment period of 52 weeks as requested by the study protocol (protocol correct completers). Patient baseline characteristics, endometrial thickness, breast density, vaginal bleeding episodes, vital signs, and adverse events were evaluated descriptively. Values of

TABLE 2. *Symptoms assessed by the Menopause Rating Scale II*

1. Hot flushes, sweating (episodes of sweating)
2. Heart discomfort (unusual sensations of heart throbbing, heart skipping, uneasiness)
3. Sleep problems (difficulties with falling asleep, difficulties with sleeping through, waking up early)
4. Depressive mood (feeling sad, on the verge of tears, lack of drive, mood swings)
5. Irritability (feeling nervous, inner tension, feeling aggressive)
6. Anxiety (inner restlessness, panicking)
7. Physical and mental exhaustion (general decrease in performance, impaired memory, decrease in concentration, forgetfulness)
8. Sexual problems (changes of sexual desire, sexual activity and satisfaction)
9. Bladder problems (voiding difficulties, pollakisuria, urinary incontinence)
10. Vaginal dryness (sensations of dryness or burning in the vagina, difficulty with sexual intercourse)
11. Joint and muscular discomfort (pain in the joints, rheumatoid complaints)

Rating of severity: none = 0; mild = 1; moderate = 2; severe = 3; extremely severe = 4.

hormone concentrations below or above the lower or upper quantification limit were replaced by the value of the lower or upper limit of quantification, respectively.

Changes in the laboratory parameters were calculated as changes between baseline and after 52 weeks of treatment. A sign rank test was performed to detect significant changes.

Special interest was put on the analyses of the mean relative changes of osteocalcin and β-CrossLaps. Sign rank tests were performed to show significant changes from baseline of the two markers of bone turnover for the patient group.

Sum scores of items 1 through 11 of the MRS II after 52 weeks of treatment were tested versus scores of the MRS II at baseline by a Wilcoxon test.

A weighted score of hot flushes was calculated for each day by multiplying the number of mild hot flushes by a factor of 1, the number of moderate hot flushes by a factor of 2, and the number of severe hot flushes by a factor of 3. For the treatment period, the weighted scores were averaged as mean values over periods of 4 weeks. Changes in the 4-week weighted score of hot flushes were statistically tested after 26 and 52 weeks of treatment by a Wilcoxon test.

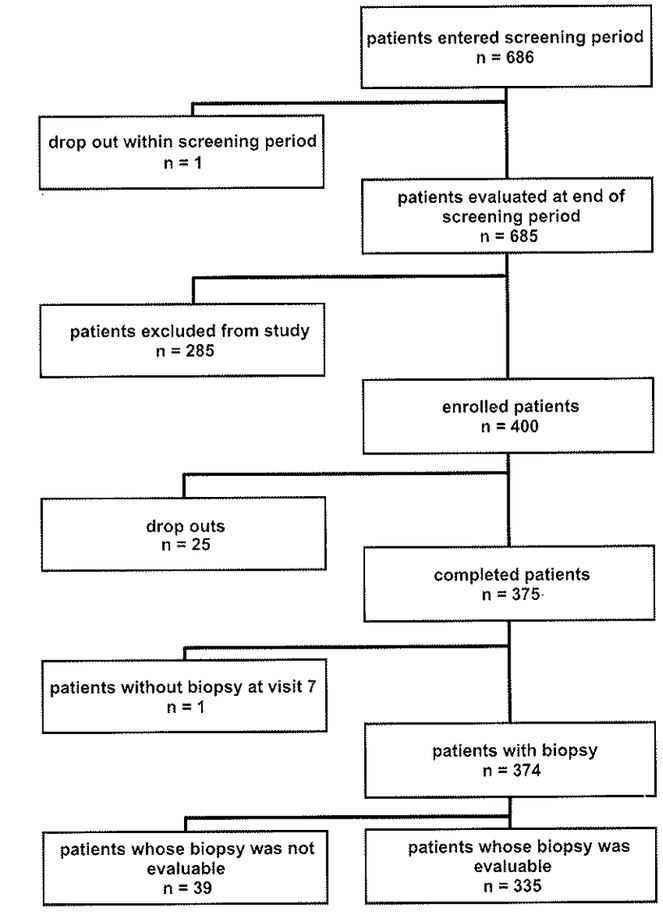

FIG. 1. Patient flow through the study from screening to completion after 52 weeks of treatment.

In general $P \leq 0.05$ was considered statistically significant. Statistical analyses and sample size calculations were accomplished with SAS Version 8.2.

RESULTS

An overview concerning disposition of the subjects is outlined in Figure 1. A total of 686 women have been screened for eligibility. One patient did not return for visit 2, and 285 women could not be included in the study. The main reasons were violations of inclusion or exclusion criteria (mostly pathological laboratory results). In five women, hyperplasia and/or more serious adverse endometrial outcomes were detected before enrollment (two cases with classification 6-1: simple hyperplastic endometrium [glands with only architectural abnormalities, including a disordered proliferative pattern and cystic glandular hyperplasia]; and one case each with classifications 5-3: endometrium with polyps [polyps with proliferative/hyperplastic features]; 7-1: carcinoma [endometrioid type]; and 8-1: others [types of hyperplasia or neoplasm not mentioned in categories 5-3, 6, or 7]). A total of 400 women were enrolled matching all inclusion and no exclusion criteria. Twenty-five premature discontinuations were recorded (16, withdrawal of consent; 7, lack of efficacy; 2, noncompliance). A total of 375 women completed the treatment period of 52 weeks as requested by the study protocol (protocol correct completers). Of these subjects, one woman refused biopsy at visit 7, and biopsy specimens from 39 women were not assessable because they were either classified as category 0-0 (no endometrial tissue obtained) or 1-0 (endometrial tissue insufficient for diagnosis) at visit 7 (n = 24) or because they were sampled more than 42 days after last intake of study medication (n = 15). Therefore, the primary endpoint could be evaluated in 335 women.

Table 3 shows the demographic characteristics of the 400 enrolled women and the 375 women who completed the treatment period of 52 weeks (protocol correct completers).

Endometrial biopsies

Of the 375 women who completed the treatment period, a third reading of biopsy samples was necessary in six cases at visit 1, in one case at visit 5, and in four cases at visit 7.

The distribution of the individual biopsy categories is shown in Figure 2. Despite the fact that six cases of hyperplasia and/or more serious adverse endometrial outcomes would have been tolerable in accordance with the CPMP guidelines *Points to Consider on*

TABLE 3. *Patient demographic characteristics*

	Mean ± SD	Minimum	Median	Maximum
Age, y				
PCC	56.45 ± 4.77	48.92	55.59	74.94
ITT	56.38 ± 4.73	48.92	55.43	74.94
Height, cm				
PCC	166.14 ± 6.38	145.00	165.00	184.00
ITT	166.09 ± 6.34	145.00	166.00	184.00
Weight, kg				
PCC	68.16 ± 8.47	44.00	68.00	88.00
ITT	68.14 ± 8.55	44.00	68.00	88.00
Body mass index, kg/m^2				
PCC	24.65 ± 2.40	18.56	24.68	29.00
ITT	24.66 ± 2.41	18.36	24.67	29.00
Menopause duration, mo				
PCC	71.27 ± 53.38	18.00	50.83	334.43
ITT	71.14 ± 52.86	18.00	50.58	334.43
17β-Estradiol, pg/mL[a]				
PCC	19.86 ± 9.38	5.00	21.50	39.40
ITT	20.19 ± 9.03	5.00	21.50	39.40
MRS II (total score)				
PCC	13.82 ± 7.49	3.00	12.00	36.00
ITT	13.92 ± 7.45	3.00	13.00	36.00
Hot flushes (4-wk weighted score)				
PCC	11.9 ± 8.9	1.9	9.1	55.9
ITT	11.9 ± 8.8	1.9	9.1	55.9

PCC, protocol correct completers (women who completed treatment; n = 375); ITT, intention-to-treat group (enrolled women; n = 400); MRS = Menopause Rating Scale.
[a]Values below the lower limit of quantification were replaced by the lower limit of quantification value.

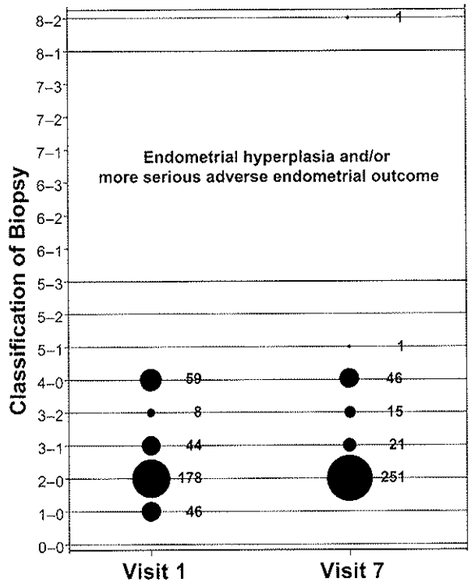

FIG. 2. Frequency of endometrial tissue classifications according to Kurman and Mazur[27] of those subjects whose biopsies were assessable (n = 335) as assessed before enrollment (visit 1) and after 52 weeks of treatment (visit 7).

Hormone Replacement Therapy for the actual sample size, *None* of the categories with pathological relevance, such as

- Endometrium with polyps (polyps with proliferative/hyperplastic features)
- Simple hyperplastic endometrium (glands with only architectural abnormalities, including a disordered proliferative pattern and cystic glandular hyperplasia)
- Complex hyperplastic endometrium (crowded, architecturally abnormal glands without cytologic atypia)
- Atypical hyperplastic endometrium (glands with both cytological and architectural abnormalities)
- Carcinoma (endometrioid type)
- Carcinoma (nonendometrioid type)
- Carcinoma (others)
- Others (types of hyperplasia or neoplasm not mentioned in categories 5-3, 6, or 7)

were detected during the whole course of the trial. Therefore, the point estimator for the probability of endometrial hyperplasia or more serious adverse endometrial outcome was 0.0 with an upper limit of 95% CI of 0.011.

Of the assessable biopsies, atrophy was the finding that dominated the classifications both at the beginning and at the end of the trial, which corresponds to the subject's age and hormonal status. The number of cases of atrophy increased in the women enrolled (178 before enrollment, 251 at the end of treatment), which, along with the absence of adverse endometrial findings as described above (see also Fig. 2), supports the expected hypothesis of endometrial safety of the CR special extract. Of the 178 women with an initially atrophic endometrium, a proliferative endometrium was diagnosed in 16 cases at treatment end. Classifications of higher degrees did not occur in these women. Of 55 women, 38 with proliferative endometrium before enrollment were classified as having an atrophic endometrium at the end of treatment. Furthermore, under treatment there were only two cases developing an endometrium of a category higher than 4-0 (proliferative endometrium). One patient was categorized as 5-1, endometrium with polyps (polyps with atrophic features), and a second patient as 8-2, others (less serious outcome not mentioned in categories 0-0 to 5-2), which in this particular case read "polypoid endometrium with necrotic proliferative features." Both of these findings were still nonpathological.

For the dropouts, no cases of hyperplasia or worse endometrial outcome were recorded. The particular biopsy categories in the group of dropouts read as follows: one, 0-0 (no endometrial tissue obtained); five, 2-0 (atrophic and/or inactive endometrium); and two, 4-0 (proliferative endometrium). Of 27 dropouts, 17 refused to undergo the final biopsy procedure. There were no cases of hyperplasia or worse endometrial outcome among the statistically nonassessable subjects (n = 15) who underwent biopsy more than 42 days after the date of last intake of study medication (the particular categories reading: five, 1-0 [no endometrial tissue obtained]; four, 2-0 [atrophic and/or inactive endometrium]; three, 3-1 [secretory endometrium, cyclic type]; and three, 4-0 [proliferative endometrium]).

Endometrial thickness

In accordance with the results of endometrial biopsies, there was no increase in endometrial thickness observed in the trial subjects. This applies to both the subjects with assessable biopsies (n = 335) and those without (n = 39) (Fig. 3). In 39 cases, biopsies were not assessable, and the main reasons for biopsies not being assessable were that the biopsy was sampled more than 42 days after last intake of

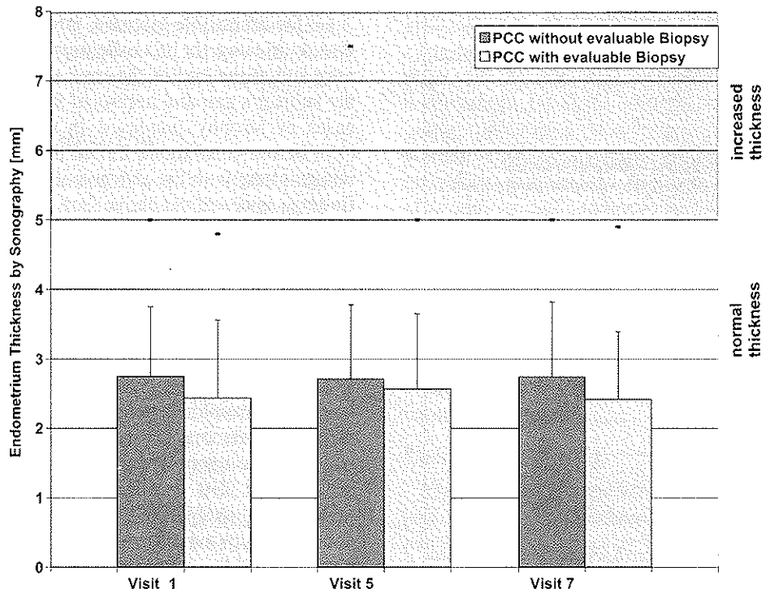

FIG. 3. Endometrial thickness was measured by ultrasonography at the screening visit and after 26 and 52 weeks in those women who completed the study (PCC = protocol correct completers) with assessable biopsies of endometrial tissue (n = 335) and those women who completed the study but biopsies of endometrial tissue were not assessable due to classifications of 0-0, 1-0, or biopsy done > 42 days after last intake of study medication (n = 39). Means ± SDs as well as maximal values of individual subjects are shown.

study medication (n = 15) or the categories 0-0 (22, no endometrial tissue obtained) and 1-0 (2, material insufficient for diagnosis). In only two cases did endometrial thickness exceed 5 mm, which is acknowledged as the upper margin of normal value. In one subject, the thickness was 7.5 mm at visit 5 (26 weeks) and the endometrial sample before hysteroscopy revealed a nonpathological category of 4-0. The thickness in the other patient was 9.0 mm measured at visit 6 (week 39). The endometrial tissue was classified as 2-0 (atrophy).

Vaginal bleeding

In total, 59 women recorded some kind of bleeding episode while under treatment (36, spotting; 8, mild bleeding; 9, moderate bleeding; and 6, strong bleeding). The serum levels of estradiol and FSH remained in the postmenopausal range (FSH, ≥35 mIU/mL; 17β-estradiol, <40 pg/mL). Of the 59 women with bleeding, 17 reported episodes lasting more than 10 days. Regardless of the bleeding intensity, there were no findings of hyperplasia; none of them developed endometrial thickness more than 5 mm. The majority of women who reported vaginal bleeding (75%) had an atrophic or inactive endometrium. For 25% of women with vaginal bleeding, a secretory (cyclic type) or proliferative endometrium was found.

Breast examination, blood pressure, heart rate, body weight

Concerning breast examination, blood pressure, heart rate, and body weight, no changes from baseline were observed.

Mammograms (breast density)

Pathologic findings on mammography were reported for only one woman, in whom an invasive breast cancer was diagnosed during the course of the trial. This breast cancer was assessed as not drug related. (For details of this case, see the section "Serious Adverse Events.") The mammogram performed on this woman before the start of the study showed no suspicious signs.

Retrospectively, mammograms for 138 women with mammograms available before and at the end of the study were centrally assessed.

There were three reasons why mammograms of only 138 women could be collected for a centralized assessment.

1. Due to legal and proprietary reasons, mammograms from Poland were excluded. Therefore, only Czech women (n = 327) were subject to centralized assessment.
2. The idea of a centralized assessment was set after the study has finished. Therefore, the procedure was not part of the patient's informed consent form. To avoid violation of laws on personal data protection, some investigators refused to provide the requested information. Therefore, a total of 139 women from nine centers could not be evaluated.
3. Some peripheral radiologists failed to provide complete mammography documentation. For some women, the mammograms before or after the study were missing. Some provided mammograms dating back even longer than 12 months before study start. Therefore, another 50 women were not assessable.

From the 138 mammograms that were evaluated according to Wolfe[29] and BI-RADS[30] classifications, there was no increase in breast density detected by mammography except in one woman (0.72%) with higher density.

Bone turnover

Concerning the concentrations of the β-CrossLaps at baseline, 80 women were assigned to a high baseline group (mean: 0.72 ± 0.21 ng/mL; minimum: 0.51 ng/mL; median: 0.66 ng/mL; maximum: 1.57 ng/mL). The medium (mean: 0.33 ± 0.08 ng/mL; minimum: 0.22 ng/mL; median: 0.33 ng/mL; maximum: 0.50 ng/mL) and the low (mean: 0.16 ± 0.05 ng/mL; minimum: 0.01 ng/mL; median: 0.18 ng/mL; maximum: 0.22 ng/mL) baseline groups consisted of 201 and 79 women, respectively. Under treatment, the women of the low baseline group showed a relative and significant increase in the β-CrossLaps of 137.78%, 166.63%, and 96.31% after 12, 26, and 52 weeks, respectively. At the same time points, the observed relative and significant increases of osteocalcin were 34.47%, 52.52%, and 36.64%, respectively.

In the medium baseline group, fewer effects on bone metabolism were observed. By contrast, in the high baseline group, a significant decrease in β-CrossLaps of about 26%, ie, a sign of antiresorptive activity, was observed when compared to baseline (relative changes from baseline after 13, 26, and 52 weeks: 9.62%, 23.36%, and 25.67%, respectively), whereas osteocalcin showed only a slight increase under treatment (relative changes from baseline after 13, 26, and 52 weeks: 7.12%, 8.25%, and 5.66%, respectively). The antiresorptive activity shown by a decrease of the marker of bone degradation (β-CrossLaps) as well as the nearly unchanged course of osteocalcin (bone formation) in subjects with levels of β-CrossLaps 0.5 ng/mL or greater is shown in Figure 4.

Hormones

Statistically, a significant yet slight increase in 17β-estradiol and decrease in LH and FSH were observed (Table 4). The slight mean increase in 17β-estradiol was due to two women with suspected ovulatory cycles who had 17β-estradiol values of 800 and 821.5 pg/mL as well as markedly decreased FSH and LH levels. Therefore, the median value is more suitable, showing a decrease of 4.6 pg/mL of 17β-estradiol. However, the extent of these changes is of no clinical significance.

Lipid metabolism

17β-Estradiol deficiency is known to result in changes of lipid metabolism, particularly in an increase in total cholesterol. The changes in the serum lipids in women under treatment are shown in Table 4. A statistically significant increase was observed for total cholesterol, HDL, LDL, and triglycerides, although these changes are clinically not relevant and are in accordance with the median decline of 17β-estradiol levels. No subjective complaints in association with these changes of lipids were reported. The LDL:HDL ratio decreased in 64.8%

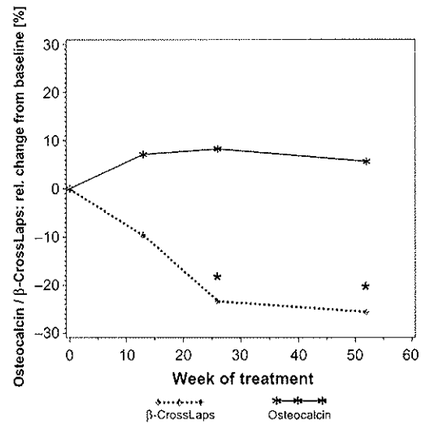

FIG. 4. Shown are the relative changes from baseline of the β-CrossLaps and osteocalcin means of the subgroup with high values (≥0.5 ng/mL) (n = 80) of β-CrossLaps at baseline and the *P* values (*significant) of the differences to baseline (sign test).

TABLE 4. *Mean changes in laboratory parameters (differences week 52 to week 0)*

	Parameter	Week 0 (mean ± SD)	Week 52 (mean ± SD)	Difference (mean ± SD)	*P*
Hormones	17β-estradiol (pg/mL)[a]	19.86 ± 9.38	22.66 ± 50.35	2.81 ± 50.38	Sig.
	FSH (mU/mL)[b]	75.05 ± 27.36	68.86 ± 33.44	−6.19 ± 29.45	Sig.
	LH (mU/mL)[a]	31.79 ± 11.48	29.64 ± 13.26	−2.15 ± 11.85	Sig.
Lipids	Cholesterol total (mg/dL)	202.91 ± 26.41	216.06 ± 36.07	13.15 ± 37.28	Sig.
	HDL (mg/dL)	64.28 ± 12.60	71.38 ± 15.52	7.11 ± 12.65	Sig.
	LDL (mg/dL)	124.14 ± 22.59	132.10 ± 30.25	7.96 ± 31.47	Sig.
	Triglycerides (mg/dL)	110.47 ± 38.75	138.93 ± 85.03	28.46 ± 84.55	Sig.
Clinical chemistry	SGOT (U/L)	21.78 ± 10.37	31.47 ± 11.67	9.69 ± 13.77	Sig.
	SGPT (U/L)	25.21 ± 11.53	28.48 ± 35.22	3.27 ± 35.53	Sig.
	γ-GT (U/L)	26.61 ± 17.47	27.44 ± 31.54	0.83 ± 32.84	NS
	Bilirubin total (mg/dL)	0.51 ± 0.25	0.56 ± 0.29	0.05 ± 0.25	Sig.
	Uric acid (mg/dL)	4.42 ± 1.19	4.42 ± 1.10	0.00 ± 1.12	NS
	Glucose (mg/dL)	98.46 ± 13.42	100.62 ± 27.78	2.16 ± 28.84	NS
Hemostasis	INR	1.01 ± 0.11	0.96 ± 0.26	−0.05 ± 0.27	Sig.

FSH, follicle-stimulating hormone; LH, luteinizing hormone HDL, high-density lipoprotein; LDL, low-density protein; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; γ-GT, gamma-glutamyl transferase; INR, international normalized ratio; Sig., significant; NS, not significant.
[a]Values below the lower limit of quantification were replaced by lower limit of quantification value.
[b]Values above the upper limit of quantification were replaced by upper limit of quantification value.

of the trial subjects at the end of the study (week 52), whereas the ratio increased in 35.2%. Only four women (1%) presented a ratio of more than 3.5, which is considered a cutoff value for increased risk of coronary heart disease.

Clinical chemistry

The results of clinical chemistry parameters, which might have pathophysiological importance in connection with 17β-estradiol deficiency, are shown in Table 4. Despite statistical significance of some of these parameters, a clinical relevance must be denied. The upper limits of noncritical values of SGPT, SGOT, and γ-GT were per protocol defined as three times above the upper limit of normal ranges. Three women showed SGPT values above this noncritical range. Two women had temporarily critical values at visit 5 that had recovered at visit 7. One woman had a critical value at visit 7. Since no follow-up was performed, the critical value was classified as still existing. Only two women had SGOT values above the noncritical range. One woman showed a critical elevation at visit 5 only, whereas the other woman had a critical value at visit 7, which was classified as still existing due to the lack of follow-up. In total, seven women had γ-GT values above the noncritical range. The noncritical range was exceeded on two subsequent measurements (visits 5 and 7) in one patient, whereas five women had critical values only at visit 5, and one woman at visit 7, which was classified as still existing due to lack of follow-up. All changes were reported as nonserious adverse events of moderate intensity and were considered unrelated to study medication. These critical and temporary increases had an impact on the mean values. For the parameters not shown in Table 4 (ie, sodium, potassium, creatinine, alkaline phosphatase, total protein, and overall hematology), no changes were observed.

Hemostasis

No clinically significant changes in the international normalized ratio were observed (Table 4).

MRS II/4-week weighted scores of hot flushes

The intensity of climacteric complaints reflected in the total score of the MRS II (items 1 through 11, Table 2) was significantly reduced by about 50% (Fig. 5). Under treatment with CR BNO 1055, the 4-week weighted score of hot flushes showed a significant decrease of 80.7% compared to baseline (Fig. 6).

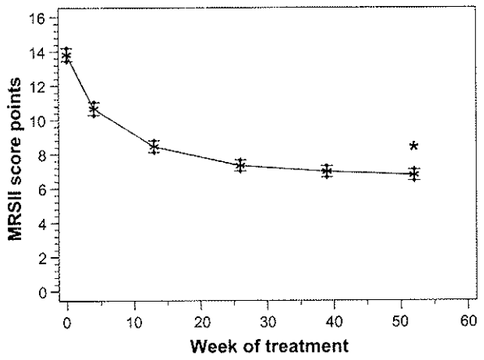

FIG. 5. Menopause Rating Scale II (MRS II), total score (items 1 through 11), and changes from baseline after 4, 13, 26, 39, and 52 weeks of treatment with CR BNO 1055 (daily dose corresponding to 40 mg of herbal drug). Mean ± SEM and *P* value (*significant) of the difference to baseline (Wilcoxon test) are shown.

Adverse events

A total of 752 adverse events (AEs) were recorded. Of those, 414 (55%) were classified as not related to the trial drug and 12 AEs (1.6%) were unclassified. The causal relationship with trial drug was assessed as possible for 318 (42%) and as probable for 8 AEs (1%). The majority (295 reports) of the possibly or probably related AEs were due to increased serum lipids (97, increased triglycerides; 95, increased total cholesterol; 101, increased LDL; 2, decreased HDL). Four gastrointestinal disorders, possibly related to the trial drug, were reported. Seven AEs, which were assessed as possibly or probably related to the trial drug, were reported in the category of reproductive system and breast disorders. Of these seven AEs, one case of false endometrial hyperplasia was documented based on an ultrasound measurement of increased endometrial thickness (9 mm), whereas the histological verification of the endometrium revealed no hyperplasia. Additionally, 20 AEs possibly/probably related to the trial drug were reported for different system organ classes (one, hypothyroidism; four, increased glucose; two, increased γ-GT; one, increased potassium; two, cervical carcinoma stage II; four, hypertension; one, phlebothrombosis; one, allergic dermatitis; two, headache; one, depression; one, influenza).

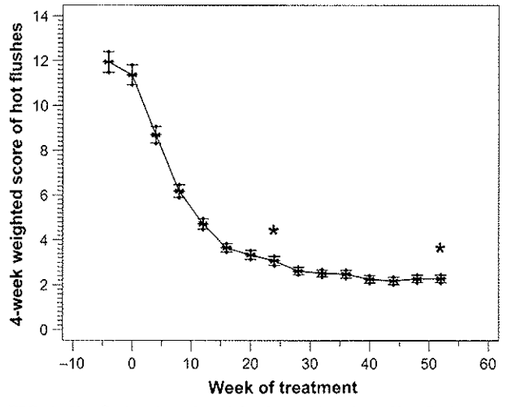

FIG. 6. Hot flushes 4-week weighted score (number of hot flushes per day multiplied by a factor according to severity: severe, 3; moderate, 2; mild, 1; sum of all days of the preceding 4 weeks at each time point and divided by number of days). Changes from baseline, means ± SEMs and *P* values (*significant) of the differences to baseline (Wilcoxon test, Bonferroni correction) are shown.

The intensity of AEs was mild in about 88% of cases, whereas moderate or severe intensity was recorded in 10.5% and 1.9%, respectively.

About 65% of those with AEs recovered without residual effects; in 30%, the AEs persisted at trial end without treatment. None of the 25 premature discontinuations was due to AEs.

Of the 752 AEs, 8 were assessed as serious adverse events (sAEs) and occurred in three women: one planned hospitalization for cholecystectomy due to cholelithiasis; one of the three women had five sAEs, four as a result of an accident (aphakia, eye hemorrhage, eyeball rupture, pathological fracture, humerus fracture) and one due to a prolonged hospitalization because of acute bronchitis; and one patient with the diagnosis of breast cancer during treatment was hospitalized and underwent lumpectomy, which was recorded as a separate sAE. The reason for surgery was a so-called interval tumor with no sign of malignancy on the mammogram performed before enrollment. Because a malignant breast tumor needs to grow for about 7 to 10 years to reach a diagnosable stage, the incidence of breast cancer in this patient can by no means be related to the trial drug. All sAEs were judged to have no causal relationship with the study medication.

DISCUSSION

This was the first time that endometrial safety for a CR extract has been proven. Endometrial biopsy is the gold standard method whenever endometrial safety is concerned. In accordance with the Committee for Proprietary Medicinal Products (CPMP) guidelines *Points to Consider on Hormone Replacement Therapy*,[24] six cases of hyperplasia and/or a more serious adverse endometrial outcome would have been tolerable for the actual trial sample size; however, no such findings were detected during the whole course of the trial. Most important, no signs of stimulatory effect of the CR special extract on endometrial thickness were observed, indicating excellent endometrial safety of the study medication CR BNO 1055.

Postmenopausal atrophic changes of the endometrium are physiological processes. Vaginal bleeding in women with an atrophic endometrium is a known event that might be due to sclerotic degenerative changes of endometrial vessels.[33] Because the number of women with an atrophic endometrium had increased during treatment, the possibility of bleeding may also have increased.

In women with a secretory or proliferative endometrium, nonovulatory cycles in irregular intervals may exist. Resting activity of estradiol could be induced by irregular follicle maturation with subsequent bleeding. Such changes with temporarily increased estradiol levels were seen in 15 women, and this resulted in higher mean estradiol levels when measured during the study. Therefore, the medians of the serum estradiol levels are more meaningful, and this value decreased.

It is well known that exogenous administration of sexual steroids, ie, of estrogens and progestins, can cause an increase in breast density,[34] which is considered one of the risk factors for the development of breast cancer.[7] Some studies reported an increase in the breast density pattern under continuous-combined HT of up to 50%.[35] An increase in breast density is due to proliferation of both the connective and the epithelial breast tissue. Dense breast tissue is considered to be associated with an increased risk of breast cancer.[29,36-38] The mammographic assessment revealed no influence on the breast density pattern in those women in whom a centralized retrospective comparison was performed. The one case of breast cancer that was diagnosed during the treatment period was judged to have no relationship to the study drug.

No clinically relevant changes of hormone levels were observed in the study. No remarkable changes in most of the parameters of clinical chemistry and hemostasis were observed. However, there was a statistically relevant increase in total cholesterol, LDL, SGOT, and SGPT recorded. On the other hand, a desired decrease in the LDL:HDL ratio was observed in 65% of women at the end of the study, with an increase in the LDL:HDL ratio in 35%. In addition, a desired increase of HDL was also observed. Nevertheless, the clinical relevance of all these laboratory findings, both positive and negative, remains questionable. Also, methodological variability in measuring lipids has to be taken into consideration. The internationally available control sera for the given parameters provide for a potentially large deviation range, eg, an international control for cholesterol values assessed as 6.5 mmol/L may vary between 5.24 and 7.86 mmol/L. Likewise, for a triglyceride value of 1.46 mmol/L, the control may vary between 1.3 and 1.6 mmol/L. Some of the unfavorable changes shown in Table 4 might be of statistical significance, yet they can hardly be considered clinically relevant. By the strictest standards, the means and values of individual cases of total cholesterol and LDL at baseline could have already been indicated for therapy with statins (cutoff values),[39] especially in women with risk factors such as positive family history, arterial hypertension, diabetes mellitus, and coronary heart disease.

Of the adverse events, which were classified by the investigators as possibly or probably related to the trial drug, most were due to an increase in total cholesterol, LDL, and triglycerides. Unfortunately, the increases in total cholesterol and LDL were recorded as separate AEs, although they actually are interrelated (amounting for approximately 60% of all possibly/probably related AEs). Virtually all the women were symptom free, and they were neither treated nor referred to specialists. No serious unexpected drug reaction was reported in this study. In addition, no causal relationship to the study drug was reported for any of the sAEs that occurred.

As for the significance of the alleviation changes observed for the MRS II scores and for the reduction in the number and intensity of hot flushes, one might note that some studies reported a statistically significant effect of up to 40% in the placebo groups as well. Despite the lack of a placebo control in this study, the MRS II score reduction of 50% and the reduction of hot flushes of about 80% are promising findings. It must be mentioned that the changes were long term in character, without signs of re-increase or oscillations and without any increasing impact on the SD.

The results obtained for bone markers are interesting. Especially when considering the extract's promising signs of effects on those women with high bone turnover combined with beneficial effects observed in some experimental studies, more research in the field of effects on the quality of bone could be one of the next steps. This could involve methods such as bone microarchitecture, densitometry, and probably even more specific and accurate assessment of combination of bone markers. In a previous clinical study as well as an experimental study in rats, beneficial effects on bone metabolism were observed.[12,40]

Considering the constantly growing population of postmenopausal women with their different needs and symptoms and the potential adverse effects of long-term HT and the newly recognized endometrial effects of phytoestrogens, the application of endometrium-safe CR extract adds an important therapeutic option in alleviating symptoms of estrogen deficiency. Possible prophylactic effects on bone add to the advantageous profile of black cohosh extracts and should be studied in greater detail.

CONCLUSIONS

In the present study with a treatment duration of 12 months, the endometrial safety of a black cohosh preparation, in this case, the special extract CR BNO 1055, was proven for the first time using the gold standard method of biopsy. The absence of an effect on breast density may indicate no increased risk of breast cancer. Additionally, the absence of effects on liver enzymes and hemostasis factor may indicate that there are no safety concerns regarding the liver with this herbal preparation. By the determination of serum surrogate parameters of bone turnover, it may be assumed that CR BNO 1055 has antiresorptive activity. Despite increased total cholesterol and LDL levels, the simultaneous HDL increase may help to avoid an increased risk of coronary heart disease. The unfavorable influence on triglycerides should be further investigated.

The 80% reduction in hot flushes represents beneficial effects on postmenopausal complaints.

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