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Influence of marketed herbal menopause preparations on MCF-7 cell proliferation

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Abstract

Objective: Given the increasing use of alternative menopause treatments, we evaluated the effect of several herbal preparations used for menopause relief on the proliferation of estrogen-sensitive breast cancer cells (MCF-7) as a means of assessing appropriateness for use in women at risk for estrogen-sensitive breast cancer.

Design: An MCF-7 cell culture model, as described previously,[1] was used to evaluate the estrogen-agonist and -antagonist activity of commercially available herbal menopause preparations containing red clover, soy, black cohosh, or a combination of herbs. Each test substance was evaluated for cytotoxic effects before conducting the proliferation assays.

Results: Commercially available products containing soy, red clover, and herbal combinations induced an increase in the MCF-7 proliferation rates, indicating an estrogen-agonistic activity in the absence of estradiol. In contrast, an isopropanolic black cohosh extract (Remifemin Menopause) did not stimulate MCF-7 growth and exerted inhibitory effects on cellular proliferation. None of the tested products enhanced estradiol-induced cell proliferation. The black cohosh preparation and one of the herbal combinations exhibited strong estrogen-antagonistic effects.

Conclusions: The lack of proliferative effects of isopropanolic black cohosh extract on estrogen-sensitive breast cancer cells in vitro suggests a favorable safety profile for use in women with a history of breast cancer. Alternatively, preparations containing red clover, soy, and combinations of various herbal ingredients may induce cell proliferation, suggesting that such herbal preparations should be used with caution in the treatment of menopause symptoms in women at risk for, or with a history of, estrogen-sensitive breast cancer.

Keywords: Black cohosh – Soy – Red clover – Breast cancer – Menopause – Herbal therapy.

The association between breast cancer and estrogen has been widely documented in the scientific literature.[2] Despite the link between estrogen use and risk of breast cancer, hormone therapy (HT), which includes estrogen and progestin supplementation, has been a popular treatment for menopause, a naturally occurring state of hormone depletion. The U.S. Preventive Services Task Force recently published a recommendation against the routine use of estrogen and progestin for the prevention of chronic conditions in postmenopausal women based, in part, on an associated risk of breast cancer.[3] In light of the recent findings regarding HT and increased cancer risk, many women are turning to alternative approaches.

Alternative therapies that have been used for menopause symptom relief include herbal preparations of black cohosh (*Cimicifuga racemosa*), red clover (*Trifolium pratense*), chaste tree berry (*Vitex agnus-castus*), and other herbs[4] or preparations containing soy isoflavones. Clinical support for the effectiveness of these ingredients varies greatly, with the most positive evidence available for the isopropanolic extract of black cohosh (Remifemin Menopause) and for soy-containing products.[5] Despite the varying degrees of scientific evidence supporting the efficacy of herbs, there is an increase in use of alternative herbal therapies

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TABLE 1. *Commercial herbal preparations*

Herb	LOT#	Active ingredients	Daily dose
Soy	HW/30C201 HW/30C203	320 mg soy extract (55 mg isoflavones and >15% saponins)	1 tablet
Red clover	PM/9KV0442 PM/9HV0578	Red clover leaf extract (40 mg isoflavone phytoestrogens)	1 tablet
Black cohosh	REM/906931	0.018–0.026 mL isopropanolic extract (40 vol.-%) from black cohosh root corresponding to 20 mg black cohosh root	2 tablets
Combination I	EV/5004H0 EV/6540K0	50 mg purified isoflavones (from pueraria lobata root and non-GMO soybean standardized extract; 100 mg kava kava root standardized extract (*Piper methysticum*); 40 mg black cohosh (*Cimicifuga racemosa*) root	1 tablet
Combination II	NP/00H704	Soy protein concentrate, standardized kudzu extract (root), standardized red clover extract (leaf, provides 80 mg isoflavone), 410 mg; standardized chastetree extract (berry), 75 mg; standardized black cohosh (*Cimicifuga racemosa*) extract (root) 40 mg	1 tablet

GMO, genetically modified organism.

for treatment of menopause symptoms, particularly in women with breast cancer.[6] Considering such widespread use, it is important to evaluate the potential of herbal preparations to exert estrogen-like effects and potentially increase the risk of estrogen-sensitive cancers.

A previous study conducted in our laboratory[1] evaluated the effects of an isopropanolic extract of black cohosh (Remifemin) on the proliferation of MCF-7 cells. Results suggest the commercially available black cohosh extract does not exert an estrogen-agonistic effect on estrogen receptor-positive breast cancer cells and, therefore, might be a safe alternative to HT in menopausal women with estrogen-sensitive disorders.

Based on these findings and the growing popularity of alternative therapies for treatment of menopausal symptoms, the current study was designed to test various commercially available herbal menopause preparations for their potential estrogen-agonistic and -antagonistic activity. Using the same experimental methods as our previous study,[1] the experiments were performed using MCF-7 cells, an established in vitro estrogen-dependent mammary tumor model.[7,8] In this model, the presence of estrogen or estrogen-like substances increases the proliferation rate of MCF-7 cells. Using rate of cell proliferation as a measure of estrogenic activity, we evaluated the activity of various commercially available herbal preparations containing soy, black cohosh, red clover, and two combinations of multiple herbs.

METHODS

Herbal preparations and extracts

Commercially available products were purchased at local retail outlets. The various herbal preparations, active ingredients, and lot numbers that were tested in the MCF-7 assay are outlined in Table 1. From each herbal preparation, an amount corresponding to the recommended daily dose was extracted with 10 mL isopropyl alcohol (40% V/V) for 30 minutes in an ultrasonic bath. The extracts were then filtrated through a 0.2 μm pore-size membrane filter and stored at 4°C.

Cells

Estrogen receptor-positive (ER^+) MCF-7 cells were purchased from American Type Culture Collection (ATCC HTB 22, Manassas, VA). They were maintained in Eagle's MEM with nonessential amino acids, 1 mM sodium pyruvate, 10 μg/mL insulin, 10% FCS, and antibiotics. HeLa cells (epithelial cervix carcinoma cells) used in the toxicity assay also were obtained from American Type Culture Collection.

Toxicity assay

To determine the cytotoxicity of the individual test substances, a fluorescence assay was performed using 4-MeUH as a fluorogenic substrate for cellular esterases. This substrate is nonfluorescent until taken up and cleaved by esterases in living cells. The toxicity assay was performed using MCF-7 cells (in complete as well as estrogen-deprived medium) and HeLa cells, as described earlier.[1] MCF-7 cells were plated at an initial cell density of 1.2×10^5 cells per well in Eagle's MEM without phenol red, supplemented with nonessential amino acids, 1 mM sodium pyruvate, 10 μg/mL insulin, and 5% FCS or charcoal-stripped FCS (CSF, Sigma C-1969). After incubation at 37°C and 5% CO_2 for 24

TABLE 2. *Cytotoxicity of tested extracts*

	Noncytotoxic dilution range[a]		
		MCF-7 cells	
Test extract[b]	HeLa cells	+ Estradiol	− Estradiol
HW/Lots: 30C201; 30C203	1:100-1:51,200[c]	1:100-1:51,200[c]	1:100-1:51,200[c]
PM/Lot 9KV0442	1:200-1:51,200[d]	1:100-1:51,200[c]	1:400-1:51,200[d]
PM/Lot 9HV0578	1:400-1:51,200[d]	1:100-1:51,200[c]	1:400-1:51,200[d]
NP/Lot 00H704	1:100-1:51,200[c]	1:100-1:51,200[c]	1:200-1:51,200[d]
REM/Lot 906931	1:100-1:51,200[c]	1:100-1:51,200[c]	1:100-1:51,200[c]
EV/Lot 5004H0	1:100-1:51,200[c]	1:200-1:51,200[d]	1:400-1:51,200[d]
EV/Lot 6540K0	1:100-1:51,200[c]	1:100-1:51,200[c]	1:200-1:51,200[d]

HeLa cells, epithelial cervix carcinoma cells; MCF-7 cells, estrogen-sensitive breast cancer cells.
[a]Dilutions given in the table represent the noncytotoxic dilution range.
[b]Tested dilution range, 1:100–1:51,200.
[c]Lower or higher dilutions have not been tested.
[d]Lower dilutions are cytotoxic.

hours, extracts were diluted in cell culture medium and added starting at a 1/100 dilution. The microtiter plates were incubated for an additional 48 hours and then centrifuged at 800 rpm for 10 minutes. The supernatants were removed and 200 μL 4-MeUH (0.1 mg/mL in PBS) were added per well. After 120 minutes, the fluorescence units per well were measured in a microtiter plate fluorometer (Fluoroskan II).

Proliferation assay with MCF-7 cells

To obtain estrogen-deprived conditions in the proliferation assay, the FCS in the cell culture medium was replaced by charcoal-stripped FCS (CSF, Sigma C-1696). A MCF-7 cell suspension (200 μL), adjusted to 5×10^4 cells/mL in test cell culture medium (Eagle's MEM without phenol red and supplemented with nonessential amino acids, 10 μg/mL insulin, 1 mM sodium pyruvate, and 5% CSF), was plated in 96-microtiter plates and incubated at 37°C and 5% CO_2 for 24 hours. After incubation, the supernatants were removed, and 150 μL fresh culture medium without insulin was added. The test extracts were diluted (6 dilution steps 1:10) in test cell culture medium without insulin and pipetted in six parallels at 50 μL/well. After 2 days of incubation (as described above), cells were pulsed with 25 μL/well [6-^{3}H]Thymidine (spec. activity 2 Ci/mMol, 0.25 μCi/well) for 8 hours. Cells were harvested according to standard methods (Cell Harvester Inotech) onto glass fiber filters and counted in a liquid scintillation counter (Wallac). This method of cell quantification is an established method of measuring *de novo* DNA synthesis.[9] As control substances, the cell culture medium and corresponding solvent dilutions were tested simultaneously. An estrogen-agonistic control substance, 17β-estradiol, was dissolved in dimethyl sulfoxide and diluted in cell culture medium to 10^{-7}M.

For evaluation of estrogen-antagonistic effects of each herbal preparation, the test design was altered by addition of constant amounts of estradiol (10^{-7}M) to each test extract dilution. Tamoxifen, a known estrogen-antagonist, was added to the cell culture at a concentration of 10^{-5}M as a positive control for estrogen-antagonistic effects.

To fully understand the effect of black cohosh on cell proliferation, isopropanol, the liquid used in the extraction process, was evaluated in the MCF-7 model. Isopropanol was prepared and filtered in the same way as the black cohosh extract and tested with and without estradiol.

Statistical analysis

The results were expressed as mean ± SD. The pharmacological data from the proliferation assays were analyzed by the Student's *t* test, and significance was assumed at $P < 0.05$.

RESULTS

Toxicity assay

The results of the cytotoxicity assays on HeLa and MCF-7 cells are summarized in Table 2. The extracts were tested at a dilution series from 1:100-1:51,200. Solvent controls were performed in each assay. Isopropyl alcohol at the maximal concentration tested had no cytotoxic effect on either cell type.

The soy (HW/30C201; HW/30C203) and black cohosh (REM/906931) extracts showed no cytotoxic activity on MCF-7 and HeLa cells in the tested dilution range from 1:100-1:51,200. Red clover (PM/9KV0442;

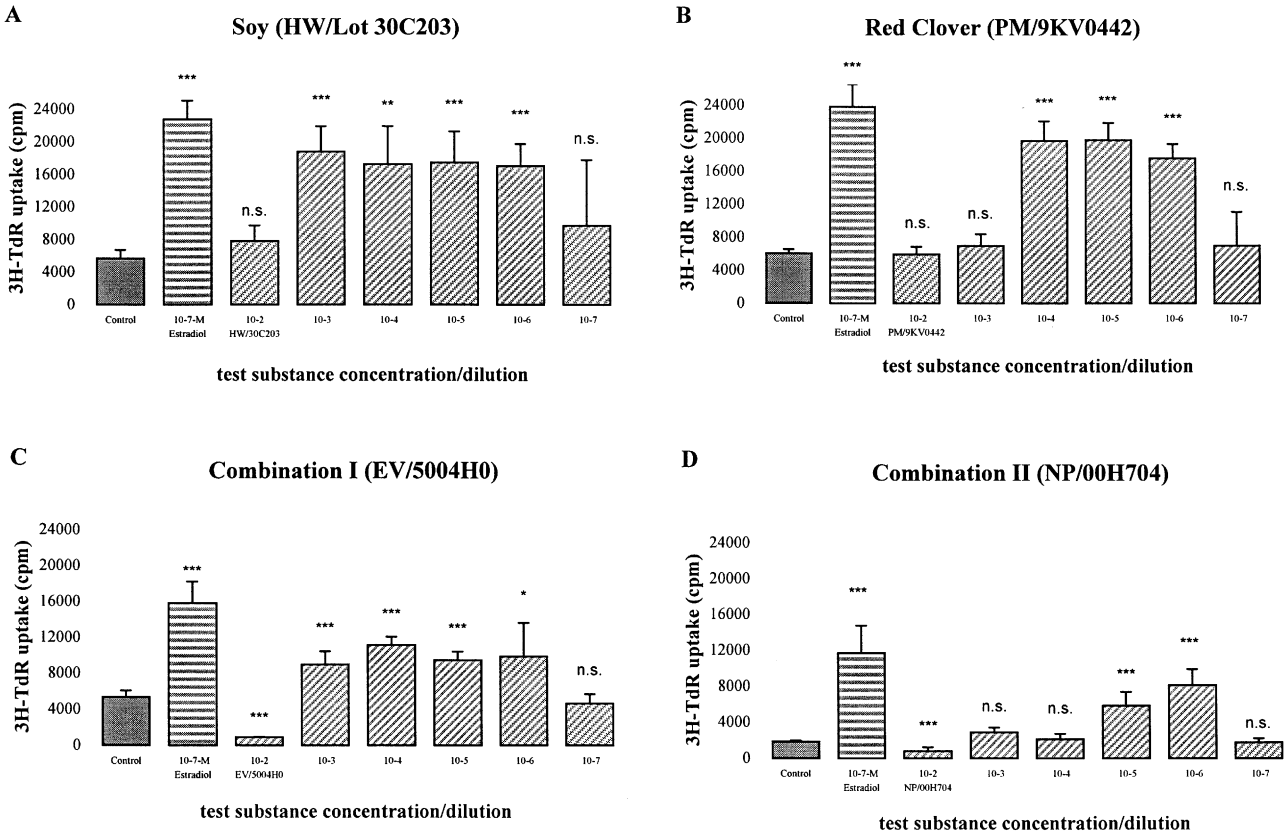

FIG. 1. Tested herbals inducing MCF-7 cell proliferation. **A**: Soy (HW/30C203); **B**: Red clover (PM/9KV0442); **C**: Herbal Combination I (EV/5004H0); **D**: Herbal Combination II (NP/00H704). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus medium controls (Student's *t* test). n.s., not significant.

PM/9HV0578) was cytotoxic at the highest concentrations (dilutions 1:100 and 1:200), and Combination II (NP/00H704) exerted toxic effects at the 1:100 dilution in MCF-7 cells only. Similarly, Combination I extract (EV/5004H0; EV/6540K0) showed no toxic effects on HeLa cells, but exerted mild, although inconsistent, cytotoxic effects on MCF-7 cells. One lot of Combination I (EV/5004H0) showed a slightly stronger cytotoxic activity than the other lot of the same combination (EV/6540K0).

Proliferation assay

The various herbal extracts were tested at a dilution series ranging from 10^{-2} to 10^{-7} in the MCF-7 proliferation assay.

Soy (HW/30C201; HW/30C203) induced significant stimulation of the MCF-7 cells. Both lots exhibited a strong proliferation increase overall dilutions from 10^{-3} to 10^{-6}, reaching incorporation rates of about 18,000 cpm (control rates: medium: 5,700 cpm; estradiol 10^{-7}M: 23,000 cpm) (Fig. 1a). Dilution of 10^{-2} did not influence proliferation in either lot, and the dilution of 10^{-7} exhibited decreased proliferation-enhancing activity. At the 10^{-5} dilution, the two soy lots exerted different proliferative effects (data not shown), an exception that was interpreted as a test artifact because the incorporation rates induced by the other dilutions of both lots were in the same range.

Red clover (PM/9KV0442; PM/9HV0578) also exerted proliferation-enhancing activity. The two lots induced significant enhancement of MCF-7 proliferation at dilutions from 10^{-4} to 10^{-6} (Fig. 1b).

Herbal Combination I induced significant stimulation of the MCF-7 cell proliferation at dilutions of 10^{-3} to 10^{-6} (Fig. 1c). For both lots (EV/5004H0; EV/6540K0), there was a decrease in the proliferation rate at the 10^{-2} dilution, a result that could be contributed to the cytotoxic activity observed for both lots in this dilution range.

The herbal Combination II (NP/00H704) also demonstrated proliferation-stimulating activity on MCF-7 cells in dilutions of 10^{-5} and 10^{-6}; however, dilutions of 10^{-3}, 10^{-4}, and 10^{-7} had no significant influence on

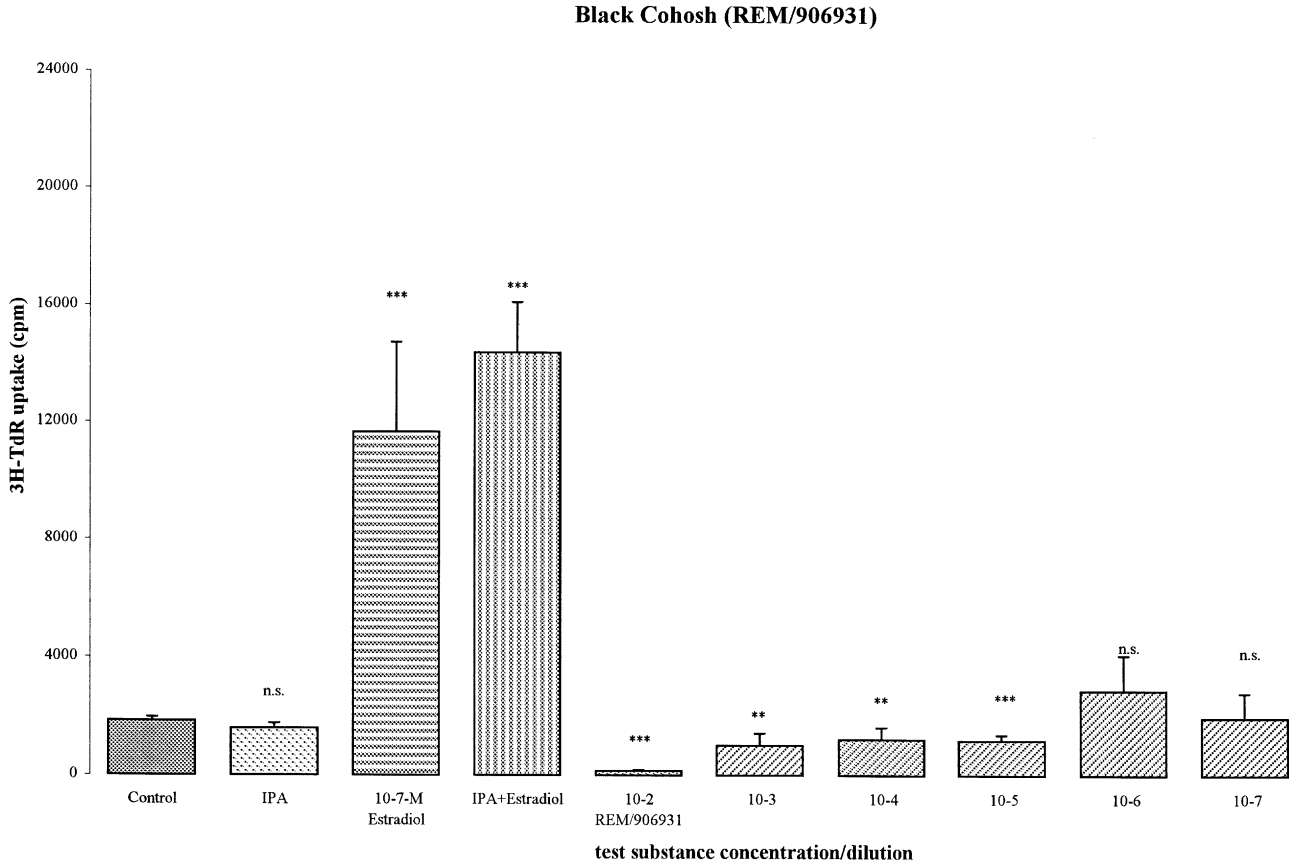

FIG. 2. Tested herbals inhibiting MCF-7 cell proliferation: black cohosh (Remifemin). IPA, isopropanol. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus medium controls (Student's *t* test). n.s., not significant.

the proliferation rates (Fig. 1d). At a dilution of 10^{-2}, Combination II (NP/00H704) induced a highly significant decrease in the incorporation rates, a result that may be due to a cytotoxic effect, as observed in the toxicity assays.

In contrast to soy, red clover, and the herbal combinations, the isopropanolic extract of black cohosh (REM/906931) did not increase cell proliferation (Fig. 2). This extract not only failed to increase the proliferation of MCF-7 cells but actually significantly inhibited proliferation at dilutions between 10^{-2} and 10^{-5}. A cytotoxic effect can be excluded because the preparation showed no toxic effects in the toxicity assay. Higher extract dilutions had no further significant influence on the proliferation rates. The isopropanol control did not influence the proliferation rate of the cells nor the proliferation-inducing effect of estradiol, suggesting the noted effects are due to the activity of black cohosh and not the extraction solvent.

Tests for estrogen-antagonistic effects demonstrate that none of the tested extracts acted synergistically with estradiol on MCF-7 proliferation and all extract preparations reduced the estrogen-induced proliferation at a dilution of 10^{-2}. The strongest estrogen-antagonistic activity was demonstrated in assays of Combination II (NP/00H704) and the isopropanolic black cohosh product (REM/906931). Combination II (NP/00H794) reduced the estrogen effect by 93% and 59% in dilutions of 10^{-2} and 10^{-3}, respectively. In dilutions of 10^{-4} to 10^{-7}, there was also a slight reduction in the estrogen-induced proliferation rates observed (Fig. 3). Comparable results were found for the black cohosh extract (REM/906931). Dilutions from 10^{-2} to 10^{-6} reduced the estrogen-induced proliferation rates. The estradiol-induced increase (21,000 cpm) was reduced by 92% (to 1,700 cpm) at a dilution of 10^{-2}, and by 60% (to 8,535 cpm) at a dilution of 10^{-3} (Fig. 4).

Soy showed a strong estrogen-antagonistic activity only at the 10^{-2} dilution. The estrogen effect was antagonized by about 65% (data not shown). Similar results were found for red clover, with the estrogen-induced incorporation rate reduced by 77% at the 10^{-2} dilution (data not shown). Likewise, neither extract of Combination I (EV/5004H0; EV/6540K0) acted syner-

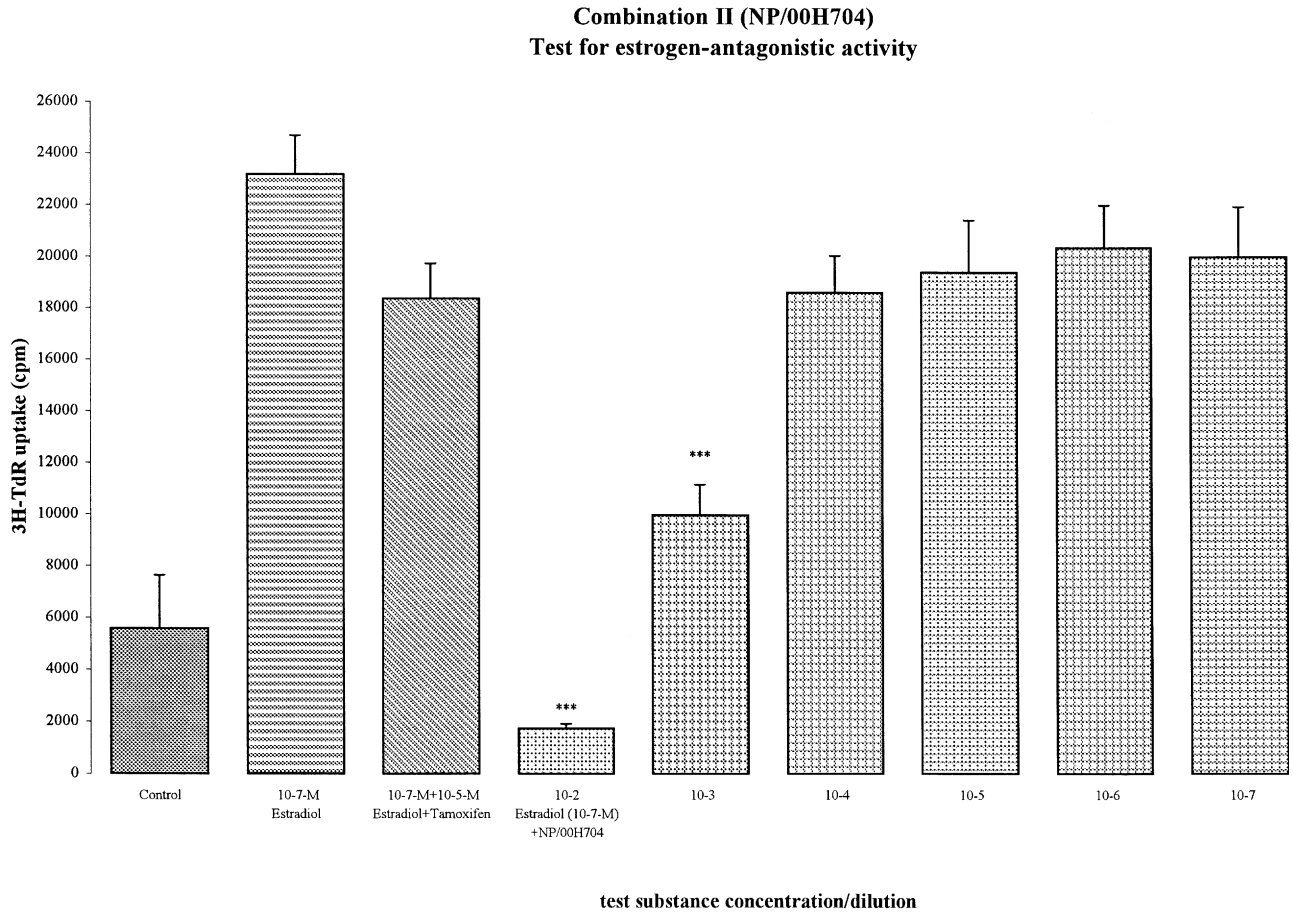

FIG. 3. Estrogen-antagonistic activity of Herbal Combination II (NP/00H704). *** $P < 0.001$ versus estradiol controls (Student's *t* test).

gistically with estradiol on MCF-7 proliferation, but both batches significantly ($P < 0.05$ vs estradiol control) reduced the estrogen-induced proliferation at a dilution of 10^{-2} (data not shown). It should be noted that red clover and the herbal Combination I showed some cytotoxic activity in this dilution range, so it is unclear whether the noted estrogen-antagonistic effects are the result of this toxic activity.

DISCUSSION

The growing use of herbal therapies for treatment of menopause symptoms necessitates study of the safety of these natural preparations. In light of the increasing interest in alternative therapies, The North American Menopause Society published a consensus opinion suggesting that, whereas phytoestrogens for the treatment of menopause symptoms are generally safe, additional research on their safety in particular subgroups of women—particularly women who have had breast cancer—is warranted.[10,11]

Black cohosh, an effective alternative therapy for treatment of menopause symptoms, does not contain classic isoflavonoid phytoestrogens.[12-14] Although it has been shown that constituents of black cohosh bind to the estrogen receptor, recent investigations suggest that its effects differ from a general estrogen-like pathway or a mechanism characteristic of classic isoflavonoid phytoestrogens.[13,15-17] Despite early reports of isoflavone formononetine in black cohosh, recent analyses have failed to detect this constituent.[15,17] Studies suggest that black cohosh does not have estrogenic effects on uterus weight or vaginal cytological parameters in mice.[16] In addition, and contrary to estrogen effects, black cohosh did not stimulate the growth of dimethyl-benzanthracene-induced mammary tumors in a study in ovariectomized rats and did not influence uterus weight and estrogen-sensitive serological parameters including luteinizing hormone, follicle-stimulating hormone, and prolactin.[13,19] The most recent clinical studies also clearly demonstrate

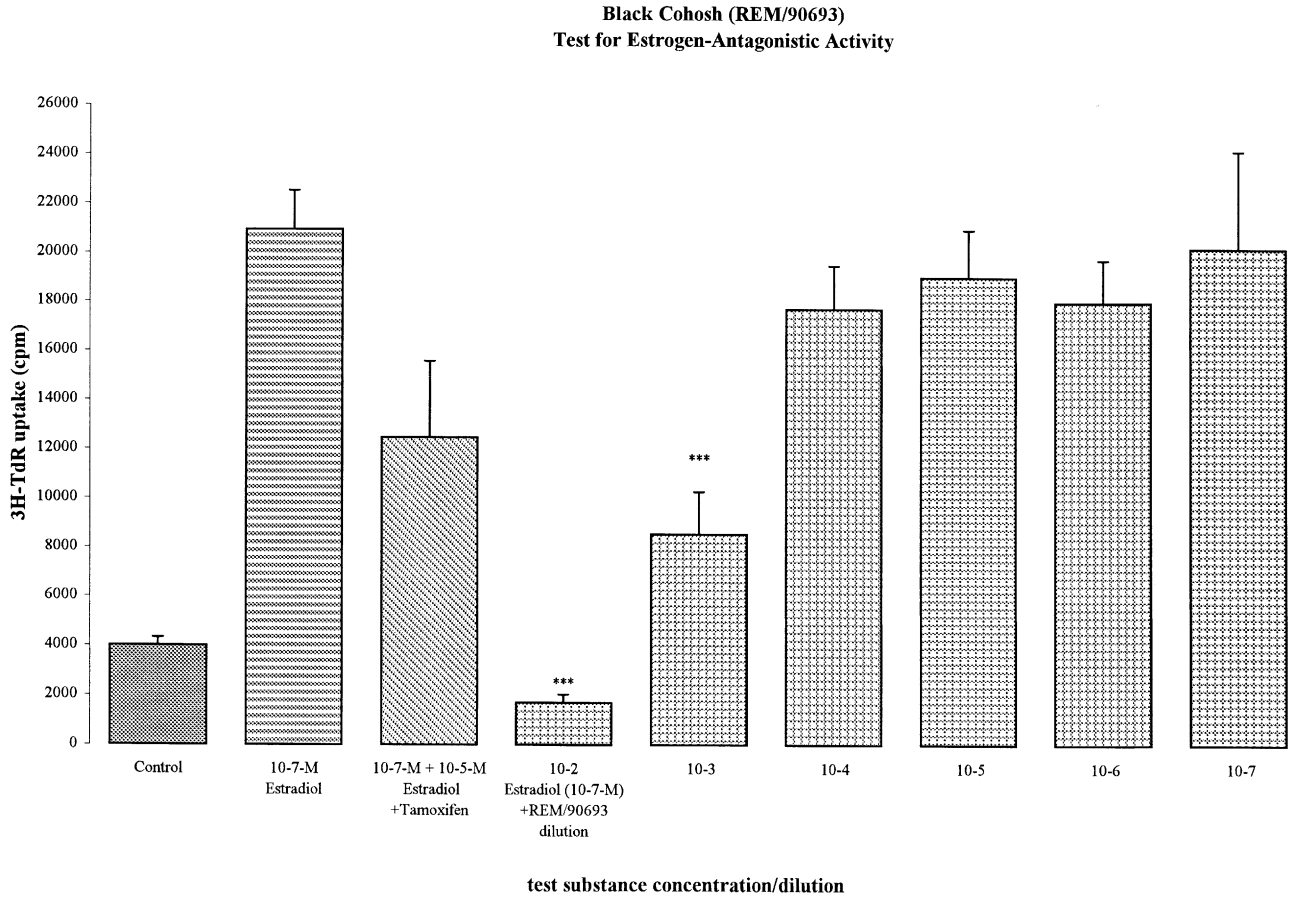

FIG. 4. Estrogen-antagonistic activity of black cohosh (Remifemin). *** $P < 0.001$ versus estradiol controls (Student's *t* test).

black cohosh's nonestrogenic influence on vaginal, cytological, and other estrogen-sensitive hormonal and serological parameters.[13,14] Taken together, all in vitro, in vivo, and clinical findings lead to the hypothesis that black cohosh acts as a "phyto-SERM," or selective estrogen receptor modulator, producing either estrogen-agonistic or -antagonistic effects, depending on the precise function and location of the target tissue.[13]

Previous work from our laboratory has demonstrated the lack of an estrogenic effect of black cohosh on estrogen-responsive (MCF-7) breast cancer cell proliferation.[1] The lack of a "systemic" estrogenic activity of black cohosh has been further demonstrated in a variety of other in vitro,[18] in vivo,[16,19] and clinical[12,14,20-22] study models.

Other herbal extracts commonly used for treating menopause symptoms may have a general estrogen-like activity. Methanolic extracts of red clover and chasteberry, for example, have significant competitive binding affinities to both α and β estrogen receptors.[23] Similarly, soy and red clover have extremely high estrogen receptor-binding affinity compared with more than 150 herbs used for a variety of health conditions.[24] Red clover also exhibits high progesterone receptor-binding activity,[24] thus mimicking the activity at the receptor level of estrogen/progesterone therapy. Being phytoestrogens, soy and red clover act through an estrogenic mechanism and may be associated with side effects similar to estrogen,[23] including an increased risk of endometrial, ovarian, and breast cancers.

To further evaluate the effects of herbal preparations on estrogen-sensitive tissues and to build upon our previous finding of the lack of estrogenic effects of black cohosh on MCF-7 cell proliferation,[1] we evaluated the in vitro estrogenic activity of red clover, soy, black cohosh, and herbal combinations from commercially available products. As described in our previous study, the human breast cancer model used in this study is an established system for detection of both estrogen-agonistic and -antagonistic effects.[1,7,8]

In evaluating the estrogen-agonistic effects, each product was tested in various dilutions under estrogen-deprived conditions. Under this system, an increase in cell proliferation indicates an estrogenic effect. In es-

trogen-deprived conditions, the preparations of soy, red clover, and the herbal combinations induced a strong increase in the MCF-7 proliferation rates, indicating estrogen-agonistic activity. The strongest effects were noted with soy, which increased the proliferation rates over a wide dilution range (10^{-3} to 10^{-6}), and red clover. In addition, the herbal combinations (I and II) showed estrogen-agonistic effects.

In contrast to the soy, red clover, and herbal combination preparations, the commercially available isopropanolic black cohosh preparation did not increase the proliferation of MCF-7 cells. In fact, the extract significantly inhibited cell proliferation.

To evaluate the estrogen-antagonistic effects of the herbal extracts used in this study, we studied the effects on an estrogen-induced increase in MCF-7 cell proliferation. In this test, dilutions of the test extract were applied to a constant estradiol exposure (10^{-7}M). As a control, we co-incubated cells with estradiol and tamoxifen, a known estrogen-antagonist commonly used for treatment of breast cancer. This control, which reduced the estrogen-induced proliferation, served as a comparison for estrogen-antagonistic effects of the herbal extracts.

In the tests for estrogen-antagonistic effects, none of the tested products acted synergistically with estradiol on MCF-7 proliferation, and all reduced the estrogen-induced proliferation at a dilution of 10^{-2}. The extracts from the commercially available preparations of black cohosh (REM/906931) and Combination II (NP/00H704) showed the strongest estrogen-antagonistic activity, reducing the estrogen-induced incorporation rates in dilutions from 10^{-2} to 10^{-3}.

Our evaluation of various commercially available herbal products commonly used for the treatment of menopause symptoms provides important data concerning the estrogen-like activity of these herbal extracts. Results from this study support in vitro[1,25] and in vivo[19] study findings suggesting that isopropanolic extracts of black cohosh do not exert estrogen-agonistic effects on estrogen-sensitive breast tissue. In contrast to the findings with black cohosh, our results demonstrate that products containing red clover, soy, and combinations of various herbal products containing isoflavonoid phytoestrogens may induce cell proliferation in estrogen-sensitive tissues.

The clinical significance of isoflavonoid phytoestrogens in the development of breast cancer, however, is unknown. In vitro and in vivo studies evaluating the estrogenic effects of genistein, an isoflavonoid phytoestrogen found in soy and red clover, on breast cancer cells have shown inconsistent results.[26,27] Different test conditions, particularly the absence or presence of estradiol in the test model, may be one reason for the variability of published results.[25] The majority of in vitro data demonstrate that genistein in concentrations less than 10 μM stimulates the proliferation of estrogen receptor-positive breast cancer cells in steroid-depleted culture conditions, whereas concentrations greater than 10 μM induce antiproliferative effects.[27-29] This biphasic effect of proliferative and antiproliferative activity may be attributed to differential effects of genistein at high and low concentrations. At low concentrations, which may be considered physiological levels, genistein exerts estrogen-like effects. However, at high concentrations, genistein exerts nonestrogen receptor-mediated effects, such as inhibiting the activity of mediators involved in the control of cell signal transduction processes.[26,27] Studies also suggest that in the presence of estradiol, genistein antagonizes the stimulatory effects of estradiol on breast cancer cell proliferation by competing with estradiol for estrogen receptor-binding.[30,31] The findings suggest that the promotion or prevention of breast cancer by isoflavonoid phytoestrogens depends on their concentration and the absence or presence of estradiol. This could have relevance for clinical application in the postmenopausal stadium, when estradiol levels are low and the estrogen-agonistic activity of phytoestrogens might be given special emphasis.

CONCLUSIONS

Based on the potential estrogen-like activity of soy and red clover, caution is warranted when using these herbal preparations for the treatment of menopause symptoms in estrogen-sensitive women.[32] Black cohosh, however, does not contain classical phytoestrogens, and our data suggest that it exerts its antiproliferative effect irrespective of the estradiol status. These data support previously reported in vitro, in vivo, and clinical trial data that suggest that black cohosh extract preparations, such as the commercially available Remifemin formulation tested in this study, may be a safe alternative to estrogen-replacement therapies. It should be noted, however, that additional studies are necessary to completely understand the mechanism of action of black cohosh and its reported effects.

In light of evidence that black cohosh and products containing isoflavones (soy and red clover) are the most effective herbal alternatives for treatment of menopause symptoms,[5] data from this study could be taken into consideration to assist in decision-making on herbal therapy use by women at risk for estrogen-sensitive breast cancer.

REFERENCES

1. Bodinet C, Freudenstein J. Influence of cimicifuga racemosa on the proliferation of estrogen receptor positive human breast cancer cells. *Breast Cancer Res Treat* 2002;76:1-10.
2. Clemons M, Goss P. Estrogen and the risk of breast cancer. *N Engl J Med* 2001;344:276-285.
3. U.S. Preventive Services Task Force. Postmenopausal hormone replacement therapy for primary prevention of chronic conditions: recommendations and rationale. *Ann Intern Med* 2002;137:834-839.
4. Fugh-Berman A, Kronenberg F. Herbs used for menopausal symptoms. *Alternative Therapies in Women's Health.* September, 2002:65-68.
5. Kronenberg F, Fugh-Berman A. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. *Ann Intern Med* 2002;13:805-813.
6. Newton KM, Buist DS, Keenan NL, Anderson LA, LaCroix AZ. Use of alternative therapies for menopause symptoms: results of a population-based survey. *Obstet Gynecol* 2002;100:18-25.
7. Schwarte AE. Wirkspektrum ausgewählter Flavonoide auf die humane Brustkrebszellinie MCF-7. Dissertation, Universität Witten-Herdecke, 1994.
8. Jones PA, Baker VA, Irwin A, Earl LK. Modulation of MCF-7 cell proliferative response by manipulation of assay conditions. *Toxicol In Vitro* 1997;11:769-773.
9. Diel P, Smolnikar K, Michna H. In vitro test systems for the evaluation of the estrogen activity of natural products. *Planta Med* 1999; 65:197-203.
10. Eden, JA. Herbal medicines for menopause: do they work and are they safe? *Med J Aust* 2001;174:63-64.
11. The role of isoflavones in menopausal health: consensus opinion of The North American Menopause Society. *Menopause* 2000;7:215-229.
12. Stoll, W. Phytopharmacon influences atrophic vaginal epithelium: double-blind study: Cimicifuga versus estrogenic substances [in German]. *Therapeutikon* 1987;1.
13. Boblitz N, Liske E, Wüstenberg, P. Black cohosh. *DAZ* 2000;24: 107-114.
14. Liske E, Hänggi W, Henneicke-von Zepelin H, Boblitz N, Wüstenberg P, Rahlfs V. Physiologic investigation of a unique extract of black cohosh (cimicifugae racemosae rhizoma): a 6-month clinical study demonstrates no systemic estrogenic effect. *J Womens Health Gend Based Med* 2002;11:163-174.
15. McCoy J, Kelly W. Survey of Cimicifuga racemosa for phytoestrogenic flavonoids. Presented at: National Meeting of the American Chemical Society; August 25-29, 1996; Orlando, FL.
16. Einer-Jensen N, Zhao J, Andersen KP, Kristoffersen K. Cimicifuga and melbrosia lack oestrogenic effects in mice and rats. *Maturitas* 1996;25:149-153.
17. Struck D, Tegtmeier M, Harnischfeger G. Flavones in extracts of Cimicifuga racemosa. *Planta Med* 1997;63:289-290.
18. Zierau O, Bodinet C, Kolba S, Wulf M, Vollmer G. Antiestrogenic activities of cimicifuga racemosa extracts. *J Steroid Biochem Mol Biol* 2002;80:125-130.
19. Freudenstein J, Dasenbrock C, Nisslein T. Lack of promotion of estrogen-dependent mammary gland tumors in vivo by an isopropanolic Cimicifuga racemosa extract. *Cancer Res* 2002;62:3448-3452.
20. Maamari R, Schreiber A. Cimicifuga racemosa and quality of life. Presented at: Annual Meeting of The North American Menopause Society; October 4-5, 2001; New Orleans, LA.
21. Lehmann-Willenbrock E, Riedel H. Clinical and endocrinological examinations concenring therapy of climacteric symptoms following hysterectomy with remaining ovaries [in German]. *Zent Bl Gynakol* 1998;110:611-618.
22. Georgiev DB, Iordanova E. Phytoestrogens: the alternative approach. *Maturitas* 1997;27(Suppl):273.
23. Liu J, Burdette JE, Xu H et al. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J Agric Food Chem* 2001;49:2472-2479.
24. Zava DT, Dollbaum CM, Blen M. Estrogen and progestin bioactivity of foods, herbs, and spices. *Proc Soc Exp Biol Med* 1998;217: 369-378.
25. Dixon-Shanies D, Shaikh N. Growth inhibition of human breast cancer cells by herbs and phytoestrogens. *Oncol Rep* 1999;6:1383-1387.
26. Messina MJ, Loprinzi CL. Soy for breast cancer survivors: a critical review of the literature. *J Nutr* 2001;131(Suppl 11):3095S-3108S.
27. Bouker KB, Hilakivi-Clarke L. Genistein: does it prevent or promote breast cancer? *Environ Health Perspect* 2000;108:701-708.
28. Hsieh CY, Santell RC, Haslam SZ, Helferich WG. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. *Cancer Res* 1998; 58:3833-3838.
29. Wang C, Kurzer MS. Effects of phytoestrogens on DANN synthesis in MCF-7 cells in the presence of estradiol or growth factors. *Nutr Cancer* 1998;31:90-100.
30. Zava DT, Duwe G. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. *Nutr Cancer* 1997;27:31-40.
31. Miodini P, Fioravanti L, Di Fronzo G, Cappelletti V. The phytooestrogens genistein and quercetin exert different effects on oestrogen receptor function. *Br J Cancer* 1999;80:1150-1155.
32. de Lemos ML. Effects of soy phytoestrogens genistein and daidzein on breast cancer growth. *Ann Pharmacother* 2001;35:1118-1121.