Chemopreventive Potential of Black Cohosh on Breast Cancer in Sprague-Dawley Rats

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Abstract. *Background/Aim: This study examines the chemopreventive potential and action of the herb black cohosh on Sprague-Dawley rats. Materials and Methods: Female Sprague-Dawley rats were treated with an extract of black cohosh enriched in triterpene glycosides (27%) at 35.7 (Group I), 7.14 (Group II), 0.714 (Group III) or 0 mg/kg b.w. for 40 weeks starting from 56 weeks of age and the incidence of benign and malignant mammary tumors was determined at the end of observation. Results: Among female rats treated at 35.7 and 7.14 mg/kg b.w. there was a dose-related reduction ($p<0.05$) of the incidence of mammary adenocarcinomas when compared to the treatment of 0.714 mg/kg b.w., with a protection index (calculated relative to the group III; PI=[total tumours ×100 animals of group III] – [total tumours ×100 animals of the group I (or group II)]/ [total tumours of group III] ×100) for mammary adenocarcinomas of 87.5 and 48.8%, respectively. Black cohosh reduced Ki-67 and cyclin D1 protein expression in fibroadenomas, by immunohistochemistry. Conclusion: Our results suggest that black cohosh may have chemopreventive potential for mammary cancer.*

The herb black cohosh (*Actaea racemosa* L., Ranuncleaceae) is a North American perennial that Native Americans have used for hundreds of years to treat gynecological, menopausal and inflammatory conditions. It also has a history of use for rheumatism, arthritis and muscle pain. In recent years, it has been used as an alternative to hormone replacement therapy to lessen the symptoms of menopause (1).

Active components in black cohosh include the triterpene glycosides and phenylpropanoids. Of more than 62 triterpene glycosides present in black cohosh (2), the triterpene glycosides actein and 23-epi-26-deoxyactein (3) constitute about 6.4% of an n-butanolic fraction of black cohosh enriched for triterpene glycosides (27%); the phenylpropanoid isoferulic acid is present at about 1.8% (4).

Studies indicate that extracts of black cohosh and isolated components inhibit the growth of human breast cancer cells (1, 5, 6), but the mechanism of action has not been defined. The growth-inhibitory effect of actein (6) and a MeOH extract of black cohosh (5) on human breast cancer cells is associated with activation of stress response pathways (7), by gene expression analysis. These agents induced two phases of the integrated stress response, either the survival or the apoptotic phase (8), depending on the duration of treatment, and for actein, also on the dose of treatment.

Case-control and animal studies substantiate the *in vitro* findings of the anticancer and chemopreventive potential of black cohosh. Rebbeck *et al.* (9) used a population-based case-control study of women to show that black cohosh extracts and Remifemin (an isopropanolic black cohosh extract) appear to reduce the incidence of breast cancer, in particular of progesterone receptor (PR)-positive tumors. A recent German case-control study (10) indicated that use of Remifemin/Remifemin Plus is weakly inversely related to incidence of invasive breast cancer, independent of estrogen receptor (ER) and PR status. This finding suggests mechanisms independent of ER-mediated pathways, such as cytotoxicity or apoptosis. The pharmacoepidemiologic observational retrospective cohort study of Zeppelin *et al.* (11) indicated that use of isopropanolic extracts, Remifemin and Remifemin Plus, was associated with prolonged recurrence-free survival after

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breast cancer. Studies of Sakurai *et al*. (12) indicated that cimigenol and cimigenol-3,15-dione have anti-tumor-initiating activity commensurate with that of epigallocatechin gallate (EGCG), suggesting a chemopreventive role for these compounds. The studies of Seidlova-Wuttke *et al*. (13) indicated that *Cimicifuga racemosa* extract BNO 1055 inhibited development, proliferation and malignancy of prostate tumors induced by subcutaneous inoculation of LNCaP cells in immunodeficient mice.

To assess chemopreventive utility, important considerations are whether, following oral administration, sufficient blood and tissue levels can be achieved, and whether an extract/compound exerts significant toxicity. Our studies indicated that treatment of Sprague-Dawley rats with actein at 35.7 mg/kg results in a peak serum level of about 2.4 μg/ml at 6 h (14). Recent pharmacokinetic studies of 23-epi-26-deoxyactein in sera of humans (15) indicated that the maximum concentration and area under the curve were proportionate to the dose, and the half-life was about 2 h for all dosages. In addition, a single dose of black cohosh was found to be safe (15).

The purpose of this study was to determine the effect of an extract of black cohosh on the spontaneous development of mammary tumors in Sprague-Dawley rats in order to assess the toxicity and chemopreventive and anticancer potential of its active components.

Materials and Methods

Materials. All solvents and reagents were reagent grade; water was distilled and deionized. Naturex, Inc. (South Hackensack, NJ, USA) generously provided the black cohosh n-butanol dry extract containing 27% triterpene glycosides, as previously described (4). In the periodic feed and water analysis for constituents and potential undesired contaminants, no presence of chemical endocrine disruptors was found.

Animal treatment and data collection.

Experimental animals and tumor model: The experimental animals were female Sprague-Dawley rats, 56 weeks old at the start of the experiment (weight: 320 g). This strain belongs to the colony used for over 30 years in the laboratory of the Cesare Maltoni Cancer Research Centre (CMCRC) of the Ramazzini Institute. More than 80,000 female Sprague Dawley rats have been studied in our laboratory, usually observed until spontaneous death. These animals have been employed for a variety of long-term experiments performed in principle in accordance with Good laboratory Practices (GLP). Whatever their use, they have been periodically weighed and examined, and submitted to systematic necropsy and histopatological examination. Such biological monitoring has provided extensive information on the expected mammary pathology. Based on the pathology observed in the historical controls over 30 years, the incidence of expected spontaneous benign and malignant tumors of different types and sites is therefore well known. Among the expected spontaneous tumors, in females, the most frequent are the mammary, which are an excellent example of the human equivalent (16-18). All types of mammary tumors observed in human pathology, and particularly all histotypes and subhistotypes of mammary carcinomas, have also been found in untreated female Sprague-Dawley rats. Among the historical controls of the last 10 years, the overall incidence of mammary carcinomas in female Sprague-Dawley rats was 8.9%, with a range of fluctuation of 2.9-14.1%. The equivalent age distribution of mammary carcinomas is very similar to those observed in women in industrialized countries (18, 19). In female Sprague-Dawley rats of our colony, local and distant metastasis of mammary cancer may occur. After weaning, the animals were distributed into the experimental groups avoiding the presence of sisters in each group.

Cages: The rats were housed in groups of five in makrolon cages (41×25×15 cm) with a stainless steel wire top; a shallow layer of white wood shavings served as bedding. Cages were identified by a card indicating the experimental number and pedigree number of each animal. All the animals used in the experiment were kept in a single room at 23±3˚C and at 40-60% relative humidity. Lighting was natural or artificial and the light/dark cycle of 12 hours was maintained. The regulation of the light/dark cycle is made by both providing natural or artificial (by lamps) light, so that the duration of the light/dark cycle is 12/12 hours. All deviations from the above mentioned values were registered.

Feed and beverages: The animals were supplied with a pellet diet used for over 30 years at the CRC/RF ('Corticella type', Dottori Piccioni Laboratory). The diet was analyzed for its nutritive components and possible pollutants (pesticides, metals, compounds with oestrogenic activity, nitrosamines and aflatoxins) throughout the study and disposed of 3 months after the date of production. Drinking water was analyzed to identify the possible presence of pollutants (bacteria and chemicals).

Treatment for chemoprevention study: In order to test the chemopreventive effect of the black cohosh extract on mammary tumors, four groups of 97, 96, 97 and 97 females were treated by oral intubation with 35.7 (the maximum tolerated dose (MTD) based on the results of preliminary toxicity studies), 7.14, 0.714 or 0 mg/kg body weight (b.w.) of extract, suspended in water, for 40 weeks, from 56 to 96 weeks of age (the window of age for higher risk of mammary cancer in this strain of rats). The plan of the experiment is shown in Table I. After stopping treatment, the animals were kept under observation until the end of the experiment (over 130 weeks of age). At 138 weeks of age, almost 12% of the experimental animals were still alive, homogeneously distributed among the groups. At this time, sacrifice of the animals still alive following a specific calendar was planned in order to distribute the sacrifices homogeneously among the groups. The sacrifices were completed in two weeks.

Necropsy: All animals of the four groups were submitted to complete necropsy encompassing all organs and tissues and every macroscopic lesion (with a part of normal tissue). A small sample of each tumor from necropsy was frozen in liquid nitrogen and then stored at –70˚C for further molecular biology studies. Samples of normal mammary gland tissue from five rats randomly selected from each group were also provided for immunohistochemical analysis.

Analyses. Histopathology: In the chemoprevention studies, all mammary tumors and mammary glands (4 levels) were trimmed following the CRCCM/RI laboratory Standard Operating Procedure (20). Trimmed specimens were processed as paraffin blocks, and 3-

Table I. *Plan of the experiment on the evaluation of the chemopreventive effects of black cohosh (BC) on the incidence of spontaneous mammary tumors in female Sprague-Dawley rats.*

Group	Animals[a]		Agent	Treatment			
	Age at start (weeks)	No.		Dose mg/kg b.w.	Route of administration	Schedule days/week	Duration
							Treatment / Biophase
I	56	97	BC	35.7	Oral by stomach	6	40 weeks / Life span tube, in water
II	56	96	BC	7.14	Oral by stomach	6	40 weeks / Life span tube, in water
III	56	97	BC	0.714	Oral by stomach	6	40 weeks / Life span tube, in water
IV	56	97	Water	0	Oral by stomach	6	40 weeks / Life span tube

[a]Only the animals alive without palpable lesions at 56 weeks of age were considered.

5 μm sections of every specimen were obtained. Sections were routinely stained with hematoxylin and eosin.

Immunohistochemical (IHC) staining for tumors and mammary glands: Tissue was labeled with a primary antibody for 90 minutes at room temperature. For tumors, the primary antibodies used were anti-cyclin D1 (cat. #2978; 1:600) and anti-KI67 (cat. #2586; 1:200) (Cell Signaling, Beverly, MA, USA); for normal mammary glands: the primary reagents were mouse anti-ER antibody (cat.#MS-354-P0; 1:150) and Her-2Neu antibody (cat. #RM-9103-S0; 1:200) (Lab Vision, Fremont, CA, USA). For the secondary reagent, we used the EnVision® detection system (DAKO Cytomation, Glostrup, Denmark A/S) according to the manufacturer's instructions. Appropriate positive and negative controls were included.

Lipid analysis: Hepatic lipids were measured as previously described (14).

Blood samples: From the last five animals alive from each group, blood samples were collected through contusion of the retrobulbar plexus with a siliconated glass Pasteur pipette, after anesthetization with ethyl ether. Blood collection was performed three days before the start of the treatment and then at 10, 20, 30, 40 weeks after the start of the experiment.

Statistical evaluation in the chemoprevention study: Using the time of the first occurrence of the particular type of mammary lesion being analyzed, a Cox proportional hazard analysis was used. Individual experimental group comparisons were carried out. For dose-response analyses, a Cox proportional hazard regression was used in which the logarithm of the hazard ratio was assumed to depend linearly on the logarithm of the administered dose rates. For the analysis of the combined endpoints of dysplasia and adenocarcinoma, for any animal with both lesions, the time to the appearance of the first lesion was used in the analysis.

Cell cultures. MDA-MB-453 (Her2 overexpressing, low ER expression) human breast cancer cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were grown in Dulbecco's Modified Eagle's medium (DMEM; Gibco BRL Life Technologies, Inc., Rockville, MD, USA) containing 10% (v/v) fetal bovine serum (FBS) (Gibco BRL) at 37˚C with 5% CO_2.

Proliferation assay. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromid (MTT) assay was used to determine the sensitivity of MDA-MB-453 human breast cancer cells to increasing concentrations of black cohosh/actein, as previously described (6).

Statistical analysis. For cell growth assays, the data are expressed as mean±standard deviation. Control and treated cells were compared using the student's *t*-test, $p<0.05$. The control and black cohosh treated rat samples for lipid analysis were also compared using the student's *t*-test, $p<0.05$.

Results

Chemoprevention. Black cohosh did not have any adverse effects on the health of the Sprague-Dawley rats when considering water and feed consumption, body weight (Figure 1A) and survival (Figure 1B).

Incidence of palpable mammary lesions clinically observed during the biophase is reported in Table II. The data show that at 96 weeks of age (end of the treatment), the incidence of palpable mammary lesions and total number of palpable mammary lesions per 100 animals were higher among the females treated at 0.714 mg/kg b.w. compared to the other groups (including the negative control group). The differences were slightly altered with aging. This trend is also represented by the cumulative prevalence of palpable mammary lesions reported in Figure 1C.

In Tables III, IV, V and VI the data on mammary gland tissues and palpable lesions histopatologically evaluated are reported. The data show: i) no difference of the incidence of fibroadenomas among the groups (Table III); ii) an increased incidence of animals bearing cellular atypia in mammary tissue and in fibroadenomas at the dose level of 0.714 mg/kg b.w. compared to the other groups (Table IV); iii) an increased incidence of animals bearing mammary adenocarcinomas and of the total number of adenocarcinomas among the females treated at 7.14 and 0.714 mg/kg b.w. compared to the animals treated at 35.7 mg/kg b.w. and to the negative controls (Table V; Figure 1D); and iv) the same trend was observed when adenocarcinomas were aggregated according to their atypical precursors (Table VI).

On the basis of these results, the potential chemopreventive effect of black cohosh on mammary adenocarcinomas may be hidden in this study by the exceptionably low incidence of mammary adenocarcinomas (1%) in the negative control

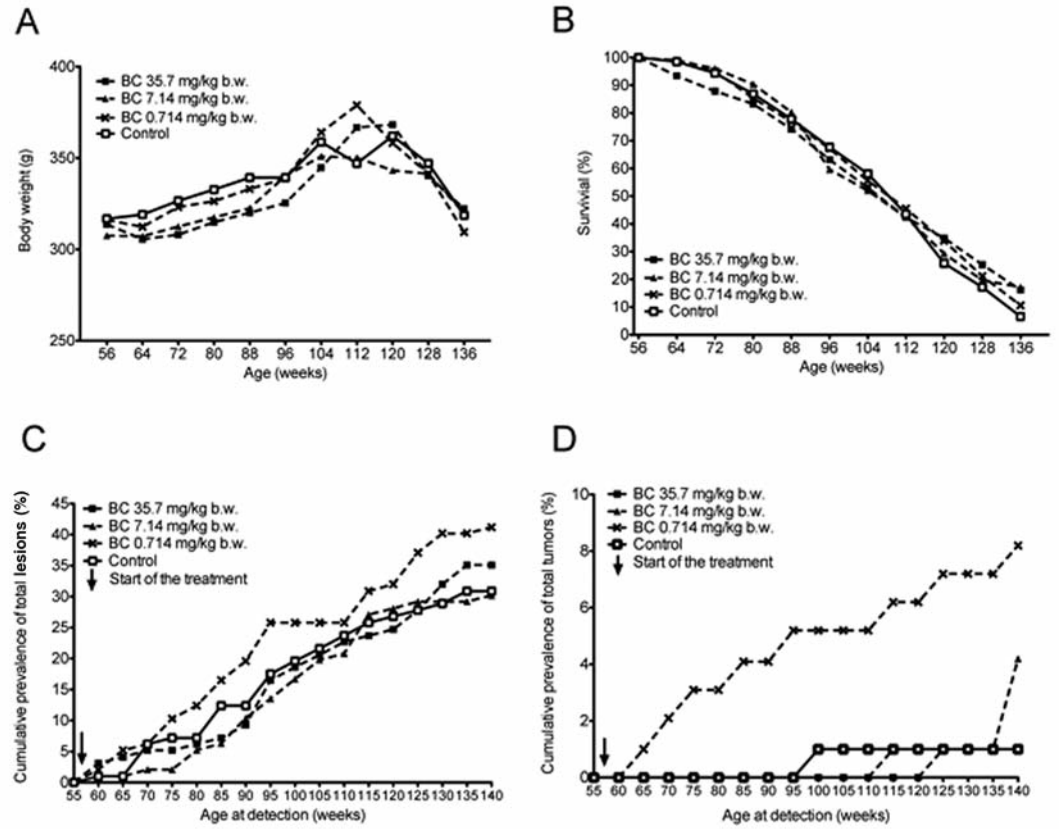

Figure 1. *Four groups of female Sprague-Dawley rats were treated with an extract of black cohosh enriched in triterpene glycosides (27%) at 35.7, 7.14, 0.714 or 0 mg/kg of body weight (b.w.) for 40 weeks, from 56 to 96 weeks of age. A: Mean body weight in female rats. B: Survival of female rats. C: Cumulative prevalence of mammary lesions in female rats clinically observed during the biophase. D: Cumulative prevalence of onset by age in female rats bearing mammary adenocarcinomas histologically evaluated. The arrow in C and D indicates the start of the treatment.*

group when compared to the incidence of the negative historical controls of our colony (overall 8.9%; range 2.9-14%). If we compare (using the observed time of occurrence) the incidence of mammary adenocarcinomas among the females treated at 35.7 or 7.14 mg/kg b.w. with the females treated at 0.714 mg/kg b.w. (as positive control group which has an incidence of mammary adenocarcinomas equivalent to that of the overall negative historical controls (8.9%), the data show: i) a significant dose-related decrease ($p<0.05$) of carcinomas, particularly in the females treated at the dose of 35.7 mg/kg b.w. ($p<0.05$); and ii) a significant dose-related decrease ($p<0.01$) of the number of aggregated adenocarcinomas plus their precursors, and specifically among the females treated at 35.7 mg/kg b.w. ($p<0.05$) or at 7.14 mg/kg b.w. ($p<0.05$).

Histopathological examination of mammary tissues and tumors. Histopathological examination of IHC stained sections of mammary tissues and H and E and IHC stained sections of fibroadenomas was performed.

IHC staining of mammary tissue. The normal mammary tissue was positive for ER in the nucleus and negative for Her2 expression (Figure 2A and B). These findings helped guide the choice of signaling pathways to explore in separate gene expression studies.

H and E and IHC staining of fbroadenomas. The fibroadenomas from rats treated with 7.14 or 35.7 mg/kg black cohosh displayed a decrease in the proportion of glandular tissue and an increase in the proportion of connective tissue in treated versus control samples (3 each) (Figure 2 C and D), whereas one fibroadenoma from rats treated with the lowest dose (0.714 mg/kg) exhibited an increase in the proportion of glandular tissue.

We also used IHC to examine the level of Ki-67 and cyclin D1 in fibroadenomas from Sprague-Dawley rats treated with 7.14 or 35.7 mg/kg black cohosh. We found a significant difference in Ki-67 and cyclin D1 staining for rats treated with black cohosh versus water (control). We counted positive cells in 10 separate fields on each slide and averaged

Table II. *Incidence of palpable mammary lesions clinically observed during the biophase (96 weeks/end of the experiment) in female Sprague-Dawley rats.*

Group	Animals no.	Dose of BC (mg/kg b.w.)	Mammary lumps							
			At 96 weeks of age				At the end of the experiment			
			Bearing animals		Total lesions		Bearing animals		Total lesions	
			No.	%	No.	Per 100 animals	No.	%	No.	Per 100 animals
I	97	35.7	14	14.4	16	16.5	30	30.9	34	35.1
II	96	7.14	12	12.5	13	13.5	26	27.1	29	30.2
III	97	0.714	20	20.6	25	25.8	29	29.9	40	41.2
IV	97	0 (control)	16	16.5	17	17.5	26	26.8	30	30.9

BC: Black cohosh extract.

the results. For Ki-67 positivity, the control rate was 5-15%, while that for treated (7.14 mg/kg) animals was <5%. For cyclin D1, the control rate was ~40%, while that for treated (7.14 mg/kg) animals was 5-15% (Figure 3).

Lipid analysis of rat serum. Analysis of the lipid content of rat serum indicated that treatment for prolonged times (2 or 20 weeks) with an extract of black cohosh at 35.7 or 0.714 mg/kg did not alter the level of lipids in the serum, except that black cohosh at 0.714 mg/kg did reduce the level of triglycerides at 20 weeks (0.69-fold, p=0.024). Treatment with black cohosh extract at 7.14 mg/kg resulted in a modest increase in the level of free fatty acids at 2 weeks (1.39-fold, p=0.026) and 20 weeks (1.46-fold, p=0.010) and triglycerides at 20 weeks (1.77-fold, p=0.04).

Effect of black cohosh on the growth of breast cancer cells. Black cohosh inhibited the growth of MDA-MB-453 Her2-overexpressing breast cancer cells with an IC_{50} value, the concentration that caused 50% inhibition of cell proliferation of approximatley 8 μg/ml compared to that for the triterpene glycoside actein of approximately 9 μg/ml (13 μM) (Figure 4). These agents were more active than the chemopreventive compounds resveratrol (IC_{50}=8 μg/ml; 35 μM) and EGCG (IC_{50}>25 μg/ml; >55 μM) (data not shown).

Discussion

We examined the effect of an extract of black cohosh on the development of spontaneous mammary tumors in Sprague-Dawley rats. We found that treatment with an extract of black cohosh enriched for triterpene glycosides (27%) at 35.7 and 7.14 mg/kg b.w. for 40 weeks (starting from 56 weeks of age) resulted in a significant dose-related reduction (p<0.05) of the incidence of mammary adenocarcinomas when compared to the incidence among the females treated at 0.714 mg/kg b.w. (considered as the positive control) and a significant dose-related decrease (p<0.01) of the number of aggregated adenocarcinomas plus their precursors. Furthermore, fibroadenomas obtained from rats treated with 35.7 and 7.14 mg/kg black cohosh displayed a decrease in the proportion of glandular tissue and an increase in the proportion of connective tissue, as well as a decrease in the level of cyclin D1 and Ki-67 protein by IHC. These findings suggest that black cohosh reduced the proliferative rate and thus the malignant potential of the tumors. It is important to note that the chemoprevention of mammary adenocarcinomas is persistent after the end of the treatment (96 weeks of age) (Figure 1D). In confirmation of our findings, our studies indicate that black cohosh inhibits the growth of MDA-MB-453 Her2-overexpressing human breast cancer cells. Taken together, these findings suggest that black cohosh may have the potential to prevent and treat mammary cancer in females.

Table III. *Incidence of benign mammary fibroadenomas in female Sprague-Dawley rats histopathologically evaluated.*

Group	Animals no.	Dose of BC (mg/kg b.w.)	Fibroadenomas			
			Bearing animals		Total tumors	
			No.	%	No.	Per 100 animals
I	97	35.7	33	34.0	41	42.3
II	96	7.14	34	35.4	41	42.7
III	97	0.714	29	29.9	41	42.3
IV	97	0 (control)	36	37.1	45	46.4

BC: Black cohosh extract.

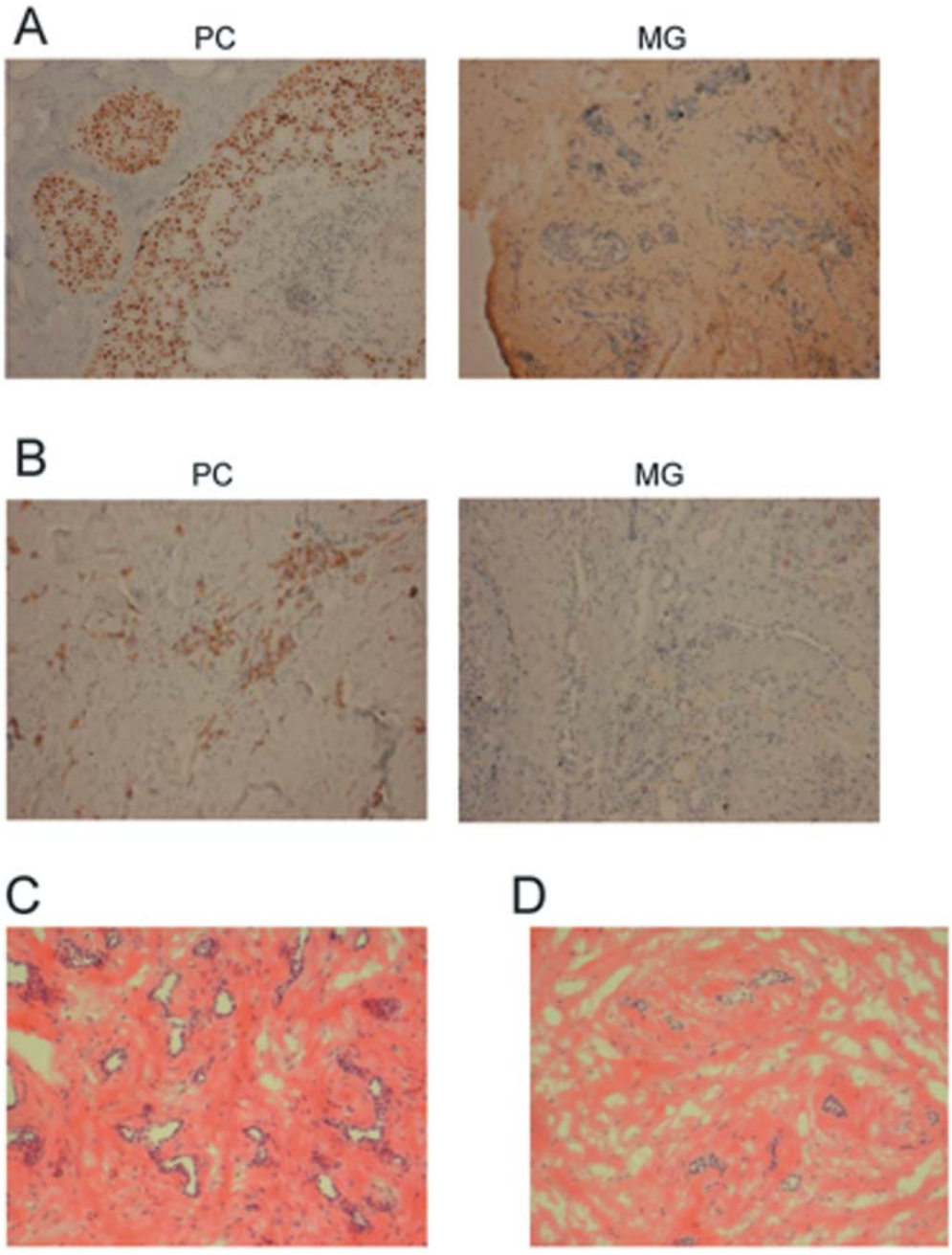

Figure 2. *Immunohistochemical staining for receptors on rat tissue: A: estrogen receptor positive control (PC) and mammary gland (MG). B: HER2 PC and HER2 MG. H&E staining of frozen sections of fibroadenomas (FA). C: Control, FA detected at age of 93 weeks, and age at death: 95 weeks; D: treated with black cohosh extract, 7.14 mg/kg. FA detected: 89 weeks; age at death:101 weeks. Magnification: 200×.*

We treated four groups of rats with increasing doses of an extract of black cohosh enriched for triterpene glycosides. We cannot explain why there was an exceptionably low incidence of mammary adenocarcinomas (1%) in the negative control group when compared to the incidence of the negative historical controls of our colony (overall 8.9%; range 2.9-14%). Rather than using these historical controls, we employed a statistical analysis of the results obtained after treating with increasing doses of black cohosh. We compared (using the observed time of occurrence) the incidence of mammary adenocarcinomas among the females treated at 35.7 or 7.14 mg/kg b.w. with the females treated at 0.714 mg/kg

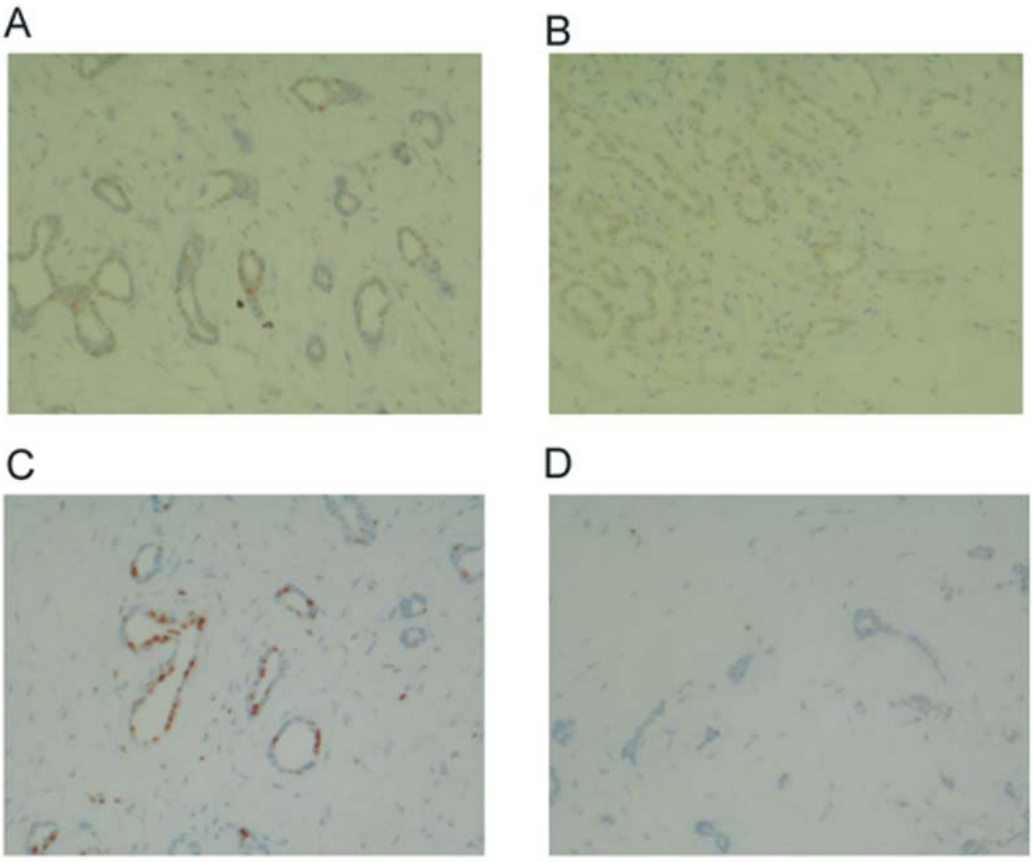

Figure 3. *Immunohistochemistry of fibroadenomas for cyclin D1 and Ki-67. Fibroadenomas as described in Fig. 2 C and D (C: control, FA detected at age of 93 weeks, and age at death: 95 weeks; D: treated with black cohosh extract, 7.14 mg/kg. FA detected: 89 weeks; death at age: 101 weeks); for cyclin D1: A: control; B: treated with black cohosh extract at 7.14 mg/kg; for Ki-67: C: control; D:, treated with black cohosh at 7.14 mg/kg. Magnification 400×.*

b.w. (as positive control group, which has an incidence of mammary adenocarcinomas equivalent to that of the overall negative historical controls (8.9%). The results indicate that black cohosh induced a significant dose-related decrease of carcinomas, especially at the dose of 35.7 mg/kg b.w, as well as of the number of aggregated adenocarcinomas plus their precursors at the dose of 35.7 mg/kg b.w. and 7.14 mg/kg b.w.

These findings are in agreement with the results of Seidlova-Wuttke *et al*. (13) that the *Cimicifuga racemosa* extract BNO 1055 (aqueous ethanol) reduced the incidence, proliferation, size and malignancy of prostate tumors induced by injection of LNCaP cells in immunodeficient mice. In their study, the treated animals developed smaller tumors and less overall tumor tissue, which was mostly confined to connective tissue. The tumors in the treated animals thus appeared to be less malignant than those in the untreated animals, indicating that black cohosh components may inhibit the progression, as well as the development of tumors.

Since the rats appeared healthy and lived for more than 40 weeks after the end of the treatment in our study, the extract at 35.7 mg/kg was not toxic. In a separate study (in press), we did, however, find that the extract induced modest liver damage and increased the level of lipids (triglycerides and free fatty acids) in liver tissue at 24 h after treatment. This increase warrants further investigation; however, it is important to note that the dose was 50 times the normal human dose (21). Our studies indicate that treatment with an extract of black cohosh (27% extract) for prolonged durations (2 or 20 weeks) at 35.7 or 0.714 mg/kg resulted in no increase and at 7.14 mg/kg, a small increase of triglycerides and free fatty acids in the serum. The findings at 0.714 mg/kg, equivalent to a normal human dose, agree with the results of Spangler *et al*. (22) that black cohosh at 60 mg per day for 3 months in humans did not alter triglyceride levels, and disagree with the results of Wuttke *et al*. (23) that black cohosh increased the level of triglycerides in humans, after 3 months at a dose of 40 mg per day.

It is important to note that animal studies indicate that black cohosh extracts do not induce toxic, mutagenic or carcinogenic effects (24, 25). However, these findings may not

Table IV. *Incidence of cellular atypia in mammary glands (CAMG) and fibroadenomas (CAFA) (precursors of adenocarcinomas) in females Sprague-Dawley rats.*

Group	Animals no.	Dose of BC (mg/kg b.w.)	Precursors of adenocarcinomas											
			CAMG				CAFA				Total			
			Bearing animals		Cellular atypia		Bearing animals		Cellular atypia		Bearing animals[a]		Cellular atypia	
			No.	%	No.	Per 100 animals	No.	%	No.	Per 100 animals	No.	%	No.	Per 100 animals
I	97	35.7	3	3.1	3	3.1	0	-	0	-	3	3.1	3	3.1
II	96	7.14	2	2.1	2	2.1	0	-	0	-	2	2.1	2	2.1
III	97	0.714	4	4.1	4	4.1	2	2.1	3	3.1	6	6.2	7	7.2
IV	97	0 (control)	2	2.1	2	2.1	2	2.1	2	2.1	3	3.1	4	4.1

[a]Animals bearing both CAMG and CAFA were considered only once. BC: Black cohosh extract.

Table V. *Incidence of mammary adenocarcinomas in female Sprague-Dawley rats.*

Group	Animals no.	Dose of BC (mg/kg b.w.)	Adenocarcinomas				
			Bearing animals		Total tumors		
			No.	%	No.	Per 100 animals	Protection Index[b]
I	97	35.7	1[a]	1.0	1	1.0	87.8
II	96	7.14	4	4.2	4	4.2	48.8
III	97	0.714	8	8.2	8	8.2	-
IV	97	0 (control)	1	1.0	1	1.0	-

[a]One animal bearing mammary carcinomas was excluded because the cancer occurred in the first week from the start of the treatment. [b]Protection index (PI) is calculated relative to the group III; PI=[total tumours ×100 animals of group III] – [total tumours ×100 animals of the group I (or group II)] / [total tumours of group III] ×100. BC: Black cohosh extract.

apply to the use of a partially purified fraction from black cohosh; in particular, black cohosh may interact with Cytochrome P450 2D6 (CYP2D6) substrates (26) and inhibit Cytochrome P450 3A4 (CYP3A4) in intestinal epithelium (27). It is of particular concern that one study associated black cohosh treatment with an increase in the incidence of lung metastases in a mouse MMTV neu model (28). There are several items pertinent to this study that may affect the relevance of the results. First, the duration of exposure lasted from adolescence until death, a treatment period that is atypical for humans taking black cohosh. Second, the MMTV-neu promoter is hormone-responsive and can respond to a number of conditions, including pregnancy, as well as glucocorticoids and progestins. Black cohosh could have activated the MMTV-neu promoter used in this mouse model, so that it is possible that the murine model of carcinogenesis may not yield effects relevant to breast cancer.

In sum, treatment of Sprague-Dawley rats with an extract of black cohosh enriched for triterpene glycosides resulted in a marked dose-related reduction of mammary adenocarcinomas. Black cohosh reduced the amount of glandular tissue and increased connective tissue, and also reduced Ki-67 and cyclin D1 protein expression in fibroadenomas. These findings suggest black cohosh may be useful in the prevention of breast cancer.

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Table VI. *Number of aggregated mammary adenocarcinomas and their precursors (cellular atypia) per 100 animals.*

Group	Animals no.	Dose of BC (mg/kg b.w.)	Adenocarcinomas plus their precursors N per 100 animals
I	97	35.7	4.1
II	96	7.14	6.3
III	97	0.714	15.5
IV	97	0 (control)	5.2

BC: Black cohosh extract.

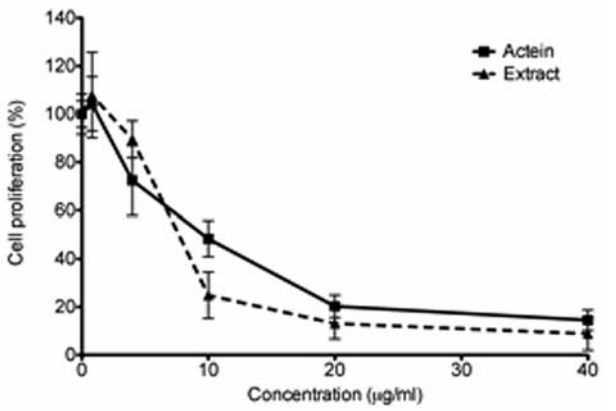

Figure 4. *Effect of A) black cohosh extract and B) actein on the growth of MDA-MB-453 breast cancer cells. Cells were treated with increasing concentrations of agents for 96 hours and the number of viable cells was determined using the MTT assay.*

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