Pharmacological mechanisms of black cohosh in Sprague–Dawley rats

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A B S T R A C T

Background: Studies indicate that extracts and purified components from black cohosh inhibit the growth of human breast cancer cells, but the molecular targets and signaling pathways have not yet been defined.

Purpose: This study examines the pharmacological mechanisms and toxicological effects in the short term of the herb black cohosh on female Sprague–Dawley rats.

Materials and methods: To assess effects on gene activity and lipid content, we treated female Sprague–Dawley rats with an extract of black cohosh enriched in triterpene glycosides (27%) at 35.7 or 0 mg/kg. Four animals for each group were sacrificed at 1, 6 and 24 h after treatment; liver tissue and serum samples were obtained for gene expression and lipid analysis.

Results: Microarray analysis of rat liver tissue indicated that black cohosh markedly downregulated mitochondrial oxidative phosphorylation genes. Phospholipid biosynthesis and remodeling, PI3-Kinase and sphingosine signaling were upregulated, driven largely by an upregulation of several isoforms of phospholipase C. Hierarchical clustering indicated that black cohosh clustered with antiproliferative compounds, specifically tubulin binding vinca alkaloids and DNA alkylators. In support of this, black cohosh repressed the expression of cyclin D1 and ID3, and inhibited the proliferation of HepG2, p53 positive, liver cancer cells. Black cohosh reduced the level of free fatty acids at 6 and 24 h and triglycerides at 6 h in the serum, but increased the free fatty acid and triglyceride content of the treated livers at 24 h.

Conclusion: Our results suggest that black cohosh warrants further study for breast cancer prevention and therapy.

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1. Introduction

The North American perennial black cohosh (Actae a racemosa L., Ranunculaceae) has been used for centuries to treat various inflammatory and gynecological conditions such as arthritis, muscle pain, and dysmenorrhea. The popular use in recent years has been as an alternative to hormone replacement therapy.

The purported active components in the plant are triterpene glycosides and phenylpropanoids [1,2]. Both of the novel classes of compounds – triterpene glycosides and phenylpropanoids – have been shown to exhibit significant anticancer effects in vitro [2]. The triterpene glycosides actein (Fig. 1) and 23-epi-26-deoxyactein are present at about 6.4% of an n-butanol fraction of black cohosh enriched for triterpene glycosides (27%) [3], which contains more than 62 triterpene glycosides [1].
In vivo studies suggest that black cohosh and triterpene glycosides have anticancer and chemopreventive activity [2]. An extract of black cohosh inhibited the development and malignancy of prostate tumors in immunodeficient mice [4]. In addition, cimigenol and cimigenol-3,15-dione inhibited mouse skin tumor promotion and had antitumor initiating activity commensurate with the chemopreventive agent epigallocatechin gallate (EGCG) [5].

The molecular targets and signaling pathways altered by black cohosh and actein have not yet been defined. Studies indicate that these agents activate two phases of the integrated stress response, the survival or apoptotic phase, depending on the dose and duration of treatment [2,6,7]. In previous studies, to gain insight into the safety, toxicity and mode of action of actein, we used the toxicological database developed by Iconix Biosciences, DrugMatrix [8]. The database compares raw gene expression data from a given organ in relation to over 650 reference compounds. Subtle changes in the liver can be used to predict toxicological and pathological effects in the liver and other tissues before these effects can be detected. The analysis indicated that actein induced stress and statin associated responses. In particular, actein altered the expression of cholesterol and fatty acid biosynthetic genes, p53 and mitochondrial oxidative phosphorylation pathway genes, as well as CCND1 and ID3. In confirmation of these effects, actein reduced free fatty acid and cholesterol content and inhibited the growth of HepG2 liver cancer cells.

Assessment of chemopreventive utility requires consideration of whether sufficient blood and tissue levels can be achieved following oral administration, and whether this compound exerts significant toxicity. Pharmacokinetic analysis indicated that actein is bioavailable; it attains a serum level of 2.4 μg/ml at 6 h [8]. In sum, these studies indicated that actein has potential to prevent and treat cancer and lipid disorders.

Pharmacokinetic and toxicity studies have also been performed for an extract of black cohosh. van Breeman et al. [9] reported that a single dose of black cohosh was found to be safe and did not induce hepatotoxicity. Pharmacokinetic analyses of 23-epi-26-deoxyactein in sera of humans after administration of a 75% ethanolic extract of black cohosh containing about 4.4% of this component indicated that the maximum concentration and area under the curve were proportionate to dose, and the half-life was about 2 h for all dosages [9].

In the framework of our project aimed to evaluate the chemopreventive effect of black cohosh extract on the incidence of spontaneous mammary tumors, both benign and malignant, in female Sprague–Dawley rats, studies on pharmacokinetic and pharmacodynamic properties of the extract and on gene expression analysis in some tissues and organs were performed. To our knowledge, no gene expression profiles of the effects of black cohosh have been reported in vivo.

The purpose of this paper is to examine the effect of an extract of black cohosh on the gene expression profiles of rat liver tissue and lipid content of rat liver tissue and serum in order to assess the toxicity, chemopreventive and anticancer potential of active components in this agent.

2. Materials and methods

2.1. Materials

All solvents and reagents were reagent grade; H2O was distilled and deionized. Naturex, Inc. (South Hackensack, NJ) generously provided the black cohosh extract containing 27% triterpene glycosides, as previously described [3]. The extract of black cohosh enriched for triterpene glycosides (27%) contained 3.4% actein and 1.8% isoferulic acid; the most abundant components were cimicifugoside (5.0%) and cimigenol arabinoside (3.7%).

2.2. Cell cultures

HepG2 (p53 positive) human liver cancer cells were obtained from the ATCC (Manassas, VA). Cells were grown in Dulbecco’s Modified Eagle’s medium (DMEM) (Gibco BRL Life Technologies, Inc., Rockville, MD) containing 10% (v/v) fetal bovine serum (FBS) (Gibco BRL) at 37 °C, 5% CO2.

2.3. Proliferation assay

The MTT assay was used to determine the sensitivity of HepG2 p53 positive human liver cancer cells to black cohosh/actein, as previously described [7].

2.4. Animal treatment and data collection

2.4.1. Experimental animals

The experimental animals were female Sprague–Dawley rats, 56 weeks old at the start of the experiment. This strain belongs to the colony used for more than 30 years in the laboratories of the Cesare Maltoni Cancer Research Centre (CMCRC) of the Ramazzini Institute. These animals have been employed for a variety of long-term experiments performed in accordance with Good Laboratory Practices (GLP). The experiment was conducted in accordance with Italian law regulating the use and humane treatment of animals for scientific purposes [10]. Regardless of use, they have been periodically weighed and examined, and submitted to systematic necropsy and histopathological examination.

2.4.2. Cages

The rats were housed in groups of four in makrolon cages (cm 41 × 25 × 15) 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

![Fig. 1. Structure of the triterpene glycoside actein.](image)
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 h was maintained. All deviations from the above mentioned values were registered.

2.4.3. Feed and beverages

Until the start of the experiment the animals were supplied with a pelleted diet used for more than 30 years at the CRC/RF (“Corticella type”, Laboratory Dottori Piccioni). The diet was analyzed for its nutritive components and possible pollutants (pesticides, metals, compounds with oestrogenic activity, nitrosamines and aflatoxins) before the start of the study. Drinking water was analyzed to identify the possible presence of pollutants (bacteria and chemicals).

2.4.4. Treatment

In order to assess short term pharmacological and toxicological effects of the black cohosh extract, two groups of 12 female Sprague Dawley rats each, 56 weeks old, were treated once with 35.7 mg/kg dosage for 24 h. The treatment and tissue samples were collected for analysis.

2.4.5. Necropsy

The following organs and tissues were collected from each animal during necropsy: brain, mammary gland (4 portions), heart, lung, liver, spleen, stomach, small intestine, large intestine, kidneys and muscle (quadriceps). During the necropsy, portions from the liver from the last 4 animals of both treated and control groups (sacrificed 24 h after the start of the experiment) were collected for analysis. Four portions of about 100 mg each were collected from the main lobe of the liver. Each portion was individually retained in a cryovial, frozen and stored at −70 °C until use. Serum collection: Immediately after sacrifice, blood was drawn from the portal vein with a sterile syringe into vials without anticlotting agents. After 10 min (the time necessary for the formation of the clot) blood was centrifuged at 1500 g for 10 min. After the separation, the serum was stored in cryogenic screw cap vials at −70 °C until use.

2.5. Analyses

2.5.1. Histopathology

Frozen liver samples were embedded in OTC and 3–5 μm cryosections were obtained. Cryosections were stained with hematoxylin and eosin and with Oil Red O. Microscopy was performed as described previously [8].

2.5.2. Lipid analysis

Hepatic lipids were measured as previously described [8].

2.5.3. Gene expression analysis

Labeled cDNA was generated from liver tissue from each study animal and hybridized to Affymetrix RG230-2 rat whole genome arrays at Columbia University, following standard Affymetrix protocols. Analyses were performed using two approaches: 1) AffyLimmaGUI: Analysis was performed using the AffyLimmaGUI package in the open-source Bioconductor suite, as previously described (7); 2) DrugMatrix®: Array data were transmitted to Iconix Pharmaceuticals as CEL files and uploaded into the Iconix database (DrugMatrix®) for Drug Signature and pathway analysis, as previously described [8].

2.5.4. Real-time RT-PCR analysis

Real-time quantitative RT-PCR methods were used to determine the nature of the RNA induced by treatment with black cohosh extract, using the Real Time PCR machine Stratagene MX3005P QPCR System, as previously described [7]. A list of primer sequences is presented in Table 1A.

2.5.5. Statistical analysis

For cell growth and real-time PCR assays, the data are expressed as mean +/− standard deviation. Control and treated cells were compared using the Student's t-test, p < 0.05. For gene expression analysis, the samples were analyzed as previously described for AffyLima analysis [7] and for Iconix Drug Matrix analysis [8]. AffyLImma Analysis: the statistical significance of differential expression was calculated using the empirical Bayesian LIMMA (LI Model for MicroArrays) method of Smyth et al. [11]: \( \log_2(\text{odds of differential expression}) \); the Bayesian natural (base e) log of the odds that the genes are differentially expressed [7]. Iconix Drug Matrix Analysis: For Iconix Pathway analysis, Fisher’s Exact Test calculated the statistical likelihood that the same number of expression changes observed in pathway genes would be observed against the same number of randomly-chosen array probe sets. For Iconix hierarchical clustering analysis, statistical analysis of the treatments in the cluster was performed using the hypergeometric distribution [8].

3. Results

3.1. Pharmacology

3.1.1. Histopathological and lipid examination of liver and kidney tissues

Liver tissues from rats treated with black cohosh (35.7 mg/kg dosage) were stained with H and E (data not shown) or with Oil Red O for lipids and counterstained with H&E (Fig. 2A, B). Lipid accumulation was not as obvious in control liver tissue as it was in the treated sample. The localization in the treated tissue occurred between the central veins (Periportal – closer

<table>
<thead>
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<th>Table 1A</th>
</tr>
</thead>
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<tr>
<td>Designed primer sequence used in RT-PCR.</td>
</tr>
<tr>
<td>Symbol</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>CCND1</td>
</tr>
<tr>
<td>ID3</td>
</tr>
</tbody>
</table>

In the Table 1A, mRNA sequences were obtained from the public GeneBank database (www.ncbi.nlm.nih.gov), and primers were designed using Primer3 software from The Massachusetts Institute of Technology (frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).
The samples displayed mild toxicity, as shown by microvesicular lipid droplets; the droplets were small, diffuse, lobular, subendothelial, and perivenule. Analysis of the lipid content of the livers revealed a 3.9 (p = 1.14 × 10^{-5}) and 4.6-fold (p = 0.00131) increase in the free fatty acid and triglyceride content, respectively, of the treated livers compared to the controls at 24 h.

For the rat kidney, a lymphoid, inflammatory infiltrate under the lining of the urinary tract was present in the treated sample, but not in the control (data not shown). We observed no tubular, glomeruli, or vascular damage or tissue inflammation. The infiltrate was not a toxic injury since glomeruli were similar in both treated and control kidneys.

### 3.1.2. Lipid content analysis of rat serum

Analysis of the lipid content of the serum revealed that treatment with an extract of black cohosh at 35.7 mg/kg reduced the level of free fatty acids at 6 h (0.71-fold, p = 0.003) and 24 h (0.68-fold, p = 0.038) and triglycerides at 6 h (0.71-fold, p = 0.038), but nonsignificantly increased the level of triglycerides at 24 h (1.2-fold, p = 0.058).

### 3.2. Gene expression analysis of rat liver tissue

We analyzed a dataset derived from the livers of female rats treated with an extract of black cohosh (35.7 mg/kg) and observed for 24 h.

#### 3.2.1. Affy–Limma analysis

After exposure for 24 h, Affy–Limma analysis indicated that the extract altered the expression of two genes (B > 0) (B = loge (Odds of differential expression); the Bayesian natural (base e) log of the odds that the genes are differentially expressed): the mitochondrial gene benzodiazequin receptor BZRP (log fold: 0.56) and the transcription factor F-box predicted and cell cycle (pp3cba). Among the most highly altered genes were those involved in: (upregulated) inflammatory response (S100a8), protein transport (Lin7a); (downregulated) cell growth and replication (lgfbp3, Id3), antipapotic activity (Birc6, predicted) and cell cycle (ppp3cb). Among the most highly altered genes were those involved in: (upregulated >2-logfold) immune (Igh-1a, predicted) and inflammatory response (Lcn2, A2m), lipid binding (Rbp2), oxidoreductase (Cyp2b15) and phosphatase (Dusp1) activity; (downregulated < -1.36-fold) xenobiotic metabolic response (Hamp), cell growth (Id1, lgfbp3), fatty acid synthesis (SCD1), transport (Sy12), metabolic process (Aldh1a4) and cell cycle (Ccn1).

#### 3.2.2. Drug matrix analysis

No match to any of the 29 Drug Signatures® derived on the RG230-2 array platform for liver was observed. A lack of compatibility with Drug Signatures® does not preclude the use of other comparative analysis tools. One such tool is pathway analysis. Considering both up and down-regulated genes in the analysis, the highest impact was observed on the Mitochondrial Oxidative Phosphorylation pathway (Table 1B). When the expression data for the genes in this pathway were overlaid on a map of the pathway (Fig. 3), it is clear that there is a profound downregulation of genes in this pathway in response to black cohosh exposure.

A general decrease in genes involved in urea and aspartate metabolism was also observed, including the mitochondrial carbamoyl phosphate synthase 1, argininosuccinate synthase, glycine amidino transferase and creatine kinase, possibly also reflecting mitochondrial damage.

When upregulated genes alone were considered, phospholipid biosynthesis and remodeling, P3-Kinase and sphingosine signaling were observed to be impacted. This was driven largely by an upregulation of several isofoms of phospholipase C, which is involved in all 3 pathways. Diacyl glycerol kinase beta was also significantly upregulated.

#### 3.2.3. Comparative analysis

A hierarchical clustering of pathway impact scores of all RG230-2 liver experiments in the database was performed alongside black cohosh (Fig. 4). Black cohosh formed part of a cluster of 51 treatments having a Pearson’s correlation coefficient of 0.58. Statistical analysis of the treatments in the cluster using the hypergeometric distribution revealed a significant representation of treatments with anti proliferative compounds, specifically tubulin binding vinca alkaloids (3 experiments, p = 0.0017) and DNA alkylators (4 experiments, p = 0.029). The repression of cyclin D1 that we previously reported (6) was corroborated in this experiment (AffyLimma: -1.37-logfold). There was a mixed effect on the apoptosis pathway, with upregulation of pro-apoptotic caspase 9 and IAP5, but downregulation of anti-apoptotic cytochrome C and BAX.
Table 2
Genes altered by treatment with black cohosh, determined by Affy-Limma analysis.

<table>
<thead>
<tr>
<th>Function</th>
<th>Affymetrix ID</th>
<th>Symbol</th>
<th>Name</th>
<th>M (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory response</td>
<td>1368494_at</td>
<td>S100a8</td>
<td>S100 calcium binding protein A8 (calgranulin A)</td>
<td>2.1727</td>
</tr>
<tr>
<td>Protein transport</td>
<td>1370108_a_at</td>
<td>Lin7a</td>
<td>lin-7 homolog a (C. elegans)</td>
<td>1.108</td>
</tr>
<tr>
<td>Receptor activity</td>
<td>1397259_at</td>
<td>RGD1309752</td>
<td>predicted</td>
<td>Similar to hypothetical protein D630010C10 (predicted)</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>1370249_at</td>
<td>Bzrp</td>
<td>Benzodiazepin receptor</td>
<td>0.565</td>
</tr>
<tr>
<td>Inactivation of MAPK activity</td>
<td>1398783_at</td>
<td>Gps1</td>
<td>G protein pathway suppressor 1</td>
<td>−0.305</td>
</tr>
<tr>
<td></td>
<td>1377143_at</td>
<td>RGD:727783</td>
<td>predicted</td>
<td>Galactose transporter</td>
</tr>
<tr>
<td></td>
<td>1382059_at</td>
<td>RGD:1359367</td>
<td>predicted</td>
<td>F-box only protein 30</td>
</tr>
<tr>
<td></td>
<td>1388991_at</td>
<td>LOC295678</td>
<td>predicted</td>
<td>NA</td>
</tr>
<tr>
<td>Regulation of DNA replication</td>
<td>1387769_a_at</td>
<td>Id3</td>
<td>Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein</td>
<td>−1.085</td>
</tr>
<tr>
<td>Apoptosis inhibitory protein</td>
<td>1374227_at</td>
<td>Birc6_predicted</td>
<td>predicted</td>
<td>Baculoviral IAP repeat-containing 6 (predicted)</td>
</tr>
<tr>
<td>Regulation of cell growth</td>
<td>1386881_at</td>
<td>Igfbp3</td>
<td>Insulin-like growth factor binding protein 3</td>
<td>−1.689</td>
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</table>

We used Affy-Limma analysis to determine the effects of actein, at a dose of 35.7 mg/kg and at 24 h, on the gene expression pattern in rat liver. Assays were performed as described in the Materials and methods section. Fold change (log) is the mean of the ratio of hybridization signals in actein treated vs. control treated cells (12 highest B values; B > −0.25).

3.3. Real-time RT-PCR

The more sensitive tool Reverse Transcription- Polymerase Chain Reaction (RT-PCR) confirmed the microarray results indicating that black cohosh suppressed the expression of cyclin D1 and ID3 at 24 h (p < 0.05) (Fig. 5A).

3.4. Growth inhibitory effect of black cohosh on liver cancer cells

Black cohosh inhibited the growth of p53 positive HepG2 liver cancer cells with an IC50 value, the concentration that caused 50% inhibition of cell proliferation, of 37 μg/ml compared to that for the triterpene glycoside actein, 26 μg/ml (38 μM) (Fig. 5B). Actein was more active than the chemopreventive compounds resveratrol: 21 μg/ml compared to that for the triterpene glycoside actein, 26 μg/ml (38 μM) (Fig. 5B). Actein was more active than the chemopreventive compounds resveratrol: 21 μg/ml (51 μM); curcumin: 25 μg/ml (68 μM); or EGCG: > 100 μM (data not shown).

4. Discussion

This study examines the pharmacological and toxicological effects of an extract of black cohosh on female Sprague-Dawley rats in the short term. We treated the rats with an extract of black cohosh enriched in triterpene glycosides (27%) at 0 or 35.7 mg/kg. Four animals for each group were sacrificed at 1, 6 and 24 h after receiving the treatment and liver tissue samples for lipid and gene expression analysis were obtained. Microarray analysis of rat liver tissue indicated that black cohosh downregulated mitochondrial oxidative phosphorylation genes and upregulated several isoforms of PLC at 24 h; by microarray and RT-PCR analysis, black cohosh reduced the expression of the cell cycle gene cyclin D1 and the inhibitor of differentiation gene ID3. In addition, black cohosh downregulated the expression of the antiapoptotic gene Birc6_predicted and clustered with antiproliferative compounds, specifically tubulin binding vinca alkaloids and DNA alkylators. In support of these findings, the extract inhibited the proliferation of HepG2, p53 positive, liver cancer cells. As such, our results suggest that black cohosh may have chemopreventive potential.

A concern may be 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. However, it is important to note that the dose was 50 times the normal human dose for treatment of menopausal symptoms [12]. In contrast, analysis of the lipid content of the serum revealed that treatment reduced the level of free fatty acids at 6 and 24 h and triglycerides at 6 h. Our findings at 35.7 mg/kg, 50 times a normal human dose, disagree with the results of Spangler et al. [13] that black cohosh at 60 mg per day for 3 months did not alter triglyceride levels in humans, and with the results of Wuttke et al. [14] that black cohosh increased the level of triglycerides in humans, after 3 months at a dose of 40 mg per day.

Animal studies indicate that black cohosh extracts do not induce toxic, mutagenic or carcinogenic effects [15]. Although black cohosh appears to be safe at doses higher than the human therapeutic doses used for treatment of menopausal symptoms, these results may not apply to the use of a partially purified fraction from black cohosh. Concerns may be that black cohosh may interact with CYP2D6 substrates [16] and also inhibit CYP3A4 in intestinal epithelium [17].

The studies of Huang et al. [18] agree with these findings since black cohosh extracts inhibited the activity of CYP450 isozymes; the triterpene glycosides exhibited weak activity. The findings of von Breman et al. [9] disagree in that oral administration of a 75% ethanolic extract of black cohosh containing about 11.4% triterpene glycosides (23-epi-26-deoxyactein: 4.4%) did not alter markers of liver function; there was no evidence of hepatic or other toxicity. Since the component 23-epi-26-deoxyactein, but no metabolites, was

Table 1B
Top 5 impacted pathways for black cohosh, 35.7 mg/kg, 24 h treatment. Both up and down-regulated genes (filtered for p < 0.05) were considered.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Impact score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial oxidative phosphorylation</td>
<td>4.01</td>
</tr>
<tr>
<td>Urea and aspartate cycle</td>
<td>1.93</td>
</tr>
<tr>
<td>P450 family</td>
<td>1.77</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>1.75</td>
</tr>
<tr>
<td>Hemes from protoporphyrin IX</td>
<td>1.64</td>
</tr>
</tbody>
</table>
detected in the serum or urine, it appears there was little metabolic conversion. This study indicates that black cohosh would most likely not cause drug botanical interactions.

We used gene expression profiling to gain an understanding of the alterations of rat liver gene expression induced by an extract of black cohosh. The data in DrugMatrix® and the signatures derived from that data were generated for purified compounds using juvenile (8–10 week-old) male rats [19]. Notably, this experiment was performed using 56 week-old female rats. It is likely that differences in biological and transcriptional responses of the rats used in this study compared to the reference dataset animals overwhelm the subtle expression signals captured by signatures. No match to any of the 29 liver signatures was observed. Using Entelos pathway analysis, the highest impact was observed on the Mitochondrial Oxidative Phosphorylation pathway (Fig. 3). The downregulation of mitochondrial genes suggests that black cohosh may cause mitochondrial damage. A disruption of mitochondrial energy generation could explain the observed lipid accumulation in the hepatocytes and also presents a potential mechanism of the hepatitis occasionally observed in human black cohosh users. Mitochondrial damage following extended use of nucleoside analogs in antiretroviral therapy has been associated with steatohepatitis and occasional liver failure [20].

In support of our findings, a study of the hepatic effects of black cohosh indicated that an ethanolic extract of black cohosh given to female Wistar rats (at doses greater than 500 mg/kg) induced hepatic mitochondrial toxicity, as evidenced by microvesicular steatosis, inhibition of beta-oxidation and the respiratory chain and resulting apoptosis [21].

Fig. 3. Pathway map of the mitochondrial oxidative phosphorylation pathway. Genes represented by probe sets on the array are shown as colored circles (p>0.05) or diamonds (p<0.05). Red indicates upregulation while green indicates downregulation of the gene.

Fig. 4. Zoomed view of hierarchical clustering heat map (UPGMA, Pearson’s correlation) of 668 liver experiments across impact scores against 135 DrugMatrix pathways.
in vitro for the induction of stress response genes that we have observed. We validated the effects on cyclin D1 and ID3 using the potential for inducing cell cycle arrest at the G1/S boundary. The upregulation of caspase 9 and IAP5 (pro-apoptotic), but repressed the expression of cell cycle genes suggesting both may induce mitochondrial damage. AffyLimma and DrugMatrix analysis indicated that both markedly downregulated mitochondrial oxidative phosphorylation genes suggesting both may induce mitochondrial damage. Affy-Limma and Drug Matrix microarray and RT-PCR analysis indicated that both repressed the expression of cell cycle (cyclin D1) and cell growth regulator (ID3) genes at 24 h. In support of this, both the extract and actein inhibited the proliferation of HepG2, p53 positive, liver cancer cells. Thus the anticancer effects of black cohosh may be due at least in part to the triterpene glycoside content. The anticancer effects of black cohosh are further supported by our observation that black cohosh clustered with antiproliferative compounds, specifically tubulin binding vinca alkaloids and DNA alkylators. Taken together, the findings suggest black cohosh warrants further study for breast cancer prevention and therapy.

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**Reviewer**

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**Fig. 5.** A) Real-time RT-PCR of rat liver after treating with black cohosh extract (35.7 mg/kg), * indicates p < 0.05; B) Effect of black cohosh on the growth of HepG2 liver cancer cells at 96 h, using the MTT assay.
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