ABSTRACT. This study was designed to assay the estrogenic activities and the antioxidant potential of ethanol extracts from the herbal dietary supplement black cohosh (Cimicifuga racemosa) relative to the natural phytoestrogen genistein. The in vivo mechanisms of action of these two natural products have not been completely elucidated, and Japanese medaka (Oryzias latipes) provides a useful organism for initial in vivo screening of natural products. While both genistein and estradiol altered ovarian and testicular steroid release and decreased circulating testosterone levels in males, neither black cohosh total extract (75-30,000 ng/fish), cimiracemoside A, 25-O-methyl-cimigenoside, actein, nor 26-deoxy-actein caused any differences in estrogenic activity compared to control fish. To assess antioxidant potential, animals were treated with natural products then challenged with 2-acetylaminofluorene (2-AAF) to induce lipid peroxidation (LPO) in the liver. Neither the total ethanol extracts
from black cohosh nor its individual components showed an inhibitory effect in 2-AAF induced LPO. However, genistein manifested potent antioxidative activity in the LPO assay, with similar potency to a high dose of α-tocopherol. In contrast to genistein, black cohosh did not exhibit traditional estrogenic effects nor significant in vivo anti-oxidant potential in this fish model system.

**KEYWORDS.** Black cohosh, genistein, lipid peroxidation, medaka, estrogen, testosterone

**INTRODUCTION**

Black cohosh (Photo 1), *Cimicifuga racemosa* (L.) Nutt. (Ranunculaceae), also known as *Actaea racemosa*, is a perennial plant indigenous to North America. It has many common names, including black snakeroot, squawroot, rattle root, rattle weed, rattle top, bugbane, cohosh and Cimicifuga.\(^1\) Black cohosh has a long and valued history as a traditional medicine used by Native Americans for the treatment of snake-bite, malaise, kidney ailments, malaria, rheumatism, sore throat, and for gynecological conditions such as menstrual irregularities and childbirth.\(^2,3\) Today in the United States, it is used to relieve the symptoms of menopause. According to McKenna and coworkers, black cohosh has become the largest-selling herbal dietary supplement in the United States for reducing symptoms associated with menopause.\(^1\)

**PHOTO 1.** Black Cohosh
The mechanism of action of black cohosh is not fully elucidated and remains somewhat controversial. Because of its reported efficacy in relieving menopausal and psychiatric symptoms, and because of the steroid-like structure of its triterpene glycoside constituents, black cohosh may act through an estrogenic mechanism. A dose-dependent increase in uterus weight with black cohosh treatment in ovariectomized rats and mice was interpreted as a suggestion that the herb has an estrogenic effect of the herb (reviewed by Foster). The finding that black cohosh has a luteinizing hormone (LH) suppressing effect in menopausal women and animal models was considered a confirmation of an estrogen-like mechanism of action. Estrogen-like effects were also observed in human breast cancer MCF-7 cells which exhibited an increase in estrogen receptor (ER) number in response to exposure to black cohosh extracts. In contrast, other studies have shown that black cohosh extracts were not estrogenic. In clinical studies hormone concentrations of LH, prolactin and estradiol, and vaginal cytology were not altered by black cohosh treatments. Other rodent studies found no changes in uterine growth or vaginal cornification in exposed animals. Black cohosh showed no estrogenic activity in Ishikawa cells, while red clover and hops exhibited estrogenic activity. Furthermore, results in three in vitro systems suggested that black cohosh antagonized estradiol induced activities including proliferation of MCF-7 cells and gene expression in the yeast estrogen screen. Neither the triterpene glycosides from black cohosh nor their enzymatically prepared aglycones bound significantly to ER-β in another estrogen receptor binding study. These controversies prompted the first aim of this study which was to evaluate the in vivo estrogenic activity of black cohosh extracts and its individual components in the Japanese medaka, a model we have successfully used in our previous study in evaluating genistein.

Although the estrogenicity of black cohosh is controversial, one of the reputed benefits of the use of natural products as dietary supplements is their potential to function as antioxidants. Oxidative stress in biological systems has been studied because of its relationship with aging and diseases such as cancer, arteriosclerosis, diabetes mellitus and halothane hepatotoxicity. Besides oxidative damage to DNA and proteins, one important toxic consequence of oxidative stress is lipid peroxidation (LPO), a ROS-initiated process that leads to oxidative damage of polyunsaturated fatty acids (PUFAs) in cellular components. Consequences of LPO affect the physical structure of biological membranes including decreased fluidity and increased permeability.
Evidence indicates that certain natural products from plants have anti-oxidant activity and hepatoprotective effects.\textsuperscript{18,19} Genistein is a potent inhibitor of hydrogen peroxide production in 12-O-tetradecanonylphorbol-13-acetate-activated human leukemia (HL-60) cells and inhibits superoxide anion generation by xanthine/xanthine oxidase.\textsuperscript{20} It also increases activities of antioxidant enzymes including catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase in skin and small intestine in mice.\textsuperscript{20} Triterpene glycosides are considered the main active chemicals in black cohosh.\textsuperscript{1,4} Studies of extracts from other species of the genus \textit{Cimicifuga} suggested some degree of protection from CCl\textsubscript{4}-induced hepatotoxicity in mice.\textsuperscript{21} However, the effect of black cohosh extracts against LPO has never been reported. Therefore, a second aim of this study was designed to determine the potential beneficial effects of genistein, black cohosh ethanol total extracts and two of the triterpene glycoside components isolated from black cohosh extracts (actein and cimiracemoside A) against LPO. An evaluation of the estrogenicity and the antioxidant potential of black cohosh extracts and component in the same model system allows an accurate comparison of its efficacy.

We have used Japanese medaka (\textit{Oryzias latipes}) to develop an \textit{in vivo} model to study the potential for black cohosh and genistein to decrease 2-acetylaminofluorene (2-AAF) induced LPO and to assess estrogenicity. Sharing similar general mechanisms of oxidative toxicity with mammalian systems, fish provide an excellent alternative system in oxidative toxicity research.\textsuperscript{17} Furthermore, medaka is a widely used and accepted model in toxicology especially in the disciplines of endocrine disruption and cancer research.\textsuperscript{14,22-24}

\section*{MATERIALS AND METHODS}

\textbf{Fish Source, Care and Handling}

Adult male Japanese medaka were acclimated for 4 days before the injection by putting each group (5 per group for LPO studies; 15 females and 15 males per group for estrogen studies) into an aerated holding container of 1.5-2 L volume. Experimental animals were maintained in balanced salt solution\textsuperscript{25} made from nanopure water at 15 hours light, 9 hours dark cycle with a water temperature of 27°C. All fish were fed twice daily with tropical flake fish food (TetraMin, Tetra Werke, Ger-
many) and brine shrimp. A complete water change was done, and the containers were cleaned every 48 hours.

**Reagents and Solutions for Injection**

N, N-dimethylformamide (DMF), 17-\(\beta\)-estradiol (E2), 2-acetylaminofluorene (2-AAF), t-butyl hydroperoxide (t-BOOH, 70% aqueous solution), butylated hydroxytoluene (BHT), and \(\alpha\)-tocopherol (\(\alpha\)T) were purchased from Sigma Chemical (St. Louis, MO, USA). Genistein (G), purity 95%, was purchased from Chromadex (Irvine, CA, USA). Black cohosh ethanol extracts (BC), cimiracemoside A (CA), 25-O-methylcimigenoside (MC), actein (A) and 26-deoxy-actein (DA) (all extracted and isolated from black cohosh extracts\(^{26}\)) injection solutions were made by adding DMF stock solutions into corn oil. The powder rhizome material of *C. racemosa* was purchased from Frontier Natural Products Co. (Norway, IA, USA; product code/lot number, 957.9012). For 2-AAF and \(\alpha\)-tocopherol the injection solutions were made by directly adding the compound into corn oil and sonicating for 1-2 hours.

**Estrogenicity Assays**

The acclimated fish were separated by sex, randomly assigned to one of the following ten treatment groups (15 females and 15 males in each group) and injected intraperitonally (i.p.) with 5 µl once every other day for 10 days (injections on day 0, 2, 4, 6, and 8): (1) solvent in corn oil as a negative control; (2) 300 ng/fish E2 in corn oil as a positive control; (3) 750 ng/fish G in corn oil; (4) 75 ng/fish total extracts from BC in corn oil; (5) 750 ng/fish total extracts from BC in corn oil; (6) 30,000 ng/fish total extracts from BC in corn oil; (7) 750 ng/fish CA in corn oil; (8) 750 ng/fish MC in corn oil; (9) 750 ng/fish A in corn oil; (10) 750 ng/fish DA in corn oil. These concentrations were selected based on the effective concentrations in previous studies.\(^{14}\) Ten days after the first injection, the fish were anesthetized with 5% 3-aminobenzoic acid ethyl ester (MS-222; Sigma). Fish length and weight were recorded following treatment, and blood, liver, and gonad samples were obtained. In order to collect enough tissue to complete all analyses, tissues collected from two fish of the same sex were pooled together as one sample. Although pooling tissues was minimized, the procedure is necessary to obtain enough material for the assays from this small species. Blood, livers, and gonads were collected as described elsewhere.\(^{14,27}\) From blood, plasma was collected and stored for analysis of circulating
E2 and testosterone (T) concentrations. Livers were homogenized and the supernatant retained for the analysis of hepatic vitellogenin (VTG) concentrations. Gonads were incubated in 200 µl Media 199 (Gibco Life Technologies; containing Hanks’ salts, L-glutamine, without sodium bicarbonate; 100 µl media/gonad) supplemented with 0.5 µg of 25-hydroxycholesterol for the analysis of ex vivo gonadal steroidogenesis or in media supplemented with testosterone, instead of 25-hydroxycholesterol, for the detection of gonadal aromatase activity. The gonads were incubated at room temperature for 48 hours, after which media was removed and stored at −80°C until analyzed. Plasma and media steroid concentrations (E2 and testosterone) were determined by enzyme immunosorbant assay, as described elsewhere.14,27 The gonad area and circumference were measured by tracing the profile of the tissue with a digitizer (Olympus digital microscope with Image Pro Plus software).

The steroid concentrations (E2 and testosterone) in plasma and the media from steroidogenesis and aromatase activity experiments were determined by EIA.28 Briefly, the plasma or media was extracted with ethyl acetate three times in glass test tubes, the pooled extracts were dried, and quantified against a standard curve of either E2 or testosterone.

In order to determine hepatic concentrations of the yolk protein precursor vitellogenin (Vtg), the pooled liver samples were homogenized, centrifuged and the supernatant was kept for analysis. Protein was determined and western blot analysis was performed using standard methods.29,14,27 The immunoblots were scanned, and the relative band intensities quantified by determining integrated optical density (IOD) corresponding to each lane of the gel using Scion Image software (Scion Corporation, based on NIH Image for MacIntosh by Wayne Rasband of NIH). An aliquot of a tissue sample containing Vtg (Positive) was run on each gel to normalize for the intensity of staining across gels.

**Effect of 2-AAF, Genistein, and Black Cohosh Extracts on LPO**

A known inhibitor of LPO, α-tocopherol, was used as a positive control.19 A carcinogenic aromatic amine, 2-AAF, was used to induce LPO.30 To confirm that 2-AAF induced LPO, fish were injected i.p. with either 4 µl/fish corn oil or 4 µl/fish of 10 mg/kg of 2-AAF (0.625 mg/ml and 0.045 mmol/kg) every other day for six days. Five of the fish
treated with 2-AAF were killed 12, 24, 48 hours after the first injection, and 24, 48 hours after the third injection. Five fish from the control group were killed 24 hours after the first or third injection. The liver samples were pooled and stored at −80°C.

To test the potential beneficial effect of genistein and black cohosh extracts against LPO, a similar dosing regime as described above was used. Fish were treated with different testing compounds by i.p. injection with 4 µl/fish of the dosing solutions once every 48 hours for ten days. At the fourth and fifth injection, half of the fish were also challenged with 2-AAF (10 mg/kg) at the same time. The fish were randomly assigned to one of the following 12 treatment groups (with 5 fish in each group): (1) blank group with no treatments; (2) corn oil control group; (3) 7.5% DMF in corn oil control group; (4) corn oil + 2-AAF (10 mg/kg) induced group; (5) 19.4 mg/kg α-tocopherol; (6) 50 mg/kg α-tocopherol; (7) 19.4 mg/kg α-tocopherol + 2-AAF (10 mg/kg); (8) 50 mg/kg α-tocopherol + 2-AAF (10 mg/kg); (9) 12.2 mg/kg genistein + 2-AAF (10 mg/kg); (10) 80 mg/kg black cohosh extracts + 2-AAF (10 mg/kg); (11) 2.0 mg/kg actein + 2-AAF (10 mg/kg); (12) 2.0 mg/kg cimiracemoside A + 2-AAF (10 mg/kg). Doses were chosen so that 2-AAF, the low dose of α-tocopherol, and genistein were the same dose with respect to molarity (0.045 mmol/kg), while the high dose of α-tocopherol was 0.12 mmol/kg. Twenty-four hours after the last injection, all fish were killed and the liver samples were obtained and stored as described above.

**Lipid Peroxidation Assay (LPO-586 Method)**

LPO in the liver was quantified by the concentration of the products of LPO, malonaldehyde (MDA) plus 4-hydroxyalkenals (4-HDA), in the supernatant of the liver homogenates, normalized by the protein concentrations in each sample obtained in the protein assay. The Bioxytech® LPO-586 kit (Oxis International, Inc., OR, USA) was used per manufacturer’s protocol with some modification. Briefly, approximately 10% (w/v) homogenate of liver was prepared by homogenizing in 400 µl phosphate buffer (20 mM, pH 7.4, containing 5 mM BHT to prevent sample from oxidation). Following centrifugation at 3,000 × g for 10 minutes, 325 µl of the LPO-586 R1 reagent, N-methyl-2-phenylindole (7.7 mM in 1:3 methanol/acetonitrile (v/v)) was added to 100 µl supernatant, followed by the addition of 75 µl of the LPO-586 R2 reagent, 15.4 M methanesulfonic acid with incubation at 45°C for 60 min.
Following centrifugation of the turbid reaction mixture at 15,000 × g for 10 min, the clear supernatant was transferred to a Costar 9017 transparent 96-well plate (Corning Inc., Corning, NY, USA) and absorbances were read at 590 nm with a plate reader (HTS 7000, Perkin Elmer, Boston, MA, USA). In order to correct for intrinsic absorbance from the tissue homogenate, a blank group was used to prepare a reaction mixture (sample blank), in which only 325 µl of 1:3 methanol/acetonitrile mixture instead of the R1 reagent was added. A standard curve using 1,1,3,3-tetra-methoxypropane (TMOP) that generates free MDA during the acid hydrolysis step was also prepared at levels of 0, 0.5, 1.0, 2.0, 3.0, and 4.0 µM. The nominal concentration of MDA + 4-HDA was calculated from the standard curve using the absorbance of sample minus that of sample blank. All samples and standards were done in triplicate.

**Statistical Analysis**

One-way analysis of variance (ANOVA) was performed using StatView, version 5.0.1 (SAS Institute Inc.) statistical software to detect differences between treatment groups. Pairwise differences were determined using Tukey-Krammer post-hoc tests, and \( p \leq 0.01 \) was considered significant.

**RESULTS**

**Plasma Steroid Levels**

Circulating steroid concentrations were not significantly affected by black cohosh extracts or its individual components. In females, E2 and genistein treatment did not alter plasma E2 level (ANOVA, \( F = 0.280, \ p = 0.978 \)) or plasma testosterone level (ANOVA, \( F = 0.527, \ p = 0.849 \)). In males, plasma E2 level did not change significantly in any of the treatment groups (ANOVA, \( F = 0.532, \ p = 0.846 \)), while plasma testosterone level was decreased significantly with E2 and genistein treatment (ANOVA, \( F = 2.623, \ p = 0.0197 \); Figure 1), but not significantly with black cohosh extracts treatment.

**Ex vivo Steroidogenesis**

Ten-day treatment with black cohosh extracts and any of the four individual components tested did not alter *ex vivo* steroid release from ei-
ther ovaries or testes incubated with 25-hydroxy cholesterol. Similar to the results in previous study, E2 in the media (pg) normalized for the profile size of the ovaries (µm$^2$) increased significantly with genistein treatment and increased 3-fold with the positive E2 group relative to controls (ANOVA, F = 5.137, p = 0.0009; Figure 2); ovarian ex vivo release of testosterone did not change in any group (ANOVA, F = 0.443, p = 0.898). A comparison of the profile size of ovarian tissue across treatment groups showed no differences (ANOVA, F = 1.315, p = 0.277).

Among males, ex vivo E2 production was not changed significantly in any of the treatment groups relative to the control group, although E2 and genistein treatment showed 1.5 and 2 fold increase, respectively (ANOVA, F = 1.340, p = 0.285). Testicular testosterone release was decreased significantly with E2 and genistein treatment, but not with black cohosh extract treatments (ANOVA, F = 3.306, p = 0.009; Figure 3). A comparison of the profile size of testicular tissue across treatment groups showed no differences with treatment (ANOVA, F = 1.298, p = 0.310).

**Aromatase Activity**

Aromatase activity was determined by the ability of ovaries and testes to convert testosterone to estradiol ex vivo. In both females and
males, gonadal aromatase activity was not affected significantly by black cohosh extracts or any of its individual components. E2 or genistein did not show significant effect either, but both showed the tendency to lower the E2 concentration in the media (ANOVA, F = 0.817, p = 0.606, for males, F = 0.951, p = 0.503).

**Liver Vitellogenin (Vtg)**

Vtg is a precursor of egg yolk which is synthesized in the liver in response to 17β-estradiol. Therefore, it is normally produced by sexually
mature females but not by males. Vtg content in male liver or plasma has been used as a biomarker of estrogen exposure in male fish. Consistent with previous study, E2 induced hepatic Vtg in both males and females (ANOVA, in females, F = 6.071, p = 0.0001, Figure 4; in males, F = 9.189, p < 0.0001, Figure 5), while neither genistein, black cohosh extracts nor its components had this effect.

Effect of 2-AAF on LPO

An initial experiment was done to confirm 2-AAF induced lipid peroxidation in medaka. 2-AAF increased LPO at both 24 and 48 hr following 3 injections (ANOVA, F = 195.6, p < 0.0001, Figure 6). LPO decreased as the time since injection increased, suggesting induction of protective enzymes.

Effect of Genistein and Black Cohosh Extracts on LPO

The treatment with 2-AAF increased the LPO in the liver significantly (Figure 7). The ten day treatment of 50 mg/kg \(\alpha\)-tocopherol alone not only decreased the LPO compared to the 2-AAF induced group, it also decreased the LPO significantly versus the corn oil control group, showing a strong beneficial effect against both constitutive and induced LPO (ANOVA, F = 20.656, p < 0.0001). However, the lower dose (19.4 mg/kg) of \(\alpha\)-tocopherol did not show any beneficial effect.
either alone or against 2-AAF induced LPO. Genistein (12.2 mg/kg) treatment had a marked ability to inhibit the 2-AAF-induced LPO at equal molar concentrations. Black cohosh extracts and cimiracemoside A did not show a significant ability to inhibit LPO when compared to the 2-AAF induced group; however, they were not significantly different from controls. Actein was the least effective inhibitor of LPO in that
its effect was not distinguishable from the 2-AAF alone treatment group. The results also showed that the addition of DMF into corn oil did not have a significant effect on LPO when compared to the corn oil control group.

**DISCUSSION**

The data presented here indicate that black cohosh extracts and its individual components tested in this study do not have estrogenic effects *in vivo* in Japanese medaka. The data also showed that E2 and genistein
had estrogenic effects on the same endocrine endpoints tested in previous study\textsuperscript{14} indicating that medaka was reliable for determining the estrogenic activity of unknown compounds. As stated in the introduction, despite the documented efficacy of black cohosh in the treatment of postmenopausal symptoms, its mechanism of pharmacological action is unknown and studies on its estrogenic effects have yielded conflicting results.

One possible mechanism can be postulated from the report that black cohosh extracts reduced LH in menopausal women and ovariectomized rats.\textsuperscript{5,6} Because symptoms such as hot flash are associated with temporally increased LH levels, it was suggested by Einer-Jensen et al.\textsuperscript{10} that black cohosh extracts may modify the frequency of LH pulses from the pituitary, and thus decrease the fluctuation in endogenous estrogen levels. This decrease in fluctuations may be important for the relief from menopausal symptoms. Duker and coworkers\textsuperscript{6} found that LH, but not FSH levels, were significantly reduced in patients receiving the extract. They also found three types of endocrine active compounds in the lipophilic extract of black cohosh using Sephadex chromatography: fraction 1 did not bind to the estrogen receptor (ER) but suppressed LH release after chronic treatment; fraction 2 bound to ER and also suppressed LH release; fraction 3 bound to ER but did not suppress LH release. However, fraction 3 did suppress LH release after a single acute injection. The authors thus speculated that fraction 3 might contain estrogendisynthetic active compounds that are rapidly metabolized so that only a transient suppressive effect on LH secretion is produced. Fraction 1, which was nonestrogenic but suppressed LH secretion, was postulated to contain \(\alpha\)-2 agonists similar to clonidine, which suppresses LH secretion without binding to ER.\textsuperscript{1,6}

The complex results from various studies, including those presented here, suggest that it would be inappropriate to define the mechanism of action of black cohosh in the traditional sense of estrogenic response through activation of the estrogen receptor. The efficacy reported in clinical trials may be mediated by a non-hormonal action. Because black cohosh extracts contain many other nutrients, a metabolic benefit of the dietary supplement is also possible. Further experiments on the components of black cohosh and other herbal dietary supplements are needed before an understanding of their mechanism of action is achieved.

Results of this study also showed that medaka were a reliable \textit{in vivo} model for screening the effects of natural products on 2-AAF induced LPO. 2-AAF is a synthesized aromatic amine that has been used as an
insecticide and has been reported to be carcinogenic in at least eight different species.\textsuperscript{30} The ten-day treatment of $\alpha$-tocopherol (50 mg/kg) and genistein (12.2 mg/kg) inhibited 2-AAF-induced LPO by approximately 30\% and 55\%, respectively, and 50 mg/kg $\alpha$-tocopherol decreased constitutive LPO by about 37\%. Black cohosh extracts did not have an anti-LPO effect even at the relatively high dose of 80 mg/kg. The results in this study are consistent with prior studies in that genistein and $\alpha$-tocopherol are effective anti-oxidants. $\alpha$-Tocopherol has been used widely as an anti-oxidant and a strong LPO inhibitor in various model systems (reviewed by Aruoma\textsuperscript{19}) and has been used in many studies as a positive control.\textsuperscript{31-35} The anti-oxidative activity of $\alpha$-tocopherol is due to its ability to scavenge the peroxyl radicals of unsaturated lipids in the membranes through its aromatic hydroxyl group. Genistein induced antioxidant enzymes in mice and inhibited both hydrogen peroxide production in 12-O-tetradecanonylphorbol-13-acetate-activated cells and superoxide anion generation by xanthine/xanthine oxidase.\textsuperscript{20} The established anti-oxidative effects of isoflavones such as genistein and daidzein, and ellagic acid (a plant phenol) were reported to be closely related to the aromatic hydroxyl groups present in their structures.\textsuperscript{36,37}

We speculate that the lack of anti-oxidative effect of black cohosh extracts is probably due to the lack of active functional groups in the molecular structure that are able to quench free radicals. In their structures, the hydroxyl group(s) in the triterpene part of actein and cimiracemoside A are not phenolic. Furthermore, it is not obvious why actein is a less effective inhibitor than cimiracemoside A because structurally they are similar. Further studies will be necessary to determine the structure activity relationships necessary for inhibition of LPO.

To our knowledge, to date there are no other studies that have investigated the anti-oxidant potential of black cohosh extracts. However, one study on a Japanese species of Cimicifuga rhizoma, Cimicifuga dahurica, showed that the methanol extracts were effective in preventing liver disorder induced by CCl$_4$ in mice. The methanol extracts used contained cycloartane type triterpenoids, furochromone and cinnamic acid derivatives that could account for the effect. Cimigenol xyloside, a cycloartane type triterpenoid, was found to exhibit a significant preventive action on the liver disorder induced by CCl$_4$, as indicated by decreased serum GOT and GPT, at a relatively high dosage of 300 mg/kg.\textsuperscript{21} Notice that in the black cohosh extract treatment group, although LPO was not significantly decreased versus the 2-AAF inducer group, they
are not significantly higher when compared to the corn oil group. Also, the dose for actein and cimiracemoside A used in this study was very low (2.0 mg/kg) compared with 300 mg/kg in mice. Therefore, it is hard to conclude that black cohosh absolutely has no beneficial effect against LPO according to the results in this study. Black cohosh was administered at a single dose in this study; future studies could use a full dose response.

In summary, Japanese medaka have proven to be a simple and effective way for testing the potential of natural products to reduce 2-AAF induced LPO in the liver and for assessing estrogenic potency. While genistein produced endocrine effects similar to the E2 positive control, black cohosh did not produce similar in vivo effects. Likewise, genistein was a potent inhibitor of lipid peroxidation while neither black cohosh nor two of its components possessed significant anti-oxidant potential.

REFERENCES


