Black Cohosh : A Review

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Abstract

Black cohosh (*Actaea racemosa* L.) is one of the most popular botanical dietary supplements for the treatment of menopausal symptoms in the United States and Europe. Despite the documented clinical efficacy of black cohosh, little is known of the mechanism of action and the active compounds responsible for the relief of menopausal symptoms. This article reviews the chemical constituents, the mechanism of action, and biological activity of black cohosh.

Introduction

Black cohosh [*Actaea racemosa* L. (synonym : *Cimicifuga racemosa*)] is a native North American plant in the buttercup family (Ranunculaceae) that is also known as black snakeroot, squawroot, rattleroot, rattleweed, bugbane or cohosh. Black cohosh grows in shady, rich soil in woods from Maine to Ontario and Wisconsin, and south to Georgia [1]. The rhizomes and roots of black cohosh have been used by Native Americans for a variety of ailments, including malaise, gynecological disorders, diarrhea, sore throat, and rheumatism [2]. In Europe, black cohosh was introduced in Germany in the late 19th century, as natural hormonal agent for treating premenstrually, dysmenorheically, and menopausally caused neurovegetative symptoms. In 1989, the German Commission E, an expert panel commissioned by the German government to address herbal products, approved black cohosh as a non-prescription medicine for the treatment of climacteric ailments such as hot flashes, heart palpitations, nervousness, vertigo, sleep disturbance, and depression [3]. Numerous clinical trials have

indicated that black cohosh preparations have a beneficial effect on the treatment of menopause [3-5]. Black cohosh is now best known in the United States and Europe as a potent alternative herbal medicine for the treatment of menopausal symptoms, and has been experiencing a dramatic increase in consumption. A variety of black cohosh preparations, including isopropanolic and ethanolic extracts, are widely available. In 1996, nearly 10 million retail units of black cohosh preparations were sold monthly in Germany, Australia, and the United States [6]. Black cohosh ranked 9th among all herbal preparations in U.S. sales on 2002 [6].

Chemical Constituents

Research on the chemical constituents of black cohosh has resulted in the isolation of three principle groups of compounds: triterpenoid glycosides, phenylpropanoid derivatives, and flavonoids [7-15].

1. Triterpenoid glycosides : Triterpenoid glycosides are the main class of compound in the rhizomes and roots of black cohosh. Highly oxygenated 9,19-cyclolartane-type triterpenoids, linked with one monosaccharide unit such as xylopyranoside or arabinoside are commonly found in *Actaea*. To date, more than 40 triterpenoid glycosides were reported such as actein, deacetylactein, 23-epi-26-deoxyactein (previously known as 27-deoxyactein). Most commercial black cohosh products are standardized for their triterpenoid glycosides content, calculated as 2.5 % 23-epi-26-deoxyactein. Beside triterpenoid glycosides, aglycones of actein, deacetylactein, and 23-epi-26-deoxyactein were also reported as acetylacteol, acteol, and 27-deoxyacetylacteol, respectively. The chemical structures of selected triterpenoid glycosides are shown in figure 1.

2. Phenylpropanoid derivatives : The majority of the phenylpropanoids in black cohosh are caffeic acid derivatives which may be present in free acid form, or in ester linked dimers with other phenolic acids such as fukiic acid or piscidic acid.

Caffeic acid derivatives, including caffeic acid, ferulic acid, and isoferulic acid, have been isolated from the roots and rhizomes of this plant. All of them can form ester-linked dimers with fukiic acid to yield fukinolic acid, cimicifugic acid A and B or with piscidic acid to yield cimicifugic acid E and F. The chemical structures of selected phenylpropanoid derivatives are shown in figure 1.

3. Flavonoids : One of these compounds, the isoflavone formononetin, was believed to be one of the active pharmacological compounds responsible for estrogenic activity of black cohosh, because it has an affinity to bind to estrogen receptors [16]. This is surprising because isoflavone formononetin is primarily found in the Fabaceae family based on chemotaxonomy. However, more recent studies have shown that black cohosh extract did not contain formononetin [17,18]. Some investigators believed that the presence of formononetin in black cohosh was due to improper taxonomic identification of the starting material or adulteration with plant parts other than the roots and rhizomes. Other studies have reported the presence of other flavonoids, such as kaempferol, biochanin A and genestein 4´-methyl ether [19]. The chemical structures of selected flavonoids are shown in figure 1.

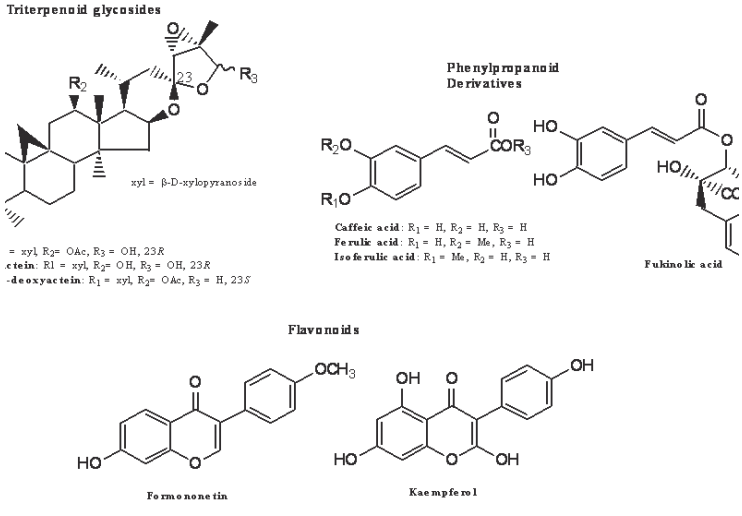

Fig.1 Chemical structures of selected triterpenoid glycosides, phenylpropanoid derivatives, and flavonoids.

In addition to the three major constituents, other constituents including tannins, resin, and fatty acids have also been reported from the rhizomes and roots of black cohosh [20].

Mechanism of Action

The mechanism of action by which black cohosh reduces hot flashes is not well understood. Early studies showed an estrogenic-like effect of black cohosh extracts in animal studies and an estrogen receptor bioassay [16,21]. Investigators suggested that black cohosh contained three synergistically acting compounds able to reduce serum luteinizing hormone (LH) levels and bind to estrogen receptors. They believed triterpenoid glycosides are responsible for the reduction in LH levels, and the isoflavone formononetin bound to the estrogen receptors. A mechanism by which black cohosh acts through estrogen receptors was proposed. However, this hypothesis has failed to convince other investigators for two reasons. First, more recent studies have reported a lack of estrogenic activity in the animal studies, cell binding assays, and cell proliferation assays [22-24]. Second, formononetin, a known estrogenic compound was not recently found in black cohosh extracts [17]. In addition, a recent analysis of the commercial product Remifemin and wild black cohosh from 13 different locations in eastern United States resulted in no detectable levels of formononetin [18].

However, some investigators still suspect that black cohosh may bind to an unidentified ERã receptor in humans due to the selective ER modulator (SERM)-like activity of black cohosh extracts [25-27]. Research to date suggests that it is unlikely that there is a direct estrogenic effect but that black cohosh may work through a central activity.

It is generally accepted that estrogens play a major role in the etiology of hot flashes because they are experienced in those periods when blood levels of estrogen are low. However, the frequency and severity of hot flashes show a poor correlation with

serum estrogen level. This suggests that other mechanisms in the nervous system may play a role [28]. Beside norepinephrine, which is the primary neurotransmitter responsible for lowering the thermoregulatory set point, serotonin (5-hydroxytryptamine, 5-HT) is another key neurotransmitter inducing hot flashes. Estrogen withdrawals are associated with decreased blood serotonin levels [29] and also with up-regulation of serotonin receptors in the hypothalamus [30]. Stimulation of certain serotonin receptors, 5-HT_{2A} receptors, may change the set point temperature, activating some autonomic functions to cool down the body, and thereby cause an increase skin temperature and sweating resulting in hot flashes [31]. Some investigators proposed that the mechanism of action of black cohosh may involve the serotonergic pathway [32]. Burdett et al. reported the presence of compounds with a strong binding to three 5-HT receptor subtypes. In this study, the isopropanolic extract was screened for the ability to bind with the 10 serotonin receptors subtype 1A, 1B, 1D, 2A, 2B, 2C, 3, 5A, 6 and 7. They found that black cohosh bound to all subtypes with great activity with 5-HT_{1A}, 5-HT_{1D}, and 5-HT_{7}. 5-HT_{1A} and 5-HT_{7} are located in the hypothalamus, where they might be in hormonal regulation of hot flashes. Investigators further conducted an experiment to distinguish the relative binding potency of various black cohosh extracts, 40% isopropanolic, 60% ethanolic and 100% methanolic extracts, for these two receptor subtypes. The methanolic extract exhibited a higher binding potency compared with other two extracts. In addition, they showed that the methanolic extract functioned as a mixed competitive ligand of 5- HT_{7} receptor. This study gave primary evidence of the serotonergic activity of black cohosh, providing possible alternative mechanism. Further studies need to be conducted to determine if black cohosh acts through the serotonergic pathway.

Some investigators have proposed a third hypothesis that black cohosh may act through binding to the dopaminergic receptors [33], although the effect of estrogen on the dopaminergic system is poorly understood [34]. This hypothesis is based on indirect evidence

and is not supported by binding assay studies. For example, De Leo et al. [35] investigated the effect of dopamine infusion on plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) after acute and chronic estrogenic withdrawal in women. They found that the dopamine infusion inhibited the plasma LH, and PRL levels, but not FSH levels. These results were very similar to those reported by Matsubara et al. [34]. Black cohosh extracts (BCEs) showed results on endocrinal hormones (LH, FSH, PRL) in animal studies similar to the results in women treated with dopamine [16,21,36]. This suggests that the biological activity on endocrinal hormone levels may be due to the presence of dopaminergic compounds in black cohosh. Further more, Johnson et al. [37] reported that a dopaminergic agonist can also cause a significant decrease in proliferation of MCF-7 cells. The inhibitory effect of black cohosh extract on the growth of estrogenic-sensitive MCF-7 cells could be due to the presence of the dopaminergic compounds in the plant [38,39]. The stimulation of MCF-7 cells growth in one study [40] could be due to loss of dopaminergic compounds and a predominance of fukinolic acid, which can stimulate the proliferation of MCF-7 cells [14]. In addition, dopamine can increase estrogen expression *in vitro* [41]. Thus, the estrogenic activity of black cohosh extract could be due to the presence of the dopaminergic compounds in the plant [21]. Recently, Jarry et al. reported direct evidence of dopaminergic activity of BCEs in a dopamine receptor ligand-binding assays [27]. Using bioassay-guided fractionation, bioactive fractions were collected by high-speed countercurrent chromatography. The structure of the purified active compounds should be available in the future. More studies need to be done to support this hypothesis.

In summary, the mechanism of action of black cohosh has not been fully understood. It is likely involved with the nervous system through central nervous activity, serotonergic, or dopaminergic pathways. However, that black cohosh acts through estrogenic pathways can not be excluded, if the unidentified ERã receptor exists.

Biological activities

Estrogenic activity

It was once believed that black cohosh acts through estrogenic-like mechanism. Much attention has been directed by researchers to test the estrogenic activity of black cohosh. The activity has been studied *in vivo* using animal models and *in vitro* using cell binding or cell proliferation assays by many research groups. The results among different studies are contradictory.

Jarry and Harnischferger [21] studied the effect of BCEs on the serum concentration of LH and FSH in ovariectomized rats. High levels of these two hormones are associated with hot flashes [42]. They found that a dichloromethane extract concentrated the triterpenoid glycosides and depressed plasma LH levels but not FSH. Jarry et al. [16] further characterized the mechanisms of action and the active constituents of black cohosh on the estrogen receptor binding assay. They identified 3 active principles in methanol extract : (i) compounds that do not bind to estrogen receptors but suppress LH levels, (ii) compounds that bind to estrogen receptors and suppress LH levels, and (iii) compounds that bind to estrogen receptors but do not suppress LH levels. One of these compounds was identified as the isoflavone formononetin, which was shown to bind to estrogen receptors but caused no reduction of effect on LH levels in ovariectomized rats. Similarly, Duker et al. [36] found that the serum LH levels in ovariectomized rats was reduced by a lipophilic but not hydrophilic BCEs. More recent research also confirmed the estrogenic activity of BCEs [40,43,44]. Harnischfeger and Cillien [43] reported that the butanol and chloroform subfractions from an alcoholic BCEs bound to estrogen receptors. Liu et al. [44] reported that the ethanolic BCEs stimulated proliferation of estrogen-receptor-sensitive human breast cancer cells, MCF-7, *in vitro* and increased the uterine weight of immature female mice. Wober et al. [45] also reported the estrogenic activity of black cohosh on the alkaline phosphatase activity in Ishikawa cells. They found that the ethanolic

extract showed a weak estrogenic effect, compared to higher activity of the isopropanolic extract. Furthermore, Kruse et al. [14] studied the estrogenic activity of individual compounds isolated from black cohosh in MCF-7 proliferation assay. They found that fukinolic acid had a stimulating effect on the proliferation rate of MCF-7 cells.

However, many more studies have reported lack of estrogenic activity of black cohosh. Einer-Jensen et al. [22] investigated the estrogenic activity of an aqueous ethanolic BCEs on uterine growth in immature mice and on vaginal cornification in ovariectomized rats. They found no estrogenic activity in terms of increasing the uterine weight and number of cornified vaginal cells as compared to a positive control group treated with estradiolbenzoate. Liu et al. [23] demonstrated that a methanolic BCEs did not bind to purified ERá and ERâ and did not increase the activity of estrogen-dependent alkaline phosphatase in Ishikawa endometrial cancer cells. Freudenstein et al. [24] evaluated the safety of a standardized isopropanolic BCEs on the stimulation of estrogen-dependent mammary gland cells induced with 7,12-dimethyl[a]anthracene in ovariectomized rats. There were no statistical differences in tumor number and size between the black cohosh treatment group and the control group, while the estrogen-treated group showed a significant increase in both number and size of tumors. These results showed a lack of mammary tumor-stimulating effect of black cohosh, indicating lack of estrogenic activity. Furthermore, Beck et al. [46] found no estrogenic activity of a standardized BCEs in an ER binding assay. These results are consistent with the data in the Klein et al. study [47], which used a methanolic BCEs. Moreover, Lupu et al. [48] also demonstrated that the aqueous methanolic BCEs did not regulate the expression of estrogen-regulated genes of the ER-positive cell line, MCF-7 and T47D, as well as the ER-negative cell line, MDA-MB-231 in the RNase protection assay, did not induce transcriptional activation of estrogen-responsive elements in the ERE-luciferase reporter assay, and did not contain estrogenic activity as determined by the

Ischikawa cell assay. Furthermore, Zhang et al. [49] investigated the estrogenic activity of ethanolic BCEs as well as the triterpenoid glycosides, actein, 26-deoxyactein, and cimiracemoside A in Japanese medaka fish. After ten day period of treatments, the estrogenic activities were determined by the measurement of plasma steroid levels, aromatase activity (the ability of gonads to convert testosterone to estradiol), and liver vitellogenin levels, a precursor of egg yolk synthesized in the liver in response to 17â-estradiol. Investigators found that neither the BCEs nor individual triterpenoid glycosides caused any change in estrogenic activity compared to control fish. Thus, they concluded that black cohosh did not exhibit any estrogenic activity.

While these studies showed a lack of estrogenic activity of black cohosh, others studies have reported that BCEs have selective ER modulator (SERM)-like activity, resulting in a positive effect on bone and blood vessels, with no effect on the breast and endometrium. Seidlova-Wuttke et al. [50] investigated the estrogenic effects of BCEs on bone, fat, and uterus of ovariectomized rat treated with 33 mg BCEs per day over 3 months. They found that the black cohosh significantly reduced bone mineral density loss and paratibial fat deposits. There was no effect on uterine weight or gene expression of E2-regulated genes. They concluded that black cohosh exhibited SERM effect in the bone and in the fatty tissue, but not in the uterus of ovariectomized rat. Further work by the same group showed the SERM effect of black cohosh in the hypothalamus by inhibiting LH secretion, as well as an effect in bone tissue, shown by the osteoporosis-preventing effect, but no effect was found in the uterus of ovariectomized rat [25]. Wuttke et al. [26] also showed the SERM effect of black cohosh in the bone and in the vagina, but without estrogenic effects in the uterus of postmenopausal women. The results of these studies further support that the mode of action of black cohosh may be to act through estrogenic activity. Moreover, Jarry et al. [27] proposed that black cohosh binds to the unidentified ERã type, which was recently discovered in fish

[51]. They found that the isopropanolic BCEs displaced estradiol from binding sites in the human endometrium cytosol preparation, but did not displace estradiol from either ERá or ERâ. This suggests that a third ER type, named ERã, may exist in the uterine cytosol. The unidentified ER may help understand why some studies showed lack of estrogenic activity of BCEs in ER cell-binding assay However, further studies are needed to prove this hypothesis. Thus, the mechanism of action of black cohosh acting via the estrogenic activity still remains unclear.

Anticancer activity

One of the side effects of hormone-replacement therapy (HRT) is cancer promotion, especially endometrial or breast cancer. To evaluate the safety of black cohosh, some studies have investigated the stimulation effect of black cohosh on estrogen-sensitive breast cancer MCF-7 cells and tumor-induction in animal models. Bodinate and Freudenstein [38] examined the isopropanolic black cohosh extract on the growth effect of MCF-7 cells. The extract did not stimulate MCF-7 growth and instead exerted inhibitory effects on cellular proliferation. Niblen and Freudenstein [52] tested the effect of isopropanolic BCEs on the transplantable endometrial adenocarcinoma cells in rats. They found that black cohosh did not stimulate the growth of the tumor cells. A study by Einbond et al. [39] also demonstrated the inhibitory effect of the ethyl acetate portion fractioned from the aqueous methanolic extract on the growth of two cell binding assays, MCF-7 and MDA-MB-453. BCEs induced cell cycle arrest at G1 with a concentration of 30 • g/ml, and at G2/M with a concentration of 60 • g/ml in MCF-7 cells. This suggests that the extract contains a mixture of components with the more active (or more abundant) components causing G1 arrest and the less active causing G2/M arrest. Triterpenoid glycosides including actein, 23-epi-26-deoxyactein, and cimiracemoside A, isolated from black cohosh also inhibited growth of MCF-7 cells and induced cell cycle arrest at G1. Furthermore, Hostanska et al.

[53] reported similar results to those in Einbond et al.'s study. Hostanska et al. found that the isopropanolic and ethanolic BCEs inhibited growth of the estrogen-receptor positive MCF-7 and estrogen-receptor negative MDA-MB231 breast cancer cells by induction of apoptosis. Hostanska et al. [54] further investigated the two major fractions, the triterpenoid glycosides, and the cinnamic acid ester, from an isopropanolic black cohosh extract, on the growth of MCF-7 cells. Both fractions inhibited cell growth by induction of apoptosis. The cinnamic acid ester fraction is more potent than triterpenoid glycosides fraction. The lack of proliferation effect of black cohosh on the estrogen-receptor sensitive MCF-7 and estrogen-receptor negative MDA-MB breast cancer cells, both *in vitro* and *in vivo*, suggests a favorable safety profile for use in women with or without a history of breast cancer. However, a long term study still has to be conducted to confirm this biological effect.

Conclusion

Although black cohosh is a potent alternative herbal medicine for the treatment of menopausal symptoms in the United States and Europe, neither the active compound nor the mechanism of action has been revealed. Previous phytochemical studies have shown that black cohosh contains three principle groups of compounds, triterpenoid glycosides, phenylpropanoid derivatives, and flavonoids, but none of these compounds has been proven to be the active compound responsible for its pharmacological activity. The evidence from *in vitro* and *in vivo* studies suggests that black cohosh possesses a central activity through serotonergic or dopaminergic pathways, instead of a hormonal effect. Further biological and chemical investigations are required to define its active compound and mechanism of action.

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