Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the risk associated to intake of food supplements containing *Cimicifuga racemosa* root/rhizome as ingredient

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Abstract

Extracts of the root or rhizome of *Cimicifuga racemosa* (L.) Nutt., also known as black cohosh, have traditionally been used for treating various conditions, including premenstrual syndrome, menopause and other gynaecological problems, uses that have been recognised by the World Health Organization (WHO) and the European Medicines Agency (EMA). In Spain, products containing *Cimicifuga racemosa* are marketed either as herbal medicinal products or as food supplements. A non-exhaustive sampling of food supplements containing *Cimicifuga racemosa* registered in the General Health Register of Food and Food Companies (RGSEAA, as per its Spanish acronym), it showed such food supplements contained highly varying accounts of *Cimicifuga racemosa*, with no uniformity in their form of expression.

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In 2006, the Spanish Agency of Medicines and Medical Devices (AEMPS) published an information note on the possible association of the use of *Cimicifuga racemosa* rhizome extract with acute liver damage, and set out a series of recommendations in this regard. In this context, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has carried out a review of the scientific evidence on the potential toxicity of food supplements containing *Cimicifuga racemosa* among its ingredients.

The effectiveness of *Cimicifuga racemosa* extracts compared to placebo in the treatment of menopausal symptoms was demonstrated in a recent meta-analysis. However, this scientific evidence has only been established for herbal medicinal products, but not for food supplements.

In terms of safety, scientific literature indicates that standardised extracts of *Cimicifuga racemosa* are generally well tolerated. With regard to any potential hepatotoxic effects of *Cimicifuga racemosa*, scientific studies to date show no evidence in this regard. However, products labelled as "black cohosh" have been associated in clinical practice with several cases of liver injury, in which data on dosing, purity, origin and preparation of the products, as well as the time period between use and onset of symptoms, were scarce. Therefore, the relationship between *Cimicifuga racemosa* consumption and liver damage is controversial. The most recent data indicate that there is no cause-effect relationship between the consumption of *Cimicifuga racemosa* food supplements and the development of liver disease. However, in the absence of essays with an appropriate methodological design analysing the safety of the comsumption of this type of supplements, it cannot be ruled out that these supplements, at the dosing and types of extract currently available on the market, are associated with hepatotoxicity and, therefore, their use is not recommended in people with impaired liver function.

In addition, health professionals, researchers and users are required to report to the appropriate institutions any serious adverse effects that they believe may be causally related to these types of supplements. In addition, regulatory authorities should develop post-marketing surveillance systems. In addition, there is a need for standardisation of food supplements containing *Cimicifuga racemosa* in terms of composition and its declaration on the label to ensure consistency of such composition.

Finally, it is necessary to promote clinical trials with an appropriate methodological design in order to draw conclusions regarding the efficacy and safety of food supplements containing *Cimicifuga racemosa*.

Key words

Cimicifuga racemosa, food supplements, menopause, hepatotoxicity.

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1. Introduction

Cimicifuga racemosa (L.) Nutt. also known as *Actae racemosa* L., is popularly known as black cohosh. It is a perennial herbaceous plant, belonging to the family *Ranunculaceae*. It has large leaves (basal leaves can measure up to 1 metre long and wide) with a thick serrated margin, forming repeated series of three leaflets. It blooms in late spring and early summer on a tall stem, forming racemes up to 50 centimetres long. Those flowers do not have petals sepals; they consist of small groups of white stamens surrounding a white stigma. They have a pronounced sweet and unpleasantly medicinal smell, which attracts flies, mosquitoes and beetles. The dry fruit is a follicle, with one carpel, containing several seeds (Richo, 2002).

Based on morphological characters and DNA studies carried, the genus *Cimicifuga* has been reclassified into the genus *Actaea* (Compton et al., 1998). The latter comprises a total of 28 taxa, distributed throughout the northern hemisphere, 8 of which are native to North America and the other 20 are found in Asia and Europe.

Cimicifuga racemosa is a well-known ethnomedicinal plant (Applequist, 2003) (Qiu et al., 2014). Its roots and rhizomes have been traditionally used by Native Americans for the treatment of different medical conditions (Predny et al., 2006). Its use in medicine was recorded in the United States Pharmacopoeia in 1830. Throughout the 19th century, it was used to treat various gynaecological disorders such as endometriosis, amenorrhoea, dysmenorrhoea, menorrhagia, infertility and severe postpartum pain, as well as to increase breast milk production (McKenna et al., 2001).

Currently, in some European countries, preparations containing *Cimicifuga racemosa* rhizome are authorised either as herbal medicinal products or as food supplements, being used primarily for management of symptoms of premenstrual tension, menopause and other gynaecological problems (Borrelli et al., 2003) (Mollá et al., 2009).

On the other hand, it is important to note that adulterations, substitutions or misidentifications have been identified in certain botanical preparations found in some American markets that have not undergone quality controls, which may pose a serious risk to consumer safety. Preparations allegedly containing *Cimicifuga racemosa* were found to have been adulterated with some other *Actaea* species, such as with blue cohosh (*Caulophyllum thalictroides* (L.) Michx.), which has been described as poisonous, due to their similar appearance and common growing sites. Besides, other species that may be misidentified as *Cimicifuga racemosa* are white cohosh, yellow cohosh, etc. (Teschke et al., 2009) (Jiang et al., 2011). Correct identification of *Cimicifuga racemosa* and differentiation from other species is therefore the critical point that ensures the quality, safety and efficacy of preparations (Beer and Neff, 2013).

In 2006, the Spanish Agency of Medicines and Medical Devices (AEMPS) received information from the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) regarding the possible association of the use of *Cimicifuga racemosa* rhizome extract with acute liver injury (EMA, 2006).

In this context, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has carried out a review of the existing scientific evidence on the possible toxicity derived from the consumption of food supplements containing *Cimicifuga racemosa* as an ingredient, with the

aim of establishing, when appropriate, a recommendation as to the dosing that could be considered safe, as well as to assess the advisability of establishing safety warnings on the labelling, with the ultimate goal of being able to take appropriate management measures if necessary.

2. Bioactive compounds from *Cimicifuga racemosa*

Cimicifuga racemosa contains many organic compounds with biological activity (Guo et al., 2017). The first characterisation of such compounds was carried out in 1827 by G.W. Mears (Li and Yu, 2006). Tannins, gallic acid, resins, gums, starches and unsorted bitter substances were obtained. In 1909, isopherulic acid, cinnamic acid derivatives, plant sterols and various fatty acids were detected. Research continued, and in the 1960s and 1970s the major bioactive compounds of the genus *Cimicifuga* were identified: triterpenoids, phenolic compounds and their metabolites, plant sterols, alkaloids and chromones. In total, about 457 compounds have been isolated, fewer showing biological activity.

2.1 Saponins

As stated above, most of the bioactive compounds of *Cimicifuga racemosa* are triterpenoids, with a highly oxidised cycloartane structure, which has three rings in the 9 and 10 positions. Based on the degree of oxidation and the system by which the rings and side chains are formed, saponins are classified into seven different subtypes. These subtypes include cimigenol-type structures. In fact, cimigenol and its glycoside derivatives and analogue substances were the first terpenoids to be isolated from the genus *Cimicifuga* in the 1960s. There is also the shengmanol type, the foetidinol type, the isodahurinol type and the pentol type (cycloartanol type). During the 1990s, several structures derived from cimigenol were isolated, including its glycosides, such as the analogues 25-anhydrocimigenol, 7,8-dedihydrocimigenol and epimers at position 24 (as in the case of cimigol).

2.2 Phenylpropanoids

Cimicifuga racemosa contains significant amounts of caffeic acid, ferulic acid and isoferulic acid; in fact, it is one of the few plants to possess the latter two compounds. In addition, it contains other phenolic acids, such as derivatives of fukic acid, including cymicifugal acids, shomasides A-E and cimiracemates A-D.

It also contains phenylpropanoid glycosides of galactose and glucose, including cimidahurin and cimidahurinine. On the other hand, two lignans can be found, the most important of which is actaealactone.

2.3 Nitrogen compounds

Various kinds of amide- and alkaloid-derived components are found in *Cimicifuga racemosa*, although the content of amide-type components is very low. Other nitrogenous compounds are derived from guanidine and include cimipronidine and cyclocimipronidine.

2.4 Chromones

Chromones from *Cimicifuga racemosa* were isolated in the 1970s, the most prominent of them being cimifugin and visnagin.

2.5 Flavonoids

The composition of *Cimicifuga racemosa* also includes flavonoids such as formononetin, an isoflavone, although some recent publications have questioned the presence of these compounds.

2.6 Other compounds

Other compounds include derivatives from plant sterols (cimisterol A), which is a 4α-methylsterol.

3. Indications, products and dosage

3.1 Indications

The World Health Organization (WHO) recognised in 2002 the use of the medicinal plant *Cimicifuga racemosa* for the treatment of climacteric symptoms such as hot flushes, heavy sweating, sleep disorders and irritability, based on available clinical data. It also recognised its use in the treatment of premenstrual syndrome and dysmenorrhoea, which is described in different pharmacopoeias and traditional medicine systems (WHO, 2002).

The North American Menopause Society also recommends the use of this medicinal plant as a treatment option for women with mild menopause-related symptoms, along with lifestyle approaches (NAMS, 2004).

Furthermore, the EMA considers that dried rhizome extracts of *Cimicifuga racemosa* have a "well-established use" for the relief of minor neurovegetative disorders associated with menopause, such as hot flushes and heavy sweating (EMA, 2018). For its part, the European Commission approves the use of extracts of *Cimicifuga racemosa* as defined in the EMA monograph for the treatment of premenstrual syndrome, dysmenorrhoea and neurovegetative disorders associated with dysmenorrhoea and menopause.

3.2 Products

The first allopathic herbal medicinal product (HMP) containing *Cimicifuga racemosa* extract was introduced in Germany in 1956 and has been extensively studied ever since. It was formulated in the form of tablets containing an isopropyl extract and in the form of a solution containing an ethanolic extract. In Germany, where herbal medicinal products are authorised under strict regulatory control, which requires proof of effectiveness, safety and state-of-the-art pharmaceutical quality, gynaecologists rate treatment with *Cimicifuga racemosa* as well-known and effective for climacteric symptoms. The German Medicines Act of 1976 obliged manufacturers to provide proof of quality, safety and effectiveness, and established a transition period for existing medicines until 1990. Between 1985 and 1987, the German Ministry of Health established standards on how to conduct clinical trials for medicinal products. In studies conducted under these conditions, a daily 127 mg dose of extract was found to be safe, although 40 mg was sufficient to produce significant positive effects (Henneicke-von Zepelin, 2017). Subsequently, two other herbal medicinal products containing alcoholic extract of *Cimicifuga racemosa* as an ingredient appeared on the market.

In 2010, the EMA's Committee on Herbal Medicinal Products (HMPC) approved the use of *Cimicifuga racemosa*, based on the literature to date, provided that the production of the extract was

of proven pharmaceutical quality, according to established standards. It did not set a limit for the duration of use, but stated that after 6 months of therapy, a medical professional should be consulted (Henneicke-von Zepelin, 2017) (EMA, 2018).

Today, *Cimicifuga racemosa* extracts are available in the United States, Canada, Europe and Australia, among others, as a food supplement or phytomedicinal product. In 2018, there were 38 medicinal products approved or registered in the European Union (HMPC, 2018).

In Belgium and Italy, its marketing as a food supplement is authorised subject to restrictions and warnings on the labelling, such as that the recommended daily dosing should not exceed 30 mg of dried rhizome, a warning to consult your doctor before use and to refrain from using it in case of liver disease. In Germany, Austria and Switzerland, in their safety assessment document for different botanical species, *Cimicifuga racemosa* is considered a medicinal plant and is listed as A (not recommended for use in food) (BVL, 2014).

In Spain, many food supplements containing *Cimicifuga racemosa* are marketed, invoking the principle of mutual recognition, provided that the product (due to its composition and presentation) cannot be classified as a medicinal product, according to Directive 2001/83/EC (EU, 2001), as amended by Directive 2004/27/EC (EU, 2004). It is important to note that the vast majority of these supplements contain not only *Cimicifuga racemosa* extract, but also a greater or lesser number of other plant extracts. The rationale for the inclusion of these additional extracts, or the role they play in the effects intended the supplement, are often not well-described. Moreover, these food supplements are available in different presentations, such as capsules, tablets, pills, tablets, liquids or powders. A non-exhaustive sampling of food supplements containing *Cimicifuga racemosa* registered in the General Health Register of Food and Food Companies (RGSEAA, as per its Spanish acronym) shows that they contain highly varying amounts of *Cimicifuga racemosa*. Translation of these amounts into recommended daily intakes has not been harmonized. For example, there are supplements which state the recommended dosing in terms of the amount of dry extract taken per day (with doses ranging from 5 to 320 mg dry of extract/day). Other supplements stating the dosing in terms of the amount of *Cimicifuga racemosa* taken per day (with dosing ranging from 17 to 90 mg of *Cimicifuga racemosa*/day). In the vast majority of them, the Drug Extract Ratio (DER), understood as the rhizome/extract ratio, is not specified, and only in a few of them the percentage of triterpene glycosides is indicated. Therefore, no comparison can be made between the dosing contained in food supplements those of and herbal medicinal products, in which its composition is well established in their Summary of Product Characteristics as well as in the EMA monograph (EMA, 2018).

In the case of herbal medicinal products, controls related to harvesting, extracting and product manufacturing and product stability are applied. These quality controls to which they are subject are performed to ensure protection against adulteration and contamination, thus guaranteeing stable levels of effectiveness and safety. However, in the case of food supplements, such stringent controls are not legally required. Often, food supplements claiming to consist of authentic North American *Cimicifuga racemosa* actually contain entirely different species of *Cimicifuga* from Asia (Jiang et al., 2006) (Ma et al., 2011). Therefore, when analysing published scientific data, it is essential to differentiate between herbal medicinal products and food supplements, as well as to differentiate between different extracts.

3.3 Dosage of *Cimicifuga racemosa* based medicinal products

In terms of dosage, the dosing recommended by various agencies differ. The proposal of the European Scientific Cooperative on Phytotherapy (ESCOP) is 40-140 mg standardised alcoholic extract (40 % isopropanolic extract or 40-60 % ethanolic extract), or equivalent oral preparations daily (ESCOP, 2003).

In contrast, the dosage proposed by EMA for herbal medicinal products in its dedicated monograph is much more restrictive (EMA, 2018):

- Dry extract (DER 5-10:1, extraction solvent ethanol 58 % V/V): 2.8 mg twice a day, by oral administration (daily dose: 5.6 mg).
- Dry extract (DER 4.5-8.5:1, extraction solvent ethanol 60 % V/V): 6.5 mg, once a day, by oral administration.
- Dry extract (DER 6-11:1, extraction solvent propan-2-ol 40 % V/V): 5 mg once a day or 2.5 mg twice a day, by oral administration (daily dose: 5 mg).

The EMA allows using these products without medical supervision for a maximum of 6 months.

Finally, the dosage recommended by the European Commission is 40 mg daily for alcoholic extracts (40-60 %) (BVL, 2014).

4. Biological activity of *Cimicifuga racemosa*

4.1 Effect on menopausal symptoms and osteoporosis

The climacteric (or menopause), characterised by major hormonal changes, is the period of transition of a woman from the reproductive stage to the non-reproductive state. During this period, the ovaries stop producing oestrogen and progesterone, and there is an increase in the production of gonadotropin hormones such as luteinising hormone (LH) and follicle-stimulating hormone (FSH). These hormonal changes can cause a wide range of vasomotor, vaginal and psychological symptoms, including hot flushes, tissue atrophy, dysfunction, sleep disturbances and emotional disturbances (Borrelli et al., 2003).

Currently, Hormone Replacement Therapy with drugs (HRT) is used in some cases to fight menopausal symptoms. In addition, many women turn to complementary and/or alternative therapies for relief of these symptoms, including plant-based preparations (Li and Yu, 2006).

In this context, the *Cimicifuga racemosa* rhizome has traditionally been used for the treatment of various gynaecological disorders, such as the relief of menopausal symptoms. The first Good Clinical Practice-compliant clinical study was conducted in 1995 in a multicentre setting in Poland, and was devoted to the question of the relationship between dosing and effectiveness. 152 patients suffering from menopausal symptoms were randomly assigned to the placebo group and to treatment groups receiving up to 127 mg of *Cimicifuga racemosa*. All doses proved to be safe and did not influence oestrogenic parameters such as luteinising hormone (LH), follicle-stimulating hormone (FSH), serum oestradiol (E2), sex hormone binding globulin (SHBG), prolactin or vaginal cytology (Liske et al., 2002). Without differentiating according to menopausal status, the extract at the 40 mg dose already showed sufficient effectiveness in terms of significant improvement. Perimenopausal women benefited more from

Cimicifuga racemosa extract at a 127 mg dosing than at a 40 mg dosing (Henneicke-von Zepelin, 2002).

The second randomised, double-blind, placebo-controlled clinical study was conducted between 2002 and 2005 in a multicentre setting in Germany. A total of 304 patients suffering from menopausal complaints participated (Osmers et al., 2005). In addition to once again demonstrating the effectiveness of *Cimicifuga racemosa*, this trial revealed that women during their first years of menopausal discomfort benefitted more from *Cimicifuga racemosa* use in terms of greater improvement in their symptoms than those who had been suffering from these problems for several years.

The two previous studies used isopropanolic extracts of *Cimicifuga racemosa*. At about the same time, the first placebo-controlled study with a product containing an ethanolic extract of *Cimicifuga racemosa* was conducted in the Czech Republic (Wuttke et al., 2003). In it, 62 patients were evaluable for effectiveness after being randomised to be given placebo, *Cimicifuga racemosa* extract or hormone therapy. In this case, the effect of the extract narrowly exceeded the significance level because the sample size was too small. However, an interesting finding of this study was that, like hormone therapy, it produced beneficial effects on serum parameters of bone metabolism and an increase in superficial vaginal cells, *Cimicifuga racemosa* produced beneficial effects on serum parameters of bone metabolism and an increase in superficial vaginal cells, but, unlike hormone therapy, had no effect on endometrial thickness.

Subsequent controlled studies published in 2005 and 2007 using isopropanolic extracts of *Cimicifuga racemosa* showed no relevant differences in effectiveness compared to oestradiol and tibolone patches, but did show greater safety of the extracts (Nappi et al., 2005) (Bai et al., 2007).

One study that included a large number of participants (736) and included data from more than 12 months was published by Uebelhack et al. (2006). It compared the effects of *Cimicifuga racemosa* extract with a combination product containing *Cimicifuga racemosa* and St. John's wort (*Hypericum perforatum*). The combination product was more effective than *Cimicifuga racemosa* extract on the primary evaluation criteria and showed additional benefit on the psychological component.

Between 2008 and 2012, some revisions were made to what had been published up to that date. Borrelli and Ernst (2008) reviewed 1 single-blind (or unpooled) trial, 2 trials conducted in women with bilateral hysterectomy and 3 trials conducted in women with drug-induced menopause, and concluded that there was no scientific evidence to conclude that *Cimicifuga racemosa* was effective in reducing menopausal symptoms. In their review, Palacio et al. (2009) concluded that in most cases *Cimicifuga racemosa* alone may not have a significant effect on climacteric symptoms, but instead could be effective in combination with other multibotanical preparations or with St. John's wort-based preparations. In 2012, Leach and Moore published a systematic review in which they reviewed 16 randomised clinical trials involving a total of 2027 perimenopausal and postmenopausal women (Leach and Moore, 2012). In all trials, *Cimicifuga racemosa*-based monopreparations in the form of tablet or capsules to be administered orally were prescribed at an average daily dose of 40 mg, for an average duration of 23.4 weeks. 11 studies compared *Cimicifuga racemosa* with placebo and 8 compared *Cimicifuga racemosa* with an active control (hormone therapy, another herbal medicinal product or other pharmaceutical product). In most of these trials, the comparison between *Cimicifuga racemosa* and placebo showed no significant differences, with inconclusive results regarding vasomotor and vulvovaginal symptoms,

menopausal symptoms and adverse effects. Therefore, in most of these trials, it could not be claimed that *Cimicifuga racemosa* extracts were effective in reducing menopausal symptoms.

These reviews were criticised by Beer and Neff (Beer and Neff, 2013) (Beer, 2015) due to the heterogeneity of the studies included (peri-menopausal and post-menopausal women, of different ethnicities, different preparations of *Cimicifuga racemosa* with varying content and containing other components in addition to *Cimicifuga racemosa*) and the inconsistency of some of them. These reviews had included all types of *Cimicifuga racemosa* preparations, whether approved as herbal medicinal products or as other types of products (food supplements or individualised preparations). In addition, its effectiveness on different types of disturbances had been analysed; while most trials assessed the effect of *Cimicifuga racemosa* on vasomotor symptoms, the parameters used to assess changes in the frequency and intensity of these were diverse and often not entirely adequate. None of these reviews took into account a differentiation by type of extract, pharmaceutical quality or indication. Beer and Neff (2013) reviewed studies published between 2000 and 2012 and were the first to conclude that only herbal medicinal products, but not food supplements, showed any evidence of effectiveness. The best evidence was provided by isopropanolic extract of *Cimicifuga racemosa*, the only extract tested in several thousand women and the only extract that provided confirmatory evidence of level 1 (highest level of evidence in Sackett's classification) and grade A recommendation (strong recommendation to act).

Recently, a systematic review and subsequent meta-analysis of 35 clinical trials was carried out, covering since the enactment of the European Union Guidelines on Good Clinical Practice in 1997 until January 2020, and involving 43 759 women, of whom 13 096 were treated with herbal medicinal products consisting of an isopropanolic extract of *Cimicifuga racemosa*. This review evidences the effectiveness of this extract compared to placebo in the treatment of neurovegetative and psychological symptoms of menopause. Effect sizes were larger when higher doses were given as monotherapy or in combination with St. John's wort. Thus, women in their early climacteric phase may benefit from an increase from 40 mg to 120 mg of drug per day. For psychological symptoms, the effects of the combination of *Cimicifuga racemosa* and St. John's wort were superior to those of *Cimicifuga racemosa* monotherapy. The effectiveness of *Cimicifuga racemosa* was comparable to that of low-dose transdermal oestradiol or tibolone. However, due to its better tolerability, *Cimicifuga racemosa* had a significantly better benefit-risk profile than tibolone (Castelo-Branco et al., 2021).

In addition to effects on hot flushes, preclinical studies have shown that extracts of *Cimicifuga racemosa* can inhibit osteoclast differentiation and activity and thus may prevent bone resorption, as well as stimulate osteoblast activity. Moreover, oxidative stress and inflammatory processes play an important role not only in vasomotor symptoms of menopause, but also in the development and progression of osteoporosis (Gordon et al., 2016) (Biglia et al., 2017). As isopropanolic extracts of *Cimicifuga racemosa* exhibit antioxidant and mitochondrial protective effects (Da et al., 2015) and anti-inflammatory effects (Yang et al., 2013), these effects may contribute to its effectiveness for vasomotor symptoms and osteoporosis risk.

As mentioned above, the review of the published literature on the effectiveness of *Cimicifuga racemosa* shows that there is scientific evidence of the effectiveness of herbal medicinal products, but not for food supplements. The effectiveness of food supplements in reducing menopausal symptoms

cannot therefore be confirmed, and trials without the methodological shortcomings shown by studies to date, which have led to inconclusive and, in some cases, contradictory results, need to be carried out in this regard.

4.2 Mechanism of action

The exact mechanism of action of *Cimicifuga racemosa* extract is unknown. Some studies suggest that several biologically active substances are responsible for its action (Wobser and Takov, 2020). Most of the available scientific evidence points to several different mechanisms of action. Because *Cimicifuga racemosa* had been used for threating menopausal symptoms caused by oestrogen deficiency, phytoestrogens were initially thought to be responsible for the effects (Seidlova-Wuttke et al., 2003). Analysis of studies carried out in cell culture, using MCF-7 breast cancer-derived cells, unfortunately yielded contradictory results (Jarry and Harnischfeger, 1985) (Liu et al., 2001) (Bodinet and Freudenstein, 2002, 2004) (Lupu et al., 2003). Discrepancies could be due to differences in the solvents used to obtain the extracts of *Cimicifuga racemosa*, or in the cell lines used in the cultures.

Currently, it has been ruled out that the impact of *Cimicifuga racemosa* on climacteric symptoms is related to the activation of oestrogen receptors (Beer and Neff, 2013). Based on data from *in vitro* studies and animal models, it appears that the effects of *Cimicifuga racemosa* extracts on vasomotor reaction (hot flushes) may be justified by its action on various dopaminergic, serotonergic and noradrenergic neurotransmitter systems (Burdette et al., 2003) (Nisslein et al., 2006) (Powell et al., 2008), all of which are involved in the onset of hot flushes, as well as on μ-opioid receptors (Rhyu et al., 2006) (Reame et al., 2008).

Some studies have focused on the actions that extracts of this plant may have on the luteinising hormone and the follicle-stimulating hormone. The results of these trials were contradictory. Thus, while some showed a decrease in both hormones, others did not detect this effect (Jarry and Harnischfeger, 1985) (Düker et al., 1991) (Freudenstein et al., 2002) (Rotem and Kaplan, 2007).

5. Safety

Scientific literature states that extracts of *Cimicifuga racemosa* are generally well tolerated (Castelo-Branco et al., 2021). Some cases of gastrointestinal problems (dyspepsia, nausea and vomiting) have been reported (Mahadi, 2005). Occasionally, particularly with high dosing of extract, profuse sweating, dizziness, headaches, hypertension or hypotension, increased menstrual bleeding, alterations of heartbeat (palpitations, sinus bradycardia, sinus tachycardia or decreased pulse rate), restlessness and visual disturbances (blurred vision) may be observed (Henneicke-von Zepelin, 2017).

Moreover, experimental studies in animal models evidence that *Cimicifuga racemosa* has no toxic, mutagenic, carcinogenic or teratogenic effects. Furthermore, there is no evidence that, in women with a history of breast cancer, intake of these extracts leads to an increased or decreased risk of breast cancer, nor to an increased risk of endometrial cancer (Henneicke-von Zepelin, 2017).

Published studies on the safety of *Cimicifuga racemosa* extracts in humans indicate that a daily dose of 40 mg (0.57 mg/kg b.w./day considering a 70 kg adult), taken for 6 months, does not lead to an increased health risk. It cannot be stated that this dose is safe to be administered to pregnant

or breastfeeding women or to children, due to the absence of information on the safe use of these extracts. In fact, the safety of its use during pregnancy and lactation has not been established.

In toxicology studies it is important to calculate the No Observed Adverse Effect Level (NOAEL), a toxicity index from which all other toxicity parameters are derived. Means the maximum concentration of a substance, found experimentally or by observation, that does not cause detectable adverse changes in the morphology, functional capacity, growth, development or life span of target organisms, distinguishable from those observed in normal (control) organisms of the same species and strain, under defined conditions of exposure, and is expressed in mg/kg b.w./day. For non-carcinogenic compounds, the goal of risk assessment is to determine the safe level of exposure for a population (risk characterisation). This threshold or level of safety should include the most sensitive individuals in the population, in order to ensure that the whole population is adequately protected, even when exposure levels reach the threshold. This degree of safety resulting from dose-response studies is often adjusted with safety factors to account for uncertainty in the toxicological or epidemiological evidence (Lema et al., 2010). In this regard, a 6-month study in rats treated with an isopropanolic extract of *Cimicifuga racemosa* defined a NOAEL value of 21.06 mg native extract/kg b.w./day (HMPC, 2010) (EMA, 2018).

5.1 Hepatotoxicity

In 2011, the first meta-analysis was conducted to analyse the liver safety of isopropanolic extracts of *Cimicifuga racemosa* (Naser et al., 2011). The results of 5 randomised and controlled double-blind clinical trials involving 1020 women aged 40-60 years (517 in the group to which an extract of *Cimicifuga racemosa* was administered and 503 in the control group) were analysed. The doses used in the treatments were in the range of 40 to 128 mg/day. The meta-analysis showed no differences between the treated groups with respect to serum enzymes relevant to the safety of liver function, even at the highest dosing and after the longest duration of exposure, and therefore concluded that there was no evidence that this extract had any negative effects on liver function. This is consistent with the safety results of the systematic review by Beer and Neff (2013) and its update (Beer, 2015), which found no cases of hepatotoxicity among the more than 12 000 patients reviewed.

The meta-analysis carried out by Castelo-Branco et al. (2021) found that treatment with *Cimicifuga racemosa*, either in monotherapy or in combination with St. John's wort, is well tolerated, with only a few minor adverse effects, with a frequency comparable to that of placebo. Clinical data revealed no evidence of hepatotoxicity. Hormone levels remained unchanged and oestrogen-sensitive tissues (e.g. breast and endometrium tissue) were not affected by the treatment.

In 2006, AEMPS received information from the EMA Committee on Herbal Medicinal Products (HMPC) regarding the possible association of the use of *Cimicifuga racemosa* rhizome extract with acute liver injury (EMA, 2006). In the information note, which was published on the AEMPS' website (AEMPS, 2006), the AEMPS explained that, although a variety of cases of acute liver injury of varying severity had been reported worldwide (34 cases reported by the national competent authorities of Member States and 8 case reports described in the literature), only 4 of them did appeared to show a reasonable temporal sequence with the intake of the product and, even in these, the causal relation-

ship could not be established with certainty. At that time, no cases had been reported to the Spanish Pharmacovigilance System. Such information note set out recommendations for both patients and healthcare professionals. The recommendations to patients literally consisted of:

- "Users of products containing *Cimicifuga racemosa* root extract should stop taking the medicine and consult their doctor if they notice any of the following symptoms: abnormal tiredness, stomach pain with nausea, cognac-coloured urine, yellowing of the skin and whites of the eyes (jaundice).
- Patients with pre-existing liver disease or liver disorders should not take these medicines; if they are already taking them, they should consult their doctor about whether it is advisable to continue the treatment.
- Users of the product who have not experienced any abnormalities and have observed a clear improvement in menopause-related symptoms can continue to use the product".

And the recommendations to health professionals consisted of:

- "Patients suspected of having acute liver injury without a clear aetiology should be questioned about their use of medicinal plants, either in the form of pharmaceutical products or purchased in herbalists' shops or other establishments, and specifically the use of products containing *Cimicifuga racemosa*.
- If association with products containing *Cimicifuga racemosa* is suspected in any case, the appropriate pharmacovigilance centre of the Autonomous Community should be notified as soon as possible".

On the other hand, according to LiverTox (2020), products labelled as containing "black cohosh" have been associated in clinical practice with more than 50 cases of clinically apparent liver injury of different degrees of severity (from an increase in serum enzymes without the presence of jaundice, to acute self-limiting hepatitis, prolonged hepatitis with cholestasis, autoimmune hepatitis and acute liver failure which required liver transplant or with a fatal outcome). The latency phase until the onset of liver injury in these cases varied from 1 to 48 weeks, but was usually 2 to 12 weeks. The typical clinical presentation was associated with jaundice and a markedly hepatocellular pattern of injury, with liver biopsy histology resembling acute viral hepatitis. Some instances of an autoimmune hepatitis-like clinical syndrome with high levels of autoantibodies and chronic hepatitis on liver biopsy have also been reported. In some cases, these food supplements appeared to have precipitated autoimmune hepatitis that was self-sustaining, and relapsed when immunosuppression was withdrawn, while in other instances hepatitis with autoimmune features resolved spontaneously after discontinuation of supplementation or after a short course of prednisone. In several cases, the product involved was retrieved and found to contain Chinese *Actaea* species instead of *Cimicifuga racemosa*.

A description of some of these cases is provided below:

Case 1. A 52-year-old woman developed fatigue and lethargy, followed by jaundice, approximately 3 months after starting a liquid herbal preparation containing *Cimicifuga racemosa*. She stopped using

it when she became ill, but subsequently developed jaundice. This preparation had been made and provided by a pharmacist, and contained fluid extracts of various botanical species, including ground ivy (*Nepeta hederacea*), goldenseal (*Hydrastis canadensis*), ginkgo (*Ginkgo biloba*), oat seeds (*Avena sativa*) and black cohosh (*Cimicifuga racemosa*). She had no history of liver disease, alcohol abuse or risk factors for viral hepatitis, and she was not taking any other medications. On medical examination, there were no signs of hepatic encephalopathy. Laboratory tests showed the following values: serum bilirubin 21.5 mg/dl, alanine aminotransferase (ALT) 1380 IU/l, alkaline phosphatase 230 IU/l and INR (prothrombin time) 3.0. Other causes of acute liver failure were said to be excluded. During the initial week, she developed evidence of hepatic failure with progressive hepatic encephalopathy and aggravated coagulation disorders, leading to liver transplantation approximately 4 weeks after admission. The explanted liver showed massive necrosis. Therefore, this is the case of a patient who developed acute liver failure of unknown cause approximately when she started taking an herbal preparation, which was said to contain *Cimicifuga racemosa*. The other components had not been involved in cases of acute liver failure, but may have contributed due to herb-herb interactions. The possibility that the liver injury is not related to *Cimicifuga racemosa* and is actually due to an adulterant or mislabelled herb, or is due to a coincidental, idiopathic or unusual viral cause of acute liver failure, cannot be thus completely excluded. However, the clinical presentation with a strongly hepatocellular pattern of injury and a gradual progression to liver failure over several weeks, even after discontinuation of the preparation, has been described in other cases of severe acute liver injury attributed to *Cimicifuga racemosa*.

Case 2. A 54-year-old woman developed fatigue and weight loss 6 months after starting to take *Cimicifuga racemosa* (1000 mg/day) to treat her menopausal symptoms. She had a history of hypothyroidism and was taking levothyroxine (100 µg/day). Fatigue persisted and she experienced the onset of forgetfulness and weight loss (4.5 kg), before seeking medical help. She had no history of liver disease, drank alcohol regularly but not excessively, and had no risk factors for viral hepatitis. On medical examination, she had tenderness over the liver, but no evidence of jaundice. Serum transaminase levels were markedly high (ALT 1003 IU/l, aspartate aminotransferase -AST- 1014 IU/l), with modest increases in alkaline phosphatase (266 IU/l) and total bilirubin (2.4 mg/dl). The patient tested negative for hepatitis A, B and C, as for herpes simplex, cytomegalovirus and Epstein-Barr virus. Autoantibodies were also negative. Ultrasound and computerized tomography of the abdomen showed no evidence of biliary obstruction or liver abnormalities. Prothrombin time was high (INR 1.4). A liver biopsy showed severe hepatocellular necrosis with panlobular inflammation and interphase hepatitis, but no fibrosis, compatible with severe acute hepatitis. Prednisone was started, but her condition worsened. After 2 weeks, serum transaminase levels were still high and total bilirubin rose to 20.6 mg/dl. She developed hepatic encephalopathy and aggravated coagulation disorders (INR 2.6). Repeated ultrasonography showed a reduction in the size of her liver. She underwent a liver transplant from a deceased donor 39 days after admission, but died in the operation table as a result of excessive bleeding. Autopsy showed a shrunken liver with extensive necrosis, minimal inflammation and regenerative nodules. In this case, the clinical presentation and course were simi-

lar to other cases of liver injury attributed to *Cimicifuga racemosa*. The longer latency to onset was atypical, but this also occurs in the latency to onset of severe hepatotoxicity with other agents that cause idiosyncratic acute liver damage, such as isoniazid and troglitazone.

Case 3. A 58-year-old woman developed fatigue and weakness while taking *Cimicifuga racemosa* (80 mg root extract per day), in addition to medications for hypertension (irbesartan 150 mg/day), hypothyroidism (levothyroxine 100 µg/day), hypercholesterolaemia (simvastatin 20 mg/day) and diabetes (insulin). She had no history of liver disease, alcohol abuse, or risk factors for viral hepatitis. The physical examination was unremarkable. However, laboratory tests showed elevations in serum ALT (318 IU/l), AST (214 IU/l) and ɣ-glutamyl transferase (GGT) (95 IU/l) with normal bilirubin and albumin levels, total protein, INR, platelet and leukocyte counts. She tested negative for hepatitis A, B and C, as well as for Epstein Barr virus and cytomegalovirus infection. Smooth muscle antibody was weakly positive (titre 1:40). A liver biopsy showed interface hepatitis and lobular inflammation with portal fibrosis, suggesting chronic hepatitis. Simvastatin was discontinued but the patient did not improve. Consequently, 3 months later, the administration of *Cimicifuga racemosa* was discontinued. Within 2 weeks, serum transaminase levels decreased and 2 months later her symptoms resolved, and liver tests results were normal. In this case, the timing of improvement in relation to discontinuation of *Cimicifuga racemosa* strongly supported its link to chronic liver injury. Drugs (e.g. methyldopa, nitrofurantoin, isoniazid) that cause idiosyncratic acute hepatitis, arising within 6 months of starting treatment, can also cause chronic hepatitis if continued long-term, sometimes with autoimmune features. However, in these cases, the time of onset of the injury may be many months or years after starting the drug, especially if it is administered intermittently.

Case 4. A 50-year-old woman was admitted for evaluation of worsening jaundice, malaise, itching and significantly elevated liver enzymes. Medical history reported chronic asthma, obstructive pulmonary disease, type 2 diabetes mellitus and iron deficiency anaemia, with no history of liver disease. Drug treatment consisted of carvedilol (with antioxidant, antihypertensive, vasodilator and antianginal properties) 12.5 mg twice daily, famotidine (anti-H2 antihistamine to fight acid reflux) 20 mg/day, albuterol (bronchodilator) and formoterol and budesonide inhalers (antiasthmatic). The patient reported taking *Cimicifuga racemosa* for 2 months before the onset of symptoms. She stated that she did not smoke, did not consume alcohol, did not abuse substances, had not received transfusions, and had no family history of liver disease. When the symptoms started, she felt fine, but she began to feel tired, nauseous and had yellowish skin.

Blood tests showed increased total bilirubin, alkaline phosphatase, AST and ALT transaminases. On admission, he had a normal INR value (1.06). After treatment with ursodeoxycholic acid, he tested positive for antimycochondrial antibodies, experienced no improvement and underwent magnetic resonance cholangiopancreatography (MRCP), which revealed bile duct narrowing. At the same time, she presented a worsening of jaundice and itching. On abdominal examination no distension or hepatosplenomegaly was observed. Alcohol or paracetamol levels were undetectable and viral serology including hepatitis A and B virus, Australia antigen, hepatitis C virus, HIV virus,

herpes simplex virus 1 and 2, Epstein-Barr virus and cytomegalovirus was negative. Other autoimmune causes were explored, but they also failed to identify the reason for the worsening symptoms. Having ruled out all possible causes, and after a differential diagnosis, cholangitis was suspected and consequently confirmed by the best available technique (MRCP). The patient was instructed to stop taking *Cimicifuga racemosa*. After withdrawal of this extract and simultaneous treatment with budenoside, a corticosteroid for the treatment of asthma, symptoms started to improve, and liver enzymes normalised within 6 months.

According to LiverTox (2020), products sold as "black cohosh" are well-established causes of clinically apparent liver damage, but the specific ingredient or component causing the damage is unclear. *Cimicifuga racemosa* does not appear to be inherently hepatotoxic, and the clinical features of the reported cases suggest that the liver damage is an idiosyncratic reaction that may be immune-mediated. As with many other herbal food supplements, unknown adulterants or ingredients not declared on the labelling could be the cause of liver damage (LiverTox, 2020).

From all available information, it can be stated that the relationship between consumption of *Cimicifuga racemosa* and liver damage is always controversial. There have been multiple reports indicating the presence of liver injury, but there are also multiple discrepancies. Data on dosing, product purity, origin and preparation of the concoctions, as well as the time relationship between use and symptom onset, are scarce. In the reported cases, most *Cimicifuga racemosa* products are over-the-counter products with various ingredients and even unknown compounds. There are many confounding variables, such as pre-existing liver damage or consumption of preparations containing multiple ingredients including plants (Brar and Marathi, 2021). To date, there is only one case where it is certain that consumption of *Cimicifuga racemosa* induced a hepatotoxic effect, but this was a multi-drug interaction problem in a patient with multiple pathologies (Teschke et al., 2009).

Although the most recent data state that there is no proven cause-effect relationship between the consumption of *Cimicifuga racemosa* preparations and the development of liver disease, and in line with the recommendations of the AEMPS (2006) for medicinal products containing this botanical species, the administration of food supplements to patients with impaired liver function is not recommended, and a doctor should be consulted immediately if symptoms suggesting liver disease (tiredness, loss of appetite, jaundice, epigastralgia with vomiting and diarrhoea or choluria) occur (EMA, 2006).

Conclusions of the Scientific Committee

Based on what has been published to date in the scientific literature, it can be concluded that there is scientific evidence of effectiveness in combating menopausal symptoms only in the case of allopathic herbal medicinal products, but not in the case of food supplements.

The standardised extracts of *Cimicifuga racemosa* described in the EMA monograph have been shown to be safe in oestrogen-sensitive tissues such as breast, uterus or tumours, and have not been shown to be hepatotoxic in clinical studies. However, in the absence of studies analysing the safety of the consumption of food supplements containing *Cimicifuga racemosa* as an ingredient with an

adequate methodological design, it cannot be ruled out that these supplements, at the dosing and types of extract currently available on the market, are related to hepatotoxicity, and therefore, the use of this type of supplement is not recommended in persons with impaired liver function. A doctor should be consulted immediately if symptoms compatible of liver disease develop.

In view of the above, it would be advisable for health professionals, researchers and citizens to report serious adverse effects that they believe may have a causal relationship with this type of food supplement to the appropriate institutions. In addition, regulatory authorities should develop post-marketing surveillance systems in order to identify, quantify, assess and prevent potential risks arising from the use of food supplements once they have been placed on the market.

Furthermore, it is clear that it is required to standardise the composition of *Cimicifuga racemosa* food supplements in terms of composition and labelling to ensure that the composition of such products is consistent and homogeneous between batches. Specifically, the designation should include: the name of the herbal substance used, the physical state of the preparation, the quantity of the preparation, the ratio to the starting material and the extract without excipients (genuine Drug Extract Ratio, or DER) and the name and composition of the extraction solvents. This would ensure unambiguous identification and allow comparison between these food supplements.

Finally, in order to obtain the scientific evidence that we still lack, both with regard to the effectiveness and the possible toxic effects of food supplements containing *Cimicifuga racemosa*, it is necessary to promote clinical trials with an appropriate methodological design, which will enable the scientific community to draw conclusions based on scientific evidence.

References

AEMPS (2006). Agencia Española de Medicamentos y Productos Sanitarios. Comunicación sobre riesgos de medicamentos para profesionales sanitarios. Nota Informativa. Extracto de la raíz de "*Cimicifuga racemosa*" y lesiones hepáticas. No 2006/06. Available at: https://www.aemps.gob.es/en/informa/notasInformativas/medicamentosUsoHumano/seguridad/2006/docs/NI_2006-06_cimicifuga.pdf [accessed: 17-11-21].

Applequist, W.L. (2003). Inappropriate advertising of dietary supplements. *New England Journal of Medicine*, 348, pp: 2255-2256.

Bai, W., Henneicke-von Zepelin, H.H., Wang, S., Zheng, S., Liu, J., Zhang, Z., Geng, L., Hu, L., Jiao, C. and Liske, E. (2007). Efficacy and tolerability of a medicinal product containing an isopropanolic black cohosh extract in Chinese women with menopausal symptoms: a randomized, double blind, parallel-controlled study versus tibolone. *Maturitas*, 58, pp: 31-41.

Beer, A. and Neff, A. (2013). Differentiated evaluation of extract-specific evidence on *Cimicifuga racemosa*'s efficacy and safety for climacteric complaints. *Evidence-Based Complementary and Alternative Medicine*, 2013, pp: 860602.

Beer, A.M. (2015). *Cimicifuga racemosa* bei klimakterischen Beschwerden. *Zeitschrift für Phytotherapie*, 36 (01), pp: 10-17.

Biglia, N., Cagnacci, A., Gambacciani, M., Lello, S., Maffei, S. and Nappi, R.E. (2017). Vasomotor symptoms in menopause: a biomarker of cardiovascular disease and other chronic diseases? *Cimateric*, 20, pp: 306-312.

Bodinet, C. and Freudenstein, J. (2002). Influence of *Cimicifuga racemosa* on the proliferation of estrogen receptor-positive human breast cancer cells. *Breast Cancer Research and Treatment*, 76, pp: 1-10.

Bodinet, C. and Freudenstein, J. (2004). Influence of marketed herbal menopause preparations on MCF-7 cell proliferation. *Menopause*, 11, pp: 281-289.

Borrelli, F. and Ernst, E. (2008). Black cohosh (*Cimicifuga racemosa*) for menopausal symptoms: a systematic review of its efficacy. *Pharmacological Research*, 58 (1), pp: 8-14.

Borrelli, F., Izzo, A.A. and Ernst, E. (2003). Pharmacological effects of *Cimicifuga racemosa* . *Life Sciences*, 73, pp: 1215-1229.

Brar, H.S. and Marathi, R. (2021). Case of cholestatic drug-induced liver injury (DILI) associated with black cohosh. *British Medical Journal Case Report*, 14, pp: e240408.

Burdette, J.E., Liu, J., Chen, S.N., Fabricant, D.S., Piersen, C.E., Barker, E.L., Pezzuto, J.M., Mesecar, A., Van Breemen, R.B., Farnsworth, N.R. and Bolton, J.L. (2003). Black cohosh acts as a mixed competitive ligand and partial agonist of the serotonin receptor. *Journal of Agricultural and Food Chemistry*, 51, pp: 5661-5670.

BVL (2014). Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. BVL-Report 8.8. List of Substances of the Competent Federal Government and Federal State Authorities, Category "Plants and plant parts". Available at: https://www.bvl.bund.de/SharedDocs/Berichte/08_Stoffliste_Bund_Bundeslaender/stofflisten_pflanzen_pflanzenteile_EN_09_2014.pdf?__blob=publicationFile&v=10 [accessed 17-11-21].

Castelo-Branco, C., Gambacciani, M., Cano, A., Minkin, M.J., Rachoń, D., Ruan, X., Beer, A.M., Schnitker, J., Henneicke-von Zepelin, H.H. and Pickartz, S. (2021). Review and meta-analysis: isopropanolic black cohosh extract iCR for menopausal symptoms - an update on the evidence. *Climacteric*, 24 (2), pp: 109-119.

Compton, J.A., Culham, A. and Jury, S.L. (1998). Reclassification of *Actaea* to include *Cimicifuga* and *Souliea* (*Ranunculaceae*): phylogeny inferred from morphology, nrDNA ITS, and cpDNA trnL-F sequence variation. *Taxon*, 47, pp: 593-634.

Da, Y., Niu, K., Wang, K., Cui, G, Wang, W., Jin, B., Sun, Y., Jing, J, Qin, L. and Bai, W. (2015). A comparison of the effects of estrogen and *Cimicifuga racemosa* on the lacrimal gland and submandibular gland in ovariectomized rats. *PLoS One*, 10, pp: e0121470.

Düker, E.M., Kopanski, L., Jarry, H. and Wuttke, W. (1991). Effects of extracts from *Cimicifuga racemosa* on gonadotropin release in menopausal women and ovariectomized rats. *Planta Medica*, 57, pp: 420-424.

EMA (2006). European Medicines Agency. EMEA Public Statement on herbal medicinal products containing *Cimicifugae racemosae* rhizoma (black cohosh, root) - serious hepatic reactions-. EMEA/269259/2006. Available at: https://www.ema.europa.eu/en/documents/public-statement/emea-public-statement-herbal-medicinal-products-containing-cimicifugae-racemosae-rhizoma-serious_en.pdf [accessed: 17-11-21].

EMA (2018). European Medicines Agency. European Union herbal monograph on *Cimicifuga racemosa* (L.) Nutt., rhizoma. EMA/HMPC/48745/2017. Available at: https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-cimicifuga-racemosa-l-nutt-rhizome-revision-1_en.pdf [accessed: 17-11-21].

ESCOP (2003). European Scientific Cooperative On Phytotherapy. Cimicifugae rhizome. In book: *ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products*. 2nd ed. Thieme, pp: 79-91.

EU (2001). Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311 of 28 November 2001, pp: 67-128.

EU (2004). Directive 2004/27/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use. OJ L 136 of 30 April 2004, pp: 34-57.

Freudenstein, J., Dasenbrock, C. and Nisslein, T. (2002). Lack of promotion of estrogen-dependent mammary gland tumors in vivo by an isopropanolic *Cimicifuga racemosa* extract. *Cancer Research*, 62, pp: 3448-3452.

Gordon, J.L., Rubinow, D.R., Thurston, R.C., Paulson, J., Schmidt, P.J. and Girdler, S.S. (2016). Cardiovascular, hemodynamic, neuroendocrine, and inflammatory markers in women with and without vasomotor symptoms. *Menopause*, 23, pp: 1189-1198.

Guo, Y., Yin, T., Wang, X., Zhang, F., Pan, G., Lv, H., Wang, X., Owoicho J.O., Zhu, Y. and Wu, H. (2017). Traditional uses, phytochemistry, pharmacology and toxicology of the genus *Cimicifuga*: A review. *Journal of Ethnopharmacology*, 209, pp: 264-282.

Henneicke-von Zepelin, H., Lin, S.Y. and Friede, M. (2002). Does the optimal dosage of *Cimicifuga* extract for climacteric complaints depend on the patient's menopausal status? *Climacteric*, 5, pp: 91.

Henneicke-von Zepelin, H.H. (2017). 60 years of *Cimicifuga racemosa* medicinal products. *Wiener Medizinische Wochenschrift*, 167, pp: 147-159.

HMPC (2010). Committee on Herbal Medicinal Products. Assessment report on *Cimicifuga racemosa* (L.) Nutt., rhizoma. EMA/HMPC/3968/2008. Available at: https://www.ema.europa.eu/en/documents/herbal-report/superseded-assessment-report-cimicifuga-racemosa-l-nutt-rhizome-first-version_en.pdf [accessed: 17-11-21].

HMPC (2018). Committee on Herbal Medicinal Products. Assessment report on *Cimicifuga racemosa* (L.) Nutt., rhizoma. EMA/HMPC/48744/2017. Available at: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-cimicifuga-racemosa-l-nutt-rhizome-revision-1_en.pdf [accessed: 17-11-21].

Jarry, H. and Harnischfeger, G. (1985). Studies on the endocrine effects of the contents of *Cimicifuga racemosa* 2. In vitro binding of compounds to estrogen receptors. *Planta Medica*, 51, pp: 316-319.

Jiang, B., Kronenberg, F., Nuntanakorn, P., Qiu, M.H. and Kennelly, E.J. (2006). Evaluation of the botanical authenticity and phytochemical profile of black cohosh products by high-performance liquid chromatography with selected ion monitoring liquid chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry*, 54, pp: 3242-3253.

Jiang, B., Ma, C., Motley, T., Kronenberg, F. and Kennelly, E.J. (2011). Phytochemical fingerprinting to thwart black cohosh adulteration: a 15 *Actaea* species analysis. *Phytochemical Analysis*, 22, pp: 339-351.

Leach, M.J. and Moore, V. (2012). Black cohosh (*Cimicifuga* spp.) for menopausal symptoms. *The Cochrane Database of Systematic Reviews*, 2012, pp: CD007244.

Lema, I., Zuk, M. and Rojas-Bracho, L. (2010). *Introducción al análisis de riesgos ambientales*. 2ª edición. México D.F. Instituto Nacional de Ecología.

Li, J.X. and Yu, Z.Y. (2006). Cimicifugae Rhizoma: From origins, bioactive constituents to clinical. *Current Medicinal Chemistry*, 13, pp: 2927-2951.

Liske, E., Hanggi, W., Henneicke-von Zepelin, H.H., Boblitz, N., Wustenberg, P. and Rahlfs, V.W. (2002). Physiological investigation of a unique extract of black cohosh (*Cimicifugae racemosae* rhizoma): a 6-month clinical study demonstrates no systemic estrogenic effect. *Journal of Women's Health and Gender-Based Medicine*, 11, pp: 163-174.

Liu, Z.P., Yu, B., Huo, J.S., Lu, C.Q. and Chen J.S. (2001). Estrogenic Effects of *Cimicifuga racemosa* (Black Cohosh) in mice and on estrogen receptors in MCF-7 Cells. *Journal of Medicinal Food*, 4, pp: 171-178.

LiverTox (2020). LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Black Cohosh. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. Available at: https://www.ncbi.nlm.nih.gov/books/NBK547990/ [accessed: 17-11-21].

Lupu, R., Mehmi, I., Atlas, E., Tsai, M.S., Pisha, E., Oketch-Rabah, H.A., Nuntanakorn, P., Kennelly, E.J. and Kronenberg, F. (2003). Black cohosh, a menopausal remedy, does not have estrogenic activity and does not promote breast cancer cell growth. *International Journal of Oncology*, 23, pp: 1407-1412.

Ma, C., Kavalier, A.R., Jiang, B. and Kennelly, E.J. (2011). Metabolic profiling of *Actaea* species extracts using high performance liquid chromatography coupled with electro-spray ionization time-of-flight mass spectrometry. *Journal of Chromatography A*, 1218, pp: 1461-1476.

Mahadi, G.B. (2005). Black cohosh (*Actaea*/*Cimicifuga racemosa*): review of the clinical data for safety and efficacy in menopausal symptoms. *Treatments in Endocrinology*, 4, pp: 177-184.

McKenna, D.J., Jones, J., Humphrey, S. and Hughes, K. (2001). Black cohosh: efficacy, safety, and use in clinical and preclinical applications. *Alternative Therapies in Health and Medicine*, 7, pp: 93-100.

Mollá, M.D., García-Sánchez, Y., Romeu, A. and Pérez-López, F.R. (2009). *Cimicifuga racemosa* treatment and health related quality of life in post-menopausal Spanish women. *Gynecogical Endocrinology*, 25, pp: 21-26.

NAMS (2004). North American Menopause Society. Treatment of menopause-associated vasomotor symptoms:

position statement of the North American Menopause Society. *Menopause*, 11, pp: 11-33.

Nappi, R.E., Malavasi, B., Brundu, B. and Facchinetti, F. (2005). Efficacy of *Cimicifuga racemosa* on climacteric complaints: a randomized study versus low-dose transdermal estradiol. *Gynecological Endocrinology*, 20, pp: 30-35.

Naser, B., Dchnitker, J., Minkin, M.J., García de Arriba, S., Nolte, K.U. and Osmers, R. (2011). Suspected black cohosh hepatotoxicity: no evidence by meta-analysis of randomized controlled clinical trials for isopropanolic black cohosh extract. *Menopause*, 18, pp: 366-375.

Nisslein, T., Koetter, U. and Freudenstein, J. (2006). *In vitro* binding of an isopropanolic extract of black cohosh to selected central nervous receptors. *Maturitas*, 54, pp: S65.

Osmers, R., Friede, M., Liske, E., Schnitker, J., Freudenstein, J. and Henneicke-von Zepelin, H.H. (2005). Efficacy and safety of isopropanolic black cohosh extract for climacteric symptoms. *Obstetrics and Gynecology*, 105, pp: 1074-1083.

Palacio, C., Masri, G. and Mooradian, A.D. (2009). Black cohosh for the management of menopausal symptoms: a systematic review of clinical trials. *Drugs and Aging*, 26, pp: 23-36.

Powell, S.L., Godecke, T., Nikolic, D., Chen, S.N., Ahn, S., Dietz, B., Farnsworth, N.R., van Breemen, R.B., Lankin, D.C., Pauli, G.F. and Bolton, J.L. (2008). In vitro serotonergic activity of black cohosh and identification of N(omega)-methylserotonin as a potential active constituent. *Journal of Agricultural and Food Chemistry*, 56, pp: 11718-11726.

Predny, M.L., De Angelis, P. and Chamberlain, J.L. (2006). Black cohosh (*Actae racemosa*): an annotated bibliography. General Technical Report SRS–97. Department of Agriculture Forest Service, Southern Research Station, pp: 99. Available at: https://www.fs.usda.gov/treesearch/pubs/26166 [accessed: 17-11-21].

Qiu, F., McAlpine, J.B., Krause, E.C., Chen, S.N. and Pauli, G.E. (2014). Pharmacognosy of black cohosh: the phytochemical and biological profile of a major botanical dietary supplement. *Progress in Chemistry of Organic Natural Products*, 99, pp: 1-68.

Reame, N.E., Lukacs, J.L., Padmanabhan, V., Eyvazzadeh, A.D., Smith, Y.R. and Zubieta, J.K. (2008). Black cohosh has central opioid activity in postmenopausal women: Evidence from naloxone blockade and positron emission tomography neuroimaging. *Menopause*, 15, pp: 832-840.

Rhyu, M.R., Lu, J., Webster, D.E., Fabricant, D.S., Farnsworth, N.R. and Wang, Z.J. (2006). Black cohosh (*Actaea racemosa*, *Cimicifuga racemosa*) behaves as a mixed competitive ligand and partial agonist at the human mu opiate receptor. *Journal of Agricultural and Food Chemistry*, 54, pp: 9852-9857.

Richo, C. (2002). *Growing at-risk medicinal herbs, Cultivation, Conservation and Ecology*. Horizon Herbs, pp: 10-27.

Rotem, C. and Kaplan, B. (2007). Phyto-Female Complex for the relief of hot flushes, night sweats and quality of sleep: randomized, controlled, double-blind pilot study. *Gynecological Endocrinology*, 23, pp: 117-122.

Seidlova-Wuttke, D., Hesse, O., Jarry, H., Christoffel, V., Spengler, B., Becker, T. and Wuttke, W. (2003). Evidence for selective estrogen receptor modulator activity in a black cohosh (*Cimicifuga racemosa*) extract: comparison with estradiol-17beta. *European Journal of Endocrinology*, 149, pp: 351-362.

Teschke, R., Bahre, R. and Genthner, A. (2009). Suspected black cohosh hepatotoxicity, challenges and pitfalls of causality assessment. *Maturitas*, 63, pp: 302-314.

Uebelhack, R., Blohmer, J.U., Graubaum, H.J., Busch, R., Gruenwald, J. and Wernecke, K.D. (2006). Black cohosh and St. John's wort for climacteric complaints: A randomized trial. *Obstetrics and Gynecology*, 107, pp: 247-255.

WHO (2002). World Health Organization. WHO monographs on selected medicinal plants. Volume 2. Geneva. Available at: http://apps.who.int/iris/bitstream/handle/10665/42052/9241545372.pdf;jsessionid=22213FE4E00014C785015CF2C5CF9BD0?sequence=2 [accessed: 17-11-21].

Wobser, R.W. and Takov, V. (2020). Black Cohosh. In: *StatPearls* [Internet]. Treasure Island. StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK470187/ [accessed: 17-11-21].

Wuttke, W., Seidlova-Wuttke, D. and Gorkow, C. (2003). The *Cimicifuga* preparation BNO 1055 vs. conjugated

estrogens in a double-blind placebo-controlled study: effects on menopause symptoms and bone markers. *Maturitas*, 44 (Suppl 1), pp: S67-S77.

Yang, C.L., Or, T.C., Ho, M.H. and Lau, A.S. (2013). Scientific basis of botanical medicine as alternative remedies for rheumatoid arthritis. *Clinical Reviews in Allergy and Immunology*, 44, pp: 284-300.