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Use of Phytoestrogens for the Treatment of Psychiatric Symptoms Associated with Menopause Transition

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Abstract

Menopause transition is recognized as a vulnerable period in women life to develop or aggravate symptoms of psychiatric disorders. Several treatments including antidepressants and hormonal restitution with estrogens have been suggested to ameliorate the symptoms. Also, in this period of life is frequent the use of other drugs to treat is also frequent the use of other drugs to treat comorbid pathologies that might even increase the risk of drug-drug interactions. Literature reports that some phytochemicals with estrogenic activity have beneficial effects during menopausal transition without collateral events. This chapter shows evidence about the use of phytoestrogens as an alternative therapy for the treatment of some psychiatric symptoms associated with the menopausal transition. Data derived from preclinical research related to the use of classical phytoestrogens (isoflavones), considering the beneficial effects, as well as adverse events, are discussed. Also, the use of polyphenols and organosulfurate compounds as an alternative for the treatment of anxiety- and depressive-like behavior as well as fibromyalgia is included. A narrative review was conducted using bibliography reporting the use of isoflavones (genistein, daidzein, equol), coumestans or lignans for the reduction of depressive-like or anxiety-like behavior. Furthermore, it is described if the use of this compounds impact in other signs of menopause, i.e. vasomotor and osteoporosis. In addition, due to the high frequency of comorbid pathologies as diabetes mellitus, dyslipidemia or metabolic syndrome with psychiatric disorders, the use of these phytochemicals is discussed.

Keywords: menopause, polyphenols, glycosinolates, phytoestrogens

1. Introduction

With the increase of life expectancy, women potentially spend the last third of their lives in post-menopause. Most of the menopausal women suffer from a variety of symptoms such as hot flashes, night sweats, mood swings, insomnia, vaginal dryness and osteoporosis [1, 2]. Also, the menopause transition could be a vulnerable period to develop some diseases related to mental disorders (i.e., anxiety and depression) and chronic non-degenerative pathologies (metabolic diseases) in addition to menopausal symptoms [3, 4]. This situation implies that women that transit in this period of life could be vulnerable to develop co-morbidities leading to a complex medical management due to the elevated cost and possible pharmacological interactions.

Hormone replacement therapy is considered the first line of treatment for menopause symptoms, mainly vasomotor and night sweats. However, many women refuse it because of their association with increased risk of breast cancer and are considering botanical products or dietary supplements as therapy because they are regarded as safer products [1, 2, 5]. For example, black cohosh is widely employed for hot flashes and mood disorders; others are compounds with estrogenic activity (phytoestrogens) as soy food products, red clover, kudzu, hops, licorice, rhubarb, yam and chasteberry [1].

The use of functional food and nutraceuticals became common to treat menopause symptoms [2]. The term “nutraceutical” combines two words—“nutrient” (a nourishing food component) and “pharmaceutical” (a medical drug). It is defined as “any substance that may be considered a food or part of it that provides medical or health benefits, including the prevention and treatment of diseases.” These products include isolated nutrients, dietary supplements and diets genetically engineered “designed” foods, botanical products and processed foods such as cereals, foods and beverages [6]. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease [6]. On the other hand, functional food according to American Dietetic Association, “functional” implies that the food possesses some identified value leading to health benefits, including reducing the risk of adverse effects for a person consuming it [7].

Nutraceuticals and functional food are classified in several manners taken into account their content of specific food, properties (anti-cancer, positive influence on blood lipid profile, anti-inflammatory, osteogenic or bone protective) or chemical structure [6]. Both nutraceuticals and functional food contain active compounds called phytochemicals that confer their properties. Phytochemicals are products of the secondary metabolism of a plant which are biologically active in humans and other animal species playing a greater beneficial role in health more than only nutritional properties. These products are part of the defense system and plants’ protection [8], and in humans, they have activity in several systems such as the digestive, immune, cardiovascular, endocrine and central nervous system [6, 8], among others. Also, phytochemicals confer organoleptic properties to vegetables and fruits [8].

Phytochemicals are classified into four groups according to their impact on health [9, 10]:

1. Terpenoids (i.e., carotenoids and phytosterols)
2. Phenols (i.e., flavonoids, phenolic acids, tannins, stilbens and curcuminoids)

3. Organosulfurate compounds (i.e., glycosinolates and isothiocyanates)
4. Nitrogen compounds (i.e., alkaloids, betalainas, indol-glycosinolates)

The distribution of phytochemicals' sources could be at the same time diffuse and copious as well as specific depending on the product, for example, carotenoids are frequently found in several products (carrots, tomato, orange, mango, pumpkin, guava), whereas glycosinolates are found only in cruciferous (broccoli, brussel sprout, cabbage, horseradish, rutabaga) [8]. The concentration of the phytochemical could vary depending on the morphological factors: skin, seed, pulp, peel, leaves, also on agronomic factors: weather, agricultural soil, technical procedure, physiological stress; and postharvest: maturity, processing and storage, among the others [8].

2. Phytochemicals: polyphenols

Polyphenols can be found in a large variety of food mainly of vegetable origin. They constitute the major group of natural compounds known (around 8000 identified) in the plant kingdom [11]. These compounds result of the secondary metabolism of carbon by the acetate-malonate and shikimate pathways. They are characterized by the presence of phenolic structures with a potent antioxidant activity that confers protection to plants against oxidative stress, ultraviolet radiation and other harmful factors of the environment such as pollutants and pathogens [12]. There are a wide variety of polyphenols but, in general, they have been classified into two groups according to their chemicals properties: flavonoids and non-flavonoids (**Figure 1**).

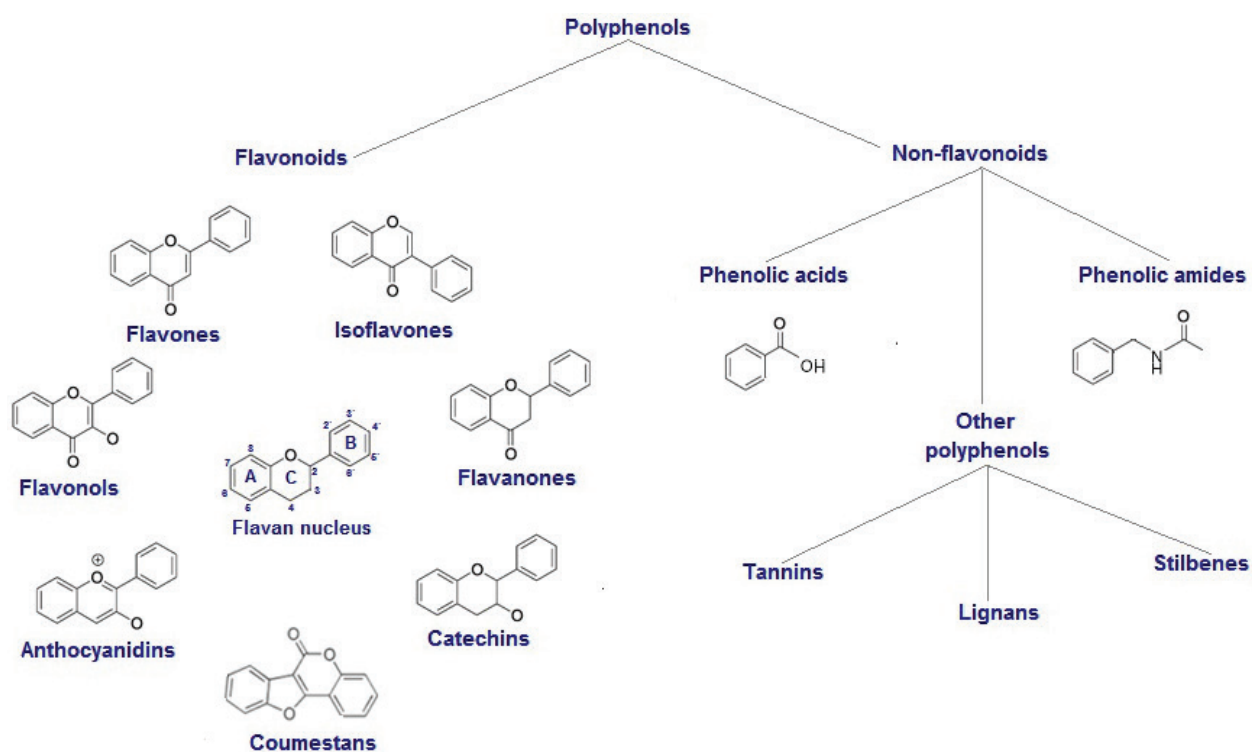


Figure 1. The scheme illustrates the main polyphenols and their division. Phytoestrogens are products of the metabolism of polyphenols: isoflavones and coumenstans are flavonoids, whereas lignans and stilbens are non-flavonoids.

The flavonoid group includes compounds whose structure contains the flavan nucleus (diphenyl propane; C:15: C3, C6, C3), such as anthocyanidins, proanthocyanidins, catechins, flavones, flavonols, flavanones, isoflavones and coumestans (**Figure 1**). The non-flavonoids group includes phenolic acids, phenolic amides and other polyphenols, such as tannins, lignans and stilbene compounds (**Figure 1**). Polyphenols, flavonoids and nonflavonoids have biological importance and are considered as nutraceuticals, not only for their antioxidant effect but also for their beneficial effects on health such as cardiovascular, diabetes and neurodegenerative protection, cancer prevention and anti-infection effect [11].

Recently, it has been determined that some polyphenols, especially isoflavones, flavonols, anthocyanidins, lignans, stilbenes and coumestans, have estrogenic activities, for which they have been denominated as phytoestrogens.

3. Phytoestrogens

Phytoestrogens are non-steroidal compounds that have a unique similarity in molecular weight and the arrangement of aromatic rings with hydroxyl groups to the cyclopentanoperhydrophenanthrene of 17β -estradiol (the most important endogenous estrogen; **Figure 2**).

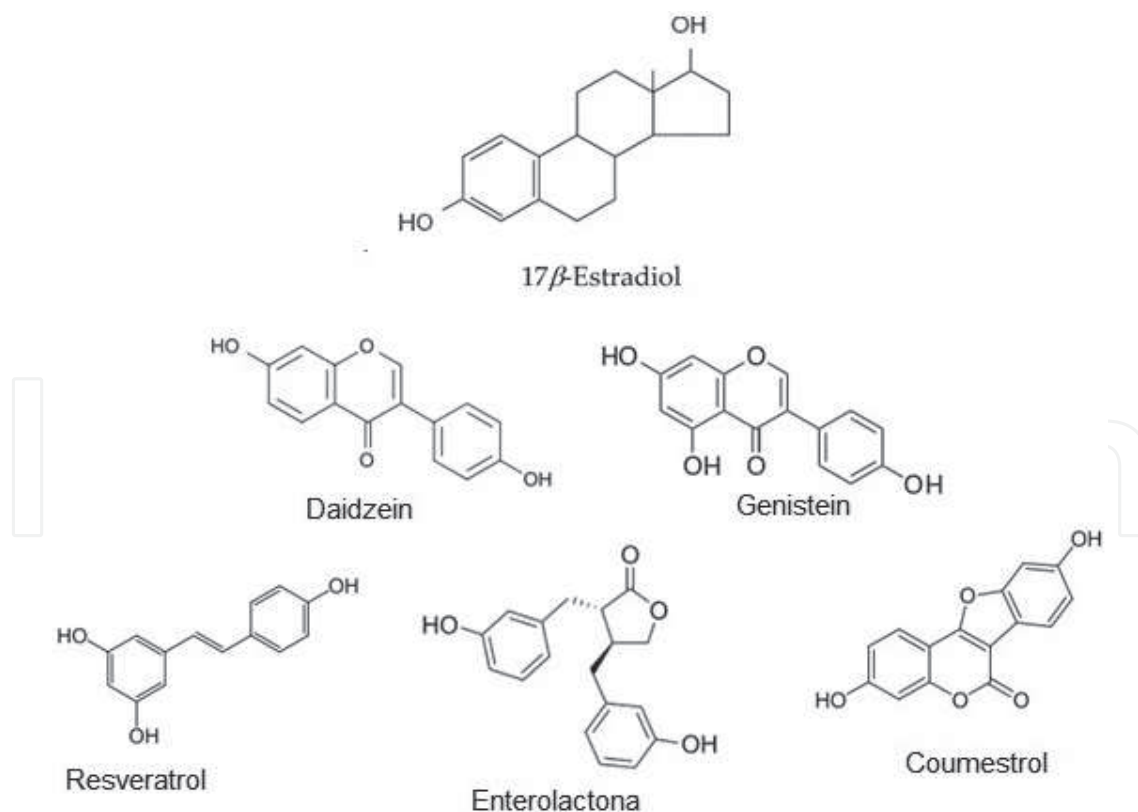


Figure 2. Phytoestrogens. The figure illustrates comparatively the chemical structure of 17β -estradiol and some phytoestrogens. Phytoestrogens are non-steroidal compounds that have a unique similarity in molecular weight and the arrangement of aromatic rings with hydroxyl groups to the cyclopentanoperhydrophenanthrene of 17β -estradiol.

The types of phytoestrogens are isoflavones, lignans, coumestans, ellagitannins and stilbens [13–15]. **Figure 3** shows the main sources of phytoestrogens and the phytochemicals with estrogenic properties. The primary sources of lignans are flaxseed, whole grain cereal and some beverages, such as coffee, tea and wine [15]. For isoflavones are legumens such as soybeans and peanuts, chickpeas and kudzu, lupine, fava, alfalfa, peanuts and chickpeas [15]. In the case of clover alfalfa and soybean sprouts, these are sources of coumestans [13]. Ellagitannins are abundant in fruits, nuts and seeds such as pomegranate, black raspberries, strawberries, walnuts and almonds [16]. The main source of stilbens such as resveratrol is the red wine and peanuts [15]. It is important to note that the beneficial actions of phytoestrogens are mostly given by the estrogenic/anti-estrogenic effect dependent on the resultant concentration of the metabolism

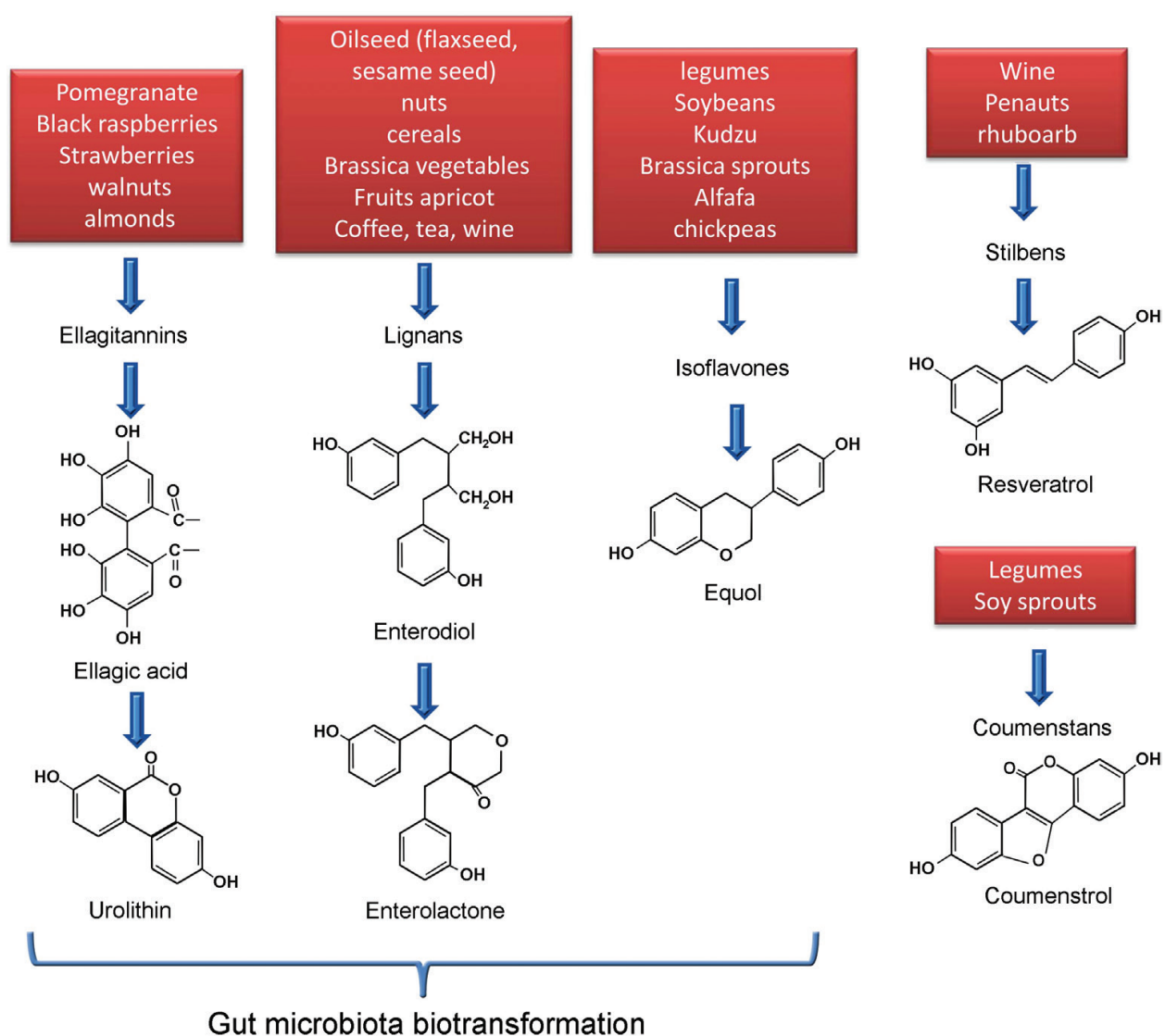


Figure 3. Phytoestrogens and their main food or beverage source. Ellagitannins, lignans and isoflavones require of microbiota biotransformation to release an active compound (urolithins A and B, enteronolactone or Equol, respectively) that produces their estrogen-like effects on different tissues. In contrast, stilbens and coumestans production appears to be independent of microbiota.

of phytoestrogens. It is known that phytoestrogens are mostly as glycosides and that only the aglycone fraction is bio-available to produce biological effects [17]. Some phytoestrogens, such as isoflavones, ellagitannins and lignans, require the action of gut bacterial enzymes to produce equol, urolithins and enterolignans, respectively [16, 18, 19]. These subproducts have more bioavailability and have more estrogenic/anti-estrogenic and anti-oxidant, anti-inflammatory and anti-proliferative activity than their precursors [20]. Furthermore, it is proposed that the biotransformation of isoflavones, ellagitannins and lignans by intestinal microbiota is essential in protection against menopausal symptoms. Also against certain chronic diseases, such as cancer, cardiovascular disease and osteoporosis [15], suggesting that the main discrepancies between a successful treatment versus lack of effect could be explained by the low bioavailability of phytoestrogens [21].

In this line, several reports indicate that coumestans—coumestrol and methoxycoumestrol—show estrogenic activity; the most active compound of isoflavones is daidzein intestinal-derived metabolite equol. However, other products such as genistein, daidzein, glycitein and their respective β -glycosides of genistein and daidzein possess estrogenic activity [13]. Ellagitannins are metabolized to urolithin A and B which showed estrogenic activity [15, 22]. Also, lignans are metabolized in the gut to produce the most estrogenic enterolignans, enterodiols and enterolactone [18].

On the other hand, the chemical similarity between 17β -estradiol and phytoestrogens confers the capability to interact with estrogen receptors (ERs). They produce effects through genomic actions with ERs alpha and beta (α , β) or non-genomic actions via membrane G-protein receptors (GPER) such as GPR30 and mER α and mER β [23]. These receptors differ in tissue distribution, ligand selectivity and transcriptional processes and, therefore, also differ in their physiological effects on its activation [24–26]. These interactions between phytoestrogens and ERs results on estrogenic and/or anti-estrogenic effects, so that phytoestrogens are considered as selective estrogen receptor modulators (SERMs) [27–29].

Several *in vitro* assays suggest that phytoestrogens, especially isoflavones, have a significant preference for ER β rather than for ER α (8- to 40-fold) [30–33]. Some authors explain this preference through steric interaction and through the difference in the attractive potential of hydrogen bonds between the ERs and phytoestrogens, which is higher in ER β [34]. It is thought, that in some way the beneficial actions of the estrogenic compounds are related to the ER β , in part because the activation of ER β has been associated with the anti-proliferative actions of phytoestrogens [35–37].

GPER activation by phytoestrogens has been related to cardiovascular, liver enzymatic and neuroprotective actions [35, 38–40]. Some research groups have proposed that the GPR30 also take part in the endocrine disrupting action of phytoestrogens [41]. Phytoestrogens also show high potency in non-genomic responses. Therefore, it is proposed that their binding affinities could be higher for mERs [42] albeit the involvement of these receptors in the phytoestrogens actions is less explored.

As it can be seen phytoestrogens also possess a complex mechanism of actions to exert their properties, for example, the rapid neuroprotective activity of resveratrol against cerebral ischemia

is mediated by ER α and ER β , and it is blocked by the estrogen receptor antagonist ICI 182 780 [36]. In contrast, Daidzein protects from excitotoxicity induced by glutamate via ER β and GPR30 [35].

4. Alternative to the hormone replacement therapy on menopause

4.1. Clinical studies

During menopause, symptoms such as body weight changes, vaginal dryness, hot flushes, sweating, sleep disturbances and loss of bone density may occur, also cognitive disturbance, mood changes and depression episodes that lead women to a poor quality of life [43]. Symptoms could vary in intensity and in some women, they could be debilitating, particularly for those women who have a previous experience of depression and anxiety in addition to vasomotor, insomnia, weight gain and stressful life events [44].

Hormone replacement therapy (HRT) is the first line of treatment followed by the treatment with selective serotonergic reuptake inhibitors antidepressants [2, 45]. Despite their benefits and efficacy for reducing most of the discomforts of menopausal women, the long-term use of HRT has been extremely controversial because of the adverse events associated. These include an increase in the risk of stroke and venous thromboembolism, an increase in endometrial, ovarian and breast cancer, in addition to the regular side effects of HRT such as headaches, weight changes, nausea and pruritus, among others. On the other hand, it has been reported that the adverse effects depend on several factors. Randomized trials have shown that the risk of presenting adverse effects is mainly given in women older than 60 years old, women during postmenopausal and after 5 years of the continued usage of the HRT.

Other therapies including non-pharmacological interventions have been recommended such as psychotherapy to address the psychological symptoms, acupuncture, physical exercise, nutritional interventions, botanical products and folk medicine, among others [44, 46].

Nevertheless, it is increasingly common for women to prefer an alternative therapy based on the intake of therapeutic compounds of natural origin instead. The alternative therapy can be based either on the consumption of food that may provide health benefits beyond nutrition (functional food) or on the administration of compounds isolated from food (nutraceuticals) with the same purpose. In this sense, phytochemicals as phytoestrogens have received much attention because of their particular health benefits which have allowed the emergence of an alternative to HRT.

Most of the therapies used to treat menopausal symptoms have been focused on alleviating vasomotor and night sweat complaints. One of the most popular therapies is based in the use of black cohosh [33], Valerian and St John's wort [33, 46] due to the lack of estrogenic effects.

Briefly, black cohosh (*Cimicifuga racemosa* L. *Synonym Actearacemosa* L.) has been used for centuries by native Americans for a variety of women's health issues [33]. It is agreed that black cohosh is not estrogenic and its mechanism of action may involve modulation of the

serotonergic system in a similar manner than antidepressants [1]. This botanical product has been tested in several trials given contradictory results. Indeed, the main effects appear to be related to vasomotor and emotional symptoms [33, 47]. For example, in a multicenter randomized, double-blind, placebo-controlled, parallel group trial, the effect of several doses of black cohosh was tested taking into consideration as a primary outcome the difference in menopausal symptoms (vasomotor, psychological and somatic), assessed by the Kupperman Menopausal Index between baseline and week 12. Secondary efficacy variables were patients' self-assessments of General Quality of Life (QoL), responder rates and safety. Compared to placebo, patients receiving black cohosh showed a significant reduction in the severity of vasomotor and psychological symptoms and improved general QoL in a dose-dependent manner from baseline to endpoint [47]. In contrast, recent clinical trials have reported adverse effects of black cohosh with no significant difference from placebo for relief of hot flashes or improving QoL in Thai women [48].

The phytoestrogens mostly used with documented effects are isoflavones. These compounds obtained from different sources have been used primarily to alleviate vasomotor symptoms, but the results appear not to be consistent on mood complaints. For example, in a metaanalysis (43 randomised controlled trials with 4364 participants) that evaluate the effect of isoflavones from soy and red clover in the treatment of hot flashes and insomnia associated with menopause, no significant difference overall was reported in the incidence of hot flashes between participants taking Promensil (a red clover extract that contains isoflavone biochanin A and formononetin) and those given placebo. In this review, four trials suggested that extracts with high levels (>30 mg/d) of genistein consistently reduced the frequency of hot flashes. Some of these trials found that phytoestrogen treatments alleviated the frequency and severity of hot flashes and night sweats when compared with placebo, but many trials were small and were determined to be at high risk of bias. A strong placebo effect was noted in most trials, with a reduction in frequency ranging from 1 to 59% with placebo. Discrepant results could be related to the amount of isoflavone in the active treatment arm, the severity of vasomotor symptoms or trial quality factors [49].

Other studies tested the effect of red clover and also found controversial results. For example, a trial of 72 women randomly divided between placebo and 40 mg dried red clover daily for 12 weeks showed a significant reduction in menopausal symptoms as measured by the Menopause Rating Scale [50]. In contrast, other clinical trials have not shown a significant difference from placebo, particularly for hot flashes relief [5]. Interestingly, red clover has proestrogens, biochanin A and formononetin, which are metabolized by CYP 450 in the gut and liver in genistein and daidzein [51], suggesting more bioavailability compared with soy products. This could be a reason why the use of red clover appears to be more effective than soy in the clinical trials [52].

Kudzu (*Pueraria lobata* Willd.) Ohwi (Fabaceae) is a traditional Chinese medicine for the treatment of the symptoms of menopause [1]; the major isoflavone in kudzu is puerarin, which is metabolized to daidzein by gut microbiota. Clinical trials with kudzu reported no significant changes in the menopausal complaints compared with control group [53].

Other compounds with estrogenic activity that differ from isoflavones have been tested, for example Hop (*Humulus lupulus* L.) (Cannabaceae). Hop extracts are in some dietary supplements

used for managing menopausal symptoms [54]. The most potent phytoestrogen in hops is the ER α -selective agonist 8-prenylnaringenin (8-PN), which is 100-fold more potent than the ER β -selective isoflavones genistein and daidzein [33]. Clinical trials suggest positive results of 8-PN in reducing the symptoms of menopause [54] however due to their high affinity to ER α ; it is important to evaluate the safety on the endometrium and other hormone-sensitive tissues [33].

Rhubard (*Rheum rhaponticum* L.) (Polygonaceae). Rhubard is a plant used for menopausal symptoms relief in Germany [54]. The extract from roots of rhubard mainly consists of rhaponticin and desoxyrhaponticin, which are converted to the resveratrol-like aglycones rhapontigenin and desoxyrhapontigenin by the microbiome. Rhapontigenin is more active than desoxyrhapontigenin, and it is suggested that P₄₅₀-catalyzed O-demethylation giving the resveratrol-catechol piceatannol might be responsible for its estrogenic activity [14, 55]. Clinical trials show that the rhubarb extract was effective and successfully decreased the Menopause Rating Scale and increased QoL [56–58].

Flaxseed (*Linum usitatissimum* L.) (Linaceae). Flaxseed is a primary source of lignans that are metabolized by the microbiota into the phytoestrogens, enterolactone and enterodiol [18]. In a randomized placebo-controlled clinical trial (90 women, 1 g/day flaxseed extract), modest but significant effects were observed in self-reporting relief of menopausal symptoms [59]. Nevertheless, a meta-analysis that included randomized clinical trials examining the efficacy of flaxseed for menopausal symptoms, concluded that there is little evidence to support the use of this dietary supplement for menopause or bone health [60].

Another product most employed in South America is maca (*Lepidium meyenii* Walp.) (Brassicaceae), it is used for hormonal balance, especially for menopausal symptoms [54]. Albeit the active phytoestrogen has not been detected, the extract of maca showed estrogenic effect increasing proliferation in MCF-7 cells. A systematic review of clinical trials concluded that evidence of the effectiveness of maca for the relief of menopausal symptoms was limited. However, because of the sample size, the number of trials and the quality of the trials, it is not pertinent to establish definite conclusions about the efficacy and safety [61].

Recently, in a placebo-controlled trial, the efficacy of resveratrol and equol supplementation was tested on menopausal women aged 50–55 years who received 200 mg of fermented soy containing 10 mg of equol and 25 mg of resveratrol (1 tablet/day) during 12 weeks. The primary outcome was the change in score on the Menopause Rating Scale, used to evaluate the severity of age-/menopause-related complaints. Additional outcomes included the Hamilton Rating Scale for Depression (HAM-D) and Nottingham Health Profile (NHP), which were used specifically to assess sleep quality. Treatment was effective to reduce Menopause Rating Scale and HAM-D scores, importantly on work and activity items and with a slight effect on anxiety-related items [62].

According to the results of meta-analyses, there is not enough clinical evidence that supports the use of phytoestrogens as HRT to alleviate all symptoms of menopause. Albeit an improvement in the quality of life is reported. An important issue to consider is the use of standardized phytoestrogens and ethnicity of the women included in the studies since these factors could be crucial to obtain a positive result for those phytoestrogens that require specific microbiota

biotransformation. Promising results are derived from isoflavones and their precursor because of non-adverse effects reported. However, in all cases, more controlled-clinical trials using large samples with women of different ethnicities are required.

4.2. Preclinical studies

In contrast to the clinical studies where the phytoestrogens were evaluated mainly to prevent or alleviate the vasomotor symptoms, in preclinical data, studies were focused on the evaluation of anxious and depressive-like behaviors. Some of the phytoestrogens are considered selective estrogen receptors modulators and are derived from the metabolism of isoflavones. Importantly, most of them showed an affinity for ER β , characteristic that may explain their effect partially as an antidepressant [63]. However, others showed an affinity for ER α or membrane receptors, making the study of their mechanism of action complex.

Early studies evaluated the effect of dietary phytoestrogens as anxiolytic-like agents [64]. The authors tested the effect of phytoestrogens in the offspring of mothers fed with a dietary soy derived phytoestrogens (600 $\mu\text{g/g}$) and found a reduction in the anxiety behavior in those animals that were maintained with soy diet when adulthood. Rodents fed with soy also showed less body weight [64]. Unfortunately, in this paper, the authors did not indicate the phytoestrogens bounded with the behavioral effect but reported high levels of equol in plasma, the more active metabolite of isoflavones found in the soy [14].

After that, several doses of a diet rich in phytoestrogens (200 or 600 ppm of phytoestrogens in diet) and equol injections (5 mg/kg) were evaluated in the forced swimming test in rats under different endocrine conditions: intact, ovariectomized and aged rats with natural ovarian failure [65]. Interestingly, the latter group resembles an animal model of "natural menopause" because rats are acyclic, and it is the only report, as far as we know, that evaluate the effect of the isoflavones under this condition. Authors showed that soy diet rich in phytoestrogens and equol produced antidepressant-like effects at the same time that induces a reduction of body weight and white adipose tissue in all endocrine conditions. After the soy-rich diet, high plasma levels of genistein, daidzein and equol were detected in those animals that received the highest dose [65]. Unfortunately, the authors did not evaluate anxiety-like behaviors.

In contrast, no effect or anxiogenic effect was reported after a rich isoflavone diet administration [66, 67]. It is important to mention that these data were generated in male rats instead of female rats where more information is needed. Briefly, when a soy-rich diet containing 150 $\mu\text{g/g}$ total isoflavones (daidzein and genistein) was dispensed to young male Lister rats, an anxiogenic effect was reported in the elevated plus-maze and social interaction test. Diet neither affected water intake nor the weight of rats but enhanced the corticosterone and vasopressin stress-response [66], suggesting an increase in sensitivity to stress. The authors explained the apparent discrepancy between results, i.e. Lund and Lephard [64] versus Hartley et al. [66] considering timing and dose used (150 $\mu\text{g/g}$ versus 600 $\mu\text{g/g}$). Other difference that could be noted is the strain of rats, Long-Evans [64] versus Lister rats [66] and the sex of the rats. The latter is an important issue since Hartley et al. [66] used intact male rats, and Lund and Lephard [64] used intact male and female rats finding positive results in anxiety in females but impaired the anxious-like state in males. Further, Patisaul et al. [67]

showed that the administration of a soy-rich (600 µg/g) diet to gonadally intact male rats produced anxiogenic effects. Furthermore, authors tested high (20 mg/kg) and low (3 mg/kg) doses of equol and resveratrol (3 and 20 mg/kg) in two animal models of anxiety in intact male rats. The authors reported that these compounds did not produce any effect after 3 days of treatment and discussed the possibility that the endocrine condition that prevails in males could be a factor to explain the lack of effect [67].

Following this idea, recently it was hypothesized that the role of isoflavones in the regulation of anxiety and depressive-like behavior depends on the endocrine status [68]. To test this hypothesis, the authors evaluated the anxiolytic- and antidepressant-like effect of isoflavone-rich diet (199.4 µg/g) in ovariectomized rats exposed, or not exposed, to estradiol replacement. Data showed that anxiolytic and antidepressant-like effects depend on the endocrine state that prevails during the treatment with isoflavones. In this case, an isoflavone diet combined with estradiol restitution promotes anxiety; in contrast, the same combination promoted an antidepressant-like effect [68]. Unfortunately, authors did not show evidence about the phytoestrogens that could be responsible for the observed effect.

The isoflavone genistein has been evaluated as an antidepressant and anxiolytic compound. This phytoestrogen also showed more affinity for ER β than ER α [69], and recent reports also suggested that it binds to ERM. Rodríguez-Landa et al. [70]) assayed several doses of this compound (0.25, 0.5 and 1.0 mg/kg, i.p.) after 4 days of administration in Wistar rats with 12-weeks after the elimination of ovaries. This model resembled a long-term period of menopause and was used to evaluate if genistein was able to induce anxiolytic-like effects as hormone replacement therapy after a long-term ovariectomy. In this study, authors showed that genistein was effective in reducing anxiety-like behavior in the black and white model after a long-term postovariectomy. Interestingly, it has been reported that E₂ is ineffective to produce behavioral effects after a long-term ovaries removal [71–73]. Therefore, the results obtained with genistein open the opportunity to use this compound as a restitution therapy. Furthermore, clinical and preclinical studies reported that genistein lacks stimulatory effects in breast and uterus [33]. Genistein also showed antidepressant-like effects in rats subjected to the forced swimming test. In this case, genistein (10 mg/kg) was administered during 14 days to ovariectomized rats and reduction of immobility behavior was observed. Data also indicated that genistein increased dopamine and restored the serotonin levels in the hippocampus at a dose of 10 mg/kg [74]. Interestingly, the effect of this compound was also tested after a subacute administration (i.e., three injections in 24 hours) and no effect was observed. This result suggested a genomic mechanism of action [74].

Another compound with estrogenic properties that has been tested is coumestrol (7,12-dihydrocoumenstan), which is considered a SERM that shows an affinity for the ER β [69]. This compound was assayed in the forced swimming test and several models of anxiety after systemic (10 µg/kg) and intracerebral administrations (2 µg/µl/slide) to ovariectomized rats. A reduction of depressive-like and anxiety-like behavior was reported after both routes of administration [63]. As it can be noted, coumestrol is effective as an anxiolytic or antidepressant compound in ovariectomized rats; however, it is necessary to evaluate its effect in young intact and acyclic female rats to establish whether the effect of this compound remains even in the presence of ovarian secretion.

Also, secoisolariciresinol (SECO) is a lignan type phytoestrogen mainly found in flaxseed that can be metabolized to enterodiol and enterolactone. This compound was administered (5, 10 and 20 mg/kg, intragastric) during 14 days to ovariectomized mice and tested in two animal models for the screening of antidepressant drugs, the tail suspension test and the forced swimming test. The authors reported an antidepressant-like effect of SECO in both behavioral tests. Furthermore, this compound restored noradrenaline brain levels and increased dopamine and serotonin concentrations without promoting a stimulatory effect on the uterus [75]. An important difference in comparison to other protocols presented here is that SECO was administered immediately after the ovariectomy. Therefore, the restitution started before a real drop in endogenous estrogen levels, suggesting a model of perimenopause rather than a menopause model.

Phytoestrogens appear to promote anxiolytic and antidepressant-like actions in animal models. However, it can be noted that the time, the dose and endocrine state are factors that may condition the effect of these estrogenic compounds. In general, acute interventions are ineffective; most of the reports indicate that more than 3 days are necessary to observe an anxiolytic or antidepressant-like effect. For isoflavones, their effect appears to depend on the time of restitution and the endocrine state of rats. In this line, the fact that their anxiolytic-like effect is observed in ovariectomized or acyclic females but not in intact rats suggests that isoflavones are working as a restitution therapy and the levels of phytoestrogens that are bioavailable after diet administration are enough to induce changes in the respective receptors like a natural estrogen.

5. Alternative sources of phytoestrogens

5.1. *Punica granatum* L. (Lythraceae)

Pomegranate is a fruit native of Western Asia and North Africa. However, it is now cultivated in most of the Mediterranean and North America region [76]. **Table 1** shows the main phytochemicals reported for pomegranate.

Over the last decades, pomegranate and pomegranate extracts have demonstrated to possess several beneficial health effects for which it is considered a functional food. Clinical and pre-clinical studies have shown that pomegranate has anti-oxidant [77], anti-inflammatory, anti-tumorigenic, anti-microbial [78], anti-obesity [79], anti-nociceptive [80], neuroprotective [81] and antidepressant-like properties [82–84]. Interestingly, most of the health benefits of pomegranate are attributed to its high content of polyphenols, which represents the 26–30% of the total weight of the fruit [85]. The main polyphenols present in the pomegranate are ellagitannins, such as punicalagin (α and β), and flavonoids such as anthocyanidins, catechins, flavonols and isoflavones [80, 86–88]. Ellagitannins are a type of hydrolyzable tannins with several hexahydroxydiphenoyl (HHDP) groups esterified to sugar moieties. When consumed, ellagitannins are easily hydrolyzed to ellagic acid in the acidic conditions of the gastric juice because of its hydrophilic nature. Therefore, on different portions of the small and large intestine, ellagitannins are transformed by bacterial metabolism to dibenzopyranone compounds called urolithins. These compounds have recently demonstrated to possess estrogenic activity on *in-vitro* assays; this particularity makes pomegranate an excellent source of phytoestrogens [16, 22, 78, 89]. Under this premise, some

Part of the pomegranate	Phytochemicals
Whole	Ellagitannins
	Anthocyanins
	Anthocyanidins
	Catechin
	Proanthocyanidins
Seed and oil	Acid derivates
	Fatty acids
	Triglycerides
	Sterols
	Terpenoids
Peel and pericarp	Ellagitannins
	Gallotannins
	Hydroxybenzoic acids
	Proanthocyanidins
	Flavonols
Leaves	Ellagitannins
	Flavones
	Flavonols
	Flavone glycosides
	Alkaloids
	Acid hydroxybenzoic
	Triterpenoids
Flavones	

Phytochemicals found in several parts of pomegranate [79, 80, 86, 87].

Table 1. Phytochemicals found in *Punica granatum* L.

research groups have sought its therapeutic potential for the treatment of symptoms in menopause. In 2012, the therapeutic effect of a 12-week schedule of pomegranate seed oil (PGS) on menopausal symptoms was investigated with a neutral response, i.e., PGS reduced menopausal symptoms, but with no significance, authors remarked the importance of evaluating the PGS for a longer period [90]. Furthermore, a systematic review reported the effect of pomegranate juice in osteoporosis, osteoarthritis, or rheumatoid arthritis. All the studies reported positive effects of pomegranate extract or juice on osteoporosis, osteoarthritis and rheumatoid arthritis [91].

Preclinical studies have reported that the juice of pomegranate reduced menopausal symptoms in animal models by inducing antidepressant-like effects and decreasing bone loss [92].

In our laboratory, an extract of pomegranate in the elevated plus-maze for the screening of anxiolytic action and the forced swimming test for an antidepressant-like effect (unpublished results) was evaluated. This extract has a high content of ellagitannins and previously showed anti-inflammatory and antinociceptive properties [80]. After 7 days of intraperitoneal administration, a reduction of anxiety-like behavior was detected and a lack of effect in the forced swimming test. The decrease of anxiety was detected at 1.0 mg/kg (see **Figure 4**). Importantly,

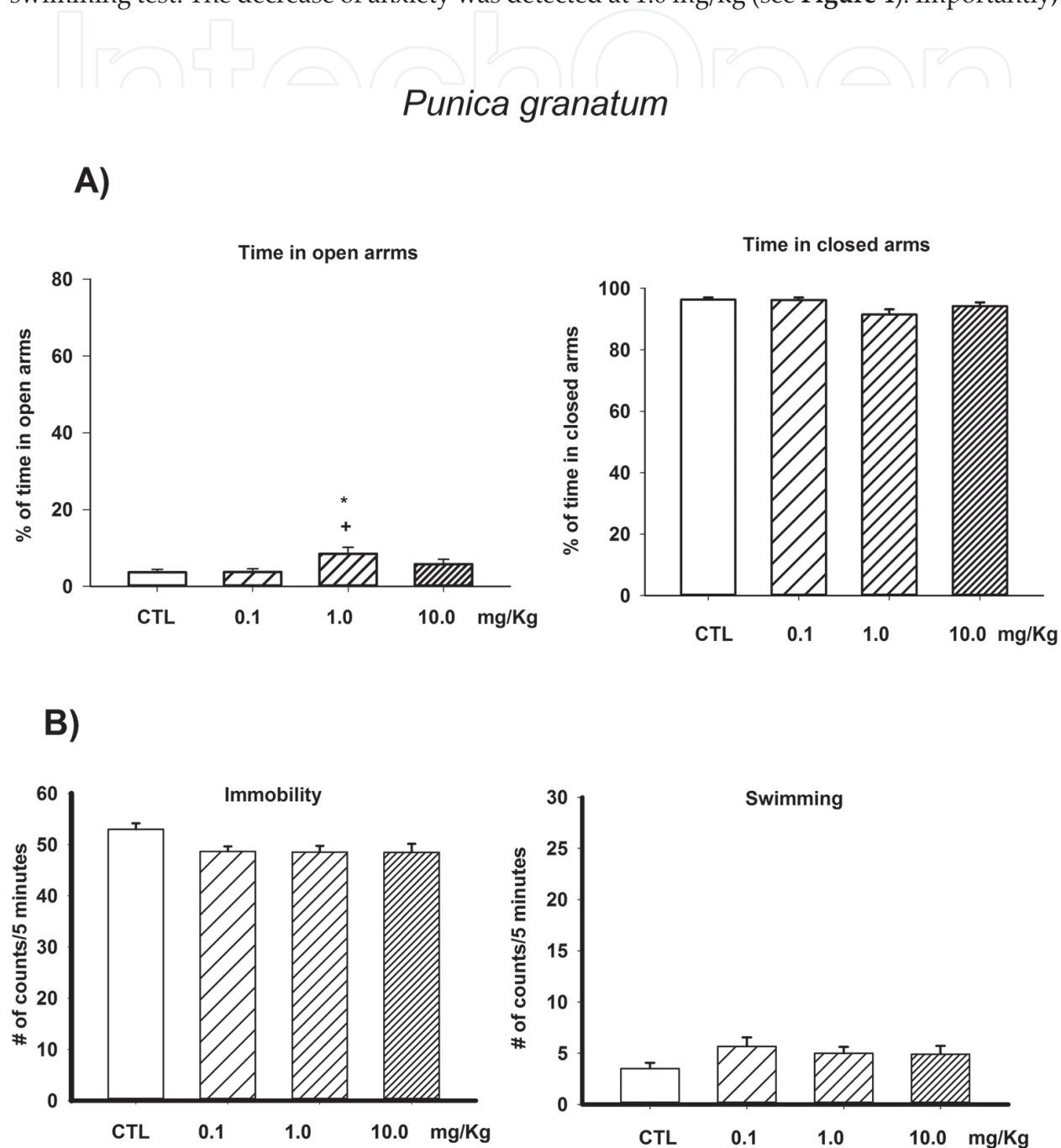


Figure 4. Effect of several doses (0.1, 1.0, 10 mg/kg, i.p) of aqueous extract of pomegranate (*Punica granatum* L.) in the elevated plus-maze (A) and the forced swimming test (panel B) after 7 days of treatment. Pomegranate extract reduced the anxiety-like behavior (panel A) but lacks antidepressant-like effect (B). Data are presented as mean \pm SE of 10–12 ovariectomized rats per group. * $p < 0.05$ versus control group; * $p < 0.05$ versus 0.1 mg/kg, One-way-ANOVA followed by Student-Newman Keuls test. Unpublished results.

the fact that the administration route was intraperitoneal suggests that other phytochemicals more than ellagitannins, which require of microbiota transformation, could be present in the extract. Future experiments may contribute to elucidate the phytochemical bounded in the anxiolytic effect of pomegranate.

5.2. *Brassica oleraceae* var. *italica*

Broccoli (*Brassica oleracea* var. *italica* Plenck) belongs to the Brassicaceae of cruciferous family where cabbage, brussels sprouts and radish can also be found [93]. It is native to the Anatolian peninsula and now is widely cultivated in other parts of the world. The phytochemical content of broccoli is shown in **Table 2**. As it can be seen, broccoli is also a source of important polyphenols, and consequently, phytoestrogens since in its composition, it can be detected quercetin, kaempferol, daidzein, antocyanins [93, 94], coumestans and lignans [45, 95].

Phytochemicals	Unity	Broccoli
β-Carotene	UI	318.56
Zeaxanthin	UI	22
Lutein	μg	1123.76
β-Cryptoxanthin	UI	0.88
Quercetin	mg	2.2
Kaempferol	mg	0.008
Myricetin	mg	3.5288
Daidzein	mg	0.0352
Anthocyanin	μg	0.01
Lignans	μg	0.528
Glucoiberin	μmol	145.2
Glucoraphanin	μmol	646.8
Progoitrin	μmol	80.15
Glucoalyssin	μmol	69.78
Gluconapin	μmol	22.88
Glucobrassicinapin	μmol	23.707
Gluconasturtiin	μmol	9.52
Glucobrassicin	μmol	308
Neo-glucobrassicin	μmol	84.48
Chlorophyll (A)	mg	0.88
Chlorophyll (B)	mg	0.748

Phytochemicals of Broccoli. Data are expressed as UI, mg, μg or μmol per 98 g of dried sample [96–117].

Table 2. Phytochemicals in different parts of *Brassica oleraceae*.

Broccoli is also a source of a potent anti-oxidant and anti-inflammatory compound called sulforaphane (1-isothiocyanato-4-methylsulfinylbutane) which is an organosulfur compound [118]. Several reports indicated that this compound might prevent depressive-like behavior induced by the inflammatory process. For example, acute sulforaphane at 3–30 mg/kg and glucorophanin (glucosinolate precursor of sulforaphane) in the diet were tested in male C57BL/6 mice. In this case, it was to determine whether these compounds were able to prevent the onset of depression-like behavior after an induction of inflammation by lipopolysaccharide administration [118]. In this chapter, authors also evaluate the effect of sulforaphane on brain-derived neurotrophic factor (BDNF) levels, synaptogenesis protein and dendritic spine density in the brain. The results showed that sulforaphane prevents the increase of TNF- α , IL-10 and microglia activation blocking the inflammation process at the same time that decreased the depressive-like behavior evaluated by two behavioral test. Interestingly, sulforaphane also reverses the reduction of BDNF expression and dendritic spines induced by inflammation process [118].

In another study, the effect of sulforaphane and glucorophanin was tested on depressive-like behavior after repeated social defeat stress using Nrf2 knock-out mice. Nrf2 is a transcription factor keap-1 system that plays a role in the inflammation and evidence has shown that both peripheral and central inflammation plays a crucial role in the pathophysiology of depression [119]. The administration of diet rich in sulforaphane and glucorophanin prevented the depressive-like behavior during adulthood; further authors showed that dietary intake of SFN-rich food during juvenile stages and adolescence could confer stress resilience in adulthood [119].

Contrasting results have also been reported, for example, Balb/c mice received sulforaphane (50 mg/kg) previous to the injection of lipopolysaccharide afterward sickness behavior (an animal model of depression), and the proinflammatory response was evaluated in the hippocampus. The authors reported that sulforaphane administration prevented the induction of pro-inflammatory mediators (IL-1 β ; IL-6, Cybb, INOS) but did not improve sickness behavior [120, 121]. Therefore, the use of sulforaphane to prevent depression-like behavior is inconclusive.

As far as we know, there is no information about the use of sulforaphane or glucorophanin in animal models of menopause. However, it has been suggested that the advantage of broccoli consumption is that some phytochemicals promote the conversion of 16- α -hydroxyestrone to 2-hydroxyestrone, the first is a carcinogenic metabolite that has been linked to breast cancer risk whereas the latter product does not exhibit estrogenic properties in breast tissue. Apparently, the ingestion of broccoli sprouts could be a good strategy for the treatment of menopause symptoms [2, 122, 123].

In our laboratory, several doses (0.1, 1.0, 10 mg/kg, i.p) of an extract of broccoli were evaluated for its anxiolytic and antidepressant-like effects after 7 days of administration to 3-week post-ovariectomized rats. The results indicated an anxiolytic—but not antidepressant-like effect (**Figure 5**, unpublished results). The phytochemical involved in this action is unknown and studies to reveal it are running.

The fact that sulforaphane exerts anti-inflammatory properties and that according to the aetiology of depression, the inflammatory process plays a major role in its aetiology, the consumption of food or nutraceuticals with sulforaphane could be suitable.

Brassica oleraceae L

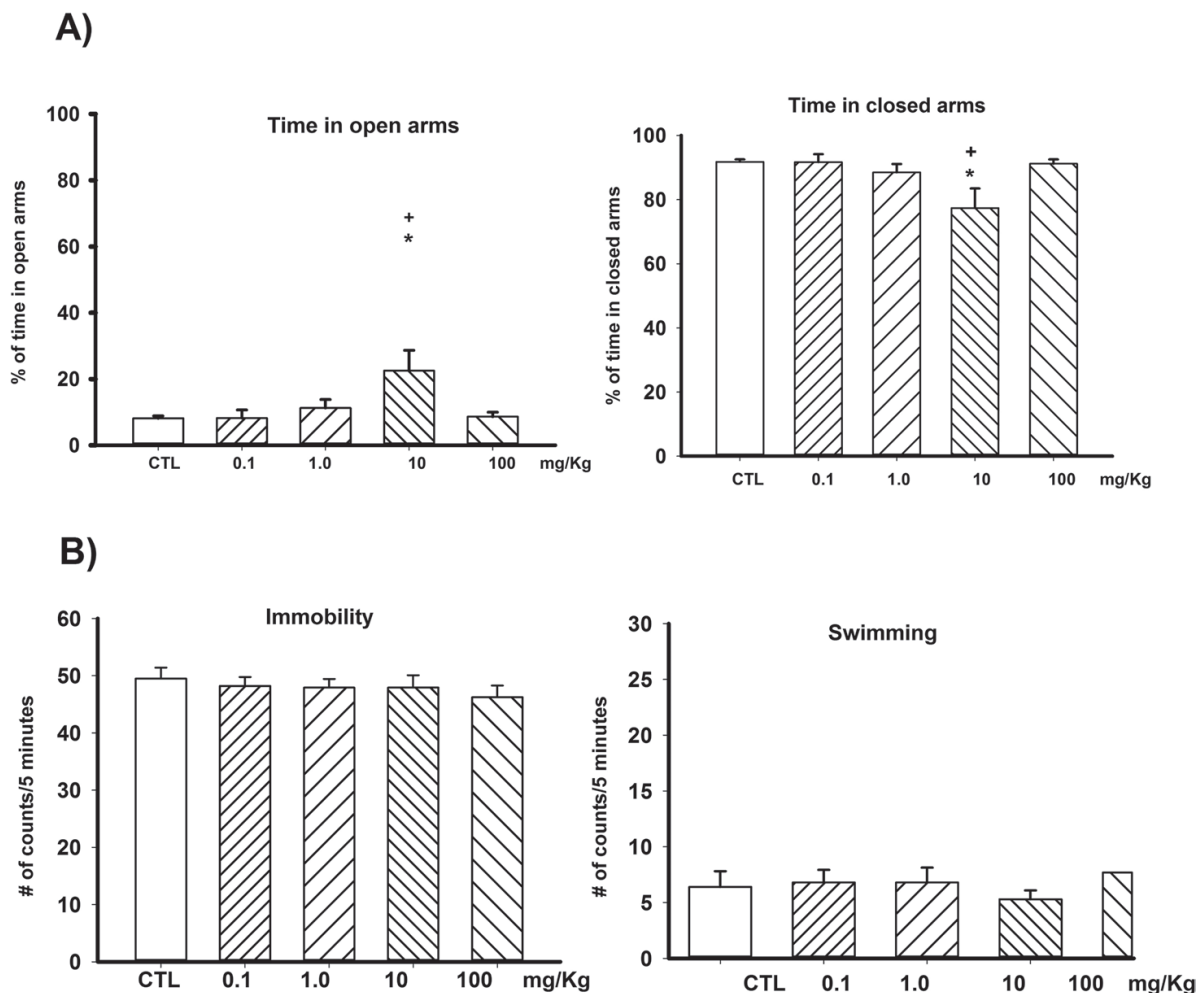


Figure 5. Effect of several doses (0.1, 1.0, 10 and 100 mg/kg, i.p) of aqueous extract of broccoli (*Brassica oleraceae* L) in the elevated plus-maze (A) and the forced swimming test (B) after 7 days of treatment. Broccoli extract reduced the anxiety-like behavior (A) but lacks antidepressant-like effect (B). Data are presented as mean \pm SE of 10–12 ovariectomized rats per group. * $p < 0.05$ versus control group; + $p < 0.05$ versus 0.1 mg/kg, One way-ANOVA followed by Student-Newman Keuls test. Unpublished results.

6. Comorbidity in menopause and alternative medicine

Fibromyalgia (FM) is a chronic, generalized pain syndrome that affects the musculoskeletal system [124]. It is characterized not only by a widespread pain observed due to the presence of multiple tender points but also by depressive behavior, fatigue and sleep disturbances without any structural or inflammatory cause [125, 126]. Studies have consistently demonstrated a female predominance of this disease [127] with a major frequency in pre- and post-menopause condition supporting that an abrupt decline or a reduced time of exposure to ovarian hormones may contribute to FM [128].

Although FM is not an inflammatory disease, it is known that a neuroinflammation process can occur, which is described as an increase in the production of interleukins at the central

nervous system level, such as IL-1 β , IL-6, IL-8, IL-10 and TNF- α , and not at the peripheral level [129]. To date, it has not been possible to establish whether their elevated levels are associated with the painful process in FM since these have also been observed in patients with depression and sleep disorders [130]. The neuroendocrine alterations observed are usually at the level of the hypothalamic-pituitary-adrenal axis where there is hyperactivation, and it has been related to deficient levels of hormones [131] that could be part of the reasons of a major frequency in menopause [128].

As it was mentioned, anthocyanidins belong to the flavonoid group of plant-derived chemicals, which have been commonly used for the treatment of chronic diseases. One randomized clinic test was done to evaluate the efficacy of this compounds (40, 80 and 120 mg/day) in the treatment of FM compared with a placebo group. The evaluation had duration of 52 weeks with each treatment given for 12 weeks, preceded by a 4-week baseline period. Authors conclude that anthocyanidins showed small but significant benefits at a dosage of 80 mg/day in the treatment of primary FM, mainly in the sleep disturbance in the presence of minor adverse effects like indigestion or nausea [132].

Chronic fatigue syndrome is also comorbid linked to early menopause and FM since it involves the muscular, nervous, hormonal and immune systems; it is often misdiagnosed as depression [133]. Isoflavones (daidzein and genistein) were capable of reversing alterations like chronic fatigue syndrome in an experimental model in mice suggesting their protective effect in this neuroimmune-endocrine disease [134].

7. Concluding remarks

1. Clinical reports show inconsistent results about the use of phytoestrogens effectiveness to treat vasomotor and psychiatric disorders associated with menopause. The biotransformation by microbiota to deliver the main active compounds appears to be fundamental to observe positive effects.
2. Preclinical data show that the effect of phytoestrogens depends on the time of administration as well as the endocrine state of rats, suggesting that these factors could also contribute to explain the inconsistency between results observed in humans.
3. More studies are necessary to evaluate if the same phytoestrogen can induce both anxiolytic- and antidepressant-like action and if their effect depends on the endocrine state.
4. Clinical and preclinical studies indicated that the use of phytoestrogens is safe due to the high antioxidant activity. However, meta-analysis studies are inconclusive. Therefore, phytoestrogens as restitution therapy should be monitored.
5. Functional food and nutraceuticals are an important source of a wide variety of phytoestrogens.

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