

Clinical Activity of the Combination of Isoflavones, Agnus Castus and Magnolia (Estromineral Serena Plus) in the Menopause

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Abstract

Introduction: The gradual menopausal decline in oestrogens impairs function of the hypothalamic and limbic systems and, therefore, thermoregulation, vasomotor stability, mood regulation and psychological wellbeing. The significantly reduced dopaminergic activity and melatonin secretion increased in hot flushes, psychological symptoms and poor sleep quality. When hormone replacement therapy (HRT) is contraindicated or refused, dietary supplements are a possible alternative.

This pilot study in menopause collected clinical data on a combination of soy isoflavones, magnolia, and agnus castus.

Method: Women in the menopause with moderate hot flushes (>5/day) and altered mood or sleep, have been treated with ESP (Estromineral Serena Plus, Meda Pharma) containing soy isoflavones, Lactobacillus sporogenes, extracts of Magnolia officinalis and Vitex agnus-castus and vitamin D3 for 12 weeks.

Kupperman Index was evaluated at baseline, after 4, 8 and 12 weeks. The doctor and the woman gave their overall opinion at the end of treatment.

Results: Fifty-three women were treated, average age of 53 years, in the menopause for 2.8 years, with prior use of HRT in 32%.

Hot flushes, night sweats, insomnia, irritability, anxiety, palpitations, vaginal dryness, dyspareunia and libido loss showed a gradual and significant improvement during ESP treatment.

Unexpected events appeared in 4 cases, 2 of which were suspended. The doctor's final opinion was good/excellent in 84% of cases.

Conclusions: This observational study provides the first data on the safety and the clinical effect of ESP in menopause disorders. The synergy of soy isoflavones, extract of Magnolia officinalis bark and extract of Vitex agnus-castus L. fruits is targeted on significantly reducing vasomotor symptoms, anxiety, irritability and insomnia. Further studies are needed to confirm these preliminary outcomes.

Keywords: Menopause, Menopausal symptoms, Hot flush, Thermal regulation, Isoflavones, Agnus castus, Magnolia, Plant extracts

Introduction

The menopause is a physiological condition which appears in women around the age of 50 and is due to the reduced production of oestrogens.

The deficiency of these hormones causes the appearance of symptoms such as hot flushes, night sweats, palpitations, insomnia, irritability, anxiety and mood changes, vaginal dryness and gradually over the long term bone mineral loss with a risk of osteoporosis, atrophy of genitourinary tissue, ageing of the skin and a higher incidence of cardiovascular disorders.

However, the endocrinological aspects of the menopause have not yet been fully explained and are complicated by the appearance of irregular cycles which characterise the pre-menopausal transition phase with regular ovulatory cycles alternating with longer or shorter anovulatory cycles [1].

Climacteric symptoms must be considered not only as disorders restricted to a poorer quality of life, but also as risk factors for osteoporosis, cardiovascular disease and cerebral impairment. In fact, the most severe hot flushes are associated with rapid bone loss and osteoporosis [2], impaired verbal memory [3], low levels of plasma antioxidant activity, increased cardiovascular reactivity to stress, impaired flow-mediated dilation [2] and systolic blood pressure [4]. In addition, vasomotor symptoms

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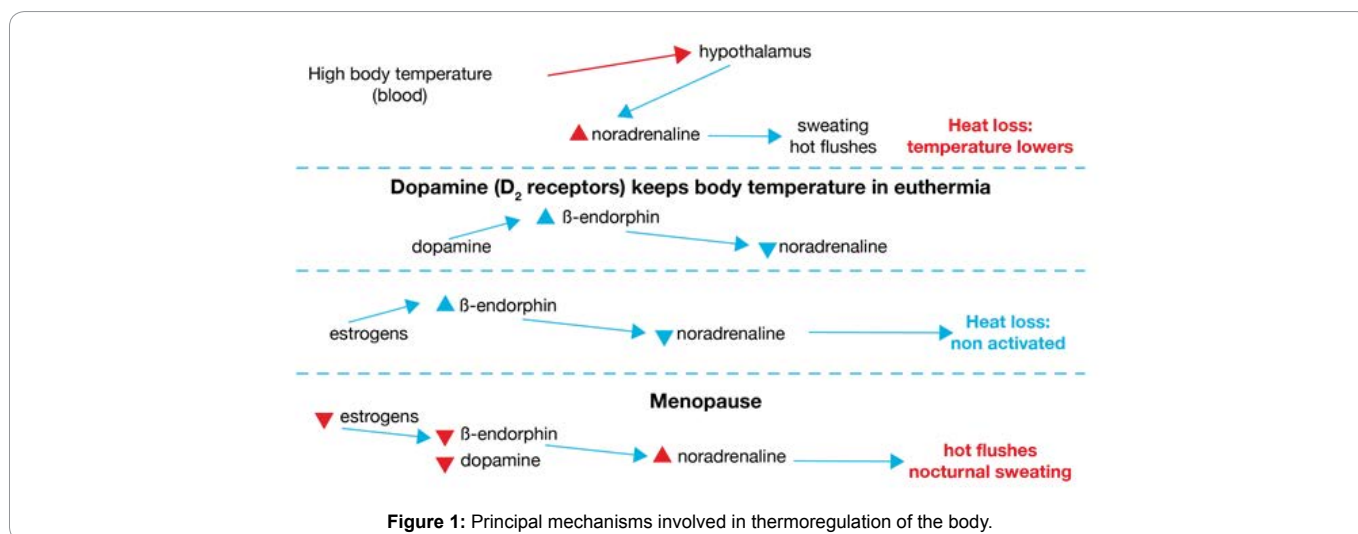


Figure 1: Principal mechanisms involved in thermoregulation of the body.

(hot flushes and night sweats) are connected with an increased risk of heart disease, which cannot be explained entirely by the risk factors for heart disease [5].

According to the different populations observed, the most common menopausal symptoms are: hot flushes (65% - 86.8%), night sweats (65% - 82.1%), insomnia (61%-67.8%), and mood changes (57% - 47.5%) [6,7].

Bearing in mind the impact of hot flushes and sweating on quality of life, linked to the change in thermoregulation, all treatments used by women in the menopause should primarily resolve these troublesome disorders.

Thermoregulation

The hypothalamus is a structure of the central nervous system which activates and integrates endocrine activity and many bodily functions and has a control function in relation to the autonomic nervous system, including maintenance of body temperature and the sleep-wake cycle, salt-water balance and food intake.

Noradrenalin release and parasympathetic nervous system regulates sweating and peripheral vasodilation promoting heat dispersion and, therefore, the lowering of body temperature. Sweating and hot flushes in the climacteric correspond to the increase of noradrenalin to lower body temperature [8,9].

In turn, dopamine is credited as an important neurotransmitter in thermoregulation, with D2 receptors involved principally in maintaining a normal body temperature. It has been observed that the dopamine agonist bromocriptine increases the activity of the endogenous opioid system of the hypothalamus (β -endorphins) on the mechanisms which regulate body temperature during the menopause [10].

In fact, β -endorphins-peptides with affinity for the μ and κ opioid receptors-also intervene in thermoregulatory homeostasis. They have opposite roles in activating dopamine transmission; β -endorphins inhibit noradrenergic neurons below the threshold for activating heat loss [11].

Oestrogens regularly modify the synthesis, release and metabolism of many mediators, including dopamine and melatonin, which modulate the function of the hypothalamic

and limbic systems, in particular dopaminergic activity. In fact, oestrogens intervene directly in dopaminergic activity by increasing the release of dopamine in the hypothalamus and the transmission of dopamine and D2 receptors. In addition, oestrogens increase the synthesis and release of β -endorphins (Figure 1) [12].

During the menopause, there is a temporary change in the hypothalamic thermoregulatory centre with a resetting of body temperature homeostasis to lower levels, which are achieved through heat dispersion via vasodilatation and sweating. In fact, the catecholaminergic and dopaminergic systems are the two systems most widely implicated in the pathogenesis of hot flushes, which are the most common and characteristic symptom of the climacteric syndrome [13,14].

Sleep-wake Cycle and Psychological Symptoms

It has been observed that melatonin levels and the time during which melatonin remains high during the night are significantly reduced with age [15]; in fact, sleep quality is linked to the quantity of melatonin secreted, especially in the elderly. Studies in perimenopausal women reveal that the decline in melatonin secreted precedes the increase in follicle-stimulating hormone (FSH) [16].

The psychological symptoms are associated with reduced dopaminergic system activity.

Although elective treatment in the menopause includes hormone replacement therapy (HRT) this has limitations in its duration of use and in the clinical conditions of the woman. In situations in which HRT is not feasible, often because of the woman's refusal, the use of preparations classified as food supplements is of interest.

Amongst these, a product has recently been developed containing soy isoflavones (SI), *Lactobacillus sporogenes* (Ls), fruit extract of *Vitex agnus-castus* L. and bark extract of *Magnolia officinalis* R et W, vitamin D3, calcium and magnesium (Estromineral Serena Plus, ESP), aimed at balancing hormone deficiencies during the climacteric and menopausal periods, preventing characteristic disorders of the menopause, since it promotes bone tissue tropism, it is adaptogen against psychological and physical stresses, it brightens mood, prevents states of anxiety and depression, and improves sleep quality.

Soy isoflavones, the absorption of which is enhanced by the presence of lactobacilli [17], have a recognised activity on vasomotor symptoms, agnus castus acts on hot flushes and psychological symptoms [7] and magnolia acts on psychoaffective symptoms, in particular anxiety, irritability and insomnia [6].

Vitamin D3, calcium and magnesium complete the formulation by strengthening bone mineralisation, in particular in association with phenomena of decalcification and/or osteoporosis.

The aim of this preliminary study was to collect clinical data on ESP activity and safety in menopausal women with moderate hot flushes and concomitant psychoaffective symptoms such as insomnia, irritability, anxiety and mood changes, which were not severe enough to require psychopharmacological therapy.

Methods

Three Italian sites took part in the open-label, observational, non-interventional pilot study. Each centre collected 20 cases. The study was conducted in accordance with the guidelines of the 1964 Declaration of Helsinki and its revisions up to 2013. Specifically, subjects gave their informed consent and could interrupt the study at any time, without having to give any reason.

Menopausal women with typical menopausal symptoms, notably hot flushes of at least moderate severity (more than 5 a day), and concomitant changes in mood or sleep that were not severe enough to require specific psychopharmacological treatment were treated with ESP (Estromineral Serena Plus, Meda Pharma) containing soy isoflavones 60 mg (genistein 30 mg and daidzein 30 mg); Lactobacillus sporogenes 1 billion spores; Magnolia officinalis bark extract 50 mg equivalent to honokiol 0.75 mg, Vitex agnus-castus L. fruit extract 40 mg equivalent to agnuside 0.2 mg, vitamin D3 5 µg, calcium 141 mg, at a dose of 1 tablet a day for 12 weeks. Women on HRT or who were simultaneously taking other products which could interfere with climacteric symptoms were excluded.

At baseline and after 4, 8 and 12 weeks the presence and severity of menopausal symptoms used to calculate the Kuppermann Index were evaluated: hot flushes, night sweats, insomnia, irritability, depressed mood (melancholy), dizziness, fatigue, joint and muscle pain, headache, palpitations and paraesthesia. At the beginning and end of treatment the following were also evaluated: vaginal dryness, pain during

sexual intercourse, anxiety, and a drop in libido. The severity of signs/symptoms was indicated as 0=none; 1=mild or barely noticeable; 2=moderate or quite persistent but not affecting normal activities; 3=marked or influencing normal activities. The Kuppermann Index was calculated by adding up the severity score of each symptom multiplied by a specific factor, more specifically multiplying the hot flushes score by 4, sweating, insomnia and irritability by 2, and dizziness, fatigue, joint and muscle pain, headache, palpitations and paraesthesia by 1.

The following evaluation was associated with the total Kuppermann Index score: <15 no treatment, 15-20 mild symptoms, 21-35 moderate symptoms (treatment with phytohormones), >35 marked symptoms (often HRT indicated). The quality of sexual activity at 12 weeks was indicated as improved, unchanged or worse compared to baseline.

At the end of treatment, the doctor's global judgment on the treatment and the woman's opinion on the acceptability of the treatment were expressed, through a semi-quantitative evaluation scale, using the following score: 0 = none; 1 = small; moderate; 2 = good; 3 = excellent.

The statistical analysis was carried out by protocol using the Kruskal-Wallis test for non-parametric data to compare each symptom and the Kuppermann Index compared to baseline, with the binominal test for the quality of sexual activity, using Excel statistical software.

Any adverse events, classed as unexpected or unwanted signs, symptoms or disease (including exacerbation of existing disease) which appeared during treatment were recorded in the appropriate session provided in the case report form for each subject.

Results

Fifty-three women were treated, with an average age of 53.0 ± 3.6 (SD) years, BMI 25.7 ± 4.2 kg/m², in the menopause for 2.8 years, with prior use of HRT in 32%. HRT was abandoned 6 months earlier than starting the ESP treatment and was due to the patients' decision.

The severity of hot flushes, night sweats, palpitations, drop in libido, vaginal dryness, pain on sexual intercourse, insomnia, irritability, anxiety, depressed mood, dizziness, fatigue, joint/muscle pain, headache and paraesthesia showed a gradual, steady and significant improvement (p<0.01) during treatment with ESP (Figures 2 and 3). The Kuppermann Index

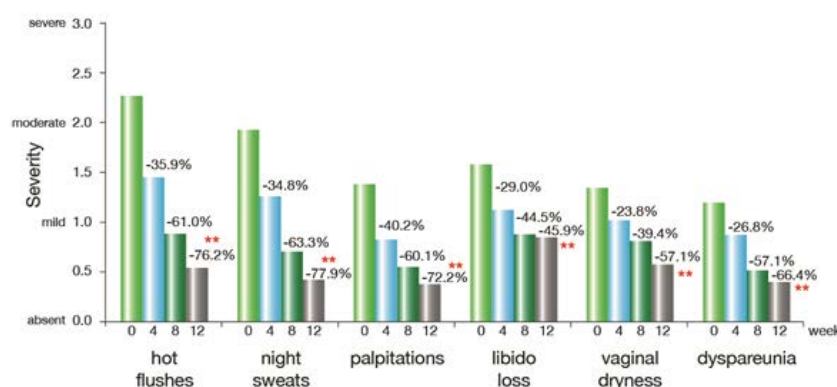


Figure 2: Typical symptoms of the menopause at baseline and during treatment with isoflavones+agnus castus+magnolia (ESP) for 12 weeks. Means ± SE. ** p<0.01 vs. baseline.

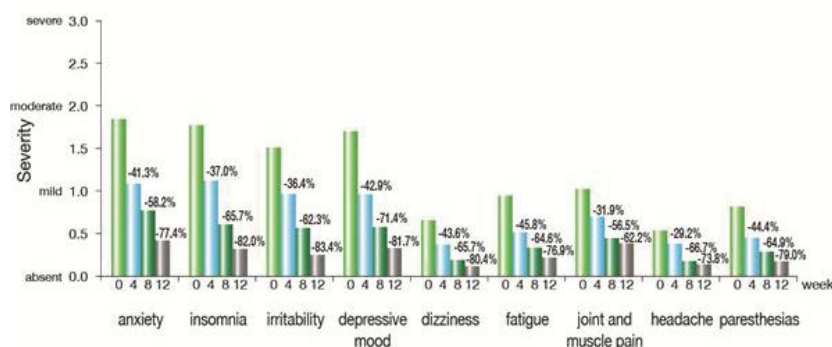


Figure 3: Psychoaffective symptoms in isoflavones+agnus castus+magnolia (ESP) menopause at baseline and during treatment with ESP for 12 weeks. Means \pm SE. ** $p < 0.01$ vs. baseline.

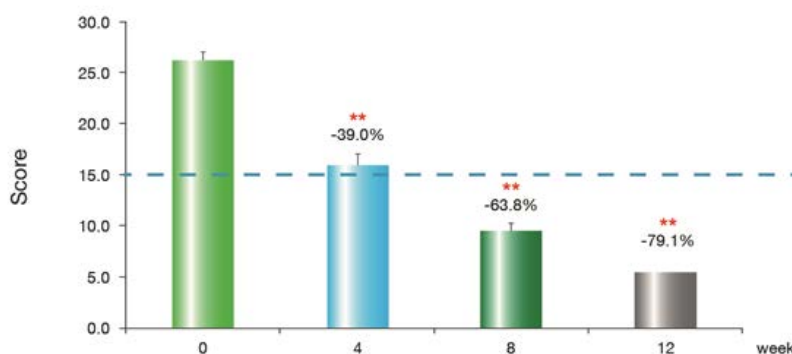


Figure 4: Kupperman Index at baseline and during treatment with isoflavones+agnus castus+magnolia (ESP) for 12 weeks. Means \pm SE; ** $p < 0.01$ vs. baseline.

was reduced statistically significantly compared to baseline ($p < 0.01$) (Figure 4).

Sexual activity at 12 weeks was improved in 69% of cases compared to baseline ($p = 0.03$).

In 4 cases (*2 of which were suspended) unexpected events appeared: gastralgia, breast tenderness, headache* and nervousness*. The doctor's final opinion was good/excellent in 84% of cases.

Discussion

The endocrinological aspects of the menopause have not yet been fully explained and are complicated by the appearance of irregular cycles which characterise the pre-menopausal transition phase and which include regular ovulatory cycles or longer anovulatory cycles, with no systematic progression from one to the other [1].

The perimenopause is characterised by a gradual decline in oestrogen levels with an increase in FSH and reduced hypothalamus-pituitary sensitivity to negative oestrogen feedback [18].

Fluctuating levels of sex steroids, in particular oestrogens, lead to an altered function of the hypothalamic and limbic systems and, as a consequence, of mood regulation, psychological wellbeing, thermoregulation and vasomotor stability.

Currently, it is believed that the aetiology of hot flushes involves a noradrenergic mechanism [19]. In symptomatic women, shrinkage of the hypothalamic thermo neutral zone has been observed, which is due, at least in part, to high levels of cerebral noradrenalin. The central noradrenergic instability associated with hot flushes could be due to the reduction in endogenous opioid activity which arises from the reduction in oestrogen levels, since hypothalamic opioidergic activity normally has an inhibitory effect on the noradrenergic neurons in the brainstem.

It has also been hypothesised that treatments which are effective on hot flushes exert their effect by increasing endogenous opioid peptide activity and consequent inhibition of the noradrenergic activity below the threshold for activating heat loss.

During the post-menopause period, the activity of the dopaminergic system is significantly lower compared to pre-menopausal women, but significantly increased by the administration of hormone therapy, with a significant and concomitant reduction in psychological symptoms [20].

This preliminary study indicates that ESP is a natural supplement which can significantly and safely reduce both moderate vasomotor and psychobehavioural disorders during the menopause. The limitations of the study outcome are due to the open uncontrolled design, considered a methodologically

correct approach as first clinical experience of a new product. On the other hand, clinical studies are not requested for marketing food supplements. The results show the ESP safety and the hot flushes reduction around 72% after 12 weeks of treatment, interesting effect as it is well known that placebo has some 20-30% efficacy in reducing hot flushes. The ESP effectiveness should be confirmed with a further extended controlled study.

The SI contained in ESP is phytoestrogens, natural plant compounds which have a mild, balanced oestrogen action due in particular to genistein and daidzein. The isoflavones can only act after they have been transformed in the intestine into aglyconic form. In ESP, this activation is supported by L.s., a lacto-fermented probiotic which produces glycosidases, enzymes which cleave the glycosidic bond in SI and release their active ingredients including genistein, daidzein and its active metabolite equol, promoting their absorption and thus increasing their efficacy [17].

The dry extract of agnus castus is a phytocomplex containing glycosides, flavonoids, terpenes and alkaloids. The main active ingredient is agnoside, an iridoid glycoside, on the basis of which the extracts are titrated. Agnus castus is a treatment considered effective for the majority of female hormonal disorders and is also used during the menopause and pre-menopause, in particular for hot flushes and an irregular menstrual cycle [7].

An epidemiological study reports that English phytotherapists prescribed extract of agnus castus fruits for a 3-month treatment of peri-menopausal disorders, and the frequency of improved subjects was 75% for hot flushes, 50% for vaginal dryness/dyspareunia and 50% for insomnia [21].

Agnus castus 50 mg in a multicomponent preparation of plant origin administered for 3 months in 50 peri and post-menopausal women helped to reduce hot flushes and night sweats in about 70% of cases, significantly superior to placebo (between 30-40%). Changes in levels of oestradiol and FSH, liver enzymes or thyroid-stimulating hormone were not observed [22].

Vitex agnus-castus, such as red clover, has binding affinity for the oestrogen receptors alpha and beta and can stimulate the expression of m-RNA for the progesterone receptor (PR expression) and 3pS2 (presenilin-2), another gene that can be induced by oestrogens [23]. Agnus castus acts as a dopamine agonist and rebalances progesterone deficiency in the corpus luteum [7]. In the extract of agnus castus fruits, diterpenes with dopaminergic activity have been identified.

The active ingredients of agnus castus act on the diencephalon-pituitary system by balancing the secretion of sex hormones and inhibiting prolactin secretion [24].

However, other mechanisms of action seem to be involved in the pharmacological activity of agnus castus.

In vitro studies demonstrate the affinity of agnus castus for opioid receptors, in particular an agonist effect with μ receptors, as well as a dose-dependent effect on the increase in melatonin secretion which could play a role in improving sleep disturbances [25,26].

Post marketing surveillance reports that the adverse events occurred during Vitex agnus castus L. treatment are mild and reversible, the most frequent being nausea, headache, gastrointestinal disturbances, menstrual disorders, acne, pruritus

and erythematous rash. No drug interactions were reported. On the basis of the data available up to now, Vitex agnus castus L is defined a safe herbal medicine [27].

Extract of magnolia calms and lifts mood, without causing drowsiness or other side effects typical of anxiolytics on prescription [28]. Magnolol and honokiol, the most important active ingredients of extract of magnolia, modulate the GABA-A receptors of the cerebral limbic system, the centre of emotions and feelings [29,30]. This explains the calming, muscle-relaxant and rebalancing activity on sleep disturbances and the consequent efficacy in fighting symptoms such as irritability, anxiety, insomnia, and mood instability. Extract of magnolia also leads to a decrease in blood cortisol, the excretion of which is linked to conditions of stress. Extract of magnolia is therefore an adaptogen, which is particularly useful in mental and physical stress conditions. Extract of magnolia in combination with isoflavones (Estromineral Serena) was more effective on anxiety and irritability and rebalance sleep disturbances in menopausal women, both in comparison with placebo and an active product [6,31].

The safe use of soy is guaranteed by its very long-term use as a foodstuff in Asian countries. SI are, in fact, included in the US FDA's GRAS (Generally Recognized As Safe) list. They are very well tolerated in adult women up to a dose of 100 mg/day in a glycosylated form [32,33]. In connection with this, it is important to remember that the reference dose established by the authorities for the use of isoflavones as supplements must not exceed 80 mg/day [34]. In ESP the quantity of isoflavones is 60 mg/day, the bioavailability of which is optimised by L.s. In a meta-analysis study on phytoestrogens safety, only the gastrointestinal effects were significantly more frequent in phytoestrogen users compared to the control group [35]. There was no difference in the incidence of gynaecological, musculoskeletal or neurological effects between treatment groups. In particular, adverse events linked to hormonal activity, such as the risk of blood loss, endometrial hyperplasia, and endometrial and breast cancer, did not show any greater incidence between women taking phytoestrogens compared to the control group.

These data confirm the results of investigational studies that have shown no correlation between phytoestrogens and the proliferation of endometrial or breast tissue [36]. The available data suggest that isoflavones do not have any negative effects, but conversely have a beneficial effect on endometrial and breast cancer: there is a low incidence of these types of cancer among Asian women (and Asian men have a low incidence of prostate cancer) probably as a result of the high consumption of soy in the diet.

SI does not increase the risk of breast or endometrial cancer, probably because of their mild oestrogen activity and simultaneously the antioestrogen activity (increase in SHBG) and because of the predominant stimulation of β -receptors, which are barely present in the organs most susceptible to oestrogen-dependent cancers.

It has been shown that a diet rich in soy isoflavones administered for a year did not change breast density [37], rather it decreased it, unlike the administration of oestradiol [35]. Breast density and endometrial thickness had not changed significantly after prolonged treatment with genistein alone or combined with

daidzein at nutraceutical doses (i.e. ≤ 80 mg/day) in two placebo-controlled studies [38,39].

The safety and manageability of ESP is also guaranteed in menopausal women on stabilised treatment with thyroid hormones for hypothyroidism, in whom there is no interference with the absorption of these hormones. Thyroid hormone replacement therapy does not require any dose adjustment with the concomitant dose of ESP and levothyroxine [40].

Current Italian legislation requires the manufacturer to notify the new food supplement to the Ministry of Health, which approves its components, doses, indications and warnings given on the label and authorises its release onto the market. Routine checks by the regulatory authorities on the actual quality of the content of the food supplement are not envisaged: these are delegated to the manufacturer, which means that there are food supplements on the market that contain the same active ingredients, but with no standardisation of the composition. This is an important aspect especially in phytotherapy where the active ingredients come from plant extracts. In this way similar formulations do not guarantee that the clinical result can be reproduced consistently, as occurs for a medicinal product. For this reason, the data described here only refer to ESP and do not apply to similar products containing the same ingredients, because the manufacturing method and quality assurance make the difference. In our opinion, it is advisable to choose a food supplement which is manufactured in accordance with the same Good Manufacturing Practices established for medicinal products, which guarantee a standardised composition and purity of the extracts, and are accompanied by clinical studies to document the therapeutic activity and safety.

Conclusion

This observational study provides the first data on the safety and the clinical effect of ESP in menopause disorders. The synergy of soy isoflavones, extract of *Magnolia officinalis* bark and extract of *Vitex agnus-castus* L. fruits is targeted on significantly reducing vasomotor symptoms, anxiety, irritability and insomnia. Further studies are needed to confirm these preliminary outcomes.

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