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Adding Agnus Castus and Magnolia to Soy Isoflavones Relieves Sleep Disturbances besides Postmenopausal Vasomotor Symptoms. Long Term Safety and Effectiveness

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Abstract: Long-term safety of a nutraceutical combination based on agnus castus and magnolia extracts combined with soy isoflavones (SI) and lactobacilli, and effectiveness on vasomotor symptoms and sleep disorders in postmenopausal women, were assessed. A controlled study was carried-out in menopausal women comparing this nutraceutical combination (ESP group) with formulation containing isoflavones alone (C group), at the dosage recommended. Kuppermann index, PSQI, and SF-36 were determined at baseline, 3, 6 and 12 months. Endometrial thickness, mammary density and liver function were evaluated at baseline and after 12 months. 180 women (100 in ESP group and 80 in C group, mean age 55.5 years, in menopause for about 36 months) were enrolled in the study. At the treatment end, mammary density, endometrial thickness, and hepatic function did not show substantial differences between groups. Kuppermann index, and particularly hot flushes, were progressively and significantly decreased in frequency and severity during ESP versus C treatment. No adverse events were observed. Agnus castus and magnolia, combined with SI + lactobacilli, can effectively and safely be used in symptomatic postmenopausal women, mainly when quality of sleep is the most disturbing complaint. Endometrium, mammary glands and liver function, were unaffected after 12 months treatment.

Keywords: isoflavones; menopause management; endometrial thickness; mammary density; liver function; plant extracts

1. Introduction

The sleep disturbances negatively affect quality of life in postmenopausal women. In large series, sleep complaints were present in almost half percentage of menopausal women and frequently associated with hot flashes or mood disorders [1,2]. Hormone replacement therapy (HRT) is the first-line therapy in case of moderate/severe menopausal symptoms [3]. However, alternative treatments are needed in case of contraindications, adverse side effects and poor compliance [4]; moreover, many women simply refuse HRT for a variety of reasons concerning fear of cancer and weight gain [5] and request a "natural" approach [6].

Therapies based on soy isoflavones (SI) are the most popular approaches: these substances have been demonstrated to alleviate climacteric symptoms at the dose between 40 and 80 mg/day. Data on SI effects show that they exert elective stimulation of β-estrogen receptors (β-ERs) with less affinity and lower potency than estrogens [7], stimulate the synthesis of SHBG [8], and that β-ERs are poorly expressed on tissues with higher risk for estrogens-dependent carcinoma. Therefore, oncological safety could be expected in long term use [9] even if data are still lacking [10,11].

SI have been combined with different oligoelements to enhance the clinical effects. It is reasonable to add SI with lactic acid bacteria in the form of spores, resistant to the gastric and biliary

secretion, to promote the action of bacterial glycosidase and to assure the bioavailability of SI [12,13]. In fact, it is well known that the absorption of SI can be very different among patients because it depends on the presence of the intestinal flora that is able to produce glycosidases and therefore to hydrolyze genistin and daidzin to the active aglycons [14,15].

The data regarding the influence of soy isoflavones on the sleep disturbances are conflicting [16,17].

Magnolia has been shown to have tranquillizer and neurotrophic properties [18], but there are few reports in relation his activity on hot flashes, mood, and sleep symptoms [19].

Furthermore, it is shown that agnus-castus increases melatonin release and interact with opioid receptors and can play a role on vasomotor symptoms and sleep diseases [7,18,20,21].

On this basis we have analyzed the effectiveness and safety of a long-term therapy with agnus castus and magnolia combined with SI and lactobacillus on vasomotor symptoms, in particular on sleep disorders, in menopausal women.

2. Materials and Methods

In a prospective, observational, controlled study, 180 postmenopausal patients in non-hormonal treatment for menopausal vasomotor symptoms gave their informed consent to participate.

The study was approved by the Ethical committee and was carried out following the principles of Helsinki Declaration.

The inclusion criteria were: age between 45 and 65 years, FSH > 30mUI/ml, E2<20 pg/ml; Kupperman score > 20 and < 30, sleep disorders score >5, non-hormonal therapy initiated by less than 30 days.

Enrolled patients were divided in two groups in relation to treatment:

- group ESP (100 women) receiving one tablet/day containing soy isoflavones 60mg, Lactobacillus sporogenes 109 spores, Magnolia officinalis extract 50mg, Vitex agnus-castus extract 40mg and vitamin D3 5µg (ESP Group)
- group C (80 women): receiving one tablet/day containing only isoflavones 60 mg (C Group).

The exclusion criteria were: soy enriched diet, BMI> 30, breast or endometrial diseases, inability to understand the study finalities and to give the informed consent.

The treatment efficacy was evaluated at baseline and every three months by:

a) Kupperman score. The scored is the result of a self-compilation questionnaire that evaluates frequency and subjective intensity of 11 among most frequent vasomotor symptoms of postmenopausal women (hot flushes, insomnia, irritability, sweating, musculoskeletal pains, headache, palpitations, fatigue, paraesthesia). The symptoms were rated as: mild (score 15-20), moderate (20-35), severe (>35). Number and daily intensity of hot flushes recorded on a self-compilation diary (0 = absent; 1=mild; 2=moderate; 3= severe; 4= very severe).

b) The Pittsburg Sleep Quality Index (PSQI) test, for the evaluation of sleep disorders, is a 19 items questionnaire about the last month's symptomatology that the patient fills in. The questionnaire generate seven composite scores, on a scale from 0 to 3; so the PSQI global score is from 0 to 21. Higher scores reflect more severe sleep diseases (the cut-off for sleep disturbances is a score >5). The results give numbers in seven categories: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction (26).

c) Short Form (SF 36) test submitted at baseline and after one year to evaluate the psycho-physics wellness parameters. The SF-36 health survey consists of 36 questions evaluating functioning and well-being. Each of the questionnaire items refer to one of the following eight different health indicators: physical functioning; role-physical, referred to the limitations in performing important life roles due to physical health; bodily pain; general health; vitality; social functioning; limitations in performing important life roles due to emotional problems; mental health, referring to the absence of anxiety and depression. The test gives results in 8 composite

scores; and a total score on a scale from 0 to 100. Lower scores show more severe impairment of psychophysics wellness parameters.

The safety of treatment was assessed at baseline (T0) and after one year. It was based on endometrial thickness, evaluated by trans-vaginal ultrasonography, mammographic density, hepatic function assessed by transaminases, bilirubin, γ-GT.

The statistical analysis was carried out with t-test of student, for paired and not paired data, with the Mann-Whitney test for the non-parametric analysis, and the Wilcoxon matched-pairs test to compare between groups and inside groups; the Chi-square test to compare the frequencies. The statistical analysis was carried out with Statistical Package for Social Sciences (SPSS) 10.0 (Chicago, IL).

3. Results

The two groups resulted homogeneous regarding clinical features as age, BMI and years of amenorrhea (ESP group: 55.0 ± 6.4 years, BMI 24.9 ± 2.9, amenorrhea 3.2 ± 2.8 years; C group: 56.0 ± 5.8 years, BMI 25.7 ± 4.3, amenorrhea 2.6 ± 7.4 years).

After 12 months of treatment mammographic density, endometrial thickness and hepatic function did not show significant differences between the ESP and C groups (table 1).

Table 1. Safety profile

	Group ESP (n.100)		**Group C (n. 80)**	
	Baseline	12 months	Baseline	12 months
Mammographic density	1.80±0.7	1.86±0.8	1.75±0.7	1.79±0.6
Endometrial thickness	3.4±0.9	3.2±0.6	3.8±1.2	3.3±1.1
ALT	18.7±6.5	20.2±4.2	19.7±2.6	21.3±3.8
AST	21.4±5.3	20.3±6.2	19.2±4.6	19.8±3.4
γGT	24.3±15.6	22.6±19.3	20.4±17.3	24.8±18.2
Bilirubin	0.46±0.22	0.40±0.26	0.48±0.34	0.42±0.23

Concerning the clinical effectiveness, we observed in ESP Group a significant decrease in number of hot flushes and in intensity of the symptoms, considered in the Kuppermann index, in both groups (figure 1).

Figure 1. Changes in severity score of hot flushes and Kuppermann index during the treatment with soy isoflavones+lactobacilli+magnolia+agnus-castus (ESP) or isoflavones alone (C). Data are expressed as mean ± SE, ** $p<0.01$ vs baseline. On top of the bars the percentage difference vs baseline is shown.

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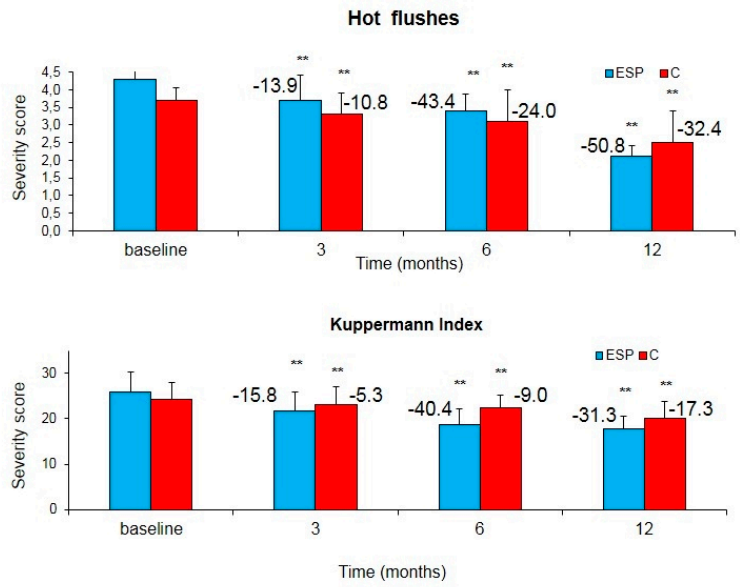
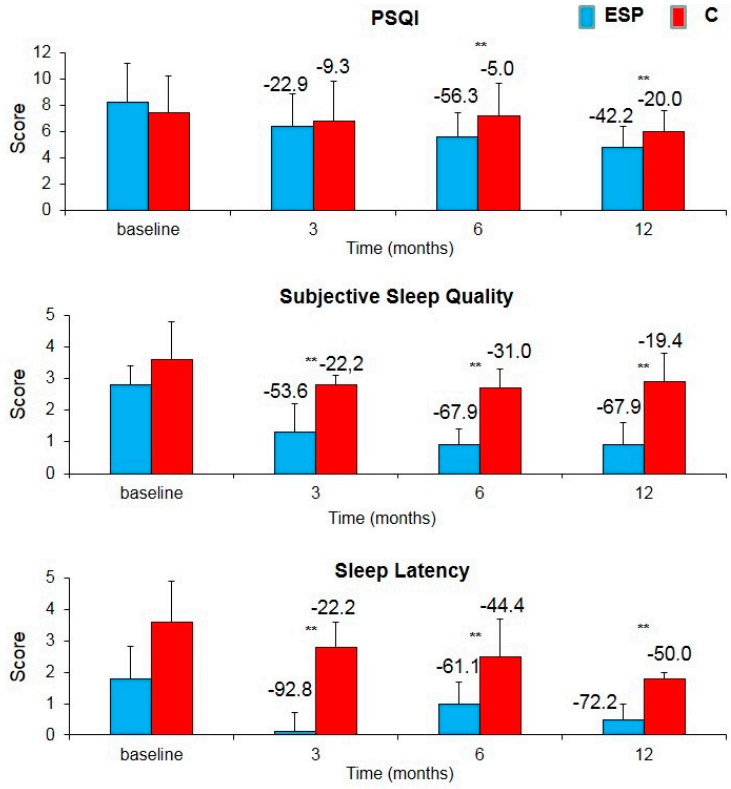


SF-36 significantly improved in ESP group from 38.0 ± 5.8 to 52.5 ± 6.3 (+38.2%), while it remained unchanged in C group from 56.0 ± 5.7 to 51.0 ± 7.3 (-8.9%) ($p < 0.01$).

A significant increase of sleep quality and psychophysics wellness parameters was observed, particularly a better subjective quality of sleep and an easier capability to fall asleep in ESP vs C group (figure 2).

Figure 2. Changes in severity score of PSQI, Subjective sleep quality and Sleep latency during the treatment with soy isoflavones+lactobacilli+magnolia+agnus-castus (ESP) or isoflavones alone (C). Data are expressed as mean ± SE, ** $p<0.01$ vs baseline. On top of the bars the percentage difference vs baseline is shown.



In fact, the high scores of PSQI parameters, reflecting more severe sleep conditions, were decreased in ESP group significantly more than C group.

4. Discussion

The postmenopausal decrease of circulating estrogens is associated with typical vasomotor symptoms among which hot flushes are well studied. Even if sleep disturbances are poorly investigated, they are very frequent and significantly impair quality of life: some sleep complaints during menopause have been found in one fourth to one half of all women as compared to approximately 15% of the general population [22], moreover they are usually associated with mood disorders, particularly depression [23].

It's well known that populations with a traditional soy rich diet have a lower incidence and a smaller intensity of vasomotor symptoms as compared to postmenopausal women who have not a similar diet [24]. Phytoestrogens consumption has been proposed as a mechanism for this difference and SI supplementation is used as an alternative to HRT for the treatment of climacteric symptoms in women with contraindications to HRT or warnings such as mammary cancer or advanced menopausal age [4,5]. However, the effect of SI is still far from being fully determined: while some studies suggest that hot flushes may benefit from SI administration [25], only few data assess the effect on sleep disturbances: in a pilot study sleep complaints as observed by polysomnographic analysis decreased from 90% to 37% in treated group versus 95% to 63% in the placebo group [26]. To increase the clinical effectiveness, SI have been combined with different substances such as vitamin and oligominerals.

The choice to evaluate the addition of Agnus Castus and Magnolia to the SI formulation + lactobacilli arose from the knowledge of their beneficial properties. Agnus-castus have been used in the treatment of many female conditions, including menstrual disorders (amenorrhoea, dysmenorrhoea), premenstrual dysphoric disorder (PMDD), corpus luteum insufficiency, hyperprolactinaemia, infertility, acne, disrupted lactation, cyclic breast pain, cyclical mastalgia and inflammatory conditions, diarrhea and flatulence [27-32]. However, the available data, regarding the use of agnus castus during menopause, are still lacking, despite early results seemed to encourage the use of agnus castus in reducing menopausal symptoms [34].

Furthermore, Magnolia showed beneficial anxiolytic effects in premenopausal women [35]. Extracts of Magnolia officinalis bark and its active constituent, honokiol, have been studied in various mouse models that have compared the activity to diazepam, without its common side effects [35,36]. Other recent study highlighted the efficacy of Magnolia extract and magnesium on psycho-affective and sleep disturbances in menopause, in addition to the effects of isoflavones on vasomotor symptoms [37].

In the present study, a combination of 60 mg SI with Lactobacillus sporogenes, agnus castus and magnolia was compared to other SI formulation. The presence of lactobacilli allows to guarantee intestinal absorption and bioavailability of the SI [12,14] but could theoretically enhance oestrogenic effects and the risk of hormone-dependent tumors [38]. Our results are reassuring as regards both effectiveness and safety, showing a progressive decrease of number and intensity of hot flushes and Kupperman index, but neither endometrial stimulation neither increase of breast density was observed. A significant part of the beneficial effect was observed during the first three-six months of therapy: such data is relevant for the patient's acceptance and guarantee of long-term compliance. The improvement of symptoms was confirmed after one year of therapy.

While vasomotor symptoms improved symmetrically in both groups, improvement of sleep global quality was more evident in the group ESP, as shown by higher score in the PSQI test compared to group C. Such improvement of sleep quality is related to the decrease of hot flushes occurring during the night, but also to the action of magnolia and agnus castus. As well known, magnolia and agno castus have neurotrophic and anxiolytic proprieties, explaining the beneficial effect on symptoms such as irritability, melancholy, headache, tiredness. A beneficial effect on these

symptoms is associated with a better quality of sleep, in particular subjective sleep satisfaction, lesser nocturnal awakening and lower nightmares frequency. The population on treatment show better subjective sleep quality and easier capability to fall asleep, reflecting a better quality of life as shown by the SF36 test results.

In conclusion, agnus castus and magnolia, in combination with SI as they are in ESP, can effectively and safely be used in symptomatic postmenopausal women, mainly when quality of sleep is the most disturbing complaint.

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