

ORIGINAL ARTICLE

The effect of red clover isoflavone supplementation over vasomotor and menopausal symptoms in postmenopausal women

Markus Lipovac¹, Peter Chedraui², Christine Gruenhut³, Anca Gocan³, Christine Kurz⁴, Benedikt Neuber¹ & Martin Imhof¹

¹Division of Obstetrics and Gynecology, General Teaching Hospital Korneuburg, Austria, ²Instituto para la Salud de la Mujer, Facultad de Ciencias Médicas, Universidad Católica de Santiago Guayaquil, Ecuador, ³Study Center Med XIX, Vienna, Austria, and ⁴Division of Obstetrics and Gynecology, Medical University, Vienna, Austria

Objective. To evaluate the effect of red clover isoflavone supplementation over vasomotor and overall menopausal symptoms in postmenopausal women. **Methods.** One hundred and nine postmenopausal women aged 40 or more were assigned to randomly receive either two daily capsules of the active compound (80 mg red clover isoflavones, Group A) or placebo of equal appearance (Group B) for a 90-day period. After a washout period of 7 days, medication was crossed over and taken for 90 days more. Daily hot flush and night sweat frequency and overall menopausal symptom intensity (Kupperman Index) were measured at baseline, 90, 97 and 187 days. **Results.** Daily hot flush/night sweat frequency and Kupperman Index values were similar in both studied groups at baseline. All indices significantly decreased after red clover phase in Group A, corresponding, respectively to a 73.5%, 72.2% and 75.4% average decrement. These decrements were significantly higher than those observed for Group B after placebo phase (8.2%, 0.9% and 6.7% respectively). In Group A, after washout and placebo phases all values significantly increased. In Group B, all indices remained similar after placebo and washout phases, however significantly dropping after red clover treatment. These values were also significantly lower than those observed in Group A after placebo phase. No side effects were encountered after treatment with the active compound or placebo. **Conclusion.** Red clover isoflavone supplementation was more effective than placebo in reducing daily vasomotor frequency and overall menopausal intensity in postmenopausal women.

Keywords: Hot flushes, red clover, isoflavones, menopausal symptoms, postmenopause

Introduction

Despite the fact that worldwide hormone therapy (HT) has proved to be effective in the alleviation of the climacteric syndrome and prevention of osteoporosis and other age related conditions [1], long term compliance is low and related to several factors, among them risk-benefit concerns [2]. Nearly a decade has passed since the Women's Health Initiative study (WHI) found that one HT regimen significantly increased the risk for cardiovascular events and breast cancer [3]. During this period physicians and patients have changed their attitude toward the use of hormonal compounds for the management of the menopause [4,5] with a

current trend toward treatment individualization [6] and the use of alternatives to estrogens [7,8].

Within the category of alternatives one can mention phytoestrogens which are plant derived molecules, basically represented by isoflavones. These exhibit estrogenic effects [9,10] and although being less potent than conventional estrogenic compounds, their selective beta-estrogenic receptor binding properties allow positive effects over various organs with a null effect over others [11]. Although soy isoflavones have been the most extensively studied, interest in those derived from red clover extracts (*Trifolium pratense*) are increasing among women and researchers. Such trend is currently supported by experimental [12–15] and clinical evidence [16–19].

Red clover supplementation has reported positive effects over menopausal symptoms [18], vaginal health [17] and lipids [19], with a promising safety profile [16]. Despite this, to date their effect over vasomotor symptoms remains controversial. The aim of the present analysis was to evaluate the effect of red clover isoflavone supplementation over vasomotor and overall menopausal symptoms in postmenopausal women.

Methods

Study design and participants

From May 2003 to November 2004 a prospective randomized, double-blind, placebo controlled trial was carried out at the Study Center Med XIX and the Department for Gynecological Endocrinology and Reproductive Medicine, General Hospital, Vienna, Austria. Primary aim was to evaluate the effects of red clover isoflavone supplementation over selected sex hormones, endometrium and depressive/anxiety symptoms in postmenopausal women. Results of this arm of the study have been previously reported [16,20]. This document presents data of the secondary objective of the initial study which was to assess vasomotor and general menopausal symptoms among participants before and after treatment.

Women were recruited as previously described [16] from the daily routine of the Menopause Ambulance of the General Hospital and The Menox Climacteric Institute, Vienna, Austria, in accordance to the following inclusion criteria: postmenopausal status (amenorrhea > 12 months), 40 years or older, moderate-severe menopausal symptoms (Kupperman index ≥ 15) with more than 5 hot flushes per day, a negative pregnancy

test, willingness to adhere to the control dates and take the prescribed preparations. Those on HT or with known isoflavone hypersensitivity were excluded. Women were informed about the research and its aims and written consent obtained. A baseline FSH >35 mIU/ml was confirmatory of postmenopausal status [16].

Participants were randomly assigned to receive either two capsules of the active compound (80 mg red clover isoflavones, Group A) or placebo of equal appearance (Group B) for a 90-day period. After a 7 day washout period, subjects switched to receive the opposite treatment for another 90 days. Number of daily hot flushes/night sweats and menopausal symptom intensity (Kupperman index) were measured at baseline, 90, 97 and 187 days. Additional examinations comprised anamnesis, medication anamnesis and height, weight and blood pressure determinations at proposed intervals. Blood pressure determinations were performed after women had been sitting for 15 min. Body mass index (BMI) was calculated as: weight (kg)/squared height (m [18]).

The study protocol was approved by the Ethikkommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt Wien—AKH.

Preparations

Red clover isoflavone capsules contained a standardized content of 40 mg aglyconic isoflavones in form of biochanin A, formononetin, genistein and daidzein. Red clover and placebo capsules were of identical design, packed in opaque containers (labeled as A or B) and blinded to investigators and participants until the end of the trial after which the code was broken.

Assessment of vasomotor and general menopausal symptoms

The Kupperman index was used to evaluate overall menopausal symptoms through the assessment of the severity of 11 menopausal symptoms (vasomotor included) occurring over the previous four weeks. Each symptom was rated according to the intensity from 0 to 3 (not present, slight, moderate and severe) in order to calculate the Kupperman index, the sum of all obtained scorings [21]. Women were requested to register the number of hot flushes and night sweats the day prior to therapy initiation and at the planned intervals.

Table I. Demographic data of studied women at baseline*

	All (n=109)	Group A (n=50)	Group B (n=59)
Age (years)	53.5 ± 7.1	54.5 ± 6.2	53.7 ± 7.8
BMI (kg/m ²)	24.7 ± 3.9	24.5 ± 3.9	24.9 ± 3.9
Hysterectomy (%)	17 (15.6)	9 (18.0)	8 (13.6)
Former HT use (%)	64 (58.7)	29 (58.0)	35 (59.3)

*Data are presented as mean ± standard deviations or percentages (n, %); BMI: body mass index; HT: hormone therapy; Group A: Red clover isoflavone; Group B: placebo.

Table II. Hot flush and night sweat frequency and Kupperman index values according to the initial assigned group

Parameters	Group A				Group B			
	Baseline	3 months Red clover	After washout	3 months Placebo	Baseline	3 months Placebo	After washout	3 months Red clover
Hot flush daily frequency	11.7 ± 4.8	3.1 ± 3.5*	7.8 ± 4.9	9.3 ± 5.2*	11.0 ± 5.1	10.1 ± 5.5 [†]	10.0 ± 5.7	3.3 ± 4.0* [†]
Mean % decrease		[73.5; 64.7 ~ 82.0]				[8.2; -2.3 ~ 19.4]		
Night sweat daily frequency	5.4 ± 2.5	1.5 ± 2.1*	3.7 ± 2.6	4.3 ± 2.6*	5.0 ± 2.8	5.0 ± 2.6 [†]	4.8 ± 2.6	1.7 ± 1.8* [†]
Mean % decrease		[72.2; 59.8 ~ 84.3]				[0.9; -11.4 ~ 13.8]		
Kupperman Index	32.5 ± 10.0	8.0 ± 6.9*	21.1 ± 12.2	26.5 ± 14.9*	34.3 ± 10.4	32.0 ± 13.2 [†]	31.9 ± 14.2	9.7 ± 9.4* [†]
Mean % decrease		[75.4; 68.2 ~ 82.7]				[6.7; -2.6 ~ 16.2]		

Data are presented as mean ± standard deviations; *p=0.0001 as compared to baseline using Wilcoxon rank test; †p=0.0001 as compared to placebo or red clover of the contrary group using the Mann–Whitney test; Values in square brackets are: mean; 95% confidence intervals.

Statistical analysis

Statistical analysis was performed on an intention-to-treat basis using SPSS software package (Version 10.0 for Windows, SPSS Inc., Chicago, IL). Data are presented as means, standard deviations, confidence intervals and percentages. The Kolmogorov Smirnov test was used to determine the normality of data distribution. Differences between groups were analyzed with the Mann–Whitney (continuous non parametric data) or the chi-square test (percentages). Changes after each treatment phase within groups were assessed using the Wilcoxon rank test. A p value < 0.05 was considered as statistically significant. Assuming that hot flush frequency would be reduced 50% in the red clover group (15% in the placebo group) a sample size of 49 individuals per group was calculated in order to achieve an 80% power at a two-sided alpha level of 0.05.

Results

During the study period a total of 113 women consented to participate. Fifty-three were randomized to group A and 60 to group B. Four women started HT and were excluded. Thus, data of 109 women who completed treatment (Group A: 50 and Group B: 59) was used for analysis. No significant differences were observed between study groups regarding basal characteristics (Table I).

Daily hot flush/night sweat frequency and Kupperman Index values were similar in both studied groups at baseline (Table II). All indices significantly decreased after red clover phase in Group A, corresponding respectively to a 73.5%, 72.2% and 75.4% average decrement. These decrements were significantly higher than those observed for Group B after placebo phase (8.2%, 0.9% and 6.7%, respectively). In Group A, after washout and placebo phases all values significantly increased (Figure 1a). In Group B, all indices remained similar after placebo and washout phases, however significantly dropping after red clover treatment (Figure 1b). These values were also significantly lower than those observed in Group A after placebo phase. No side effects were encountered after treatment with the active compound or the placebo group.

Discussion

Risk-benefit issues raised after the publication of the WHI results have changed physicians' and women's attitude toward HT use [4]. Indeed, nowadays women simply just do not want to take hormonal compounds. As a consequence, current trend is to individualize treatment and focus on alternatives for the menopause [4,5,22]. This tendency seems to be more pronounced among those with contraindications or with high risk situations.

Phytoestrogenic compounds are among the available alternative treatments for the menopause. These are plant derived molecules mainly represented by isoflavones with estrogenic like effects [9–11]. Despite the fact being less potent than conventional

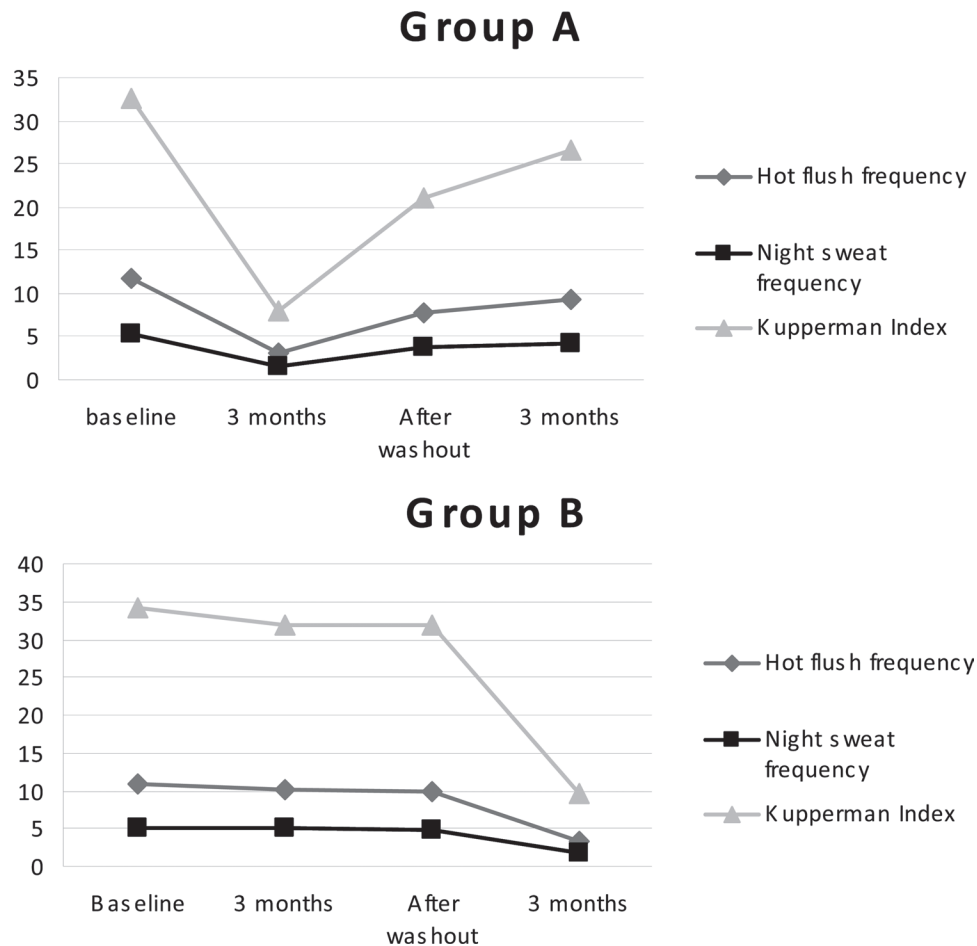


Figure 1. (a) After 3 months of red clover supplementation daily hot flush/night sweat frequency and Kupperman index values significantly decreased. A significant rise after the placebo phase was observed. (b) No significant decrease in daily hot flush/night sweat frequency and Kupperman index values after placebo phase. After the red clover phase all indices significantly decreased.

estrogenic compounds, their selective beta-estrogenic receptor binding properties allow beneficial effects on specific organs or systems [11]. In this sense, interest in red clover isoflavones (a type of phytoestrogen) has grown significantly in the past decade with reports evidencing positive effects over menopausal symptoms [18,23], vaginal health [17], serum lipids [19] and a promising safety profile [16]. Nevertheless, their effect over vasomotor symptoms (hot flushes and night sweats) remains to date a controversial issue [24].

Vasomotor symptoms are the most common menopausal symptom experienced by climacteric women and a leading reason to seek health care advice [25]. Indeed these symptoms are highly prevalent among mid-aged women and have a negative impact on their quality of life [26]. Moreover, recent reports seem to support the fact that vasomotor symptoms may significantly increase cardiovascular and osteoporosis risk [27,28]. Under this scenario, treatment is mostly warranted. Bearing this in mind we aimed at re-assessing data of the original Austrian red clover trial specifically in terms of hot flushes, night sweats and general menopausal symptoms. Re-analysis found that daily vasomotor frequency and menopausal symptom intensity (Kupperman Index) significantly decreased three months after red clover supplementation. This decrease was significantly higher than that observed after placebo use in Group B. Interestingly, in the red clover group all values significantly increased after washout and placebo phases. In group originally assigned to placebo, red clover treatment produced a significant decrease in all indices, to values which in fact were also significantly lower than those

observed after placebo in Group A. To mention is the fact that all baseline values were similar in both studied groups. In the red clover group, average decrement for all three indices was 74%. These findings are similar to those reported by Hidalgo et al. [18]; although daily hot flush/night sweat frequency was not taken into account (only presence and severity) and placebo effect was somewhat higher in the Ecuadorian series. Although both trials (Austrian and Ecuadorian) used 80 mg/day of red clover, positive effects over hot flushes, menopausal symptoms and sexuality have also been reported after four months using 40 mg/day in Brazilian women [29].

Superiority of red clover supplementation over placebo in the treatment of vasomotor and menopausal symptoms was clearly demonstrated in the present series. Despite this, as mentioned above, data addressing the effect of alternative therapies (red clover isoflavones included) over vasomotor symptoms are quite controversial, with some systematic reviews and meta-analysis indicating benefits [23,30] others not [31,32]. Discrepancies found in the literature using red clover, and in general with any isoflavone compound (soy included), seem to rely on several identified aspects which have been related to: the active compound (content, standardization, and bioavailability), methodology (sample size, type of study), and individual differences (dietary habits, absorption and metabolism) [18,24,33]. Pooling data for meta-analysis becomes difficult under these circumstances. Content and standardization of the active compound is important. Indeed, Reiter et al. [34] reported that only 5 out of 19 food supplements displayed the amount of content specified in

the label. Worthy to mention in this regard is that the red clover supplement used in the Austrian and Ecuadorian trials has reported a high level of standardization and isoflavone content [11]. Moreover it has been reported that products containing red clover extracts may contain more than 20 identified and 22 quantitatively measured compounds aside from the usually classically mentioned daidzein, genistein, formononetin and biochanin A [35]. Clinical effects of these additional compounds remain to be determined but could well explain some of the contradictory results found in the literature. Equol production among isoflavone consumers is another important aspect to be taken into account when it comes to results. S-equol is produced from the biotransformation of the soy isoflavone daidzein [36]. Future isoflavone research should include screening for equol production prior to treatment.

Finally the present series found that vasomotor symptom improvement correlated with a concomitant decrease in depressive and anxiety symptoms [20]. This is an important issue as depressive symptoms are highly prevalent in menopausal women and relate to severe menopausal symptoms [37]; and vice-versa women with severe vasomotor symptoms display more depression [38]. In this regard, red clover isoflavones may well be exerting a positive effect over mood through hot flush improvement. However, a direct or combined effect of the active compound over mood cannot be totally ruled out, for which more research is warranted. In any case, red clover treatment in women presenting both conditions seems promising.

In conclusion, red clover isoflavone supplementation was more effective than placebo in reducing daily vasomotor frequency and overall menopausal intensity in postmenopausal women. The identification of women that may clinically respond better to red clover isoflavones is warranted for future research.

Acknowledgement

Authors would like to thank women who participated in this study.

Declaration of interest

The authors declared no conflicts of interest.

References

- Bhavnani BR, Strickler RC. Menopausal hormone therapy. *J Obstet Gynaecol Can* 2005;27:137–162.
- Castelo-Branco C, Rostro F. Management of menopause. *Minerva Ginecol* 2006;58:137–152.
- Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, et al.; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288:321–333.
- Blümel JE, Castelo-Branco C, Chedraui PA, Binfa L, Dowlani B, Gómez MS, Sarrá S. Patients' and clinicians' attitudes after the Women's Health Initiative study. *Menopause* 2004;11:57–61.
- Ettinger B, Grady D, Tosteson AN, Pressman A, Macer JL. Effect of the Women's Health Initiative on women's decisions to discontinue postmenopausal hormone therapy. *Obstet Gynecol* 2003;102:1225–1232.
- Pérez-López FR, Chedraui P, Gilbert JJ, Pérez-Roncero G. Cardiovascular risk in menopausal women and prevalent related co-morbid conditions: facing the post-Women's Health Initiative era. *Fertil Steril* 2009;92:1171–1186.
- Lazar F Jr, Costa-Paiva L, Morais SS, Pedro AO, Pinto-Neto AM. The attitude of gynecologists in São Paulo, Brazil 3 years after the Women's Health Initiative study. *Maturitas* 2007;56:129–141.
- Nassar AH, Abd Essamad HM, Awwad JT, Khoury NG, Usta IM. Gynecologists' attitudes towards hormone therapy in the post "Women's Health Initiative" study era. *Maturitas* 2005;52:18–25.
- Miksicek RJ. Commonly occurring plant flavonoids have estrogenic activity. *Mol Pharmacol* 1993;44:37–43.
- Miksicek RJ. Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. *J Steroid Biochem Mol Biol* 1994;49:153–160.
- Dornstauder E, Jisa E, Unterrieder I, Krenn L, Kubelka W, Jungbauer A. Estrogenic activity of two standardized red clover extracts (Menoflavon) intended for large scale use in hormone replacement therapy. *J Steroid Biochem Mol Biol* 2001;78:67–75.
- Simoncini T, Fornari L, Mannella P, Caruso A, Garibaldi S, Baldacci C, Genazzani AR. Activation of nitric oxide synthesis in human endothelial cells by red clover extracts. *Menopause* 2005;12:69–77.
- Simoncini T, Garibaldi S, Fu XD, Pisaneschi S, Begliuomini S, Baldacci C, Lenzi E, et al. Effects of phytoestrogens derived from red clover on atherogenic adhesion molecules in human endothelial cells. *Menopause* 2008;15:542–550.
- Mueller M, Jungbauer A. Red clover extract: a putative source for simultaneous treatment of menopausal disorders and the metabolic syndrome. *Menopause* 2008;15:1120–1131.
- Adaikan PG, Srilatha B, Wheat AJ. Efficacy of red clover isoflavones in the menopausal rabbit model. *Fertil Steril* 2009;92:2008–2013.
- Imhof M, Gocan A, Reithmayr F, Lipovac M, Schimitzke C, Chedraui P, Huber J. Effects of a red clover extract (MF11RCE) on endometrium and sex hormones in postmenopausal women. *Maturitas* 2006;55:76–81.
- Chedraui P, Hidalgo L, San Miguel G, Morocho N, Ross S. Red clover extract (MF11RCE) supplementation and postmenopausal vaginal and sexual health. *Int J Gynaecol Obstet* 2006;95:296–297.
- Hidalgo LA, Chedraui PA, Morocho N, Ross S, San Miguel G. The effect of red clover isoflavones on menopausal symptoms, lipids and vaginal cytology in menopausal women: a randomized, double-blind, placebo-controlled study. *Gynecol Endocrinol* 2005;21:257–264.
- Chedraui P, San Miguel G, Hidalgo L, Morocho N, Ross S. Effect of Trifolium pratense-derived isoflavones on the lipid profile of postmenopausal women with increased body mass index. *Gynecol Endocrinol* 2008;24:620–624.
- Lipovac M, Chedraui P, Gruenhut C, Gocan A, Stammmer M, Imhof M. Improvement of postmenopausal depressive and anxiety symptoms after treatment with isoflavones derived from red clover extracts. *Maturitas* 2010;65:258–261.
- Kupperman HS, Wetchler BB, Blatt MH. Contemporary therapy of the menopausal syndrome. *J Am Med Assoc* 1959;171:1627–1637.
- Schonberg MA, Wee CC. Menopausal symptom management and prevention counseling after the Women's Health Initiative among women seen in an internal medicine practice. *J Womens Health (Larchmt)* 2005;14:507–514.
- Coon JT, Pittler MH, Ernst E. Trifolium pratense isoflavones in the treatment of menopausal hot flushes: a systematic review and meta-analysis. *Phytomedicine* 2007;14:153–159.
- de Cremoux P, This P, Leclercq G, Jacquot Y. Controversies concerning the use of phytoestrogens in menopause management: bioavailability and metabolism. *Maturitas* 2010;65:334–339.
- Nachtigall LE, Baber RJ, Barentsen R, Durand N, Panay N, Pitkin J, van de Weijer PH, Wysocki S. Complementary and hormonal therapy for vasomotor symptom relief: a conservative clinical approach. *J Obstet Gynaecol Can* 2006;28:279–289.
- Blümel JE, Chedraui P, Baron G, Belzares E, Bencosme A, Calle A, Danckers L, et al.; for the Collaborative Group for Research of the Climacteric in Latin America (REDLINC). A large multinational study of vasomotor symptom prevalence, duration, and impact on quality of life in middle-aged women. *Menopause* 2011;18:778–785.
- Gambacciani M, Pepe A. Vasomotor symptoms and cardiovascular risk. *Climacteric* 2009;12 Suppl 1:32–35.
- Pinkerton JV, Stovall DW. Is there an association between vasomotor symptoms and both low bone density and cardiovascular risk? *Menopause* 2009;16:219–223.
- Giorno CD, Fonseca AM, Bagnoli VR, Assis JS, Soares Jr JM, Baracat EC. [Effects of Trifolium pratense on the climacteric and sexual symptoms in postmenopause]. *Rev Assoc Med Bras* 2010;56:558–562.
- Jacobs A, Wegewitz U, Sommerfeld C, Grossklaus R, Lampen A. Efficacy of isoflavones in relieving vasomotor menopausal symptoms - A systematic review. *Mol Nutr Food Res* 2009;53:1084–1097.
- Kelley KW, Carroll DG. Evaluating the evidence for over-the-counter alternatives for relief of hot flashes in menopausal women. *J Am Pharm Assoc (2003)* 2010;50:e106–e115.

32. Lethaby AE, Brown J, Marjoribanks J, Kronenberg F, Roberts H, Eden J. Phytoestrogens for vasomotor menopausal symptoms. *Cochrane Database Syst Rev* 2007;CD001395.
33. Leclercq G, de Cremoux P, This P, Jacquot Y. Lack of sufficient information on the specificity and selectivity of commercial phytoestrogens preparations for therapeutic purposes. *Maturitas* 2011;68:56–64.
34. Reiter E, Beck V, Medjakovic S, Mueller M, Jungbauer A. Comparison of hormonal activity of isoflavone-containing supplements used to treat menopausal complaints. *Menopause* 2009;16:1049–1060.
35. Booth NL, Overk CR, Yao P, Burdette JE, Nikolic D, Chen SN, Bolton JL, et al. The chemical and biologic profile of a red clover (*Trifolium pratense* L.) phase II clinical extract. *J Altern Complement Med* 2006;12:133–139.
36. Jackson RL, Greiwe JS, Desai PB, Schwen RJ. Single-dose and steady-state pharmacokinetic studies of S-equol, a potent nonhormonal, estrogen receptor β -agonist being developed for the treatment of menopausal symptoms. *Menopause* 2011;18:185–193.
37. Chedraui P, Morales B, Hidalgo L. Depression and related risk factors among climacteric women *Climacteric* 2008;11:125.
38. Juang KD, Wang SJ, Lu SR, Lee SJ, Fuh JL. Hot flashes are associated with psychological symptoms of anxiety and depression in peri- and post- but not premenopausal women. *Maturitas* 2005;52:119–126.