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Improvement of postmenopausal depressive and anxiety symptoms after treatment with isoflavones derived from red clover extracts

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ABSTRACT

Objective: To evaluate the effect of isoflavones derived from red clover extracts (MF11RCE) over anxiety and depressive symptoms among postmenopausal women.
Methods: One hundred and nine postmenopausal women aged 40 or more were randomly assigned to receive two daily capsules of MF11RCE (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. Anxiety and depressive symptoms were measured at baseline, 90 and 187 days with the Hospital Anxiety and Depression Scale (HADS) and Zung's Self Rating Depression Scale (SDS).
Results: After receiving the MF11RCE compound the total HADS (anxiety and depression subscale scores also) and the total SDS scores decreased significantly. This effect was equivalent to a 76.9% reduction in the total HADS score (76% for anxiety and 78.3% for depression) and an 80.6% reduction in the total SDS score. After placebo, total HADS (anxiety and depression subscale also) and total SDS scores also decreased significantly in comparison to baseline but only equivalent to an average 21.7% decline.
Conclusion: Red clover derived isoflavones (MF11RCE) were effective in reducing depressive and anxiety symptoms among postmenopausal women.

1. Introduction

Depression, anxiety and mood disorders are very common and debilitating diseases with significant personal, social and economical consequences [1,2]. Women are at higher risk for depressive disorders when compared to men. Hence lifetime prevalence of depression in women is about 21% as compared to 13% in men [3,4]. Peri- and postmenopausal years are times subject to many stressors (i.e. family, social, work, health-related, economic and sexual), that seem to increase within this population the frequency of mood disorders [5–8]. Moreover, the climacteric syndrome (vasomotor symptoms) has been found to have a direct relationship over the prevalence of mood symptoms [9–11] and vice versa depressive symptoms may increase hot flashes [12]. The detection of emotional disorders among climacteric women is an important, although difficult task. As assessed with several quality of life (QoL) scales an important amount of climacteric women display mood and depressive symptoms [13–16]. Indeed, more than 70% of perimenopausal women present easy crying, irritability and symptoms of unhappiness [13], and more than 60% of postmenopausal ones suffer of anxiety, depression and loss of memory [16].

Although the benefits of hormone therapy (HT) for the alleviation of the climacteric syndrome and prevention of osteoporosis and other age related conditions are well known worldwide [17], long term compliance is low and related to several factors, among them risk-benefit concerns [18]. In this sense, the use of HT decreased furthermore after the unexpected findings of the Women's Health Initiative study (WHI) in which one HT regimen significantly increased the risk for cardiovascular events and breast cancer [19]. Due to this, physicians and patients have changed their attitude toward the use of HT for the management of the menopause [20,21], focusing their interest toward alternatives to estrogens [22,23]. Within this category one can mention phytoestrogens which are plant derived molecules, mainly represented by isoflavones, exhibiting estrogenic effects [24,25]. Although they are less potent than conventional estrogenic compounds, their selective β estrogenic receptor binding properties allow positive effects over various organs (bone, vagina, brain) with null effect over the

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uterus or breast [26]. Although isoflavones derived from soy have been the most extensively studied, interest in those obtained from red clover extracts (*Trifolium pratense*), a type of phytoestrogen, are increasing among women and researchers. Such tendency is currently supported by experimental [27–30] and clinical evidence [31–34].

Although the positive effects of red clover isoflavones have been observed over menopausal symptoms [33,35], vaginal [32] and cardiovascular health [34], with a promising safety profile [31], to date the effect of isoflavones (red clover or soy derived) over female climacteric mood disorders, remains to be determined. Hence, the aim of this study was to evaluate the effect of isoflavones derived from red clover extracts (MF11RCE) over anxiety and depressive symptoms among postmenopausal women.

2. Methods

2.1. Subjects

From May 2003 to November 2004, a prospective randomized, double-blind, placebo controlled trial was carried out by the Study Center Med XIX, and the Department for Gynecological Endocrinology and Reproductive Medicine, General Hospital, Vienna, Austria, primarily aimed to evaluate the effects of a non-prescription red clover extract (MF11RCE) on selected sex hormones and endometrium in postmenopausal women. Results of this arm of the study have been previously reported [31]. As a secondary objective of the initial study depressive and anxiety symptoms were also assessed among participants, results which are presented in this document.

As previously described [31] one-hundred and thirteen women were recruited from the daily routine of the Menopause Ambulance of the General Hospital and The Menox Climacteric Institute, Vienna, Austria. Inclusion criteria were: postmenopausal status (amenorrhea >12 months), 40 years or older, negative pregnancy test, willingness for adherence to the control dates, and to take the prescribed preparations, presenting with moderate to severe menopausal symptoms (Kupperman index ≥15). Women under HT or with a known isoflavone hypersensitivity were excluded. A written informed consent was obtained from all patients.

Participants were randomly assigned to one of two groups to receive two capsules of either MF11RCE (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. Anxiety and depressive symptoms were measured at baseline, 90 and 187 days with the Hospital Anxiety and Depression Scale (HADS) and Zung's Self Rating Depression Scale (SDS). Additional examinations comprised anamnesis, medication anamnesis, body weight and blood pressure determinations at same intervals. These examinations were performed before and after both treatment phases. Blood pressure determinations were performed after women had been sitting for 15 min. Body mass index (BMI) was calculated as [weight (kg)/square of height (m)] [33].

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

2.2. Preparations

Each MF11RCE (isoflavone red clover extracts) capsule had a standardized content of 40 mg aglyconic isoflavones in form of biochanin A, formononetin, genistein and daidzein. MF11RCE and placebo capsules of identical design were provided by Melbrosin International (Produktions und Vertriebs GmbH & Co KG, Vienna, Austria) 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.

Table 1
Demographic and anamnetic data.

	Group A ($n=50$)	Group B ($n=59$)	Overall ($n=109$)
Mean age (years)	54.5 ± 6.2[a]	53.7 ± 7.8	53.5 ± 7.1
Mean BMI	24.5 ± 3.9	24.9 ± 3.9	24.7 ± 3.9
Hysterectomy (%)	18.0	13.6	15.6
Former HRT (%)	58.0	59.3	58.7

[a] Mean ± standard deviation.

2.3. Assessment of depressive and anxiety symptoms

2.3.1. The Hospital Anxiety and Depression Scale (HADS)

The HADS is a questionnaire commonly used to assess levels of anxiety and depression. It is composed of 14 items presented as statements which the subject rates based on their experience over the past week and divided in two subscales: anxiety and depression (7 items each). Each item is rated on a four-point scale (0–3). Thus the sum of each graded item renders total HADS and subscale scores [36].

2.3.2. The Zung's Self Rating Depression Scale (SDS)

The SDS was designed to assess the level of depression in patients diagnosed with depressive disorders. It is a short self-administered survey to quantify the depressed status of a patient. There are 16 items on the scale that rate the four common characteristics of depression: the pervasive effect, the physiological equivalents, other disturbances, and psychomotor activities. There are ten positively worded and ten negatively worded questions. Each question is scored on a scale of 1–4 (a little of the time, some of the time, good part of the time, most of the time). The sum of all graded items renders a total SDS score [37].

2.4. Statistical analysis

Statistical analysis was performed on an intention-to-treat basis using SPSS (Version 11.0 for Windows, SPSS Inc., Chicago, IL, USA). Data is presented as means, standard deviations and percentages. Due to different absolute values at the baseline points of the two phases, comparison was performed with regard to the observed changes. Differences between groups for continuous and categorical data were compared with the non-paired Student's T and the chi-square tests respectively. Changes within each of the treatment phases were assessed using paired Student's T test. A p value <0.05 was considered statistically significant.

3. Results

A total of 113 women were enrolled in this study. Fifty-three were randomized to group A and 60 to group B. Four women started HT and were excluded. Thus, 109 women were accepted for evaluation, 50 assigned to group A and 59 to group B. Mean age of the participants was 53.5 ± 7.1 years (Group A 54.5 ± 6.2 years vs. Group B 53.7 ± 7.8 years). No significant differences were observed between study groups regarding basal characteristics (Table 1). No side effects were encountered after treatment with the active compound or the placebo group.

After receiving the MF11RCE compound the total HADS (anxiety and depression domain scores also) and the total SDS scores decreased significantly compared to baseline. This effect was equivalent to a 76.9% reduction in the total HADS score (76% for anxiety and 78.3% for depression) and an 80.6% reduction in the total SDS

Table 2
The HADS and SDS scorings after each treatment phase.

	Baseline	After placebo	After MF11RCE
The Hospital Anxiety and Depression Scale (HADS)			
Anxiety	9.98 ± 4.68[a]	8.05 ± 4.76*	2.40 ± 2.53*,†
Depression	6.91 ± 4.02	5.23 ± 3.65*	1.50 ± 2.06*,†
Total HADS	16.89 ± 8.45	13.28 ± 8.00*	3.91 ± 4.26*,†
The Zung's Self Rating Depression Scale (SDS)			
Total SDS	12.24 ± 7.39	9.57 ± 7.01*	2.37 ± 3.97*,†

[a] Mean ± standard deviation.
* $p < 0.001$ as compared to baseline.
† $p < 0.001$ as compared to placebo.

score (Table 2). After placebo, total HADS (anxiety and depression subscale also) and total SDS scores also decreased significantly in comparison to baseline but only equivalent to an average 21.7% decline.

4. Discussion

Epidemiological data indicate that women are at higher risk for mood disorders in comparison to men [3,4], with an increased prevalence among those peri- and postmenopausal [13,14,16]. This situation could be, at least in part, explained by increasing estrogenic deficiency [38] and to the intensity of menopausal symptoms. Indeed, the presence of hot flashes is a risk factor for psychological symptoms (anxiety and depression) in peri- and postmenopausal women [9,39]. Among middle aged women, depressive symptoms have been related to the severity of somatic menopausal symptoms [40]. Aches and stiff joints have also correlated to negative mood [41]. In any case, increased menopausal symptoms (anxiety and depressive included) impair female QoL [42,43].

Estrogens exert their positive effects within the brain through selective β estrogenic receptor binding [44] and interactions with the dopaminergic, serotonergic, and cholinergic systems, and brain regions crucial to higher cognitive function and mood [38]. This basis has long validated HT for the improvement of menopausal symptoms, depressive and anxiety ones included. Despite this, the findings of the WHI study have significantly decreased HT use worldwide [19], with an increased tendency among women and physicians for the use of alternatives [21]. Among these alternatives one can mention phytoestrogens, mainly represented by isoflavones, which basically display their positive effects over various organs (brain included) through selective β estrogenic receptor binding [26]. Interest among women and researchers is currently increasing in relation to isoflavones derived from red clover extracts (*T. pratense*), a type of phytoestrogen. This fact has been supported by experimental [27–30] and clinical evidence [31–34]. Despite this, up-to-date data supporting the effect of isoflavones over mood disorders among climacteric women is still lacking. What can be found in the literature is the positive effect of soy and red clover isoflavones over depressive and anxiety symptoms included in menopausal symptom questionnaires but not as assessed with specific anxiety and depressive inventories. For instance one can mention the positive effect over menopausal symptoms including sleeping disorders, anxiety and depression observed among 190 postmenopausal Spanish women receiving 17.5 mg soy isoflavones [45]. Therefore, to the best of our knowledge our data may be the first (at least for red clover isoflavones) to determine their positive clinical effect over postmenopausal depressive and anxiety symptoms as assessed with the HADS and SDS inventories. In the present series, the HADS and SDS scorings were significantly decreased (improvement) in comparison to baseline and to placebo. Despite this, important to mention is the fact that in this series hot flushes also significantly decreased (80%) in the actively treated arm as compared to null effect in the placebo group (Data not shown). As already mentioned depressive symptoms among middle aged women relates to the severity of somatic menopausal symptoms [40]; hence red clover isoflavones may be welling 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. More research is warranted in this regard.

Experimental data supporting our clinical evidence is also scarce. Despite this, the protective effects of red clover isoflavones against LPS-induced injury in dopaminergic neurons [46], through inhibition of microglia activation and pro-inflammatory factor generation, have recently been determined. Equally red clover isoflavones protected human cortical neurons against glutamate toxicity [47] and oxidative stress [48], which could have been the result of their antioxidant and estrogenic actions.

Experimental data regarding soy isoflavones seem to be confined to anxiety issues. Levels of BAD (a proapoptotic member of Bcl-2 protein family) were significantly decreased in the frontal cortex and the medial basal hypothalamus (MBH); but significantly increased in the amygdala in animals fed with a Phyto-600 diet. In these rats, levels of beta III tubulin were significantly increased in amygdala, frontal cortex, hippocampus and MBH compared to phyto-free values, providing evidence for the neuroprotective potential of soy isoflavones at these sites, implying that their consumption may be beneficial on learning and memory, anxiety-related behaviors, and recovery from trauma [49].

Equol, the main active product of daidzein metabolism, has the unique and important ability to specifically bind 5 alpha-dihydrotestosterone, and in turn inhibit the action of this potent androgen. In rodents, the specific influence of dietary soy phytoestrogens on consumptive, learning and memory, and anxiety-related behaviors has been identified [50]. Additionally in adult male rats, a phyto-rich diet produced anxiolytic effects as assessed in the elevated plus maze as compared to phyto-free fed ones [51]. These findings support the biological actions of phytoestrogens on brain and behavior [51]. In another study, phytoestrogens produced anxiolytic effects in both male and female Long–Evans rats. Additionally, phytoestrogens decreased body weight but increased consumption of food and/or water [52].

Finally although clinical data regarding phytoestrogens and mood disorders is still scarce, the present series determined that red clover derived isoflavones (MF11RCE) were effective in reducing depressive and anxiety symptoms among postmenopausal women. More clinical and experimental research in this regard is warranted.

Conflict of interest

The authors of the manuscript "Improvement of postmenopausal depressive and anxiety symptoms after treatment with isoflavones derived from red clover extracts" declare no conflict of interests.

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