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Effects of a red clover extract (MF11RCE) on endometrium and sex hormones in postmenopausal women

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Abstract

Objective: To evaluate the effects of a non-prescription red clover extract (MF11 RCE, Melbrosin International, Vienna, Austria) on selected sex hormones and endometrium in postmenopausal women.
Patients and methods: One-hundred and nine postmenopausal women with an age ≥40 years were randomly assigned to one of two groups either two capsules of MF11RCE (80 mg isoflavone) per day for a 90 day period, or placebo of equal design. After a 7 day washout period, medication was crossed-over for another 90 days.
Results: Combined evaluation demonstrated that supplementation with MF11RCE (verum), in contrast to placebo, significantly increased plasma testosterone levels and decreased endometrial thickness.
Conclusion: MF11RCE exerts a moderate effect on testosterone levels in postmenopausal women, while estradiol levels remained unchanged. The observed reduction of endometrial thickness provides further support for a safe role for isoflavones in terms of endometrial hyperplasia.

Keywords: Isoflavones; Phytoestrogens; Red clover; Menopause; Sex hormones; Endometrium

1. Introduction

As a result of increased life expectancy, women nowadays spend more than one third of their lives in a state of estrogen (E) deprivation, leading to a number of adverse experiences and long-term changes. Estrogen replacement therapy (ERT) by administration

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of 17β-estradiol (E_2), conjugated equine estrogens or 17α-ethinylestradiol has been shown to be effective in a number of these disorders and diseases. However, ERT has become controversial due to suspected risks of breast cancer, cardio-vascular diseases, and strokes [1]. Furthermore, 10% of western women have conditions that are contraindications to ERT, including a history of E-dependent malignancy, liver disease, thromboembolic disorders, and severe migraine [2].

Epidemiological data have shown that the severity and frequency of a number of hormone-related complaints and diseases like vasomotor menopausal symptoms [3], osteoporosis [4], or E-dependent tumors [5] is significantly lower in Asian women when compared to women from Europe and the USA. These differences are, at least in part, attributed to the traditional diets prevalent in the respective regions. Asian nutrition comprises a high proportion of plant food, particularly soy, which is a rich source of so-called phytoestrogens, a group of phenolic compounds present in a number of plants, which bind to E receptors alpha (ER-α) and beta (ER-β) and exhibit E-like activity. Phytoestrogens are considered a highly interesting alternative to ERT, which is particularly true for a subgroup of phytoestrogens, isoflavones. Isoflavones exhibit estrogenic activity by ER-activation and anti-estrogenic activity by competitive binding to ER and inhibiting E_2. Moreover, they have a significantly higher affinity to ER-β than to ER-α [6]. Due to the heterogenous distribution of the two ER subforms, isoflavone activity is stronger in tissues rich in ER-β, e.g. bone, capillary epithelium, skin, and central nervous system [7].

Modern Western feeding habits lead to low isoflavone uptake. Thus, an increasing number of preparations and food supplementation products to manage menopausal symptoms have been developed, many of which are based on soy [8,9].

More recently, red clover (*Trifolium pratense*) has gained increasing interest as a rich source of isoflavones. Similar to soy, red clover contains the isoflavones genistein, daidzein, biochanin A, and formononetin, though in a different distribution. In red clover, the predominant isoflavones are biochanin A and formononetin [10], the *O*-methylated precursors, which are demethylated in the gut to genistein and daidzein [11].

The present study examined the effects of a daily dose of 80 mg red clover-derived isoflavones (MF11RCE) on sex hormones, gonadotropins and endometrial thickness. The study was performed using MF11RCE red clover extract.

2. Materials and methods

2.1. Subjects

This prospective randomized, double-blind, placebo-controlled trial was carried out by the Study Center Med XIX, Vienna, Austria, and the Department for Gynecological Endocrinology and Reproductive Medicine, General Hospital, Vienna, Austria. The study protocol was approved by the Ethikkommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt Wien—AKH.

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, moderate to severe menopausal symptoms (Kupperman index ≥ 15). Women under constant ERT or with a known isoflavone hypersensitivity were not accepted. Written informed consent was obtained from all patients.

Participants were randomly assigned to one of two groups to receive two capsules of either MF11RCE (verum) or placebo for a 90 day period. After a 7 day washout period, subjects switched to receive the opposite treatment for another 90 days.

2.2. Preparations

Verum was MF11RCE red clover extract, with a standardized content of 40 mg aglyconic isoflavones per capsule in form of biochanin A, formononetin, genistein and daidzein. Verum and placebo capsules of identical design were provided by Melbrosin International (Vienna, Austria) in opaque containers, labeled as A or B and blinded to investigators and participants during the study period, after which the code was broken.

2.3. Examinations

Examinations comprised anamnesis, medication anamnesis, body weight, height, blood pressure and a transvaginal ultrasound. Fasting blood samples were taken for analysis. 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)].

Ultrasound examination was performed with a Toshiba SSA304A using a PVF-621VT Endovaginal Transducer (Toshiba Medical Systems, Wiener Neudorf, Austria). Serum parameters were assayed with an Immulite 2000 automatic immunoanalyzer using chemiluminiscent technique (Diagnostics Products Corporation, Los Angeles, CA, USA).

2.4. Outcome measures

Serum levels of testosterone (T), 17β-estradiol (E_2), follicle stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone binding globulin (SHBG); endometrial thickness.

2.5. Statistical analysis

Statistical analysis was performed on an intention-to-treat basis using SPSS 11.0 software (SPSS Inc., Chicago, IL). Due to different absolute values at the baseline points of the two phases, comparison was performed with regard to the observed changes. Differences between verum and placebo phases were assessed by Wilcoxon rank test. Changes within each of the treatment phases were assessed using paired *T*-test. A p-value <0.05 was considered statistically significant.

3. Results

Initially, 113 women were enrolled in this study. Fifty-three were randomized to group A and 60 to group B. Four women started ERT 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 (54.5 ± 6.2 years in group A, and 53.7 ± 7.8 years in group B). Body mass index (BMI) was virtually identical in both groups (Table 1).

The results of the baseline measures are shown in Table 2. None of the differences between the groups was significant.

Table 1
Demographic and anamnetic data

	Group A (n = 50)	Group B (n = 59)	Overall (n = 109)
Mean age (years)	54.5 ± 6.2	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

Table 2
Baseline measures

	Group A (n = 50)	Group B (n = 59)	Overall (n = 109)
E_2 (pg/ml)	36.30	38.31	37.39
T (ng/ml)	0.43	0.63	0.54
LH (mlU/ml)	30.64	29.92	30.25
FSH (mlU/ml)	60.31	60.45	60.38
SHBG (nmol/l)	61.44	57.69	59.41
	Group A (n = 41)	Group B (n = 51)	Overall (n = 92)
Endometrium (mm)	4.3	3.4	3.8

Data are presented as mean values.

Table 3
Changes with MF11RCE

	Before MF11RCE	After MF11RCE	Change (%)
T (ng/ml)	0.54 ± 0.26	0.66 ± 0.29	22.12
Endometrium (mm)	3.8 ± 1.9	3.2 ± 1.5	−14.69

Data are presented as mean ± S.D.

The evaluation of changes with verum and placebo versus baseline revealed a significant increase in T of about 22% (p < 0.001) and a significant decrease in endometrial thickness of 14.7% (p < 0.001) with verum, but not with placebo (Table 3). Surprisingly, a significant reduction of LH was found for placebo (p = 0.04), but not for verum. No effects were found for E_2, FSH, and SHBG (for details see Table 4).

Overall comparison of verum versus placebo by Wilcoxon rank test showed that serum T-levels were significantly increased with verum and endometrial thickness was significantly decreased with verum (p = 0.003 for T and 0.001 for endometrium), as compared to placebo. In the overall calculation, the decrease of FSH with placebo was not significant (p = 0.069).

Table 4
T-tests for mean changes vs. baseline

	MF11RCE		Placebo	
	Mean change	p-Value	Mean change	p-Value
E_2 (pg/ml)	1.85	0.781	−5.87	0.360
T (ng/ml)	0.12	0.000	−0.02	0.634
LH (mlU/ml)	−0.43	0.741	−2.45	0.040
FSH (mlU/ml)	6.54	0.781	1.76	0.342
SHBG (nmol/l)	−3.70	0.078	−3.58	0.107
Endometrium (mm)	−0.55	0.000	−0.18	0.145

Table 5
Wilcoxon-test MF11RCE vs. placebo

	N	Negative ranks	Positive ranks	*p*-Value
E_2	109	40	43	0.861
T	109	61	36	0.003
LH	109	64	44	0.861
FSH	109	62	47	0.069
SHBG	109	58	51	0.824
Endometrium	92	15	45	0.001

No differences were observed in terms of E_2, FSH, and SHBG. All rankings and *p*-values are provided in Table 5.

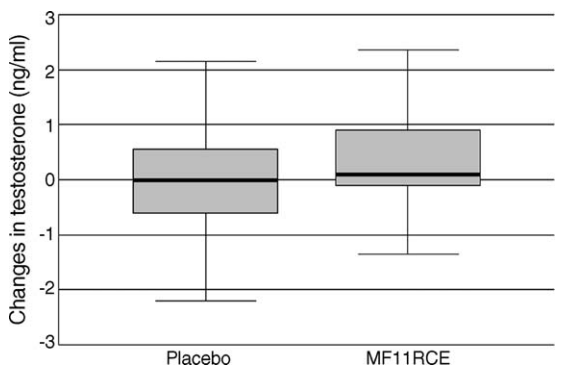

Fig. 1. Changes in T serum levels after 90 days of treatment with placebo and MF11RCE, respectively.

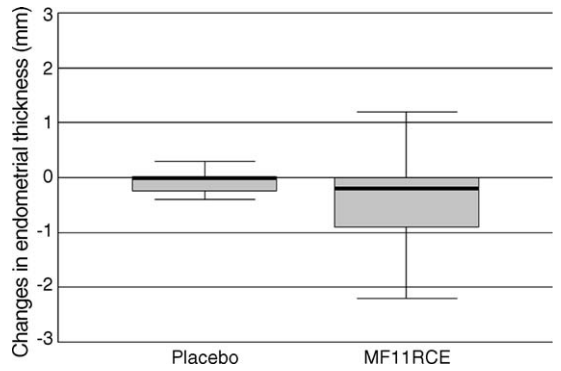

Fig. 2. Changes in endometrial thickness after 90 days of treatment with placebo and MF11RCE, respectively.

4. Discussion

The present double-blind, randomized, cross-over study demonstrated a significant effect of 80 mg per day red clover-derived isoflavones over T-levels and endometrial thickness (Figs. 1 and 2), while exerting no effect over E_2, FSH, and SHBG levels when compared to baseline or placebo. LH demonstrated an unexpected significant decrease on placebo, but not on verum.

The reduction of endometrial thickness is an interesting finding in the context of safety considerations, which is a major concern in the treatment of menopausal disorders, particularly in terms of suspected adverse effects of ERT. Isoflavones have been suggested as a promising alternative because they seem to avoid undesired estrogen-related effects.

In the last years, a number of studies have addressed endometrium in the context of isoflavone supplementation. Despite variances in duration, dose, and isoflavone source, all these studies coincide in the failure to demonstrate endometrial stimulation, as determined by transvaginal ultrasound [12–18], histologic examination of biopsy specimens [17,19,20], or immunohistochemical search for proliferation marker Ki-67 [17,21]. Soy isoflavones were tested versus placebo when given alone or in combination with conjugated equine estrogens (CEE) and lead to no isoflavone-related changes in endometrial thickness [22]. These results suggest safety in regard to the endometrium.

Only recently, the results of a 5-year randomized double-blind study with 150 mg isoflavones per day found that 6 of 154 patients developed endometrial hyperplasia, compared with none of 165 patients on placebo [23], leading the authors to the conclusion that long-term treatment with soy phytoestrogens was associated with an increased occurrence of endometrial hyperplasia. However, the dose was rather high and hyperplasia was first detected after 5 years.

Despite their estrogenic activity, isoflavones have not been associated with increased cancer risks [24]. An epidemiological meta-study has concluded that isoflavones do not promote, but rather reduce the risk of cancer in the breast [25], another E-sensitive tissue. A recent phase I pharmacokinetic study found no evidence of acute toxicity, significant side effects, or adverse events for a red clover extract at 40–120 mg/day on a short-time base [26], and a review of the recent literature concluded that isoflavones consumed orally and in doses below 2 mg/kg body weight per day should be considered safe for most population groups [27].

The finding of an increase in serum T is interesting in regards to common age- and menopause-related conditions. Circulating T and preandrogens fall with increasing age, and levels decrease continuously to about 50% before menopause compared to 20-year-old women. Thus, T depletion is not menopause-specific. However, it is increasingly recognized that the decline in androgens also plays a significant role in affecting perimenopausal and menopausal symptomatology and quality of life [28]. Testosterone exerts profound effects on mood and mental state, possibly through interactions with various neurotransmitter systems [29] and is associated with greater well-being and with reduced anxiety and depression [30]. Lower T levels are related to depression [31] which is a major symptom in peri- and postmenopause. The risk of depression triples in perimenopausal women [32].

However, the observed influence on T requires further confirmation, since it is in contrast to a recent 8 week dietary intervention study with soy that found no effect on sex hormones [33].

Although a number of studies of diverging isoflavone source (soy or red clover), dosage (20–160 mg per day), duration (from 10 weeks to 1 year) and design have addressed sex hormone levels in postmenopausal women after isoflavone intake, significant effects were reported only rarely.

In terms of E_2, gonadotropins and SHBG, our findings are basically in accordance with the negative results reported by others. In case of E_2, no significant changes have been found [12,33,34], even after 1 year of isoflavone-supplementation [35]. One study reported a trend toward lower E_2 after 2 mg/kg high-isoflavone diet [19]. FSH levels remained unaffected by isoflavones [12,15,34], even with 160 mg for 12 weeks [36] or after treatment for 1 year [4]. Only one study found a decrease in FSH, accompanied by an increase with placebo [37]. Concerning LH, reports were also negative [4,34,35]. Most studies have also failed to demonstrate an effect on SHBG [11,12,36,37]. However, in a randomized cross-over study with different isoflavone doses, the high-dose group showed a small significant increase [19] and another study suggested that phytoestrogens may increase SHBG in subjects with low SHBG concentrations [38].

The evaluation of isoflavone activity is challenged by a number of factors that play a role in metabolization and absorption, resulting in an individual variability in isoflavone excretion with up to a 1000-fold variation [39]. These factors comprise habitual foods, route of administration, chemical form of administered isoflavones, variability in composition of gut flora, intestinal transit time, redox potential of the colon, and genetic differences in metabolism [40].

A comparison of clinical studies is additionally complicated by the wide range of designs in terms of isoflavone source, form of ingested agent, isoflavone content and composition, dosage, and duration, just to name some. Application studies of non-prescription remedies face the fact that virtually no two remedies have the same composition and that producer statements on isoflavone content are often unreliable [41].

The isoflavone content of MF11RCE is highly standardized, due to a procedure specifically developed for the quantification of the *O*-methylated isoflavones biochanin A and formononetin, which are the predominant isoflavones in red clover [10]. The estrogenicity of MF11RCE has been evaluated by assay. Transactivational potency was equivalent to 78 μg E_2 per gram MF11RCE for ER-β, and 17 μg for ER-α [42]. The present study demonstrated that a daily dose of 80 mg red clover-derived isoflavones increased T and reduced endometrial thickness in postmenopausal women, while exerting no effect over E_2-levels. This study provides further evidence for a role of isoflavones in the treatment of menopausal disorders and for their safety in terms of endometrial hyperplasia and is reassuring in regard to contraindications to ERT. Future research will have to further elucidate the role of red clover-derived isoflavones and their metabolites.

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