Nonhormonal management of postmenopausal women: effects of a red clover based isoflavones supplementation on climacteric syndrome and cardiovascular risk serum profile

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Summary

Purpose of investigation: The aim of this prospective randomized study was to evaluate a red clover based isoflavones supplementation in the treatment of climacteric syndrome and its effects on cardiovascular risk serum profile. *Materials and Methods:* The study included 150 healthy postmenopausal women that were randomly assigned to receive phytoestrogens tablets, amounting in a total daily intake of 60.8 mg red clover isoflavones plus 19.2 mg soy isoflavones (n = 75), or placebo (n = 75). The authors evaluated teh following: daily number of hot flushes and Kupperman Index at baseline and after one and three months; serum total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen, and antithrombin III (ATIII) at baseline and after three and six months. *Results:* One hundred twenty-eight patients completed the study: 67 in the active group and 61 in the placebo group. The treatment led to a progressive significant reduction ($p < 0.05$) of the number of hot flushes in the active group compared to placebo already after one month, while Kupperman Index was statistically reduced after three months. No significant variation in total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, PT, PTT, fibrinogen, and ATIII were found. *Conclusion:* The present findings suggest that a red clover based isoflavones supplementation in healthy postmenopausal women is promptly effective on climacteric syndrome, improves neurovegetative symptoms, safe on cardiovascular risk serum profile, and does not modify lipids and coagulation.

Key words: Menopause; Phytoestrogens; Isoflavones; Red clover; Soy; Climacteric syndrome; Serum lipid; Clotting profile.

Introduction

Isoflavones are the main class of phytoestrogens, compounds naturally found in soy and in a wide variety of other plants, and provided with chemical and biological similarity to estradiol, with great affinity for estrogen receptors and both agonist and antagonist properties [1,2]. Also, red clover (Trifolium pratense), a wild plant by legume family, contains a high amount of the four most important isoflavones (genistein, daidzein, biochanin A, and formononetin) as opposed to soy which only contains the first two [3] and, moreover, their isoflavones are fast- and long-acting with high bioavailability and high receptorial affinity [4-6].

Isoflavones are mainly present as inactive glycosides (glycone isoflavones) and become active compounds (aglycone isoflavones) after removal of the sugar residue by gut bacterial beta-glycosidases [7], therefore, their association with milk ferments with glycosidase activity showed useful [8] and fructo-oligosaccharides showed to modify intestinal bioavailability of isoflavones [9]. Red clover extracts contains aglycone (active) isoflavones, as opposed to soy that contains isoflavones in glycoside (inactive) form requiring intestinal cleavage / activation [10].

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Phytoestrogens intake and supplementation, despite very reduced effect compared to endogenous estrogens (in the order of 1,000 times less than estradiol), seem to have a role in the support of postmenopausal women by improving climacteric syndrome [11]. In particular, isoflavones may reduce the incidence and severity of neurovegetative symptoms, specially hot flushes and whole vasomotor disorders [12-18]. Consumption of as little as 30 mg of soy isoflavones, in soy protein or as an extract, reduces vasomotor menopausal symptoms by 30% - 50% (10% - 20% including the placebo effect), even if nowadays 40 - 80 mg are suggested, with larger efficacy for severe symptomatology and fractionate daily dose [19]. In fact, other evidences suggest that isoflavones do not significantly relieve menopausal vasomotor symptoms any better than placebo [20].

Moreover, postmenopausal women are a population at increased risk for coronary artery disease due to the serum changes in lipoprotein metabolism that accompany the loss of endogenous estrogen secretion [21], including elevated total and low-density lipoprotein (LDL)-cholesterol and decreased high-density lipoprotein (HDL)-cholesterol [22]. Phytoestrogens / isoflavones effects on cardiovascular risk serum profile are currently unclear because of conflicting data concerning the effects on serum

Table 1 – *Effects of isoflavones 80 mg daily supplementation on climacteric syndrome (mean value ± SD)*

	$T_{0\ (baseline)}$	$T_{1\ (1\ month\ of\ treatment)}$	$T_{2\ (3\ months\ of\ treatment)}$
Hot flashes (per day)	9.2 ± 9.7	5.4 ± 7.0*	3.4 ± 5.9*
Kupperman Index	11.5 ± 7.2	8.8 ± 5.7	7.5 ± 5.6*

p<0.05 vs. baseline (*)

Table 2 – *Effects of isoflavones 80 mg daily supplementation on serum lipids (mean value ± SD)*

	$T_{0\ (baseline)}$	$T_{2\ (3\ months\ of\ treatment)}$	$T_{3\ (6\ months\ of\ treatment)}$
Total cholesterol	218.7 ± 40.6	211.6 ± 36.5	216.1 ± 33.9
LDL-cholesterol	139.7 ± 40.2	133.6 ± 37.1	132.6 ± 34.7
HDL-cholesterol	62.7 ± 17.5	65.9 ± 14.1	66.2 ± 16.2
Triglycerides	90.7 ± 39.9	96.2 ± 39.3	94.5 ± 37.1

p>0.05 vs. baseline at T2 and T3

lipids and clotting profile [23-26]: some studies showed a benefic effect lowering total and LDL-cholesterol and increasing HDL-cholesterol [27-29]; other studies should show a positive effect on blood pressure and coagulation pathways [30], even if not significant modifications on clotting profile are reported, contrary to what is demonstrated with estrogen replacement therapy [31].

The aim of this prospective randomized study in a healthy postmenopausal population was to evaluate a red clover based isoflavones oral supplementation in the treatment of climacteric syndrome, by improving the neurovegetative symptoms, and its safety on cardiovascular risk serum profile, and its impact on serum lipids and clotting profile.

Materials and Methods

One hundred fifty healthy postmenopausal women were enrolled in this prospective randomized study between May and September 2012. Subjects were randomly assigned to two groups: active group (n = 75), daily receiving oral isoflavones (80 mg); placebo group (n = 75), daily receiving oral calcium (500 mg) and vitamin D3 (400 IU).

Investigational patients received two tablets each containing: 8% red clover (Trifolium pratense) extract isoflavones (30.4 mg) plus 40% soy (Glycine soy) extract isoflavones (9.6 mg), amounting in a total daily intake of isoflavones of 80 mg. In particular, red clover isoflavones contained were: genistein 1.0%, daidzein 2.0%, biochanin A 0.7%, formononetin 8.5%, ononin 0.8%, and sissotrin 0.4%.

The patients were selected according to the following inclusion criteria: age ≥ 45 years; clinical (≥ 12 months amenorrhea) and hormonal (serum estradiol < 110 pmol/l, serum follicle stimulating hormone (FSH) > 30 IU/l) diagnosis of postmenopausal condition; climacteric syndrome with neurovegetative symptoms and vasomotor disorders (≥ 20 hot flushes per week). The exclusion criteria were: early menopause (< 45 years); body mass index (BMI) > 28 kg/m^2; use of hormone replacement therapy (HRT) less than six months before enrolment; dyslipidaemia and use of interfering drugs; coagulation pathways disorders, use of interfering drugs; high dietary soy intake.

The authors assessed neurovegetative symptoms by daily hot flushes frequency and Kupperman Index at baseline (T_0), and after one (T_1) and three months (T_2). The Kupperman Index covers 11 menopausal symptoms: hot flashes, paresthesia, insomnia, nervousness, melancholia, vertigo, weakness, arthralgia or myalgia, headache, palpitations, and formication (each symptom rated 0-3 and weighted 4 for hot flashes and 2 for paresthesias, insomnia, and nervousness) [32].

Serum lipids evaluated were total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides, at baseline and after three (T_2) and six months (T_3); clotting profile was defined by prothrombin time (PT) (sec, %, INR), partial thromboplastin time (PTT) (sec, ratio), fibrinogen, and antithrombin III (ATIII) at baseline and after three (T_2) and six months (T_3).

All values are presented as the mean ± standard deviation (SD). The statistical analysis was used by paired and non-paired Student *t* test. The level of statistical significance was set at $p < 0.05$.

Results

One hundred twenty-eight patients completed the one-year study: 67 in the active group and 61 in the placebo group (drop-out active group 10.7%, drop-out placebo group 18.7%: total drop-out 14.7%). Eight patients [two in active group (2.7%) and six in placebo group (8.0%)] dropped out of the study for persistent symptomatology, one patient in active group (1.3%) deferred therapy for gastric intolerance and 13 patients as a whole (8.7%) left follow-up.

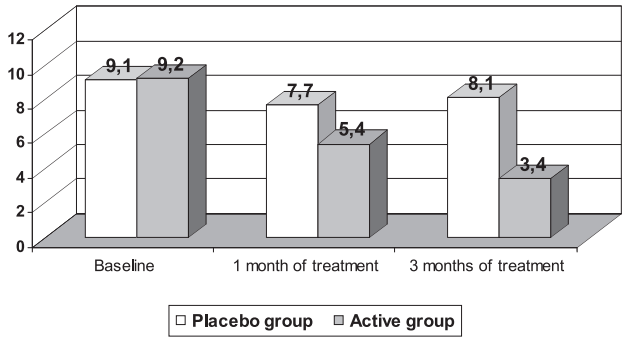
The women enrolled had mean age of 54.6 ± 5.0 years and BMI of 25.9 ± 1.7 kg/m^2; menopause mean age was 49.7 ± 4.5 years and mean duration of menopause 5.5 ± 5.4 years. The clinic baseline characteristics of the subjects of two groups did not significantly differ ($p > 0.05$).

The results of the study are detailed in Tables 1-3 and Figures 1 and 2. In substance, the isoflavones treatment led to a progressive significant reduction ($p < 0.05$) of the number of hot flushes already after one month (from 9.2 ± 9.7 to 5.4 ± 7.0), while Kupperman Index was statistically reduced after three months (from 11.5 ± 7.2 to 7.5 ± 5.6) (Table 1). An identical superiority trend is reg-

Table 3 – *Effects of isoflavones 80 mg daily supplementation on clotting profile (mean value ± SD).*

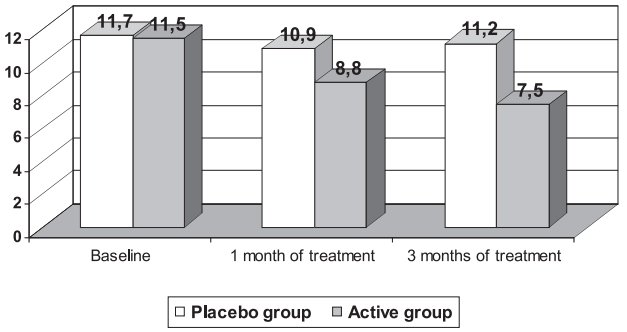
	$T_{0\ (baseline)}$	$T_{2\ (3\ months\ of\ treatment)}$	$T_{3\ (6\ months\ of\ treatment)}$
PT (sec)	11.5 ± 1.0	11.7 ± 1.1	11.5 ± 1.2
PT (%)	99.6 ± 11.4	97.8 ± 11.5	99.5 ± 13.3
PT (INR)	1.03 ± 0.09	1.03 ± 0.08	1.02 ± 0.09
PTT (sec)	24.2 ± 4.3	27.0 ± 5.2	25.9 ± 4.8
PTT (ratio)	0.87 ± 0.10	0.90 ± 0.10	0.89 ± 0.11
Fibrinogen (mg/dl)	317.3 ± 89.6	307.8 ± 71.8	296.9 ± 62.9
ATIII (mg/dl)	102.6 ± 18.7	101.9 ± 18.1	101.2 ± 19.5

p > 0.05 vs. baseline at T2 and T3



p < 0.05 vs. baseline and placebo at one and three months

Figure 1 – Effects of isoflavones 80 mg daily supplementation on climacteric syndrome: number (per day) of hot flashes (mean value)



p < 0.05 vs. baseline and placebo at three months

Figure 2 – Effects of isoflavones 80 mg daily supplementation on climacteric syndrome: Kupperman Index (mean value)

istered comparing these results to those of placebo group (Figures 1 and 2).

On the contrary, no significant variation in total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, PT, PTT, fibrinogen, and ATIII were found either at three (T_2) or six months (T_3) of treatment (Tables 2,3).

Discussion

Isoflavones (genistein, daidzein, glycitein, biochanin A, and formononetin), phytoestrogens naturally found in soy and a wide variety of other plants, have an ever more important and definite role in postmenopausal care for their chemical-structural similarity (phenolic ring and distance between hydroxyl groups) and biological-receptorial affinity with estradiol, greater for beta (ER-beta) than for alpha estrogens receptors (ER-alpha), overall with both agonist and antagonist properties [1,2]. Isoflavones, therefore, could be a valid choice in postmenopausal women with mild-moderate climacteric syndrome and/or contraindications or decline of classic HRT and, currently, numerous isoflavone preparations derived from soy or red clover are available as dietary supplement to treat menopausal disorders.

The North American Menopause Society (NAMS) recently performed an interesting review of randomized controlled trials (RCTs) on soy isoflavones treatment of postmenopausal vasomotor symptoms (14 RCTs; n=1422; dose range 40-160 mg/day) showing a significant improvement in the isoflavone arms compared to placebo in 11/14 trials (decrease in daily frequency of hot flashes 24% - 60%), with a dose of at least 50 - 60 mg/day for at least 12 weeks for a significant symptom improvement (but without linear dose/duration response relationship) [2]. Similarly, a meta-analysis favored soy isoflavones over placebo, even if the marked heterogeneity of the studies led to conclude that the efficacy on hot flushes could not be established with certainty [33]. On the contrary, some papers did not show comparable significant improvement on postmenopausal symptoms: a review of 17 soy isoflavone RCTs with conflicting results [34]; a meta-analysis barely favorable to isoflavones over placebo [35].

Red clover also, a wild plant by legume family whose flowers are usually dried for therapeutic use, is a rich and complete source of phytoestrogens since contains a high amount of the four most important isoflavones: genistein and daidzein plus biochanin A and formononetin (methylated precursors of genistein and daidzein), as opposed to soy which only contains the first two [3]. This high content of isoflavones, as well as the shorter time for maximal plasmatic concentration (T-max) and longer plasma half-live (T-1/2) than soy extracts [5], plus the higher transactivational potency for ER-beta than ER-alpha [4,6], suggest red clover as a source of "high-quality" isoflavones.

A systematic literature review (1951-2006) searching all RCTs of monosupplementation with red clover isoflavones accounted five trials suitable for meta-analysis; this analysis indicates a marginally significant reduction ($p = 0.05$) in hot flush frequency in the active treatment group (40 - 82 mg daily) compared with the placebo group [36]. On the contrary, a randomized double-blind clinical trial showed a reduction of vasomotor symptoms after 12-month intervention for red clover (57%) not significantly different from placebo (63%) [37], and a meta-analysis failed to show efficacy for red clover extracts over placebo [33].

Moreover, isoflavones impact on cardiovascular risk serum profile is still not fully clear because of conflicting data concerning the effects on serum lipids and clotting profile [23-26]. A meta-analysis of 38 studies concluded that consumption of 31 - 47 g soy protein/day could reduce plasma concentrations of both total cholesterol and LDL-cholesterol [38], while a review of 22 RCTs showed that soy reduced plasma concentrations of LDL-cholesterol by approximately 3% on average, with no significant effects on HDL-cholesterol, triglycerides, lipoprotein(a), or blood pressure [39].

This prospective randomized study showed that 80 mg isoflavones supplementation for the treatment of postmenopausal neurovegetative symptoms (60.8 mg of red clover isoflavones plus 19.2 mg of soy isoflavones) leads to a progressive significant reduction ($p < 0.05$) of daily hot flushes frequency (after only a month of treatment) and Kupperman Index (after three months) in the active group compared to baseline (63.0% and 34.8% after three months, respectively) and placebo. Moreover, this treatment not significantly affected total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, PT, PTT, fibrinogen and ATIII (also after six months of treatment). Therefore, the present findings suggest that the oral supplementation of healthy postmenopausal women with a red clover based isoflavones is promptly effective on climacteric syndrome relieving neurovegetative symptoms, and safe on cardiovascular risk serum profile not modifying serum lipids and clotting profile.

Larger trials with standardized clinical data (types/combinations of isoflavones, dose/duration of treatment) are needed to better define the therapeutic role of isoflavones on climacteric syndrome and their overall safety on postmenopausal women. There is an indication for studies considering "pure" genistein supplementation, investigating clinical differences between different isoflavones sources (soy, red clover, etc.), and estimating isoflavone aglycone values.

Finally, equol is a crucial isoflavone metabolite produced from daidzein by intestinal bacteria with a high affinity for ER-alpha [40], therefore, the clinical (added) value of equol production also requires further study, also comparing women whose intestinal bacteria able to convert daidzein to equol (equol producers) with those without that ability (equol non-producers) [2].

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