Original Article

EFFECTS OF TRIFOLIUM PRATENSE ON CLIMACTERIC AND SEXUAL SYMPTOMS IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Objective. To evaluate the effects of treatment with Trifolium pratense on climacteric symptoms and sexual satisfaction in postmenopausal women.

Methods. This is a prospective, randomized, double-blind, placebo-controlled study. Initially, 120 women aged 45-65 years with menopausal symptoms, more than 12-month amenorrhea and no treatment in the past six months were selected. The participants were then divided into two groups: TG – receiving 40 mg Trifolium pratense, 1 capsule/day; PG – receiving placebo capsules containing lactose (control), 1 capsule/day. The duration of treatment was 12 months. The patients underwent clinical and laboratory evaluation before treatment and at four, eight and 12 months of treatment. The Kupperman Menopausal Index and the Golombok Rust Inventory of Sexual Satisfaction (GRISS) were used. At the end of the study, each group comprised 50 patients.

Results. According to the Kupperman Menopausal Index, there was significant improvement in menopausal symptoms after four months of treatment, especially in relation to hot flashes, when compared to baseline data in both groups. However, no significant differences were observed between groups. There was no improvement in sexual satisfaction after treatment.

Conclusion. A 12-month treatment with a daily dose of 40 mg Trifolium pratense did not yield a significant improvement in menopausal symptoms and sexual satisfaction.

Key words: Menopause. Sexuality. Trifolium pratense. Hot flashes.

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Introduction

Climacteric corresponds to the period of life during which women gradually loose their reproductive capacity as a result of aging. This period, according to the World Health Organization, commonly occurs between the ages of 40 and 65. It is closely related to loss of activity of ovarian follicles, with consequent estrogen deficiency[1,2].

Approximately 70% of women report some type of symptom during the climacteric period. In general, these symptoms are attributed to estrogen deprivation. The most common complaints are vasomotor symptoms and night sweats. In addition, vaginal dryness, dyspareunia and urinary urgency, which are associated with urogenital atrophy, may interfere with the sex life and quality of life of postmenopausal women[2,3]. Some women also experience cognitive impairment, insomnia, depression, irritability, fatigue, psychological symptoms, and increased risk for osteoporosis and cardiovascular disease[3,4].

To minimize the impact of ovarian failure on women's health, hormone therapy is often recommended. However, the results of several studies, including the Women's Health Initiative (WHI)[5] and the Million Women Study[6], have raised serious concerns regarding the use and safety of hormone replacement therapy (HRT) in relation to cardiovascular events and breast cancer in long treatment[7]. These studies have significantly influenced the decline in the prescription of HRT in the United States, which dropped from 91 million users in 2001 to 57 million in 2003[8]. For this reason, many researchers have sought alternative therapies, such as the use of phytoestrogens to relieve menopausal symptoms[9,10].

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Phytohormones comprise several compounds that can be extracted from plants and, when purified, can enhance their activity in the body as well as improve their bioavailability[9]. Phytoestrogens are substances with chemical structure and function similar to that of estrogens and have been shown to bind to estrogen receptors due to the presence of a phenolic ring[9,10,11].

Phytoestrogens are classified into four main groups: sterols, terpenoids, saponins, and phenols. The last group consists of isoflavones, lignans, coumestans, flavanols, flavones, and chalcones[11,12].Phytoestrogens are classified into four main groups: sterols, terpenoids, saponins, and phenols. The last group consists of isoflavones, lignans, coumestans, flavanols, flavones, and chalcones[13]. Among isoflavones, the main biologically active compounds are genistein, daidzein, biochanin A, and formononetin[11,12].

A source of isoflavones is red clover (Trifolium pratense)[14,15], which has been used as a therapeutic agent in various diseases and also for menopausal symptoms[16]. In addition to isoflavones, this drug contains cyanogenic glycosides and coumarin derivatives which may reduce blood clotting[16,17]. Trifolium pratense has been used mainly in the treatment of vasomotor symptoms, but data in the literature on its actual effects are still scarce.

In a systematic review and meta-analysis including 17 clinical trials involving Trifolium pratense, only five studies were randomized and placebo-controlled. The authors reported that Trifolium pratense would be effective for treating hot flashes, over a six-month to one-year period, in a dose ranging from 40 to 80 mg/day[18]. The other studies were excluded due to methodological problems, incomplete results or because they did not use a control group[19, 20, 21, 22, 23].

In relation to sexual dysfunction, the effect of estrogen is well defined, especially concerning improvement in atrophy of the female genital tract[24]. Chedraui et al., in a randomized, double-blind, placebo-controlled study, observed an improvement in the vaginal epithelium with 80 mg/day of Trifolium pratense compared to placebo. In addition, participants reported reduced dyspareunia and vaginal dryness and increased libido[25].

The objective of this study was to evaluate the effects of treatment with Trifolium pratense on climacteric and sexual symptoms in postmenopausal women.

Methods

The study was conducted at the Division of Endocrine Gynecology and Climacteric, Discipline of Gynecology, Department of Obstetrics and Gynecology, School of Medicine of Universidade de São Paulo (FMUSP), Brazil. The treatment consisted of a daily dose of 40 mg Trifolium pratense, which is considered the minimum effective dose to control vasomotor symptoms in postmenopausal women[18].

Of the 500 women recruited in our climacteric outpatient clinic, 120 participants aged 45 to 65 years with menopausal symptoms were selected. All participants were informed about the study protocol before agreeing to participate.

During an interview, the participants were informed about the procedures, risks and benefits involved in the study and provided written informed consent.

This study was approved by the Research Ethics Committee of FMUSP Hospital das Clínicas (no. 153/4), Brazil. Inclusion criteria were: more than 12-month amenorrhea; menopausal symptoms; follicle-stimulating hormone (FSH) > 30 mIU/mL; estradiol < 30 pg/mL.

Exclusion criteria were: diabetes mellitus; cardiovascular disease; hypersensitivity to drugs used in the study; estrogen-dependent cancer; liver failure; nephropathy; systemic lupus erythematosus; porphyria; altered cervicovaginal cytology; osteoporosis (standard deviation > -2.5); endometrial thickness > 6 mm; uterine volume > 200 cm^3; BI-RADS category 3, 4 or 5 mammograms; hormone treatment with sex steroids or phytoestrogens in the past six months.

This is a prospective, randomized, double-blind, placebo-controlled study. The duration of treatment was 12 months. Four visits were performed: before treatment (randomization) and at four, eight and 12 months of treatment. The study was conducted from December 2005 to December 2008.

Blinding of drugs

The bottles were identified as four-digit numbers separated by a slash, as follows: 01/01, where the first number indicates the patient and the second indicates the month. Example: patient 01 should always receive samples identified with these numbers: 01/01; 01/02; 01/12.

Randomization

Women included in the study (n = 120) were randomized into two groups using a computer program. Sample blinding codes were disclosed after completion of treatment for all patients.

End of study

Of the 120 participants, 20 failed to complete the study due to personal reasons (n = 12) or because they did not take more than 80% of the drug (n = 8). Thus, the final sample was composed of 100 women, who were equally divided into the following groups: Trifolium group (TG) – receiving 40 mg Trifolium pratense, 1 capsule/day (n = 50); placebo group (PG) – receiving placebo capsules containing lactose, 1 capsule/day (n = 50).

Kupperman menopausal index

This index measures the sum of symptoms reported by women during the climacteric period, which is classified as mild, moderate and severe according to each item evaluated. Symptoms are classified as mild when the cumulative score is up to 19, moderate from 20 to 35, and severe above 35. Vasomotor symptoms followed

the patterns described by Kuppermann[26]. This index was assessed at each visit, i.e., before treatment and at four, eight and 12 months of treatment.

Hormone measurements

During selection, 12-hour fasting blood samples were withdrawn by venipuncture. Levels of FSH and estradiol were also assessed before patients were included in the study.

Golombok rust inventory of sexual satisfaction – female version (griss)

Subjective evaluation of the sexuality of sexually active women was performed using the GRISS[27], a reproducible questionnaire already validated in Brazil[28].

This inventory consists of a questionnaire with 28 questions that evaluate how women relate to their own body and to their partner's, the level of interest in sexual activity, quality of communication with the partner, sexual frequency, and the ability to achieve orgasm.

The answers vary according to intensity, from none to the highest intensity observed or experienced by women in relation to the questions under each topic of the assessment instrument[28].

For data analysis, among the five response options, "always" and "usually" were considered affirmative; "never", "almost never" and "occasionally" were considered negative answers.

Statistical analysis

The initial sample size calculation was based on hot flashes (beta = 10%, power = 80%) for 38 patients per group (76). Bartlett's test was used to assess sample homogeneity. Student's t test was used to compare TG and PG results at different time points. To assess the influence of time (before and after four, eight and 12 months), two-way ANOVA was applied to each group. Significance level was set at 5% ($p < 0.05$).

Results

After analyzing the clinical data, no significant differences were observed between TG and PG in the following parameters: age at menopause, time since menopause, initial weight, and body mass index (BMI) before treatment (Table 1).

Overall results of the Kupperman Menopausal Index were similar between groups at the following time points: before treatment (TG = 25.34±10.17 and PG = 25.12±9.02, p = 0.65); four months (TG = 16.96±9.16 and PG = 17.22±8.34, p =0.45); eight months (TG = 13.16±9.55 and PG = 13.3±7.06, p = 0.39); 12 months (TG = 11.12±8.68 and PG = 12.01±9.01, p = 0.87). Throughout treatment, symptoms improved after four, eight and 12 months of treatment ($p < 0.01$) in both groups according to two-way ANOVA.

Table 2 describes the status of vasomotor symptoms during treatment, revealing similar behavior in both groups.

Table 3 provides the mean and standard deviation of GRISS scores in both study groups. There were no significant differences before and after treatment in both groups.

No side effects were observed during treatment.

Table 1 - Clinical characteristics of patients per study group

	TG (n = 50)	PG (n = 50)	p
Age	55.78 ± 4.93	55.14 ± 4.97	0.51
Age at menopause	48.74 ± 3.79	48.90 ± 4.49	0.84
Time since menopause	6.96 ± 4.93	5.86 ± 4.60	0.25
Initial weight	72.45 ± 14.38	68.54 ± 12.71	0.59
Initial BMI	29.29 ± 6.32	28.38 ± 4.44	0.41

BMI = body mass index; TG = Trifolium pratense group; PG = placebo group. Student's t test was used for statistical analysis.

Table 2 – Status of vasomotor symptoms during treatment per study group

Vasomotor symptoms	TG (n = 50)	PG (n = 50)	p***
Baseline	8.72 ± 3.68*	8.10 ± 3.88**	0.56
4 months	5.76 ± 3.89	4.52 ± 3.63	0.67
8 months	3.96 ± 3.69	3.08 ± 3.31	0.72
12 months	3.44 ± 3.68	3.01 ± 3.96	0.54

TG = Trifolium pratense group. PG = placebo group
*p<0.001 compared with time points 4, 8 and 12 months in TG.
**p<0.001 compared with time points 4, 8 and 12 months in PG. Two-way ANOVA was applied to previous analyses in this table.
***Student's t test was used to compare TG and PG results at the same evaluation time point.

Table 3 – Scores for the Golombok Rust Inventory of Sexual Satisfaction (GRISS) during treatment per study group

GRISS	TG (n = 50)	PG (n = 50)	p
Baseline	38.64 ± 16.82	39.44 ± 16.24	0.45
4 months	38.32 ± 17.59	39.64 ± 16.81	0.49
8 months	36.94 ± 17.51	37.40 ± 17.61	0.53
12 months	34.54 ± 18.18	34.78 ± 17.94	0.78

TG = Trifolium pratense group; PG = placebo group.
Two-way ANOVA and Student's t test were used to compare the results.

Discussion

It is estimated that approximately 75% of postmenopausal women have some symptoms related to estrogen deficiency, and an average of 40% will seek medical attention to relieve menopausal symptoms, especially hot flashes, night sweats, vaginal dryness, and sleep

disturbances[3, 6]. Such symptoms can negatively affect daily activities and the quality of life of these women[11]. For this reason, therapy to ameliorate these symptoms is an important factor in this phase.

Traditionally, HRT is the main form of treatment for symptoms associated with postmenopausal hypoestrogenism. However, some studies report negative effects of this therapy, including the Heart and Estrogen-progestin Replacement Study (HERS II)[29], the Women's Health Initiative (WHI)[5], and the Million Women Study[6]. In those studies, a greater risk of cardiovascular disease and breast cancer was observed with the traditional estrogen-progestin replacement therapy. For this reason, several authors have sought alternatives to reduce menopausal symptoms, especially vasomotor symptoms[16, 17, 18, 22].

In recent years, there has been a growing interest in the use of phytoestrogens for the relief of vasomotor symptoms in postmenopausal women[7,8]. Therefore, we chose to study Trifolium pratense in this population. However, our study revealed no significant differences in the effects of Trifolium pratense on menopausal symptoms, as assessed by the Kupperman Menopausal Index, and on sexual satisfaction, as assessed by the GRISS, compared to patients receiving placebo after one year of treatment. At first glance, these findings seem to conflict with data from the literature. We point out that this is a randomized, double-blind, placebo-controlled study, which is different from most publications on the subject[16-22].

Lethaby et al.[15], in a meta-analysis to assess vasomotor symptoms with Trifolium pratense, observed that more than 70% of studies could not be included due to methodological issues. The minimum effective dose was 40 mg daily (the same used in our study). However, other studies reported improvement with 80 mg per day. Therefore, the use of the minimum dose may be a possible explanation for the failure to identify greater effects of the drug over placebo in our study[15]. Nevertheless, it is worth noting that, during the preparation of this study protocol, other studies showed that the daily dose of 40 mg would be sufficient to control hot flashes[29-30].

We also highlight that treatment expectations, either positive or negative, are modulating factors that influence perception, behavior and even the biological process[6]. The findings justify a placebo effect in reducing symptoms during the study. This fact might explain the improvement in menopausal symptoms observed in both groups during the study. Furthermore, our group expected that any improvement in vasomotor symptoms would have an impact on sexuality, and for this reason we applied the GRISS[28]. This questionnaire has been validated in Brazil[28] and other countries to assess the presence and severity of sexual problems. Some authors have demonstrated its reproducibility in the assessment of anorgasmia, vaginismus, and decreased sexual satisfaction[28]. For these reasons, this instrument was used to assess participants in the present study. However, no significant differences were observed in the improvement in questionnaire scores compared to placebo.

Conclusion

A 12-month treatment with a daily dose of 40 mg Trifolium pratense did not yield a significant improvement in menopausal symptoms and sexual satisfaction.

Conflict of interest: No conflicts of interest declared concerning the publication of this article

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Artigo recebido: 10/03/10
Aceito para publicação: 07/06/10