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Original Article

Effects of standardized phytoestrogen on Taiwanese menopausal women

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Abstract

Objective: To investigate the effects of standardized soy extract on climacteric symptoms, lipid profiles, bone markers, and serum isoflavone concentration in healthy Taiwanese postmenopausal women.
Materials and Methods: A multicenter, open-labeled, randomized, prospective, comparative study design was used. A total of 130 outpatients who had undergone natural menopause were randomly administered either 70 mg or 35 mg soy extract daily for 24 weeks.
Results: The evidence suggests that the soy extract treatment that was administered to both groups for 1 month could help reduce climacteric scores (reductions of 19.66% [$p < 0.01$] and 18.85% [$p < 0.01$] in the 35 mg and 70 mg groups compared with baseline, respectively), and the efficacy was more potent after 6 months of treatment. Soy isoflavone significantly reduced the total cholesterol (reductions of 4.50% [$p < 0.01$] and 3.06% [$p < 0.05$] in the 35 mg and 70 mg groups, respectively) and low density lipoprotein cholesterol levels (reductions of 4.67% [$p < 0.05$] and 5.09% [$p < 0.05$] in the 35 mg and 70 mg groups, respectively) in patients with total cholesterol > 200 mg/dL after 6 months of treatment. In patients with high bone turnover (urinary deoxypyridinoline/creatinine > 7.4 nM/mM), soy extract treatment reduced the deoxypyridinoline /creatinine level by 10.53% ($p < 0.05$) and 11.58% ($p < 0.05$) in the 35 mg and 70 mg groups, respectively. Serum levels of isoflavone increased in both groups after 6 months of treatment.
Conclusion: Soy extract is highly efficacious at relieving menopausal symptoms and demonstrates a positive effect on the cardiovascular system and skeleton.

Keywords: bone markers; climacteric symptoms; lipid profiles; menopause; phytoestrogen

Introduction

During menopause, the decline in ovarian hormones (particularly estrogen) may result in unpleasant effects, such as hot flushes and vaginal atrophy, that adversely affect quality of life and may increase the risk of osteoporosis and coronary heart disease [1]. Hormone therapy (HT) is recognized as the most effective treatment for the relief of the climacteric symptoms of menopause [2,3]. Despite the clinical benefits attributed to the use of HT in postmenopausal women, compliance ranges from 10–50% due to the undesirable side effects. Therefore, alternative therapies with the same benefits of estrogen, but lower side effects, are needed for women who refuse to use, are not compliant with, or have contraindications for HT.

Phytoestrogens, or plant-based compounds with estrogenic activity, received a great deal of attention after the publication

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of epidemiological data describing how they could be used as a way to manage the symptoms of menopause and associated diseases [4,5]. Isoflavones are the major type of phytoestrogen that is of current interest, while the most important isoflavones are genistein, daidzein, glycitein, and biochanin A. The structures of these isoflavones resemble estrogen. Isoflavones exhibit both estrogenic and antiestrogenic activities depending on the concentrations of circulating endogenous estrogen, estrogen receptors, and intracellular coregulators [6,7]. Isoflavones seem to have a higher binding affinity for estrogen receptor-β (ERβ) than ERα [8]. They are often referred to as natural selective estrogen receptor modulators (SERMs) [5,9]. These different affinities could explain their antiestrogenic effects on uterine and breast tissues [10] and their estrogenic effects on bone [11] and blood vessels [12]. An inverse association between soy intake and breast cancer risk has been reported [13]. The normal Western adult's self-reported intake of soy isoflavones is around 5 mg/day and the serum isoflavone level ranges from 50–100 nM [14] compared with > 50 mg/day intake and 3–5 mcM serum isoflavone plasma concentration in the average Japanese adult [15].

Many randomized controlled clinical trials have been conducted to evaluate the effects of soy isoflavones on vasomotor symptoms and cholesterol [16–22]. However, little clinical data on the effects of isoflavones on bone mineral density can be found in the literature [5]. Previously reported contradictory results could be due to the large heterogeneity of the soy derivatives that were studied. In this study, we used a standardized purified isoflavone extract in capsule form that is readily available as a dietary supplement. Its clinical benefits had been previously reported [23,24]. Because the background level of serum isoflavones is high in Taiwanese adults (generally between the levels measured in Japanese and Westerner patients because soy is a part of the habitual diet in Taiwan; unpublished data), and because historically there is a high drop-out rate from placebo control groups due to ineffectiveness, we did not include a placebo control group in this study. Instead, high-performance liquid chromatography (HPLC) analysis of the plasma isoflavone concentration was used to evaluate any changes after using the dietary supplement. Therefore, in this multicenter, open-label, randomized, prospective study, the objective was to evaluate the efficacy and safety of isoflavones (35 mg or 70 mg daily) for alleviating climacteric symptoms and their effects on lipid profiles and bone markers in Taiwanese postmenopausal women.

Materials and methods

Patients

A group of 130 healthy postmenopausal women, each with an intact uterus, was recruited from the gynecology out-patient clinics of four medical centers in Taipei city. The inclusion criteria required that all patients be women >45 years of age with established menopause, which was diagnosed as amenorrhea for at least 3 months, a follicle stimulating hormone (FSH) level > 40 mIU/L, and a plasma estradiol level < 25 pg/L. No patients had a history of liver disease, breast cancer, endometrial cancer, thrombophlebitis, thromboembolic disorders related to estrogen use, myocardial infarction, ischemic heart disease, chronic renal disease, cerebrovascular accident, uncontrolled hypertension, diabetes, metabolic bone disease, or had received any hormone treatment within the previous 3 months. This study was approved by the Joint Institutional Review Board (Taiwan) and informed written consent was obtained from all of the patients enrolled in this study.

Treatment

This was a multicenter, open-label, randomized, comparative, outpatient study. All patients participated in a 14-day prestudy evaluation period followed by 6 months of treatment. In all, 130 postmenopausal women were enrolled in this study. A computer-generated randomized code stratification system was used according to the medical center of enrollment. One group ($n = 65$) received 35 mg of soy extract (Phyto Soya, a standardized isoflavone supplement prepared from soy extract that contains 17.5 mg/cap soy isoflavones consisting of 5.25 mg glycitin, 8.75 mg daidzein, and 3.5 mg genistein; Arkopharma CARROS FRANCE) that was split into two equal doses and orally consumed twice daily. Another group ($n = 65$) received 70 mg of soy extract that was also split into two equal daily doses. The treatment period was 6 months, and the women received follow-up examinations after the first, third, and sixth months.

Laboratory methods

During the 6-month treatment period, the patients were asked to participate in follow-up visits at baseline and 24 weeks after taking the soy extract. The follow-up examination consisted of laboratory examinations, including hematology, determination of blood chemistry and lipid profiles, hormonal study, transvaginal sonography (TVS), and determination of the isoflavone serum concentration. Urine and serum samples were also collected for bone marker tests, and blood samples were collected for laboratory tests. Lipid levels (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides [TG]) were measured using an automatic analyzer (Hitachi-7600; Nakashi, Japan).

To assess bone resorption, a second-void fasting urine sample was taken in the early morning in order to determine the concentration of deoxypyridinoline (Dpd) using a commercial enzyme immunoassay (EIA) kit (Metra Biosystem, Catalog No. 104827, Palo Alto, CA, USA). To assess bone formation, a serum specimen was collected to measure the bone-specific alkaline phosphatase (BAP) level using a commercial EIA kit (Metra Biosystem).

Serum isoflavone concentrations

Serum samples were deproteinated using 1% TCA. After centrifugation, the clear supernatant was subjected to acid

hydrolysis with 2 M HCl at 100°C for 2 hours. The hydrolysate was neutralized and passed through a reverse-phase C18 column (Waters, Milford, MA.). Isoflavonoid compounds were eluted using 2 mL methanol. The elution was dried under a nitrogen flow. The dried material was redissolved in 100 μL methanol and subjected to HPLC analysis that was carried out on a GOLD system chromatograph (Beckman, Fullerton, CA, USA). This system consists of a solvent delivery module (model 126) and an ultraviolet detector (model 166). The column configuration consisted of a Nova-Pak C18 (4 μm, 3.9 × 150 mm ID) reversed-phase column coupled to a Nova-Pak C18 (4 μm, 3.9 × 20 mm ID) guard column (both from Waters, Milford, MA). The gradient elution was carried out at a flow rate at 0.8 mL/minute and a column temperature of 20°C. The system consisted of acetonitrile and 10% acetic acid in water (v/v). The gradient profile for separation was optimized as 15/15/25/70/70/15% acetonitrile in 10% acetic acid at 0/2/2.5/14.5/17.5/25 minutes, respectively. The substances were detected and quantified at 260 nm.

Clinical assessment

Each patient provided a complete medical history, including past medical history, surgical procedures (especially related to the reproductive and/or endocrine organs), and obstetrical history. The baseline visit was scheduled for 14 days after the screening visit. Study visits were scheduled after first, third, and sixth months of treatment. Menopausal symptoms were recorded by the patients using a modified Greene Climacteric Scale (GCS) and were scored at each clinical visit. The GCS is a brief but comprehensive and valid measure of climacteric symptomatology. Menopausal symptoms were categorized according to four main groups: psychological, somatic, vasomotor, and urogenital atrophy. Each group was evaluated as follows: 0 = none, 1 = a little, 2 = quite a bit, and 3 = extremely. The original criterion for the urogenital atrophy scale is "loss of interest in sex". The modified GCS adds three more items—urinary tract complaints/frequency, incontinence, and vaginal dryness—in order to cover urogenital symptoms. Complete physical examinations were performed at the baseline visit and after the completion of 6 months of treatment. Analyses of any adverse events (AEs) were performed after the first, third, and sixth months of treatment. Diary cards were maintained by each patient and used to record the frequency of hot flushes. Efficacy was evaluated as a comparison of the changes in frequency and severity of menopausal symptoms, lipid profiles, and bone markers within each group (self-comparison, baseline, and 6 months after beginning treatment), as well as between the two treatment groups.

Statistical analysis

All measurements were listed and/or tabulated, and means and standard deviations, if appropriate, were calculated. Differences between baseline and 6 months after, as well as differences between the two treatment groups, were compared using either the paired t test (within group), unpaired t test (between groups), or ANOVA using the baseline as a covariate for continuous variables, or using the Mantel-Haenszel method for categorical data. All statistical tests were two-sided, and a p value < 0.05 was considered statistically significant. HPLC data were analyzed using independent and paired t tests and with linear regression using a statistical software package (SPSS 10.0 for Windows, Microsoft).

Results

Altogether, 130 patients were randomly assigned to the two treatment groups (65 per treatment arm). These patients were well balanced with respect to baseline characteristics. Table 1 lists the demographic and baseline features of the study groups. No differences were statistically significant between the two treatment groups. One hundred and seven patients (57 patients in the 35 mg and 50 patients in the 70 mg group) completed the study. The drop-out rate was 17.7%. Most of the discontinued patients were those who were lost on follow-up ($n = 7$) or those who experienced a lack of efficacy ($n = 12$).

Climacteric symptoms

Table 2 shows the comparisons of the symptoms according to the modified GCS between the two treatment groups. Climacteric symptom scores (items 1–24) at baseline between the two treatment groups were comparable (15.05 ± 7.14 for the 35 mg/day group and 15.60 ± 9.45 for the 70 mg/day group; $p = 0.73$). As the results show, the scores were significantly reduced in both treatment groups after 1 month of treatment, and the efficacy was maintained throughout the 6 months of treatment. After the first month of treatment with soy extract, the total climacteric symptoms score was reduced by 3.84 ± 5.73 points from baseline (decreased by 19.66 ± 53.80% from baseline) in the 35 mg/day group and reduced by 2.78 ± 6.25 points from baseline (decreased by 18.85 ± 45.66% from baseline) in the 70 mg/day group. There was a statistically significant net change in the total climacteric symptoms scores within the groups after 1, 3, and 6

Table 1
Summary of patient demographics.

Characteristics	35 mg SSE ($n = 65$)	70 mg SSE ($n = 65$)	p
Age (y)	52.47 ± 4.74	51.35 ± 4.69	0.133
Height (cm)	156.14 ± 5.26	157.16 ± 4.79	0.146
Weight (kg)	55.33 ± 7.28	55.51 ± 8.02	0.838
BMI (kg/m^2)	22.80 ± 2.63	22.48 ± 3.00	0.450
Years since menopause	3.90 ± 3.76	3.35 ± 4.12	0.550
E2 (pg/mL)	8.86 ± 5.64	9.95 ± 6.41	0.227
FSH (mIU/L)	74.65 ± 17.28	77.27 ± 17.50	0.313
Systolic blood pressure (mmHg)	120.49 ± 19.20	116.33 ± 14.48	0.141
Diastolic blood pressure (mmHg)	76.35 ± 11.50	73.94 ± 9.49	0.176
Heart rate (beats/min)	74.26 ± 9.67	73.16 ± 8.59	0.429

BMI = body mass index; SSE = standardized soy extract. All values are the means ± SD.
p = difference between the two groups.

Table 2
Effect of treatment on menopausal symptoms (modified Greene Climacteric Scale).

	Baseline	1 months	3 months	6 months
Treatment				
35 mg SSE ($n = 57$)	15.05 ± 7.14	11.21 ± 6.47	10.63 ± 7.65	10.12 ± 7.89
70 mg SSE ($n = 50$)	15.60 ± 9.45	12.82 ± 9.89	11.74 ± 9.46	11.00 ± 8.90
Change from baseline				
35 mg SSE ($n = 57$)	—	−3.84 ± 5.73	−4.42 ± 6.65	−4.93 ± 7.37
70 mg SSE ($n = 50$)	—	−2.78 ± 6.25	−3.86 ± 7.21	−4.60 ± 6.67
% change from baseline				
35 mg SSE ($n = 57$)	—	−19.66 ± 53.80	−21.80 ± 59.00	−30.17 ± 48.91
70 mg SSE ($n = 50$)	—	−18.85 ± 45.66	−25.63 ± 52.28	−28.86 ± 42.97

SSE = standardized soy extract.
All values are the means ± SD. All values are significantly different from baseline ($p < 0.01$).

months of treatment. However, no statistically significant difference was observed between the two treatment groups.

Table 3 shows the comparisons of the individual symptoms according to the modified GCS between the two treatment groups. No statistically significant difference was observed between groups ($p = 0.506$).

Lipid profiles

No statistically significant differences were observed in any item at baseline between the two treatment groups. After 6 months of treatment, none of the lipid profiles were significantly different from baseline within each group. As shown in Table 4, in the subgroup analysis of patients with TC >200 mg/dL ($n = 69$), TC was reduced by 11.30 ± 23.73 mg/dL (decreased by 4.50 ± 10.29% from baseline) in the 35 mg/day group and by 7.90 ± 18.60 mg/dL (decreased by 3.06 ± 7.61% from baseline) in the 70 mg/day group after 6 months of treatment; however, no statistically significant difference was found between the two treatment groups ($p = 0.79$). HDL was reduced by 2.63 ± 9.19 mg/dL (decreased by 2.56 ± 11.07% from baseline) in the 35 mg/day group and increased by 0.34 ± 7.87 mg/dL (increased by 1.79 ± 12.03% from baseline) in the 70 mg/day group, but this difference was not significant ($p = 0.08$ and 0.82, respectively). LDL showed statistically significant changes within each treatment group after 6 months of treatment: LDL was reduced by 8.45 ± 22.97 mg/dL (decreased by 4.67 ± 14.64% from baseline) in the 35 mg/day group and by 8.97 ± 22.49 mg/dL (decreased by 5.09 ± 13.30% from baseline) in the 70 mg/day group. There was no statistically significant difference between the two treatment groups ($p = 0.93$).

In the subgroup analysis of patients with TG >103 mg/dL ($n = 39$), there was no statistically significant difference in TG between the two groups at baseline ($p = 0.99$). TG was significantly reduced by 22.45 ± 38.03 mg/dL (decreased by 14.20 ± 25.75% from baseline) in the 35-mg/day group and by 28.00 ± 50.97 mg/dL (decreased by 17.23 ± 30.46% from baseline) in the 70 mg/day group after 6 months of treatment. No statistically significant difference was found between the two treatment groups ($p = 0.70$).

Bone markers

Table 5 shows the relative changes from baseline of the bone resorption and formation marker (urinary Dpd/creatinine [Cr] and serum BAP) levels after short periods of soy extract treatment. The baseline levels of Dpd/Cr and BAP were comparable between the two treatment groups ($p = 0.42$ and 0.79, respectively). At 6 months, there was no statistically significant difference within or between groups. In the subgroup analysis of the patients with Dpd/Cr > 7.4 nM/mM ($n = 44$), a reduction in bone resorption activity was shown. The cut-off point of Dpd/Cr for choosing a subgroup followed the reference range that was provided with the commercial assay kit (as described in the Materials and Methods section) and was validated in our laboratory. The baseline level of Dpd/Cr between the two subgroups was comparable (8.93 ± 1.22 nM/mM for the 35 mg/day group and

Table 3
Observed data on the individual symptoms in terms of the modified Greene Climacteric Scale.

	35 mg SSE ($n = 57$)				70 mg SSE mg[#] ($n = 50$)			
	Baseline	6 months	Change (%)	p	Baseline	6 months	Change (%)	p
P (1–11)	6.67 ± 3.62	4.09 ± 4.05	−40.65 ± 49.68	<0.01	7.42 ± 5.61	5.18 ± 4.89	−30.52 ± 61.67	<0.01
S (12–18)	4.83 ± 3.06	3.02 ± 2.66	−21.32 ± 95.65	<0.01	4.78 ± 3.03	3.02 ± 2.57	−22.30 ± 79.00	<0.01
V (19–20)	1.65 ± 1.55	0.74 ± 1.01	−43.60 ± 75.84	<0.01	1.78 ± 1.67	0.82 ± 1.04	−39.00 ± 71.22	<0.01
UG (21–24)	3.21 ± 2.31	2.26 ± 2.22	−13.91 ± 88.19	<0.01	2.60 ± 2.02	1.82 ± 1.60	−25.71 ± 42.70	<0.01

P = psychological scale; S = somatic scale; V = vasomotor scale; UG = urogenital scale; SSE = standardized soy extract. All values are the means ± SD.
p = difference from baseline (within the group).
[#] No statistically significant difference was observed between the two treatment groups ($p = 0.506$).

Table 4
Lipid profile of postmenopausal women during the 6 months of treatment with soy extract.

Lipids	Baseline (mg/dL)	6 mo (mg/dL)	Change (%)	p	Baseline (mg/dL)	6 mo (mg/dL)	Change (%)	p
35 mg SSE ($n = 57$)					70 mg SSE ($n = 50$)			
TC	215.40 ± 33.42	209.19 ± 30.00	−2.03 ± 11.88	0.06	207.30 ± 32.77	205.08 ± 28.80	−0.32 ± 10.43	0.46
HDL-C	64.81 ± 14.86	62.89 ± 13.65	−1.38 ± 16.81	0.14	63.70 ± 14.82	64.14 ± 13.76	1.81 ± 12.08	0.71
LDL-C	139.65 ± 31.58	135.37 ± 28.79	−1.41 ± 16.44	0.16	132.14 ± 30.11	129.44 ± 26.27	−0.15 ± 16.35	0.39
TG	101.21 ± 43.81	99.89 ± 44.25	4.63 ± 45.16	0.81	92.10 ± 50.68	85.80 ± 43.24	1.02 ± 36.19	0.24
TC subgroup ($n = 69$)								
35 mg SSE ($n = 40$)					70 mg SSE ($n = 29$)			
TC	232.33 ± 23.33	221.03 ± 25.48	−4.50 ± 10.29	0.00	227.38 ± 25.25	219.48 ± 21.12	−3.06 ± 7.61	0.03
HDL-C	67.98 ± 14.27	65.35 ± 12.99	−2.56 ± 11.07	0.08	64.00 ± 13.79	64.34 ± 11.86	1.79 ± 12.03	0.82
LDL-C	153.53 ± 25.88	145.08 ± 26.82	−4.67 ± 14.64	0.03	150.86 ± 23.11	141.90 ± 23.02	−5.09 ± 13.30	0.04
TG	100.98 ± 40.13	99.15 ± 40.00	5.55 ± 49.88	0.78	97.72 ± 47.51	89.76 ± 35.07	0.38 ± 34.47	0.19
TG subgroup ($n = 39$)								
35 mg SSE ($n = 22$)					70 mg SSE ($n = 17$)			
TC	221.00 ± 31.64	209.77 ± 33.68	−4.66 ± 11.45	0.05	211.82 ± 38.28	206.47 ± 35.85	−3.06 ± 7.31	0.25
HDL-C	56.64 ± 13.55	57.55 ± 15.10	2.28 ± 16.26	0.62	53.53 ± 12.71	55.47 ± 12.59	1.79 ± 12.03	0.14
LDL-C	149.00 ± 31.84	139.73 ± 32.73	−4.97 ± 17.44	0.11	138.06 ± 33.01	133.00 ± 32.93	−5.09 ± 13.30	0.32
TG	146.23 ± 34.07	123.77 ± 39.41	−14.20 ± 25.75	0.01	146.47 ± 49.03	118.47 ± 52.09	−17.23 ± 30.46	0.04

TC subgroup: total cholesterol > 200 mg/dL; TG subgroup: triglyceride > 103 mg/dL; SSE = standardized soy extract. All values are the means ± SD.
$p < 0.05$: significantly different from baseline (within the group).

9.06 ± 1.18 nM/mM for the 70 mg/day group; $p = 0.73$). After 6 months, a decrease of 10.53 ± 22.58% from baseline was observed in the Dpd bone resorption marker level in the 35 mg/day group and a decrease of 11.58 ± 19.55% was noted in the 70 mg/day group. However, no statistically significant difference between the two treatment groups was seen ($p = 0.82$).

In the subgroup analysis of the patients with a BAP level > 18 U/L, the baseline BAP levels of the two groups were comparable (24.71 ± 4.60 U/L in the 35 mg/day group and 27.02 ± 9.00 U/L in the 70 mg/day group; $p = 0.15$). The cut-off point of BAP for choosing a subgroup followed the reference range provided with the commercial assay kit (as described in Materials and Methods section) and was validated in our laboratory. No statistically significant difference in the net change in BAP (from baseline) between ($p = 0.18$) or within the two treatment groups ($p = 0.81$ for the 35 mg group; $p = 0.87$ for the 70 mg group) was seen during the treatment period.

HPLC data

The baseline levels of daidzein and genistein were comparable between the two treatment groups ($p = 0.41$ and 0.69, respectively). The genistein serum level was higher than the daidzein level due to the subjects' dietary soy composition (unpublished data). Table 6 shows that the mean serum levels of daidzein in both the 35 and 70 mg soy extract treatment groups increased significantly (3.7- and 4.4-fold, respectively; $p < 0.01$) after 6 months after treatment. Serum levels of genistein increased by 1.2-fold in the low-dosage group and 1.1-fold in the high-dosage group ($p = 0.03$; 0.04), respectively. There was no statistically significant difference between the two treatment groups after the treatment period (all $p > 0.1$).

Table 5
Bone turnover markers in postmenopausal women during short-term treatment with soy extract.

		Baseline	6 months	Change (%)	p
Dpd/Cr (nM/mM)	35 mg SSE	7.32 ± 1.91	7.44 ± 2.05	7.08 ± 39.16	0.70
	70 mg SSE	7.22 ± 1.70	7.17 ± 1.62	2.38 ± 24.41	0.84
BAP (U/L)	35 mg SSE	22.10 ± 6.13	22.93 ± 7.10	6.33 ± 30.33	0.27
	70 mg SSE	23.33 ± 9.42	23.91 ± 8.81	5.90 ± 23.46	0.48
Subgroup Dpd/Cr (nM/mM) > 7.4 ($n = 44$)					
Dpd/Cr (nM/mM)	35 mg SSE ($n = 27$)	8.93 ± 1.22	7.97 ± 2.20	−10.53 ± 22.58	0.02
	70 mg SSE ($n = 17$)	9.06 ± 1.18	7.96 ± 1.74	−11.58 ± 19.55	0.02
BAP (U/L)	35 mg SSE ($n = 27$)	22.88 ± 5.59	22.86 ± 5.27	1.24 ± 15.38	0.98
	70 mg SSE ($n = 17$)	25.80 ± 7.77	24.88 ± 8.12	−2.02 ± 18.58	0.52
Subgroup BAP (U/L) >18 ($n = 76$)					
Dpd/Cr (nM/mM)	35 mg SSE ($n = 42$)	7.44 ± 2.08	7.39 ± 2.11	−0.24 ± 18.28	0.88
	70 mg SSE ($n = 34$)	7.64 ± 1.74	7.30 ± 1.52	−1.68 ± 21.74	0.24
BAP (U/L)	35 mg SSE ($n = 42$)	24.71 ± 4.60	24.55 ± 6.07	−0.24 ± 18.28	0.81
	70 mg SSE ($n = 34$)	27.02 ± 9.00	26.85 ± 8.89	−0.71 ± 19.46	0.87

Dpd = deoxypyridinoline; Cr = creatinine; BAP = bone-specific alkaline phosphatase; SSE = standardized soy extract. All values are the means ± SD.

Table 6
Serum isoflavone (mean ± SD ng/mL) levels at baseline and after 24 weeks of soy extract treatment.

	35 mg SSE ($n = 57$)		70 mg SSE ($n = 50$)	
	Baseline	6 months	Baseline	6 months
Daidzein	126 ± 23	473 ± 46*	100 ± 20	444 ± 57*
Genistein	287 ± 23	351 ± 32*	316 ± 31	415 ± 45*

SSE = standardized soy extract; SD = standard deviation.
*Significantly different from baseline: $p < 0.05$.

Clinical assessment

There were no statistically significant changes from baseline in terms of body weight, blood pressure, pulse rate, endometrial thickness, or laboratory data within or between the two treatment groups. In terms of the frequencies of spotting and bleeding analysis, most patients did not demonstrate spotting or bleeding during each cycle during treatment. No significant changes were noted in the vaginal epithelium or endothelium using transvaginal sonography (TVS).

Safety

Fifteen incidences of AEs (7 in the 35 mg/day group and 8 in the 70 mg/day group) were reported. Five and six patients reported soy extract-related AEs in the 35 and 70-mg/day groups, respectively ($p = 0.811$). The soy extract-related AEs were mostly mild. The most frequently reported soy extract-related AE was nausea (3 in the 35 mg group and 3 in the 70 mg group), followed by breast tenderness (1 in the 35 mg group and 3 in the 70 mg group). All of the AEs were standardized using the CORSTART coding system.

Discussion

Previous data on the clinical efficacy of isoflavone are conflicting and difficult to analyze because of variations in the examined populations, duration of exposure to dietary isoflavone, study designs, and responsiveness in postmenopausal women to isoflavone supplementation. Furthermore, these discrepancies may be related to the specific sources of the isoflavones and individual metabolisms. Washburn et al [25] suggested that having a consistent circulating level of isoflavone may be more efficacious than a higher single dose.

We did not have a placebo control group in this study because the background level of plasma isoflavones in Taiwanese adults is high (unpublished data), and experientially there is a high drop-out rate from placebo control groups due to ineffectiveness. In addition, the limitations of the small number of patients and the short duration of follow-up in this study should be recognized. Therefore, we used HPLC to analyze the plasma isoflavone concentration in order to evaluate changes following dietary supplementation.

This study presents, for the first time, the serum levels of isoflavones in Taiwanese postmenopausal women. After administering a twice-daily standardized soy extract dietary supplement for 6 months, a substantial increase in the serum isoflavone level was achieved. Furthermore, the increased isoflavone level reflects the measurement of daidzein and genistein in the intervention material (i.e., soy extract). However, no dose-dependent response in terms of daidzein or genistein serum levels was observed in the 35 or 70 mg groups. This may be due to the high background level of serum isoflavone in Taiwanese women, as well as the constant clearance of isoflavones. Variations in absorption also played an important role. Further kinetic studies may explain these conflicting results.

This study confirms that soy extract, when administered at a daily dose of 35 or 70 mg, effectively and safely relieves climacteric-related symptoms in postmenopausal women. These results are in line with those of Albert et al [23] and Evelyne et al [24]. The significant reduction in climacteric symptoms with the use of soy extract was superior to the placebo effect. However, there was no statistically significant difference between the 35 and 70 mg groups ($p = 0.506$), which was partly due to similar plasma concentrations. Jou et al [26] suggested that a dose $\geq$ 70 mg/day is needed to provide earlier onset in the improvement of somatic symptoms.

Based on previously reported positive findings, a health claim for the role of soy supplements in reducing serum cholesterol was granted by the United States Food and Drug Administration. The American Heart Association also recommends the consumption of soy protein and isoflavones in order to reduce the risk of coronary heart disease (CHD). Researchers believe that isoflavones are responsible for most of the hypocholesterolemic effects of soy supplements [27,28]. The potential mechanisms include: (1) direct phytoestrogenic effects; (2) increased arterial compliance; and (3) direct antioxidant properties. Although the lipid-lowering effects of isoflavones are modest and may not achieve target lipid parameters, it is possible that isoflavones may contribute to a lower risk of CHD if consumed over many years in conjunction with other lipid-lowering strategies [28]. An improvement in blood lipid levels has been noted to be related to the initial degree of hyperlipidemia in each patient [27,29,30]. However, due to our small sample size and the length of treatment, some true correlations might not have been observed. Herein, a statistically significant reduction of TG, as well as TC and LDL, was demonstrated in hypertriglycemic and hypocholesterolemic patients. This is contradictory to the usual results of oral estrogen intake, in which isoflavones cause a decrease in TG. Further studies are warranted on the use of isoflavones in combination with other lipid-lowering medicines for the management of hyperlipidemia. The National Institutes of Health's National Center for Complementary and Alternative Medicine is currently supporting Professor Howard N. Hodis of the University of Southern California, who is conducting the Women's Isoflavone Soy Health (WISH) trial. This ongoing trial will last 2.5 years and will help to determine if isoflavone-containing soy protein treatments reduce the progression of atherosclerosis in postmenopausal women. Changes in the intimal media thickness of the carotid artery will be the primary endpoint. We should await the arrival of more data before rushing to judgment about the effects of isoflavone.

Kritz-Silverstein et al [31] indicated that the bone-sparing effects of short-term supplemental isoflavones would be more apparent in high bone-turnover patients. Most of the patients included in this study were in the normal range of bone turnover. Although a statistically significant reduction in Dpd/Cr was seen in the high-resorption subgroup, there is a need for further studies on the long-term effects on bone activity, including the study of high-dose isoflavones in rapid bone-losing menopausal women.

Conclusion

This study assessed the effects of standardized isoflavone supplement soy extract on climacteric symptoms, lipid profiles, and bone markers in postmenopausal women in Taiwan. Based on the results obtained in this preliminary study, soy extract demonstrates a good efficacy for relieving menopausal symptoms, in addition to good tolerance and compliance. The data also suggest the positive effects of soy extract on the cardiovascular system and skeleton. It could, therefore, be used by women who suffer from menopausal symptoms and who choose not to take HT for personal or medical reasons.

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