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Short communication

The effect of synthetic genistein on menopause symptom management in healthy postmenopausal women: A multi-center, randomized, placebo-controlled study

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ABSTRACT

Objective: To evaluate the efficacy of synthetic genistein for reducing the frequency and severity of hot flushes.
Study design: A 12 week randomized double-blind, placebo-controlled study in which 84 postmenopausal women received placebo or a single 30 mg dose of synthetic genistein.

Outcome measures primary: percentage change in the number of daily hot flushes from pre-treatment to week 12. Secondary: duration and severity of daily hot flushes, Greene Climacteric Scale score, serum follicle stimulating hormone (FSH), 17β-estradiol and endometrial thickness.
Results: Genistein supplemented subjects completing at least 4 weeks on trial ($n = 40$) demonstrated a 51% reduction (9.4–4.7/day) in the number of hot flushes by week 12 compared to a 27% reduction in the placebo group (9.9–7.1/day) ($p = 0.026$). Subjects in the genistein group also reported significantly fewer hot flushes per day ($p = 0.010$) and a decrease in total duration of hot flushes per day ($p = 0.009$) at week 12 versus placebo. Subjects on genistein ($n = 32$) completing 12 weeks on trial demonstrated a 51% reduction (9.7–4.7/day) in the number of hot flushes by week 12 ($p = 0.049$) compared to 30% reduction in the placebo group (9.8–7.0/day) and had fewer hot flushes per day and a decrease in total duration of hot flushes per day at week 12 compared to placebo ($p = 0.020$ and $p = 0.017$, respectively). There were no differences between groups in Greene Climacteric Scale, FSH, 17β-estradiol, endometrial thickness or adverse events.
Conclusions: The current study provides the first evidence that a single daily dose of 30 mg of synthetic genistein reduces hot flush frequency and duration.

1. Introduction

In most developed countries, substantial numbers (~50–70%) of menopausal women experience hot flushes and it is the most common reason given by women for seeking treatment for menopausal symptoms [1]. Hormone therapy effectively alleviates menopausal symptoms but its use is advocated with caution because of its well-known side effects including an increased risk of stroke and breast cancer [2]. Furthermore, many women would prefer to address their symptoms without resorting to the use of pharmaceuticals. Thus, there is need for a safe and effective nonhormonal alternative for the treatment for hot flushes. One of the most highly investigated proposed alternatives is soy and soybean isoflavones.

Isoflavones are classified as phytoestrogens because they bind to estrogen receptors (ER) and affect estrogen-regulated gene products [3]. They are also classified as selective estrogen receptor modulators because they preferentially bind to and transactivate ERα in comparison to ERβ [4]. The three soybean isoflavones are genistein (4′,5,7-trihydroxyisoflavone), daidzein (4′,7-dihydroxyisoflavone), and glycitein (7,4′-dihydroxy-6-methoxyisoflavone) although in the soybean itself they occur primarily in their respective β-glycoside forms (genistin, daidzin, and glycitin). Genistein, daidzein, and glycitein represent approximately 50, 40, and 10% of the total soybean isoflavone content, respectively [5].

Since 1995, dozens of clinical trials have evaluated the efficacy of isoflavone-containing products for relieving menopausal symptoms. Several reviews and statistical analyses of this research have been published. Some, such as a meta-analysis by Howes et al. [6] found that isoflavones were modestly efficacious whereas others have determined that the data were not sufficiently definitive to allow such a conclusion to be reached [7,8]. Several explanations for the inconsistent data have been proposed including

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variation in baseline hot flush frequency (i.e., some work suggests initial hot flush frequency is positively related to efficacy) [9] the huge inter-individual differences in isoflavone metabolism [10] and the differing genistein content of the intervention products [11]. Support for this last explanation is particularly intriguing and is consistent with knowledge that the estrogenic potency of genistein is greater than that of daidzein and glycitein [12]. Furthermore, each of the three trials that evaluated the ability of isolated genistein to alleviate hot flushes found it to be efficacious [13–15]. However, in each case, the baseline hot flush frequency was much lower than is generally recommended for these types of trials. In addition, no genistein-only trials involved North American women.

Therefore, the objective of the current study was to investigate the efficacy of a single 30 mg daily dose of 99% pure, synthetic genistein in reducing the frequency and severity of hot flushes in Canadian postmenopausal women. This dose was selected because it is twice the threshold genistein exposure proposed to be needed for hot flushes alleviation [11] but still compatible with the amount of genistein to which Asian women consuming a traditional diet are exposed [16].

2. Materials and methods

2.1. Study sample

This study was conducted in accordance with Good Clinical Practice Guidelines and the ethical principles of the Declaration of Helsinki (2000). The study protocol and materials were approved by Institutional Review Board (Aurora, ON), and all subjects gave written informed consent prior to participation.

This was a randomized, double-blind, placebo-controlled, 12-week study in healthy postmenopausal women, conducted at five study sites in southwestern Ontario, Canada. To be eligible, subjects had to have a minimum of 40 hot flushes per week, be between the ages of 40 and 65 and be in a physiological state of natural or surgical menopause. In the case of natural menopause, women were required to be amenorrheic for $\geq$3 months and have serum FSH levels greater than 35 IU/ml whereas surgically menopausal women had to be >42 days post surgery. Subjects were ineligible from participating in the study if clinical or laboratory abnormalities were identified by the Medical Director; had used conventional hormone therapy or selective estrogen receptor modulators within 4 weeks of study start; had known allergy or hypersensitivity to soy, peanuts, purified isoflavones, genistein, lactose and/or cow's milk; had consumed soy products within 4 weeks prior to the screening visit; reported unpredictable vaginal bleeding (i.e., leiomyoma or endometrial polyps), uterine fibroids or endometriosis that required treatment; untreated polycystic ovary syndrome (PCOS); history of abnormal pap smear; use of gonadotropin agonists within 24 weeks; glucocorticoids or chronic high dose (>7.5 mg/day) prednisone or equivalent for the past 12 weeks.

2.2. Study products

The intervention product tested (geniVida®: DSM Nutritional Products) is a patented, 99% pure, synthetic, aglycone genistein with an extensive safety package of toxicology [17–20] and human pharmacokinetic [21–24] studies. Lot SI06068001 of genistein was used in this study and the analytical results were: purity 99.1%, water 0.1%, heavy metals < 10 ppm, arsenic < 1 ppm, lead < 2 ppm. Genistein was administered as a 30 mg dose in a two-piece hard shell capsule as was the placebo which was microcrystalline cellulose. The genistein and placebo capsules (manufactured by Manhattan Drug Co., Hillsdale, NJ) were filled into sealed plastic bottles of 60 capsules with identical labels except for the randomization and visit numbers. Subjects were instructed to take the genistein or placebo capsule once daily with breakfast.

2.3. Randomization and supplementation

Subjects were randomly assigned to one of two treatment groups in blocks of six and a treatment code was randomly allocated in the order in which a subject was enrolled. Each treatment code was associated with either the genistein or placebo. In order to protect blinding, genistein and placebo capsules were packaged in identical bottles with identical labels with each label containing an individual unique randomization number. Sealed envelopes for each randomization number were maintained, with each envelope containing treatment assignment for the individual subject. The patient, Medical Directors and research staff were blinded to the treatment assignment for the duration of the trial.

2.4. Study protocol

At screening eligibility was determined based on the inclusion and exclusion criteria. Peripheral blood was collected to determine clinical chemistry, hematology measures and FSH and a urine pregnancy test performed. Medical history, concomitant therapies, and soy product consumption were reviewed and, biometric measurements and vital signs recorded and the Greene Climacteric Scale administered. The Greene Climacteric Scale is designed to provide a score for the psychological, vasomotor, somatic, depression and anxiety of the subject [25]. Subjects were given a diary to record daily hot flushes. Eligible subjects had an ultrasound measurement to determine endometrial thickness.

At baseline and at weeks 4, 8 and 12 biometric measurements and vital signs were recorded and soy product consumption, concomitant therapies and adverse events reviewed and the Greene Climacteric Scale administered. The investigational products and subject treatment diary were dispensed to each participant to record daily hot flushes and product use at weeks 4 and 8. Concomitant therapies, adverse events and treatment diary of each participant were reviewed as a measurement of treatment compliance. At week 12 an ultrasound measurement to determine endometrial thickness was performed.

A follow-up telephone call was conducted 14 days after the end of study visit to assess any changes in the subject's health status.

2.5. Statistical analysis

The sample size calculation was based on results by Crisafulli et al. [13] from which variability could be estimated for the percentage change in hot flushes. Assuming a standard deviation of 50% and allowing for a 20% rate of withdrawal, 42 subjects per group were required to detect a clinically important difference of 35% at the 5% level of significance (two-sided) with 80% power. On analysis testing for the effect of study site on the primary endpoint, it was found that adjustments for study site were not needed.

The statistical analysis was a modified intent-to-treat analysis in which all subjects receiving the test product for a period of four weeks were included in the efficacy analysis, and all subjects taking at least one dose of the test product were included in an analysis of safety. A per protocol analysis of the results was also conducted for both efficacy and safety endpoints and included all subjects completing 12 weeks of treatment. Where subjects terminated early, data from the withdrawal date were used as study completion data. The distribution of baseline characteristics in the two groups was compared descriptively. Treatment group comparisons for primary and secondary outcomes, the percentage change in the number of hot flushes, the change in the duration and severity of hot flushes, the change in Greene Climacteric Scale scores, endome-

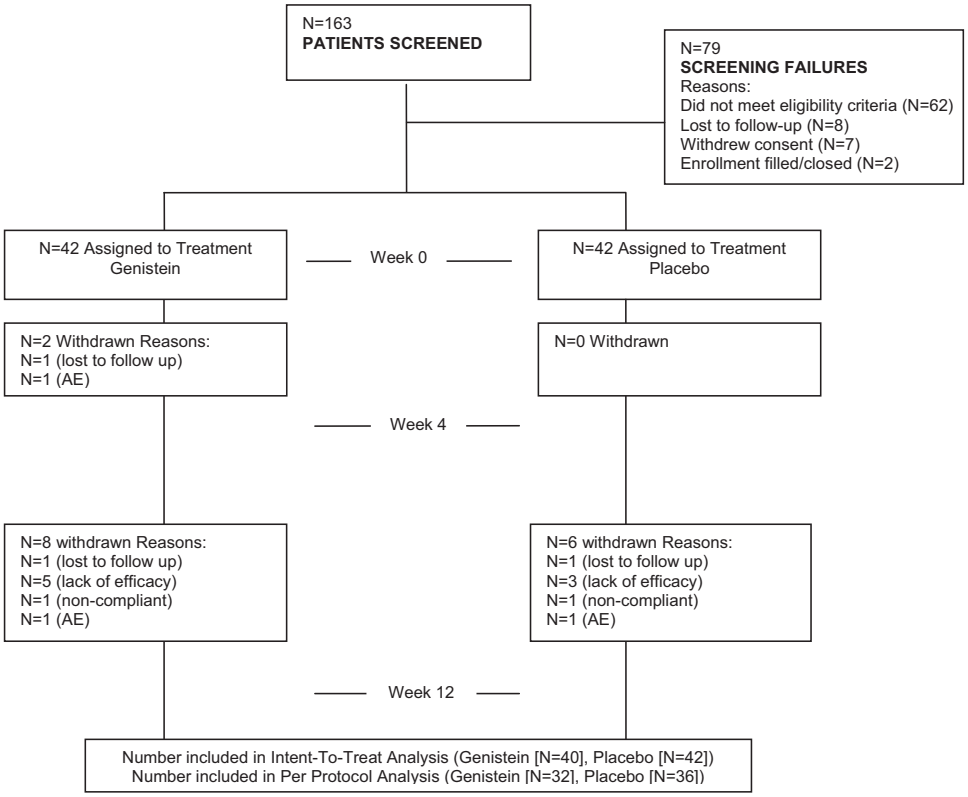

Fig. 1. Flow chart describing the disposition of study subjects during the trial (AE represents adverse events).

trial thickness, serum FSH and 17β-estradiol concentrations were analysed using analysis of covariance (ANCOVA). Descriptive statistics present the mean values and associated standard deviations for all available data by treatment groups. Calculations of within group changes were made using data for subjects having both baseline and applicable endpoint values. A *t*-test was used to determine probability values for within group differences.

An unpaired *t*-test was used to determine differences between groups with respect to treatment compliance. Differences in compliance to treatment regimens were determined using a Student's *t*-test. Safety data and adverse events were summarized by treatment group. Between group differences in the number of withdrawals and the number of individuals experiencing adverse events were compared using a chi-square test for comparing proportions. SAS version 9.1 was used to conduct the statistical analysis. Probability values $p < 0.05$ were considered statistically significant.

3. Results

3.1. Characteristics of the study population

A total of 84 subjects were randomized (Fig. 1). Two subjects (2.4%) withdrew prior to week 4 and by week 12 a total of sixteen subjects (19%) withdrew from the study. The number of withdrawals did not differ between groups ($p = 0.353$). There was an equal distribution of women with surgical (36.6% vs. 31.0%) and natural menopause (63.4% vs. 69.1) in both the genistein and the placebo groups. Only two women (one in each group) were classified as perimenopausal (3 to < 12 months of amenorrhea) with the remainder being postmenopausal (≥12 months of amenorrhea). The baseline characteristics of both genistein and placebo groups were similar (Table 1). Compliance was high and similar between the genistein (96.5%) and placebo (98.8%) groups.

3.2. Plasma genistein

Subjects on genistein completing the 12 week treatment exhibited significantly higher mean levels of fasting plasma total genistein (188.27 ± 129.71 ng/mL) compared to subjects on placebo

Table 1
Screening demographics and characteristics of subjects on genistein or placebo.

	Study group	
	Genistein ($n = 41$)	Placebo ($n = 42$)
Female	41/41 (100.0)[a]	42/42 (100.0)
Age {mean ± SD}[b]	53.39 ± 5.05	53.50 ± 4.44
Height {mean ± SD}	161.8 ± 5.8	161.9 ± 6.1
Tobacco use[c]		
Current	10/40 (25.0)	7/41 (17.1)
Former	9/40 (22.5)	9/41 (22.0)
Never	21/40 (52.5)	25/41 (61.0)
Alcohol use[c]		
Daily	5/40 (12.5)	4/41 (9.8)
Non-drinker	11/40 (27.5)	10/41 (24.4)
Social-occassional	17/40 (42.5)	16/41 (39.0)
Weekly or a few/week	7/40 (17.5)	11/41 (26.8)
Years since menopause[d]		
1–5 years	16/40 (40.0)	20/41 (48.8)
6–10 years	13/40 (32.5)	10/41 (24.4)
>10 years	11/40 (27.5)	11/41 (26.8)
Menopause status		
Natural	26/41 (63.4)	29/42 (69.1)
Surgical	15/41 (36.6)	13/42 (31.0)
BMI (kg/m^2) {mean ± SD}	25.5 ± 3.8	26.4 ± 3.8

[a] Frequency/number (percentage).
[b] Mean ± SD.
[c] Two subjects (one on genistein, one on placebo) did not report tobacco and alcohol use.
[d] Two subjects (one on genistein, one on placebo) were not able to recall number of years since natural menopause.

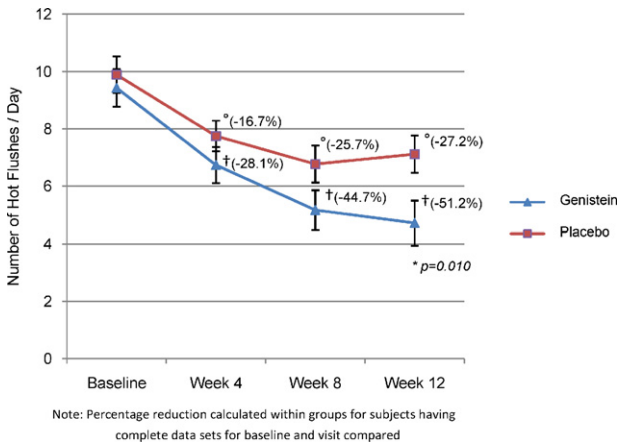

Fig. 2. Mean ± SEM number of hot flushes in subjects on genistein or placebo groups at baseline, week 4, week 8 and week 12 (*$p < 0.05$ vs. placebo (p value represents the difference between treatment groups with respect to the total number of hot flushes), † mean percent change from baseline for genistein,° mean percent change from baseline for placebo, intent-to-treat analysis).

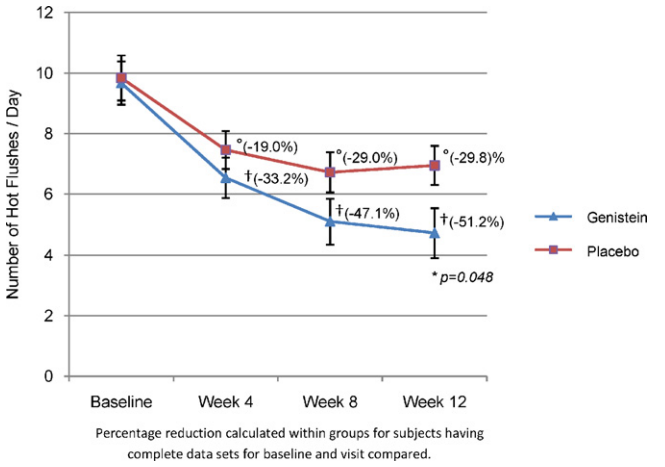

Fig. 3. Mean ± SEM number of hot flushes in subjects who completed the 12 week study on genistein or placebo at baseline, week 4, week 8 and week 12 (*$p < 0.05$ vs. placebo (p value represent the difference between treatment groups with respect to the total number of hot flushes), † mean percent change from baseline for genistein,° mean percent change from baseline for placebo, per-protocol analysis).

(11.00 ± 18.48 ng/mL) ($p < 0.001$). Mean plasma unconjugated genistein was also significantly different between the genistein (0.72 ± 1.15 71 ng/mL) and the placebo groups (0.07 ± 0.18 71 ng/mL) ($p = 0.004$).

3.3. Hot flushes

Subjects on genistein (intent-to-treat analysis) demonstrated significantly fewer mean hot flushes/day (4.7 vs. 7.1/day, $p = 0.010$) (Fig. 2) than placebo and a significantly decreased mean total duration of hot flushes per day ($p = 0.009$) at week 12 compared to subjects on placebo (Table 2), however there was no difference in the mean duration per hot flush [2.21 ± 2.04 min (genistein) vs. 2.68 ± 1.78 min (placebo)] (data not shown). Subjects on genistein demonstrated a trend toward a greater percent reduction in the mean number of hot flushes from baseline to week 4 (28% vs. 17%, $p = 0.150$) and week 8 (45% vs. 26% $p = 0.067$) but this difference was not statistically significant until week 12 (38% vs. 19%, p = 0.026) (mean reduction in number of hot flushes −2.85 vs. −2.13, −4.49 vs. −2.94 and −5.22 vs. −2.96, respectively) (Fig. 2). Analysis incorporating study site did not impact the results. The 32 subjects on genistein who completed the 12 week study (per-protocol-analysis) reported a significant decrease ($p = 0.020$) in the mean number of hot flushes at week 12 (4.7 vs. 7.0) when compared to the 36 completers on placebo. Further, subjects on genistein also demonstrated a greater percent reduction in the mean number of hot flushes from baseline to weeks 4, 8 and 12 of the study compared to placebo (33% vs. 19%, $p = 0.098$); 47% vs. 29% ($p = 0.064$; and 51% vs. 30%, $p = 0.049$, respectively) (Fig. 3).

3.4. Greene Climacteric Scale

There were no statistically significant differences between groups in any parameters or the total score on the Greene Climacteric Scale (Table 3).

3.5. Vaginal bleeding, endometrial thickness, FSH and 17β-estradiol

At week 12, there was no treatment effect on mean endometrial thickness (4.89 ± 2.76 vs. 4.18 ± 1.92 mm) or vaginal bleeding (data not shown), mean serum FSH and mean 17β-estradiol between subjects on genistein as compared to those on placebo (Table 4).

3.6. Adverse events

Results for the safety parameters are presented only for subjects with at least one measurable outcome, who were known to start treatment and returned for at least one follow up visit.

Table 2
Total hot flush duration and average severity of hot flushes in subjects on genistein or placebo during the 12 week study.

	Study group			
	Genistein ($n = 40$)	Placebo ($n = 42$)	p value[a]	p value[b]
Total hot flush duration (min/day)				
Week 0 (baseline)	[40] 23.63 (17.79)[c]	[42] 37.95 (48.42)		
Week 4[d]	[37] 15.64 (11.39)	[41] 23.56 (24.64)	0.550	0.638
Week 8[e]	[30] 12.43 (11.37)	[37] 22.91 (25.79)	0.162	0.256
Week 12[f]	[28] 11.86 (11.82)	[34] 22.65 (22.47) (22.47)	0.009	0.029
Average severity of hot flush (scale 0–3; 0 = none 3 = severe)				
Week 0 (baseline)	[40] 1.86 (0.43)	[41] 2.06 (0.39)		
Week 4[d]	[36] 1.77 (0.47)	[41] 2.02 (0.49)	0.383	0.361
Week 8[e]	[30] 1.61 (0.48)	[37] 2.04 (0.56)	0.053	0.047
Week 12[f]	[28] 1.70 (0.58)	[34] 1.96 (0.57)	0.500	0.663

[a] Statistical analyses were performed using analysis of covariance (ANCOVA). p-Values less than 0.05 are significant.
[b] Statistical analyses were performed using analysis of covariance (ANCOVA) adjusting for site. p-Values less than 0.05 are significant.
[c] [n] mean (SD) are presented.
[d] 3 subjects on genistein and 1 subject on placebo did not provide complete entries.
[e] 5 subjects on genistein and 4 subjects on placebo did not provide complete entries.
[f] 2 subjects on genistein and 2 subjects on placebo did not provide complete entries.

Table 3
Greene Climacteric Scale scores for subjects on genistein or placebo.

	Study group		
	Genistein ($n = 40$)	Placebo ($n = 42$)	p value[a]
Psychological (1–11)			
Screening	[40] 9.65 (5.97)[b]	[42] 10.38 (6.89)	
Week 0 (baseline)	[37] 9.08 (5.90)	[42] 10.45 (7.46)	
Week 4	[39] 6.59 (6.50)	[41] 8.61 (6.63)	0.248
Week 8	[34] 6.38 (4.20)	[39] 8.15 (6.06)	0.484
Week 12	[31] 5.48 (3.91)	[37] 7.65 (6.68)	0.182
Somatic (12–18)			
Screening	[39] 3.59 (2.64)	[41] 4.00 (3.29)	
Week 0 (baseline)	[39] 3.36 (2.69)	[41] 4.17 (3.19)	
Week 4	[39] 2.28 (1.97)	[42] 3.26 (3.16)	0.254
Week 8	[35] 2.51 (2.23)	[38] 2.71 (2.74)	0.617
Week 12	[30] 2.30 (1.95)	[37] 2.73 (3.00)	0.608
Vasomotor (19–20)			
Screening	[40] 4.80 (1.26)	[42] 4.57 (1.23)	
Week 0 (baseline)	[40] 4.05 (1.58)	[42] 4.43 (1.64)	
Week 4	[39] 2.85 (1.63)	[42] 3.64 (1.86)	0.084
Week 8	[35] 2.74 (1.40)	[39] 3.46 (1.89)	0.099
Week 12	[31] 2.39 (1.48)	[37] 3.22 (1.83)	0.099
Anxiety (1–6)			
Screening	[40] 5.05 (3.10)	[42] 5.93 (3.83)	
Week 0 (baseline)	[38] 4.79 (3.13)	[42] 5.76 (3.84)	
Week 4	[39] 3.64 (3.38)	[41] 4.56 (3.34)	0.581
Week 8	[35] 3.43 (2.63)	[39] 4.54 (3.03)	0.250
Week 12	[31] 3.00 (2.25)	[37] 4.32 (3.34)	0.142
Depression (7–11)			
Screening	[40] 4.60 (3.40)	[42] 4.45 (3.48)	
Week 0 (baseline)	[39] 4.36 (3.19)	[42] 4.83 (3.74)	
Week 4	[39] 2.95 (3.35)	[42] 4.19 (3.56)	0.070
Week 8	[34] 2.94 (2.13)	[39] 3.62 (3.25)	0.543
Week 12	[31] 2.48 (2.06)	[37] 3.35 (3.55)	0.389
Sex (21)			
Screening	[39] 1.67 (1.08)	[42] 1.40 (1.04)	
Week 0 (baseline)	[39] 1.46 (1.17)	[42] 1.43 (1.04)	
Week 4	[38] 1.26 (1.03)	[42] 1.29 (1.02)	0.829
Week 8	[34] 1.18 (1.00)	[39] 1.23 (0.96)	0.907
Week 12	[30] 1.00 (0.91)	[37] 1.05 (1.00)	0.923
Total score (1–21)			
Screening	[38] 19.29 (7.96)	[41] 20.10 (10.12)	
Week 0 (baseline)	[36] 17.61 (9.17)	[41] 19.93 (10.41)	
Week 4	[38] 12.84 (9.61)	[41] 16.68 (10.72)	0.202
Week 8	[33] 12.73 (6.94)	[38] 15.21 (9.00)	0.694
Week 12	[29] 11.28 (6.63)	[37] 14.65 (10.66)	0.243

[a] Statistical analysis was performed using analysis of covariance (ANCOVA). p-Values less than 0.05 are significant.
[b] [n] mean (SD) are presented.

An analysis was conducted on all subjects taking at least one dose of study product (intent-to-treat). A total of 72 and 79 adverse events were reported by subjects in the genistein and placebo groups, respectively. Twenty nine of the 41 subjects (70.7%) on genistein and 33 of the 42 subjects (78.6%) on placebo experienced one or more adverse events. There was no significant difference between subjects on genistein or placebo with respect to the number of subjects experiencing at least one adverse event ($p = 0.885$). There were 39 adverse events that were assessed as being possibly related to product by the Medical Director. There were no adverse events assessed by the Medical Director as probably or most probably related to product. Of the 39 adverse events possibly related, 19 were found to be in subjects on genistein and 20 in subjects on placebo. Of the 19 adverse events possibly related to genistein, 14 were mild in intensity, 4 were moderate in intensity and 1 was severe in intensity (increase in hot flushes) (Table 5). Six of the adverse events experienced by subjects on placebo were categorized as moderate in intensity with the remaining 14 categorized as mild in intensity (Table 5). There were no serious adverse events that occurred during the course of the study. Breast soreness and or tenderness, significant increase in hot flushes, spotting, bloating and recurrent headaches were reported by subjects in both groups but there were no differences between groups (Table 5). There were also no significant changes in routine biochemistry, liver function, or hematology results in all subjects taking at least one dose of study product.

Table 4
Mean FSH and 17β-estradiolconcentrations and endometrial thickness in subjects on genistein or placebo groups at baseline and week 12 of treatment.

	Study group		
	Genistein ($n = 40$)	Placebo ($n = 42$)	p value[a]
FSH (IU/L)			
Screening	[39] 77.18 (28.43)[b]	[42] 75.29 (28.18)	
Week 0 (Baseline)	[39] 76.21 (26.47)	[39] 72.56 (26.69)	
Week 12	[36] 71.91 (23.53)	[39] 73.13 (29.22)	0.225
17β-Estradiol(pmol/L)			
Week 0 (Baseline)	[39] 114.4 (93.1)	[39] 107.2 (68.1)	
Week 12	[36] 131.1 (189.0)	[40] 109.2 (55.9)	0.504
Endometrial thickness (mm)			
Screening	[25] 4.28 (1.98)	[28] 3.66 (1.21)	
Week 12	[14] 4.89 (2.76)	[18] 4.18 (1.92)	0.548

[a] Statistical analysis was performed using analysis of covariance (ANCOVA). p-Values less than 0.05 are significant.
[b] [n] mean (SD) are presented.

4. Discussion

The objective of the current study was to evaluate the efficacy of synthetic aglycone genistein in alleviating menopausal symptoms in healthy postmenopausal women. The trial duration (12 weeks) was consistent with the recommendations of the US Food and Drug Administration (FDA). The FDA also recommends enrolling women with ≥7 hot flushes/day. Although the eligibility criteria in this study allowed for women to be enrolled with slightly less than this amount (~6/day), the mean baseline hot flush frequency (mean ± SD) in the genistein and placebo groups was 9.43 ± 4.01 and 9.89 ± 4.25, respectively. Thus, this study represents one of the few trials evaluating isoflavones to conform to the FDA guidelines and the only genistein-only trial to do so.

The intention to treat analysis showed that women on genistein demonstrated a greater reduction in daily hot flushes compared with the placebo group, from 9.4 to 4.7 per compared to 9.9–7.1. Women in the genistein group completing 12 weeks on the trial experienced a mean 51% decrease in hot flush frequency, which was statistically significantly different from the mean 30% reduction in the placebo group. These results are similar to those observed in a 12-week study by Crisafulli et al. [13] in which postmenopausal Italian women were given 54 mg/day genistein, but the reduction was less than that reported in another Italian study which used the same genistein dose (15). However, in the latter study, in comparison to baseline the actual mean reduction in hot flush frequency in the genistein group was ~41% (hot flushes increased slightly in the placebo group). Thus, the results of these three genistein studies are similar and supportive of efficacy. In contrast, in another Italian genistein study 90 mg/day had no effect on menopausal symptoms when all women were included in the analysis (14). However, there was a significant decrease in symptoms among women ($n = 41$) with a mean hot flush score (frequency times severity) ≥9 (31.25 vs. 20%, $p = 0.02$). Some previous work [6,9] but not all [11] has suggested isoflavone efficacy to be related to baseline hot flush frequency. Nevertheless, the evidence overall indicates a role for genistein in alleviating menopausal symptoms.

In contrast to frequency, genistein was without effect on hot flush severity. In the only one of the three genistein-only studies to separately evaluate this parameter, D'Anna et al. [15] found that 54 mg/day genistein significantly reduced mean severity by about 25% in comparison to baseline. Whether the larger genistein dose used in that trial contributed to the differing results is unknown.

Table 5
Adverse events experienced by subjects on genistein or placebo and classified as possibly related to the study products.

Event	Genistein	Placebo
Breast-sore, tender	2	3
Severity	Mild (2)	Mild (3)
Action	None	None
Cramps/cramps and bleeding	Cramps (2) cramps and bleeding (1)	0
Severity	Mild (2) moderate (1)	N/A
Action	None	N/A
Hot flushes-significant increase in	1	1
Severity	Severe	Moderate
Action	Test article discontinued	Con med required
Spotting	2	1
Severity	Mild (2)	Mild
Action	Test article interrupted for 3 days	None
Vaginal bleeding	1	0
Severity	Moderate (1)	MildN/A
Action	Test article discontinued	N/A
Vaginal odor – strong	1	0
Severity	Moderate	N/A
Action	None	N/A
Bloating	2	6
Severity	Mild	Mild (4) moderate (2)
Action	Test article discontinued (1) None(1)	None
Heart burn	0	1
Severity	N/A	Moderate
Action	N/A	Con med required
Nausea/recurrent headaches	Nausea (4) recurrent headaches (1)	Recurrent headaches (1)
Severity	Moderate (1) mild (3)	Mild
Action	None (3) test article discontinued (1)	Con med required
Stomach ache	0	1
Severity	N/A	Mild
Action	N/A	None
Stomach upset/GI upset	0	3
Severity	N/A	Mild (2) moderate (1)
Action	N/A	None
Frequent urination	1	0
Severity	Mild	N/A
Action	Test article discontinued	N/A
Insomnia	0	1
Severity	N/A	Mild
Action	N/A	None
Body itching	1	0
Severity	Mild	N/A
Action	None	N/A
Increasingly emotional	0	1
Severity	N/A	Mild
Action	N/A	None
Increase in perspiration	0	1
Severity	N/A	Moderate
Action	N/A	Test article discontinued

However, it is important to note that there was no decrease in the severity of hot flushes in either the placebo or genistein group suggesting that this parameter may not have been captured effectively in the current study.

The current study is the first to document the effects of genistein on the Greene Climacteric Scale, which is a standard scale for the measurement of climacteric symptoms [25]. There were no significant changes although the reduction in the vasomotor symptoms in the Greene Climacteric Scale closely reflected the reductions in hot flush frequency in the genistein and the placebo groups. Evidently, the Green Climacteric Scale includes many menopausal symptoms unaffected by genistein.

That efficacy was achieved in the current study despite genistein being administered in a single dose is especially noteworthy. Dividing the dose would have likely led to higher sustained circulating genistein levels but requiring two doses in free living populations could compromise effectiveness since compliance is inversely related to the frequency of prescribed dose [26].

There is little direct basis for comparing the efficacy of different non-hormonal treatments for the alleviation of hot flushes. In one study that included multiple treatments, neither black cohosh nor red clover was more effective than placebo at alleviating hot flushes [27]. In another study that included black cohosh and counseling to increase soy intake, neither treatment alleviated hot flushes, but on average subjects consumed only about one serving of soy per day – far too little to provide the estimated 50 mg of isoflavones needed to alleviate hot flushes [28]. In a review by Nelson [29], soy extracts and red clover were shown to reduce the number of hot flushes per day by 1.15 and 0.44, respectively, versus 2.6 for oral estrogen. However, the isoflavone data were based on only 5 trials. Perhaps the most relevant data come from a review by Williamson-Hughes et al. [11], which reported that genistein-rich isoflavone products were more consistently efficacious than those low in genistein. However, this conclusion was based solely on whether the results of an individual study were statistically significant, not on the degree to which hot flushes were reduced. Certainly, it is well accepted that at least in regard to receptor binding and transactivation, genistein is the most potent isoflavone [30]. Nevertheless, only a large multi-arm study in which different treatments are directly compared can determine definitively which isoflavones and isoflavone-rich products are most efficacious.

Importantly, no differences in adverse events were noted between women in the placebo and genistein groups. Also, there were no differences between groups in routine biochemistry, liver function, hematology, circulating levels of FSH and 17β-estradiol and endometrial thickness. These results are generally consistent with the published literature for isoflavones [31–33]. However, a 5-year Italian study by Unfer et al. [34] found that 90 mg/day of mixed isoflavones led to a slight increase in the mean incidence of simple endometrial hyperplasia in postmenopausal women. Simple hyperplasia very infrequently progresses to cancer and is a reversible condition [35]. Furthermore, this study [35] has been criticized on several grounds [36,37]. Also, although not a design flaw, the lack of hyperplasia in the placebo group suggests the results may have occurred by chance. If for example, the percentage of women developing hyperplasia in the placebo group in the study by Unfer et al. [34] was the same as that in the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial, differences between the placebo and isoflavones would certainly not have been statistically significant [38]. Furthermore, all of the data indicate that there are no stimulatory effects of genistein or isoflavones on endometrial tissue for the first three years of use [34,39].

The findings in this study add to the published data supporting the safety of genistein. The current body of evidence includes four animal toxicology studies in two species [17–20], two dose tolerance studies in young adults [21,22] and one multiple oral dose

study in postmenopausal women [24]. The chronic safety studies in rats and dogs [17,19], are accepted by the US Food and Drug Administration as alternatives to long-term population studies in humans and required for approval as a dietary supplement and as a GRAS food additive. Support for the safety of genistein also comes from studies in which postmenopausal women were administered 54 mg/day genistein for up to three years [13,15,33]. This research shows adverse events did not differ between the genistein and placebo groups and that no effects on safety-related endpoints were noted including breast tissue density, endometrial thickness and thyroid function. As hot flushes dissipate over time it is not likely that women will require consuming genistein for >3 years

The most controversial issue involving isoflavone exposure concerns the potential risk these molecules pose to breast cancer patients and women at high risk of developing this disease. Concerns are based on the stimulatory effects of genistein on the growth of mammary tumors in ovariectomized, athymic mice implanted with estrogen-receptor positive human breast cancer cell [40]. No trials have examined the effects of isoflavones from soyfoods or supplements on tumor recurrence in breast cancer patients, but the clinical data indicate that in contrast to menopausal hormone therapy, isoflavone exposure does not adversely affect markers of breast cancer risk such as breast tissue density and breast cell proliferation [41]. Further, recent epidemiologic data indicate that the post-diagnosis consumption of isoflavone-rich soyfoods actually improves prognosis and does not interfere with the efficacy of tamoxifen [42,43]. Also, three-year data show 54 mg/day genistein does not affect breast tissue density and actually maintained BRCA1 and BRCA2 expression whereas expression decreased in the placebo group [33]. Thus, the weight of the evidence indicates isoflavone exposure is safe. Nevertheless, because long term definitive safety data are not available, breast cancer patients should discuss use of genistein-rich products with their primary healthcare provider.

5. Conclusions

The current study provides the first evidence that 30 mg/day synthetic genistein taken in a single dose reduces menopausal symptoms without producing adverse effects. Based on the large body of safety data, including research in two animal species and in studies up to three years in duration in humans, synthetic genistein may be viewed as a safe alternative to estrogen for short term use for the alleviation of menopause-related hot flushes.

Contributors

James Elliott, Bob Berman and Najla Guthrie contributed to the objectives and design of the study. James Elliott and Bob Berman had no role in the execution of the study or the statistical analyses. Mal Evans was involved in the execution of the study and both Mal Evans and Prachi Sharma contributed to the writing of the study manuscript. James Elliott and Robert Berman helped with the editing and review of the document.

Competing interest

The authors Elliott and Berman are employees of DSM Nutritional Products, Inc. the company which funded the study. The other authors have no competing interest.

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Ethical approval

The study was conditionally approved by DSM Nutritional Products, Inc.

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