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Review

Isoflavones from red clover (Promensil®) significantly reduce menopausal hot flush symptoms compared with placebo

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Abstract

Objectives: To investigate the effectiveness and safety of a red clover isoflavone dietary supplement (Promensil, Novogen Ltd., Australia) versus placebo on the change in hot flush frequency in postmenopausal women. *Methods:* In this randomized, double blind, placebo-controlled trial 30 women with more than 12 months amenorrhoea and experiencing more than five flushes per day were enrolled. All received single blind placebo tablets for 4 weeks and were subsequently randomized to either placebo or 80 mg isoflavones for a further 12 weeks. Efficacy was measured by the decrease in number of hot flushes per day and changes in Greene Climacteric Scale Score. *Results:* During the first 4 weeks of placebo the frequency of hot flushes decreased by 16%. During the subsequent double blind phase, a further, statistically significant decrease of 44% was seen in isoflavones group ($P < 0.01$), whereas no further reduction occurred within the placebo group. The Greene score decreased in the active group by 13% and remained unchanged in the placebo group. *Conclusion:* In this study, treatment with 80 mg isoflavones (Promensil) per day resulted in a significant reduction in hot flushes from baseline. At the end of the study there was a significant decrease in hot flushes of 44% between the active and placebo group, demonstrating the effectiveness of Promensil in the management of hot flushes.

Keywords: Menopause; Isoflavone; Phytoestrogen; Hot flush

1. Introduction

Menopausal symptoms, especially hot flushes, have been reported to vary between countries. Postmenopausal women in Europe and North America report an incidence of hot flushes as high as 70–80%, while women in Japan, China and Southeast Asia, report the rate of 25 [1], 18 [2] and 14% [3], respectively [4]. It has been speculated that these differences may be due to estrogenic plant compounds in their diets. Traditional diets in South American, Mediterranean and Asian countries are high in many different varieties of legumes [5]. One such group of plant compounds, which are present in legumes, is the isoflavones. Isoflavones are molecules with similar spatial chemical structures to steroids. They have been shown in vitro to bind and interact with the

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estrogen receptor (ER), predominantly the ER beta form of the receptor, and exert a weak estrogenic effect [6]. Isoflavones are now being extracted and produced as dietary supplements for a variety of indications, some of which are currently treated predominantly with steroidal estrogens. The study medication (Promensil) is a standardized extract of red clover isoflavones that has passed independent testing for label accuracy. Each tablet delivers 40 mg of formononetin, daidzein, biochanin and genistein [7] in the biologically active aglycone forms.

Randomized placebo-controlled studies are necessary for proof of any pharmacological effect. This is especially true when testing products for indications that have a potentially high placebo effect. There have been conflicting reports on the efficacy of such products for the treatment of hot flushes [8–11]. Differences could be attributed to poor study design with low patient numbers, lack of control of dietary intake of isoflavones and inclusion of patients with too few hot flushes per day at baseline. Recently studies have adopted more stringent selection criteria [12,13], such as more than five hot flushes per day at randomization, a criterion that has also been used in hormone replacement therapy (HRT) studies [14].

This study was designed to investigate the efficacy in reduction of the number of hot flushes in Dutch postmenopausal women with a standardized isoflavone preparation. Hot flushes are the principal menopausal symptom of concern in Dutch postmenopausal women [15]. It has also been established that in The Netherlands the diet is generally low in legumes [16], which may make it easier to control the dietary isoflavone intake of the participants.

2. Methods

2.1. Study design

This study was double blind, randomized placebo-controlled trial with a single blind, 4 weeks, placebo screening phase. The local Ethics Committee approved the protocol and participants gave informed consent before the start. Participants were given a list of 'foods to avoid', which included legumes and isoflavone supplements. They were instructed to record the number of hot flushes each day throughout the study on a diary and to score a list of 21 symptoms [17] as non-existent, mild, moderate or severe. The baseline hot flush count was calculated as the average count of the last 7 days from the 4 weeks screening phase. Only the number of hot flushes were used as an inclusion criteria, not the severity of hot flushes. Women with an average of more than five hot flushes per day were then randomized to either two tablets (Promensil, 40 mg) or two placebo tablets, and were instructed to take these tablets every morning for the entire 12 weeks. Both types of tablets (active and placebo) were in identical in size, color, and weight. During the 4 weeks run-in phase only placebo tablets were used (single blinded).

The isoflavones in the active tablets were manufactured from three varieties of red clover using a standardized extraction and blending process to obtain a proprietary ratio of daidzein, genistein, biochanin and formononetin. A 24 h urine specimen was collected at screening (visit 1), before randomization (visit 2) and at study end (visit 3). All study medication for the second phase of the study was numbered by the Hospital Pharmacy. Each batch number was put in a blank sealed envelope. All envelopes were shuffled and then again numbered from 1 to 36 on the outside and handed over to the study nurse who gave out envelopes in successive numbers. Participants took their envelope to the pharmacy where the number in the envelope was matched with the batch number on the medication. Inclusion would stop at either 30 participants included or after 6 months of active screening. In total 30 numbers were given out after 6 months screening.

2.2. Study participants

Thirty symptomatic postmenopausal women, aged 49–65 years were recruited into the study. Postmenopause was defined as at least 12 months amenorrhoea. Women were excluded if they received HRT or antibiotics within 12 weeks of study entry, had undiagnosed vaginal bleeding,

active liver or renal disease, a history of allergy for foodstuffs or a previous history of malignancy, cardiovascular disease or thromboembolism.

2.3. Urinary isoflavone analysis

The total volume of the 24 h specimen was measured and recorded before a 100 ml aliquot was collected and stored at −8 °C. The frozen samples were shipped on dry ice and assayed at Novogen Laboratories (North Ryde, Australia) using high performance liquid chromatography [18].

2.4. Statistical analysis

Study data were analyzed using Statistics for Windows 5.1 (Statsoft Inc., Tulsa, OK, USA) and SAS for Windows. Differences in baseline parameters were tested using two sample *t*-tests. Fisher's Exact test was used to test the proportions of participants in each treatment group who were above or below the overall median percentage change in hot flush count from the week prior to randomization to week 4, 8 and 12 (study end). The median was used because the data were not normally distributed, and the median therefore, provided a more accurate representation of the data. Efficacy data recorded after randomization were used for analysis. The evaluable sample included 11 datasets from the placebo group and 15 datasets from the isoflavones group. Analysis was according to Intent-to-Treat (ITT).

3. Results

Forty-two women were screened and 30 were randomized during the 6 months screening period (16 to active treatment and 14 to placebo). Six participants were ineligible due to <5 hot flushes per day, four did not return to the clinic and two recorded inadequate data on the diaries. The age, weight, height and body mass index (BMI) of the randomized group are shown in Table 1 and show no significant differences between the two groups at randomization. Three women withdrew from the isoflavone group and three from the placebo group. Principal reason for withdrawal was lack of efficacy. Tolerability was generally good and the active group showed no more side effects than the placebo group.

3.1. Hot flushes

There was no difference in the hot flush count between the two groups at baseline. The primary endpoint assessed was the reduction in hot flushes from baseline to 12 weeks treatment. In the single blind run-in, for all patients, there was a median decrease in the number of hot flushes from 6 to 5 (−16.7%). When the results from the 4 weeks run-in were grouped according to the prospective treatment or placebo arm in the double blind phase, there was no statistically significant difference in decrease of hot flushes for the group later randomized to isoflavones than for the group later randomized to placebo group.

The median values for the percentage change throughout the 12-week study for the placebo and isoflavones treated groups of the double blind phase are shown in Fig. 1.

The median percentage change of hot flushes for placebo remained close to baseline over the 12 weeks, while the median change for isoflavones reduced by 44% over the same period. The sharpest decline occurred between weeks 1 and 3 with a decrease of −33%. Hot flushes continued to decrease to a maximum of −56% at week 10, and then leveled at −44% at weeks 11 and 12. Fisher's exact test demonstrated significance at

Table 1
Baseline characteristics of study participants

	Placebo ($n=14$)	80 mg Promensil ($n=16$)	P[a]
Age at trial start	52.5 ± 5.2 years	54.2 ± 7.4 years	0.2
Weight	67.8 ± 8.8 kg	70.6 ± 15.2 kg	0.56
Height	165.2 ± 6.7 cm	163.4 ± 5.8 cm	0.44
BMI	24.8 ± 3	26.4 ± 5.4	0.35

Values are given as mean ± SD.
[a] P values calculated from Student's *t*-test.

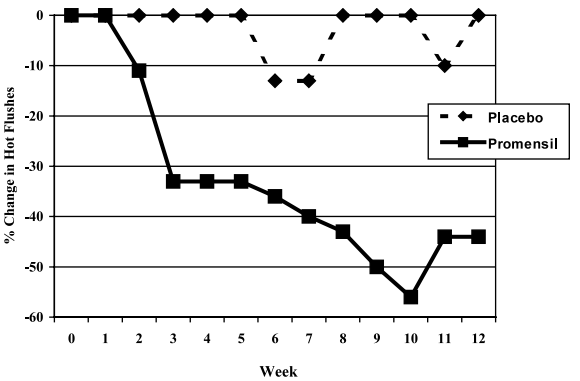

Fig. 1. Median percentage change in hot flushes during the 12 weeks double blind phase*. *Median values for all data at W4 = 0, W8 = −22.6 and W12 = −26.7%. The difference in the proportion of patients above and below the median was tested at weeks 4, 8 and 12. It was significant using the Fisher's Exact Test between placebo and active at weeks 8 and 12 ($P = 0.0154$).

week 8 for a median of −22.5% change in hot flushes from baseline ($P = 0.0154$) and week 12 for the median of −26.7% change ($P = 0.0154$). Only two of 11 (18%) placebo participants responded below the median, while 11 of 15 (73%) isoflavone users responded at week 12. The mean values for the hot flush count are shown in Table 2.

3.2. Greene score

Overall menopausal symptoms, as measured by the Greene score, were observed to be slightly reduced in the active group while the overall score for the placebo group slightly increased (Table 2). The difference between groups was not large enough to reach a level of significance.

3.3. Urinary isoflavone excretion

Total isoflavone excretion did not change significantly among women in the placebo group, however excretion of total isoflavone increased significantly for women in the active treatment group from baseline to study end ($P = 0.0005$); and from randomization to study end ($P = 0.027$) (Table 3).

Data was graphed for the individual and total isoflavone levels against the mean hot flush count for the week at which urine was collected. There were three urine collections per participant. Total urinary isoflavone excretion (mg/24 h) values against the hot flush count (per 24 h) are shown as Fig. 2. Statistical or correlation analysis was not appropriate because the data were not independent. Graphical representation of each individual isoflavone against the hot flush count revealed similar trends but are nor shown.

4. Discussion

This study demonstrates that following 12 weeks treatment with 80 mg isoflavones (Promensil) the hot flush count reduced by 44% while there was no further change in hot flush frequency of the placebo group. The difference was statistically significant at both weeks 8 and 12. Other menopausal symptom studies have revealed a high

Table 2
Change in Greene score and hot flush count

	Randomization	Week 4	Week 8	Week 12
Greene score				
Placebo ($n = 11$)	13.75 ± 9.5	15.08 ± 12.87	13.45 ± 11.6	14.55 ± 11.8
Promensil ($n = 15$)	12.5 ± 11.2	11.73 ± 8.06	11.27 ± 8.5	10.9 ± 9.89
Hot flushes				
Placebo ($n = 11$)	5.75 ± 5	5.32 ± 3.3	5.9 ± 4.9	6.04 ± 5.5
Promensil ($n = 15$)	5.43 ± 2.6	4.53 ± 3.4	3.74 ± 2.9	3.35 ± 3

Data are presented as mean ± SD. The Greene score is a 21-symptom measure, which does not include the number of hot flushes. Hot flushes are shown separately and are the weekly average of the number of hot flushes per day.

Table 3
Mean isoflavone excretion (mg/24 h) by treatment group at baseline, week 4, and end of study (week 12)

	Baseline	Randomization	*P*	Week 12	*P*
Placebo (± SD)	0.3 ± 0.6	1.1 ± 2	NS	2.7 ± 4.2	NS
Promensil (± SD)	0.3 ± 0.5	1.8 ± 3.3	NS	5.2 ± 3.8	0.0005[a]

SD, standard deviation; NS, not significant from baseline.
[a] Significant difference from baseline (Student's *t*-test).

placebo effect [20]. Use of a placebo tablet during the run-in phase to identify participants that might be partial to its influence has been suggested as a way of normalizing the study group by accounting for potential spurious inaccuracies at the outset. The handling of the study in this way may allow one to better concentrate on the outcomes as treatment specific effects. This strategy has been employed in studies of hypertension [21], diabetes [22], asthma [23], premenstrual syndrome [24], and psychological studies [25]. The lack of placebo effect during the double blind treatment phase may be explained by the 16% reduction of hot flushes occurring during this type of run-in phase which is equivalent to the level of the placebo effect on hot flush reduction in other studies using non-prescription therapies [8,10].

While only 18% of the placebo participants reported less severe hot flushes after 12 weeks, 73% of the Promensil participants reported an improvement at weeks 8 and 12. These results were confirmed by the study of Jeri and Romana [12] where the treatment group reported a statistically significant reduction of 43.9% in the frequency of hot flushes per day compared to 5.5% in the control group. Other factors, which may have contributed to the results of the current study when compared to other studies that were done with isoflavones, are that in this study 80 mg of isoflavones per day was used, while in other studies 40 mg was given. Also the entry criteria of more than five hot flushes per day rather than three might have been of influence. The only study using a soy preparation and showing a statistically significant result did include women with at least seven hot flushes per day [26]. Drawbacks in prior negative, placebo-controlled, trials with Promensil [8,9] might have been that in these studies mildly symptomatic or perimenopausal individuals were included and that the dietary intake of isoflavones was not controlled. We believe the latter to be an important point as in order to attribute any improvement of the measured symptom; subjects must not be allowed to obtain isoflavones externally during the placebo phase. Part of the apparent placebo effect in many previous trials of hot flush incidence has been attributed to inadvertent or deliberate intake of isoflavone-rich foods during the trial period, even when a higher dose of 160 mg per day is used [9]. In this trial, the absence of a placebo effect at weeks 4, 8, and 12 is consistent with this proposition as the intake of isoflavone-containing foods was strictly controlled in the placebo group and in any case the Dutch population have low background levels of isoflavones in their diet. Studies of prescription products generally are not affected by these considerations, as potential participants in placebo groups would not have access to the test compounds from the natural environment. A further potential confounding factor was raised in a report of a recent estrogen trial, which reported that perimenopausal women have a higher placebo effect than postmenopausal women [27]. Such issues must be taken into consideration when designing any study assessing the effectiveness of dietary supplementation. Results of previous clinical studies assessing the effects of isoflavones on menopausal symptoms have been varied but there has been epidemiological, anecdotal and clinical evidence to support the use of isoflavones for hot flushes. Although, the effects in this regard have not been as profound as those observed with HRT, it is important that there has been no evidence of adverse estrogenic effects on the endometrium with red clover isoflavones

[18,28], alleviating concerns of undesirable side effects associated with these prescription therapies. A recent single case report of a woman diagnosed with endometrial cancer and a history of taking supplements (some possibly containing phytoestrogens) suggested that additional research is needed concerning endometrial safety [19].

Since isoflavone bioavailability varies between individuals and access to food-sourced isoflavones is difficult to control, a more accurate way of demonstrating the physiological effectiveness of isoflavone-based therapies may be through analyzing excretion (as a reflection of intake) versus specific symptom or laboratory parameters. The graph shows that participants, who absorbed and metabolized greater amounts of isoflavones, had lower hot flush counts. This has also been reported in another red clover study [8] and in a more recent Japanese community-based prospective study [29]. Different reasons may account for the variability in absorption [30]. Some studies have shown that people with higher dietary intakes of carbohydrates and less fat produced higher levels of metabolites [31]. Gut flora are also an important part of isoflavone metabolism as bioavailability depends on an individual's gut microflora [32]. It would be interesting to assess in larger future studies the relationship between isoflavone correlations and menopausal symptoms. This study indicates the view that the use of a red clover isoflavone dietary supplement is effective in alleviating the acute hot flush symptoms of menopause. While isoflavones can be obtained from the diet, dietary modification is a difficult option because of the need to ingest large quantities of legume food plants and the variability in intake. Although, a range of isoflavone dietary supplements are now available, those produced from red clover offer the benefit of containing all four important isoflavones and Promensil provides these in a form standardized with respect to concentration and ratio.

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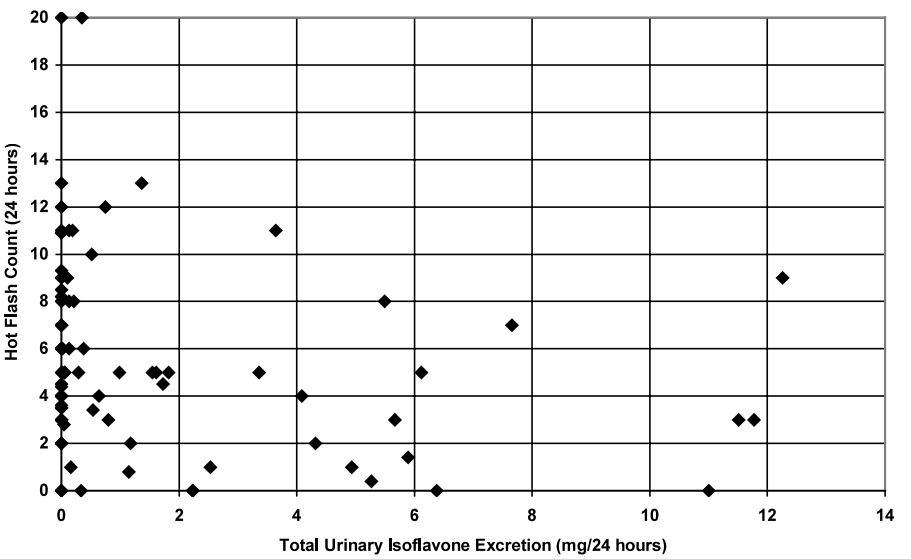

Fig. 2. Graph of hot flush count over 24 h and total urinary isoflavone excretion (mg/24 h). Three urine samples per patient at three points throughout the study.

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