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# *Trifolium pratense* isoflavones in the treatment of menopausal hot flushes: A systematic review and meta-analysis

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## Abstract

*Objective*: To critically assess the evidence of supplements containing *Trifolium pratense* (red clover) isoflavones in the reduction of hot flush frequency in menopausal women.

*Data sources*: Systematic literature searches were performed in (Medline (1951 – April 2006), Embase (1974 – April 2006), CINAHL (1982 – April 2006), Amed (1985 – April 2006) and The Cochrane Library (Issue 2, 2006). Reference lists located were checked for further relevant publications. Experts in the field and manufacturers of identified products were contacted for unpublished material. No language restrictions were imposed.

*Review methods*: Studies were selected according to predefined inclusion and exclusion criteria. All randomized clinical trials of monopreparations containing *T. pratense* isoflavones for treating hot flushes were included. Study selection, data extraction and validation were performed by at least two reviewers with disagreements being settled by discussion. Weighted means and 95% confidence intervals were calculated and sensitivity analyses were performed.

*Results*: Seventeen potentially relevant articles were retrieved for further evaluation. Five were suitable for inclusion in the meta-analysis. The meta-analysis indicates a reduction in hot flush frequency in the active treatment group (40–82 mg daily) compared with the placebo group (weighted mean difference −1.5 hot flushes daily; 95% CI −2.94 to 0.03; $p = 0.05$).

*Conclusion*: There is evidence of a marginally significant effect of *T. pratense* isoflavones for treating hot flushes in menopausal women. Whether the size of this effect can be considered clinically relevant is unclear. Whereas there is no apparent evidence of adverse events during short-term use, there are no available data on the safety of long-term administration.



## Introduction

Since the early termination of the Women's Health Initiative study of hormone replacement therapy due to an increase in overall health risk, phytoestrogens have become increasingly popular in the management of women's reproductive health. This follows the observation that women in Asia, who consume large quantities of isoflavones in their diet, have a lower incidence of menopausal symptoms, cardiovascular disease, osteoporosis and hormone related cancers (Anderson et al., 1999).

*Trifolium pratense* (red clover) is a medicinal herb, traditionally used in the treatment of chronic skin

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diseases and whooping cough (Ernst et al., 2006), that contains at least four estrogenic isoflavones: formononetin, biochanin A, daidzen and genistein (Capasso et al., 2003; Setchell et al., 2001). Supplements containing isoflavones derived from *T. pratense* isoflavones are promoted worldwide for the treatment of menopausal symptoms and the maintenance of health and welfare after the menopause (Ernst et al., 2006).

The objective of this systematic review and meta-analysis is to critically assess the evidence of supplements containing *T. pratense* (red clover) isoflavones for reducing hot flush frequency in menopausal women.

## Methods

### Identification of clinical trials

In order to identify clinical trials involving isoflavones derived from *T. pratense* in the treatment of hot flushes in menopausal women, systematic literature searches were conducted in the following electronic databases from their respective inception: Medline (1951 – April 2006), Embase (1974 – April 2006), CINAHL (1982 – April 2006), Amed (1985 – April 2006) and The Cochrane Library (Issue 2, 2006). The search terms used were: red clover, isoflavones, *Trifolium pratense*, cow clover, meadow clover, purple clover, beebread, trefoil, isoflavonoids, daidzen, genistein, formononetin, biochanin, legume, menopause, promensil, rimostil, phytoestrogens, hot flush and hot flash. Further relevant papers were located by hand-searching the reference lists of all papers and departmental files and contacting experts in the field. Hand searching of retrieved references and Internet searches revealed four manufacturers/distributors of *T. pratense* isoflavone products, who were contacted and asked to supply any published or unpublished material.

### Inclusion and exclusion criteria

All randomized controlled trials of oral administration of *T. pratense* isoflavones for treating hot flushes in healthy menopausal women were included. Trials were included if they tested oral preparations containing *T. pratense* isoflavones as the only active component (mono-preparation). Trials were included if they were controlled against placebo or comparator treatment. No restrictions on the language of publication were imposed.

### Data extraction and quality assessment

Data relating to sample size, study design, inclusion criteria, concurrent dietary advice, intervention and control, treatment duration, primary outcome measures, funding source and results were extracted according to predefined criteria (JTC, EE, MHP). The methodological quality of each included clinical trial was assessed using the Jadad scoring system (Jadad et al., 1996), which ranges from 0 (poorest) to 5 (best) and quantifies the likelihood of bias, based on the description of randomisation, blinding and withdrawals. Data on adverse events were extracted from all identified papers. Data extraction and quality assessment were performed independently by one reviewer and validated by a second with any disagreements being settled by discussion (JTC, EE, MHP).

### Analysis

Frequency of hot flushes per day compared with baseline was defined as the primary outcome measure. The change between baseline and endpoint was used to assess differences between the active and placebo groups. Weighted means and 95% confidence intervals were calculated using standard meta-analysis software (RevMan 4.2; Update Software Ltd, Oxford, UK) that uses the inverse of the variance to assign a weight to the mean of the within-study treatment effect. None of the studies reported sufficient information to allow us to directly calculate the variance of the pre-intervention to post-intervention change. The variance of the change can be imputed by assuming a correlation of 0.4 between pre- and post-intervention values (Follmann et al., 1992). Summary estimates were calculated using a random effects model. The $\chi^2$ test for heterogeneity was performed to determine whether the distribution of the results was compatible with the assumption that inter-trial differences were attributable to chance variation alone. A funnel plot was performed to assess publication bias. Sensitivity analyses tested the robustness of the results (MHP).

## Results

A total of 17 potentially relevant clinical trials were identified from the literature searches (Fig. 1) and retrieved for more detailed evaluation (JTC, EE). All trials were published in English and all except one were identified through database searches. Five papers described the administration of *T. pratense* isoflavones to women for the management of hot flushes and were included in the meta-analysis. Twelve studies were excluded from the meta-analysis for the following reasons: not concerned with treating hot flushes (Atkinson et al., 2004a, b; Clifton-Bligh et al., 2001; Hale et al., 2001; Howes et al., 2000; Ingram et al., 2001; Knudson et al., 2001; Nestel et al., 1999; Samman et al., 1999) full results not available (Tice et al., 2003) and no control group (Abernethy et al., 2001; Nachtigall et al., 1999).

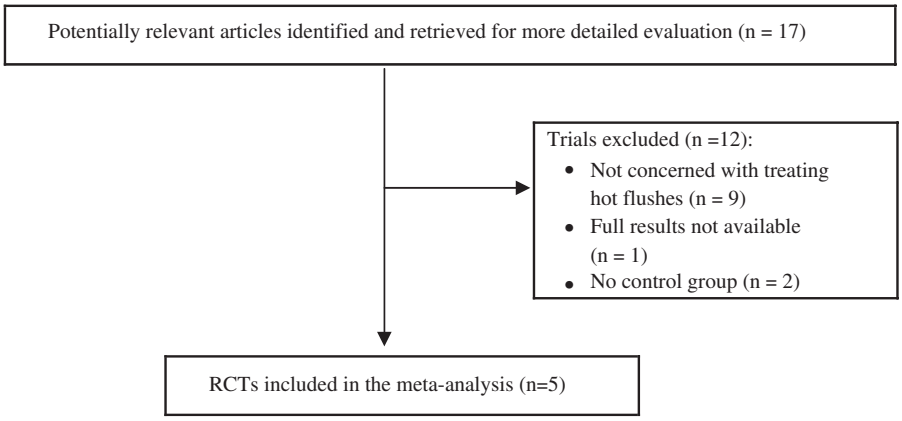


**Fig. 1.** Flowchart of trial selection process.

Characteristics of the five randomized clinical trials (Baber et al., 1999; Jeri, 2002; Knight et al., 1999; Tice et al., 2003; van de Weijer and Barentsen, 2002) meeting the inclusion criteria are summarised in Table 1. All trials used the same standardized preparation (Promensil®) in recommended doses of 40–80 mg/day and assessed hot flush frequency using patient diaries. In two trials comparisons with other standardised doses were also performed: Rimostil® at 57 mg/day (Tice et al., 2003) and Promensil® at 160 mg/day (Knight et al., 1999).

The meta-analysis for hot flush frequency is shown in Fig. 2. Meta-analysis of all trials indicates a difference of borderline statistical significance between women receiving active treatment and those receiving placebo (weighted mean difference −1.45 hot flushes/day; 95% CI −2.94 to 0.03; $p = 0.05$). The $\chi^2$ test for heterogeneity ($p = 0.09$) indicates that the observed differences in results between trials could be caused by factors other than chance. However, since the number of trials was small, it was not felt appropriate to perform formal sensitivity analyses to explore these factors. The sample size ($n = 5$ trials) was not adequate to produce a meaningful funnel plot.

## Sensitivity analysis

Two trials included two groups of patients on active treatment. In the study by Knight et al. (1999), 12 patients received 40 mg/day isoflavones (included in the main meta-analysis) and 13 received 160 mg/day isoflavones. When the data from the higher dose group was included in the sensitivity analysis, the difference between *T. pratense* isoflavones and placebo became slightly larger (weighted mean difference −1.63 hot flushes/day; 95% CI −2.97 to −0.28; $p < 0.02$). Tice et al. (2003) included one group treated with 82 mg/day isoflavones (Promensil; Novogen Ltd, Australia) and one group treated with 57 mg/day isoflavones (Rimostil; Novogen Ltd, Australia). When the data from the group treated with Rimostil was included in the analysis rather than the group receiving Promensil, there was no significant difference between active and placebo treatment (weighted mean difference −1.17 hot flushes/day; 95% CI −2.98 to 0.65; $p = 0.21$).

Using a fixed effects model for the main analysis produced a weighted mean difference of 1.66 hot flushes per day (95% CI −2.49 to −0.84; $p < 0.0001$). Using a correlation factor (Follmann et al., 1992) of 0.5 also had a small impact on the results (weighted mean difference −1.42 hot flushes daily; 95% CI −2.92 to 0.08; $p = 0.06$, random effects model).

## Adverse events

Of the 17 papers describing administration of *T. pratense* isoflavones to human subjects, three included data on adverse events. Ingram et al. (2001) reported that three subjects had experienced adverse events during treatment [cystitis (1; placebo), psoriasis (1; 40 mg daily *T. pratense* isoflavones), thrush (1; 80 mg dialy *T. pratense* isoflavones)]. All were described as mild and no information regarding causality was provided. In the study by Samman et al. (1999) one subject withdrew from the study because the tablets made her feel tense. No further details are provided. Cold or upper respiratory tract infection, headache, myalgia, nausea, arthralgia, and diarrhea were reported by Tice et al. (2003) Twelve reports did not refer to the occurrence of adverse events at all; a general statement such as 'no adverse events reported' was included in two papers (Nachtigall et al., 1999; van de Weijer and Barentsen, 2002). In five trials routine blood tests were performed and these were unchanged. Two trials measured changes in endometrial thickness during treatment and found no significant effects (Clifton-Bligh et al., 2001; Nachtigall et al., 1999). A large randomised clinical trial of the effects of *T. pratense* isoflavones on breast density found no significant difference in changes in breast density after 12 months treatment at recommended doses (Atkinson et al., 2004a).

**Table 1.** Randomized controlled trials of *Trifolium pratense* isoflavones for treating vasomotor symptoms in peri- and post-menopausal women: study characteristics

| First author (Ref.) | Design Jadad score | *N* (randomized/ analysed) | Daily dose (mg) | Control | Trial inclusion criteria | | | Dietary advice | Single blind placebo run-in/ treatment duration | Baseline hot flush frequency, mean + SD (per day) (*T. pratense*/placebo) | Final hot flush frequency, mean + SD (per day) (*T. pratense*/placebo) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hot flush frequency | Duration of amenorrhea (months) | Serum FSH level | | | | |
| van de Weijer [28]§ | Parallel 5 | 30/26 | 80* | Placebo | ⩾ 5/day | ⩾ 12 | Not known | Patients were given a list of isoflavone-rich foods to avoid | 4/12 weeks | 5.43 ± 2.6/ 5.75 ± 5.0 | 3.35 ± 3/ 6.04 ± 5.5 |
| Jeri [16] | Parallel 2 | 30/30 | 40* | Placebo | ⩾ 5/day | > 12 | > 30 mIU/ml | Patients were non-vegetarian and were asked to avoid soy or other estrogen active plant products | None/16 weeks | 7.0 ± 1.94/ 5.7 ± 1.55 | 3.6 ± 1.16/ 5.1 ± 1.16 |
| Baber [5] | Crossover 4 | 51/42/46‡ | 40* | Placebo | > 3/day | > 6 | > 30 mIU/ml | Patients were asked to maintain a normal diet | 1/12 weeks | 5.4 ± 17.95/ 5.49 ± 19.26 | 4.22 ± 20.86/ 3.72 ± 18.79 |
| Knight [17]§ | Parallel 5 | 37/37 | 40* or 160* | Placebo | ⩾ 3/day | ⩾ 6 | > 40 1U/l | Patients were non-vegetarian and asked not to change their dietary habits | None/12 weeks | 6.9 ± 2.1/ 8.6 ± 4.6 (40 mg) 9.0 ± 5.2/ 8.6 ± 4.6 (160 mg) | 4.9 ± 4.8/ 5.8 ± 4.5 (40 mg) 5.9 ± 4.6/ 5.8 ± 4.5 (160 mg) |
| Tice [27]§ | Parallel 5 | 252/246 | 82* or 57† | Placebo | ⩾ 35/ week | > 6 | > 30 mIU/ml | Patients were non-vegetarian and consumed soy products < 1/week | 2/12 weeks | 8.46 ± 5.75/ 7.80 ± 2.36 (82 mg) 8.08 ± 2.96/ 7.80 ± 2.36 (57 mg) | 5.04 ± 4.06/ 5.04 ± 3.47 (82 mg) 5.23 ± 4.10/ 5.04 ± 3.47 (57 mg) |

*Promensil; Novogen Ltd, Australia.
†Rimostil; Novogen Ltd, Australia.
‡51 patients were randomized into this trial; data are available for 42 and 46 patients for red clover and placebo, respectively.
§Funded at least partly by Novogen Ltd.

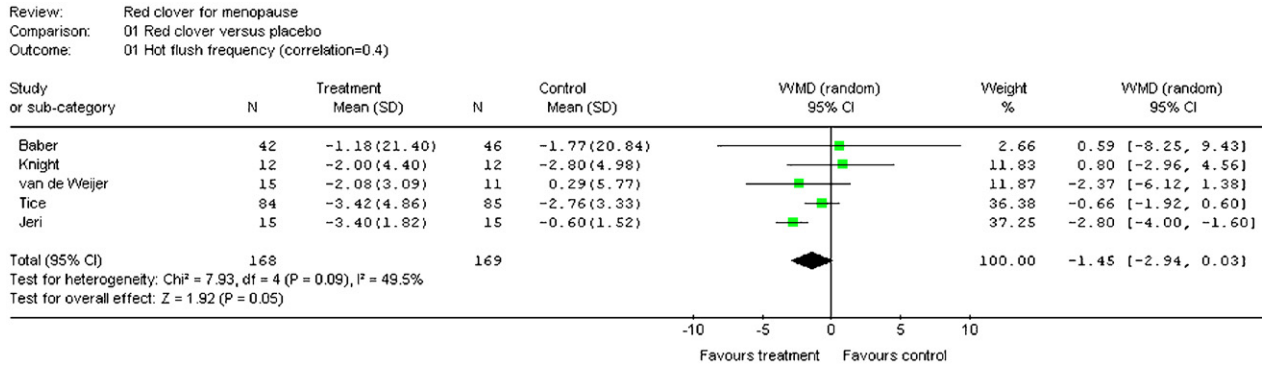


**Fig. 2.** Effects of *Trifolium pratense* isoflavones on the number of daily hot flushes in peri- and post-menopausal women. The mean differences of the change from baseline are given with 95% confidence intervals. The vertical line represents no difference between *T. pratense* and placebo.

## Discussion

There is some evidence in support of the use of *T. pratense* isoflavones in the treatment of hot flushes in menopausal women. The women included in these trials were experiencing an average of five to nine hot flushes per day. Supplementation for 12–16 weeks reduced hot flush frequency by approximately one hot flush per day. Whether the size of this effect would be considered clinically important is unclear.

The test for heterogeneity suggests that the observed differences between studies might be caused by factors other than chance although the power of the test is small. Possible factors include the differences in study design and sample size, differences in the type of dietary advice provided, the definition of hot flush used, genetic and cultural differences in study populations and the inclusion of peri-menopausal and post-menopausal women.

Our result is in contrast with another systematic review and meta-analysis which concluded that data from red clover trials showed no improvement in hot flush frequency (Krebs et al., 2004). The reason for this apparent discrepancy is probably due to differences in the methodology used. In this paper (Krebs et al., 2004), only final values for hot flush frequency at the end of treatment (with either *T. pratense* or placebo) were compared and led to its main conclusion. Differences in baseline values after randomisation were not taken into account; a more accurate method is to compare changes in hot flush frequency from baseline between each group. Our analysis included these considerations, showing data, which are suggestive of differences in effect between *T. pratense* isoflavones and placebo.

Three of the five trials included women who had been amenorrhagic for 6 months or more and therefore also included peri-menopausal women (Baber et al., 1999; Knight et al., 1999; Tice et al., 2003). There is evidence to suggest that women who are peri-menopausal may contribute to a higher placebo response rate in trials of hot flushes (Simon et al., 2001). Trials involving peri-menopausal women therefore require larger patient populations to demonstrate a difference between treatments. It is not clear whether sample size calculations were performed for all these trials, but the sample sizes are for the most part small ($< 50$). It is interesting to note that the two trials which included only women who had been amenorrhagic for 12 months or longer (post-menopausal) produced the largest treatment effects in favor of *T. pratense* isoflavones (Jeri, 2002; van de Weijer and Barentsen, 2002).

There is a paucity of data on the safety of *T. pratense* isoflavones. We were unable to locate any long-term safety data. Epidemiological observations suggest that the consumption of soy-derived isoflavones is protective against endometrial and breast cancer (Anderson et al., 1999), whilst hormone replacement therapy has been associated with an increased risk of breast cancer. There was no evidence of a proliferative effect on either endometrial or breast tissue in the trials identified within this review. Moreover, two of the trials measured endometrial thickness and found no significant increase and a large trial of breast density found no significant changes associated with consumption of *T. pratense* isoflavones. Whether these results can be extrapolated to women taking isoflavones as an alternative to hormone replacement therapy on a long-term basis is unclear.

We found no direct evidence of herb-drug interactions with *T. pratense* isoflavones. However, there is indirect evidence (from animal and in vitro studies) for herb–drug interactions with drugs which are substrates for the P450 CYP3A4 isozymes (Budzinski et al., 2000), oestrogen replacement therapy and oral contraceptives (Burdette et al., 2002). *T. pratense* is known to contain several coumarins which may interfere with blood clotting and have the potential for serious herb–drug interactions with drugs such as warfarin. The supplements used during these trials are assayed by the manufacturer to ensure that there are no coumarins present and there is some evidence that short-term

supplementation does not lead to adverse changes in factors involved in coagulation and platelet activation (Nestel, 2003), although this may not be the case for all *T. pratense* preparations.

Most of the identified clinical trials were funded, at least in part, by the manufacturer of the most commonly available products. This illustrates the need for independent replication of the results, but also the difficulty in obtaining funding for clinical trials of commercial products from independent sources. We did not receive a reply from the three other manufacturers/distributors, which were contacted and asked to provide published and unpublished data.

In conclusion, there is evidence of a marginally significant effect of *T. pratense* isoflavones for treating hot flushes in menopausal women. Whether the size of this effect would be considered clinically relevant is unclear. Whereas there is no apparent evidence of adverse events during short-term use, there are no available data on the safety of long-term administration.

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