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Effects of a standardised extract of Trifolium pratense (Promensil) at a dosage of 80 mg in the treatment of menopausal hot flushes: A systematic review and meta-analysis



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ABSTRACT

Objective: To critically assess the evidence for a specific standardised extract of Trifolium pratense isoflavones (Promensil) at a dosage of 80 mg/day in the treatment of menopausal hot flushes. *Data sources:* Systematic literature searches were performed in Medline, Scopus, CINAHL Plus, Cochrane,

AMED and InforRMIT and citations obtained from 1996 to March 2016. Reference lists were checked; corresponding authors contacted and the grey literature searched for additional publications.

Review methods: Studies were selected according to predefined inclusion and exclusion criteria. All randomised clinical trials of a specific standardised extract of Trifolium pratense isoflavones (Promensil) used as a mono-component at 80 mg/day and measuring vasomotor symptoms were included. The data extraction and quality assessment were performed independently by one reviewer and validated by a second with any disagreements being settled by discussion. Weighted mean differences and 95% confidence intervals were calculated for continuous data using the fixed-effects model.

Results: Twenty potentially relevant papers were identified, with only five studies meeting the inclusion criteria. The meta-analysis demonstrated a statistical and clinically relevant reduction in hot flush frequency in the active treatment group compared to placebo. Weighted mean difference 3.63 hot flushes per day: [95% CI 2.70–4.56]; p < 0.00001). Due to a lack of homogeneity *a priori* defined sub-group analyses were performed demonstrating a substantive difference between cross-over and parallel-arm clinical trial designs.

Conclusion: There is evidence for a statistical and clinically significant benefit for using a specific standardised extract of red clover isoflavones (Promensil) at 80 mg/day for treating hot flushes in menopausal women across the 3 studies included in the meta-analysis. The preparation was safe over the short-term duration of the studies (3 months).

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Introduction

It has been estimated that the vasomotor effects of menopause; hot flushes and night sweats, affect up to 80% of menopausal women in Western countries to varying degrees (Freeman and Sherif, 2007). Although hormone replacement therapy is highly efficacious for the treatment of menopausal symptoms, many women are concerned about its use since the findings of the Women's Health Initiative, which highlighted significant safety concerns (Haas et al., 2004; Rossouw et al., 2002). This combination of factors has led to increased interest in phytoestrogens from both soy and red clover (Posadzki et al., 2013) as a potentially safer approach. The phytoestrogens identified in red clover are isoflavones (formononetin, biochanin A, diadzen and genistein) which are able to act as selective oestrogen receptor modulators (Beck et al., 2005; Brzezinski and Debi, 1999; Hwang et al., 2006).

Several systematic reviews and meta-analyses have been conducted on clinical trials using red clover for the reduction of menopausal symptoms. Overall, these studies suggest a very small positive effect with uncertain clinical relevance (Chen et al., 2015; Coon et al., 2007; Gartoulla and Han, 2014; Ghazanfarpour et al., 2015; Howes et al., 2006; Jacobs et al., 2009; Krebs et al., 2004; Nelson et al., 2006).



Abbreviations: CI, confidence intervals; GMP, good manufacturing practice; WMD, weighted mean differences.

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The comparison of red clover from varied sources is problematic and is part of a wider problem in the review of herbal medicines. Erroneous generalisations can be made about the pharmacological activity of specific plants by comparing different preparative forms and presuming they are the same without providing evidence that they have similar chemical profiles and biological activity (Myers and Smith, 1997).

Additionally, the dosage of red clover in many of the earlier clinical trials may have been too low. Two more recently published trials (Hidalgo et al., 2005; Lipovac et al., 2011) used double the dose of earlier trials (80 mg daily dose) and demonstrated substantial positive results. Estrogenic effects of phytoestrogens have been suggested to occur when a steady plasma concentration of isoflavones of 50–800 ng/ml is reached, this value is similar to that of the Asian population regularly consuming a soy-rich diet (Beck et al., 2005). The chosen investigative dose of 80 mg per day has been shown to be sufficient to raise plasma isoflavones to a level comparable with the plasma isoflavone content of populations consuming a soy rich diet (Howes et al., 2002).

Each of the isoflavones in red clover has a variable effect on the activation or antagonism of the oestrogen receptors (Bolego et al., 2003). The biological activity in red clover cannot be seen simply as the total quantity of isoflavones, but the individual constituent profile. A chemical analysis of a red clover extract isolated 22 separate compounds within the raw material (Booth et al., 2006) giving a unique chemical profile which is dependent upon its extraction method. Different extraction methods using the same batch of Trifolium pratense have been confirmed to show considerable differences in estrogenic activity (Piersen et al., 2004). It is not surprising, therefore, that the hormonal activity of different red clover products also showed differences in estrogenic activity (Beck et al., 2003).

This difference may be due partly to the low quality of certain preparations (Howes et al., 2002), or in part to the amount of aglycones within the extract in comparison to glycosylated isoflavones (Howes et al., 2002; Beck et al., 2005). Aglycone-rich isoflavone supplements are thought to be more bioavailable than glycoside-rich products. The Promensil/Menoflavon extract contains mainly aglycones, which may be more easily absorbed than other red clover preparations (Beck et al., 2005).

Overall, these findings demonstrate that it is critically important for assessment of red clover extracts that the pharmacological activity is determined by comparing clinical trial results from the same standardised extract and not by mixing extracts of differing pharmacological activity. For this reason the present study is limited to the investigation of one specific standardised red clover extract (Promensil) which is marketed widely in the United States, Europe and Australia.

The objective of this systematic review and meta-analysis is to critically assess the evidence for a specific standardised extract of Trifolium pratense (Promensil) at a dosage of 80 mg/day in the treatment of menopausal hot flushes.

Methods

The systematic review and meta-analysis were conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

Search strategy

Systematic literature searches were conducted from 1996 to March 2016 using the following databases: Medline, Scopus, CINAHL Plus, Cochrane, AMED and InforRMIT. Free-text search terms were used for all databases in various combination: red clover; Trifolium; pratense; MF11RCE; Promensil; Rimostil; Menoflavon; phytoestrogen*; menopaus*; hot flush; hot flash; vasomotor; climacteric; postmenopaus*; perimenopaus*; post-meno*; peri-meno*; night sweat*; quality of life; QOL. Additional MeSH terms searched include: Trifolium*; Plant preparations and menopaus*. In addition to database searches, hand searches of the grey literature were carried out and corresponding authors of all identified papers were contacted to determine if they were aware of any unpublished datasets.

Inclusion and exclusion criteria

All randomised controlled clinical trials of Promensil use for vasomotor symptoms in healthy menopausal women at a dose of 80 mg or higher were included. Due to the high incidence of placebo effect in this population, only studies that were placebo controlled were considered. Trials were included if they were controlled against placebo or comparator treatment. The outcome measures for the studies necessitated a measure of vasomotor symptoms. Trials were included if they tested oral preparations containing the specific T. *pratense* isoflavones extract (Promensil) as the only active component (mono-component). No restrictions on the language of publication were imposed.

Data extraction and assessment of methodological quality

The included studies were extracted into data tables comprising intervention and control, study design, sample size, study population, inclusion and exclusion criteria, concurrent dietary advice, treatment duration, primary and secondary outcome measurements, results, adverse events and funding source. The quality of the studies was assessed following the CONSORT guidelines (Schulz et al., 2010). Additionally, assessment of bias was conducted following the Cochrane risk of bias assessment tool (Higgins and Green., 2011). The data extraction and quality assessment were performed independently by one reviewer (VV) and validated by a second (SPM) with any disagreements being settled by discussion.

Pharmacological quality

To ensure that the preparation used in the studies had a consistent extraction process and was appropriate for comparison, the holder of the international rights to the standardised red clover extract marketed as Promensil (Pharmacare Laboratories Pty Ltd, Sydney, Australia) were contacted and asked for independent verification.

Data analysis

Hot flush score was chosen as the primary outcome measure. Other measurements of vasomotor symptoms were considered secondary outcome measures.

Insufficient data was provided in the selected literature to determine the specific variance of the change in mean value from baseline to the end of the intervention. The variance of the change between the mean value from baseline to the end of these studies was imputed using a method developed by Follmann et al. (1992) and outlined in Higgins and Green (2011).

Meta-analysis was carried out using the Review Manager (RevMan) software (Version 5.3.5. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012). We quantitatively combined the results of the studies that were suitable for statistical pooling and calculated the weighted mean differences (WMD) and 95% confidence intervals (CI) for continuous data using the fixed-effects model. The change between baseline and endpoint means was used to assess differences between the red clover and



Fig. 1. Flowchart of study selection.

placebo groups. None of the included studies reported the variance of this change to allow us to directly calculate a correlation coefficient. The variance of the change was imputed, set at 0.4 following Coon et al. (2007) and tested for sensitivity (Higgins and Green, 2011). The chi-squared test for heterogeneity was performed to determine whether the distribution of the results was compatible with the assumption that differences between trials were due to chance variation alone. For small numbers of trials it is recognised that the power of this test is low and the p value was set at less than 0.01. Where heterogeneity was found a predetermined sub-group analysis looking at cross-over studies and parallel-arm studies was undertaken. To evaluate potential publication bias if the search returned adequate studies we intended to construct a funnel plot for the primary outcome of change in hot flush score, using effect size as a measure, and examine it visually for asymmetry.

Results

Included studies

The search strategy and study selection is outlined in Fig. 1. In total 2414 papers were found of which 112 abstracts or full text articles were selected for further evaluation. Twenty studies using 80 mg Promensil were identified. Of these only four concerned vasomotor symptoms in menopausal women and were included in the analysis. One additional study was identified that trialled Promensil at a dose of 40 mg and 160 mg (Knight et al., 1999). Hand searching of retrieved references for other relevant studies was conducted, with one conference paper identified (Nachtigall et al., 1999), this was later dismissed due to being a 40 mg study. Additionally, relevant journals were hand-searched where available. A systematic search of all material published by each of the authors on the identified studies revealed no further relevant papers. Authors of identified studies were contacted where possible for further information or knowledge of any unpublished research in this area.



Key: Green = low risk of bias; yellow = unclear risk of bias; red = high risk of bias



Characteristics of the five studies (Hidalgo et al., 2005; Knight et al., 1999; Lipovac et al., 2011; Tice et al., 2003; Van De Weijer and Barentsen, 2002) meeting the inclusion criteria are summarised in Table 1. One study (Knight et al., 1999) had one arm using a dose of 160 mg and was included to ensure that all studies of 80 mg or greater were included.

Two of the studies used a cross-over design (Hidalgo et al., 2005; Lipovac et al., 2011) and three used a parallel-arm design (Knight et al., 1999; Tice et al., 2003; Van De Weijer and Barentsen, 2002). All the studies were of good methodological quality with Van de Weijer and Barentsen having the weakest methodology according to the CONSORT guidelines and also evidenced in the Cochrane risk of bias assessment as shown in Fig. 2.

Constituent profile

Three studies (Knight et al., 1999; Tice et al., 2003; Van De Weijer and Barentsen, 2002) used the product Promensil (Pharmacare) and two studies (Hidalgo et al., 2005; Lipovac et al., 2011) used the product Menoflavon (Melbrosin International). Pharmacare provided the authors with a letter titled "Declaration on Red Clover" from Linnea SA, a raw materials supplier in Switzerland, dated 19th October 2012. This letter states that the raw material supplied to Pharmacare is the same raw material supplied to Melbrosin and that Linnea SA has produced the Red Clover Extract since 2001, in compliance with an Authorisation License issued by the Swiss Health Authority (Swissmedic) released upon good manufacturing practice (GMP) system acceptance. In addition Pharmacare provided certificates of analysis of retention time for both Promensil Double Strength (80 mg) and Menoflavon Forte (80 mg) undertaken by an independent laboratory (Chemika, Girraween Australia) in 2013, demonstrating that the four isoflavone peaks in the sample are concordant. One study (Knight et al., 1999) was conducted prior to 2001, therefore the primary meta-analysis was undertaken on the four studies that used exactly the same preparation. An additional meta-analysis was undertaken with the inclusion of the Knight et al. to assess its impact if any on the results.

A HPLC chromatogram of HPLC chromatogram of ethanolic extract of double strength Promensil tablets demonstrating the main four *Trifolium pretense* isoflavones in red clover (Daidzein, Genistein, Formononetin, and Biochanin A) is given in Fig. 3.

The study by Tice et al. in addition to Promensil and placebo also included a third arm of another red clover product called Rimostil, which has a different concentration of isoflavones and isoflavone profile compared to Promensil (Tice et al., 2004). This arm was excluded from the analysis.

Table 1

Characteristics of included studies.

First	Study design	N = enrolled/	Study population	Trial inclusion criteria		Trial exclusion criteria	Dietary advice	Hadad score	
author/date		analysed	(subjects)	Hot flush score	Amenorrhoea	FSH		during study	
Hidalgo, 2005	Placebo controlled crossover trial - 90 days treatment/7day washout/crossover-90 days Dose-80 mg Menoflavin	60/53	Latin American population over 40 years & postmenopausal with mod-severe menopausal symptoms. Mean age 51.3 ± 3.5 years (69.7% were >50 years)	Kupperman index score >15	>12 mths	>30 mIU/ml	-On HT or taking isoflavone supplements -Taking thyroid mx or hx thyroid disease -Taking mx for blood lipid control	Not given	5
Lipovac, 2011	Placebo controlled crossover trial - 90 days treatment/7 day washout/crossover-90 days Dose-80 mg Menoflavin	113/109	Austrian population over 40 years & postmenopausal with mod-severe menopausal symptoms. Mean age 53.5 ± 7.1 years	Kupperman index score >15 >5 hot flushes/day	>12 mths	>35 mIU/ml	-On HT or with known isoflavone hypersensitivity	Not given	4 – no randomisation method given
Tice, 2003	Placebo controlled 3-arm parallel design – 2 weeks placebo run-in/ 12 weeks treatment Dose-82 mg Promensil OR 57 mg Rimostil	252/246	North American population 45–60 years & postmenopausal with mod-severe menopausal symptoms. Mean age 52.3 ± 3.4 years	>35 hot flushes/ week	>2 mths consecutively Or bilateral oophorectomy	>30 mIU/ml	 -Vegetarian/ or soy >1 x week -Taking mx affecting isoflavone absorption -HT or menopause treatment < 3mths prior to enrolment -Significant GIT disease -Consumed <80% of tablets in 2-week placebo phase 	No dietary advice specified, however, excluded if vegetarian or had soy >1 per week	5
Van de Weijer, 2002	Placebo controlled parallel design, diet controlled 4 weeks placebo run-in/ 12 weeks treatment Dose-80 mg Promensil	30/24	Dutch postmenopausal women. Mean age 54.2±7.4 years	>5 hot flushes/day	>12 mths	Not specified	-HT < 12 weeks prior to enrolment -Undiagnosed vaginal bleeding -Acute liver or renal disease -History of malignancy, CVD or thromboembolism	Given list of foods to avoid including legumes & isoflavone supplements	4 – insufficient explanation of participant withdrawls
Knight, 1999	Placebo controlled 3-arm parallel design, diet controlled 1 week placebo run-in/ 12 weeks treatment Dose-40 mg Promensil OR 160 mg Promensil	37/35	Australian postmenopausal population with >3 hot flushes/day. Age range 50.6–60 years	>3 hot flushes/day	>6 mths OR bilateral oophorectomy	>40 mIU/ml	-HT < 6weeks prior to enrolment -Recent hx bowel, liver or gallbladder disease -Diabetes requiring medication -Malignance (excluding skin) -Women with contraindications to HT use -Vegetarians or regular soy users -Mx that may raise liver enzymes	Non-vegetarian participants asked not to alter diet during study	5

Table 2Red clover standardised extract studies outcome data.

Paper	Comparison	Number	Hot flush number	Kupperman Index
Lipovac, 2011 ^a	Menoflavon 80 mg	109	7.57 (±5.16)	23.26 (±11.86)
	Placebo	109	$-0.2(\pm 5.8)$	-1.23 (±14.92)
Hidalgo, 2005	Menoflavon 80 mg	53	-	21.3 (±7.10)
	Placebo	53	-	6.3(±7.40)
Tice, 2003 ^b	Promensil 82 mg	84	3.4 (±4.97)	-
	Placebo	85	2.8(±3.32)	-
Van de Weijer, 2002	Promensil 80 mg	15	2.08 (±3.09)	-
	Placebo	11	-0.29 (±5.77)	-
Knight, 1999 ^c	Promensil 160 mg	13	3.1 (±5.39)	-
	Placebo	12	2.8 (±4.98)	-

Key: Promensil and Menoflavon are the same red clover extract. Outcomes given as mean change (±SD);

^a Outcome data for Lipovac et al., 2011 was given for groups A and B depending on cross-over order and was transformed using the calculator function in RevMan 5.3.5;

^b Outcome data in Tice et al., 2003 was given as mean and 95% confidence intervals and was transformed according to the method outlined in Higgins JPT and Green S (2011);

^c Data for Knight et al., 1999 is given only for the 160 mg dosage.



Fig. 3. HPLC chromatogram red clover extract.



Key: The mean difference with change from baseline are given with 95% confidence intervals (CI). The vertical line represents no difference between red clover and placebo control. The black diamond indicates the summary measure

Fig. 4. Effects of red clover versus placebo on hot flush scores.

Hot flush score

All the five studies with one exception (Hidalgo et al., 2005) contained a hot flush score. The corresponding author of this study (Dr Chedraui) responded to an email request for data pointing out that while presence and intensity of hot flushes was scored the study did not record a daily number. The study outcome measures used in the meta-analyses are given in Table 2.

The meta-analysis for hot flush score is shown in Fig. 4. The three trials using an 80 mg dose that reported hot flush scores included in this meta-analysis indicates a statistically significant difference between women receiving red clover and those receiving placebo (weighted mean difference -3.63 hot flushes per day: [95% CI 2.70-4.56]; p < 0.00001). This difference is clinically significant and represents an approximate decrease in hot flushes between 30-50%. To test the extent of its contribution to the result the inclusion of Knight et al., (1999) does not alter these results substantively (weighted mean difference -3.46 hot flushes per day: [95% CI 2.56-4.37]; p < 0.00001).

The chi-squared test for heterogeneity was very low (p < 0.00001) indicating that the studies are not homogenous.

Sub-Group 1: Cross-Over Studies Red Clover Mean Difference Mean Differenc Placeb Study or Subgroup Mean SD Total Mean SD Total Weight 5.35 109 100.0% IV, Fixed, 95% C IV, Fixed, 95% CI 3.21 3.76 -6.52 [-7.75, -5.29 Total (95% CI) 109 109 100.0% -6.52 [-7.75, -5.29] -Heterogeneity: Not applicable Test for overall effect: Z = 10.41 (P < 0.00001) Freatment Sub-Group 2: Parallel-Arm Studies Red Clos Mean Differenc Mean Diffe Study or Subgroup Mean SD IV, Fiz ked, 95% C Tice 2003 Van de Weijer 2002 5.1 4.15 3.35 3 84 15 5 6.01 3.25 5.5 85 11 91.1% 0.10 [-1.02, 1.22] 8.9% -2.66 [-6.25, 0.93] Total (95% CI) 96 100.0% -0.15 [-1.22, 0.93] Heterogeneity: Chi^a = 2.07, df = 1 (P = 0.15); l^a = Test for overall effect: Z = 0.27 (P = 0.79) -10

Fig. 5. Effects of red clover versus placebo on hot flush scores (sub-group analysis).



Fig. 6. Effects of red clover versus placebo on Kupperman Index.

Following a pre-determined protocol, sub-group analyses dividing the studies on design (cross-over and parallel-arm) were undertaken and are shown in Fig. 5. The cross-over group had only one study with a hot-flush score (Lipovac et al., 2011) which demonstrated a statistically significant difference between red clover and placebo (mean difference -7.77 hot flushes per day: [95% CI 6.31– 9.23]; p < 0.00001). In comparison, the two parallel-arm studies (Tice et al., 2004; Van De Weijer and Barentsen, 2002) showed no significant difference between red clover and placebo (weighted mean difference 0.78 hot flush per day; [95% CI -0.42-1.99]; p = 0.20). Again the addition of the fifth study (Knight et al., 1999) did not alter the results substantially (weighted mean difference 0.74 hot flush per day; [95% CI -0.41-1.90]; p = 0.21). The major difference between the results of the sub-groups was considered likely to explain the lack of homogeneity of the studies selected.

A funnel plot analysis to determine publication bias was not considered meaningful with so few studies (n = 5).

Kupperman Index

A meta-analysis was undertaken on the Kupperman Index (Kupperman et al., 1953) which was used in the two cross-over studies (Hidalgo et al., 2005; Lipovac et al., 2011) and is given in Fig. 6. This index scores the severity of 11 menopausal symptoms (including hot flushes) over the previous four weeks. Each symptom is rated according to intensity from 0 to 3 (not present, slight,

moderate and severe) in order to calculate the Kupperman Index, the sum of all obtained scoring is calculated with a maximum of 33 points. The meta-analysis of the two trials with comparable chemical profile and Kupperman Index indicates a statistically significant difference between women receiving red clover and those receiving placebo (weighted mean difference 21.8 [95% CI 18.77–24.83]; p < 0.00001). This difference is also clinically significant, representing a reduction of 66% from maximal scored symptoms.

Adverse events

Four of the five included studies reported adverse events with the exception of Knight et al. (1999). Van De Weijer and Barentsen (2002) reported that tolerability was generally good and the active group showed no more side effects than the placebo group, but provided no further details. Tice et al. (2004) reported self-limiting adverse effects in 31 participants (37%) of the red clover group and 33 participants (39%) of the placebo group. These events included upper respiratory tract infections (9 cases active; 14 placebo); headache (5 active; 11 placebo); myalgia (10 active; 7 placebo); nausea (4 active; 4 placebo); arthralgia (5 active; 6 placebo); and diarrhoea (2 active; 3 placebo). There was no statistically significant difference for any of these conditions between the active treatment and placebo, Hidalgo et al. (2005) reported two adverse events, both headaches, of which one was during the placebo phase and one during the red clover phase. Lipovac et al. (2011) reported that no side effects occurred in either group.

Discussion

This systematic review and meta-analysis focused on a specific standardised extract of *Trifolium pratense* isoflavones (Promensil) at a dosage of 80 mg per day. Four clinical trials out of the five studies identified used a product with a confirmed comparable extraction process. The fifth clinical study (Knight et al., 1999) could not be confirmed as using a comparable extraction process and was excluded from the primary analysis. It was, however, included in an additional analysis to ensure that its exclusion did not bias the results of the primary analysis.

The primary meta-analysis assessing the effect on hot flush scores contained three trials using the standardised extract at 80 mg per day. It demonstrated a statistically significant difference between women receiving red clover and those receiving placebo. This equated to a reduction of 3.63 hot flushes per day in a population that was experiencing a mean of 7–12 per day at baseline. A reduction between 30–50% in hot flush frequency is clinically significant.

The addition of the fifth study to this analysis did not alter the results as the trial had only small numbers (n=25) and received a low weighting (5%).

The four studies comprised two parallel-arm studies (Tice et al., 2004; Van De Weijer and Barentsen, 2002) and two cross-over studies (Hidalgo et al., 2005; Lipovac et al., 2011). All the studies were of similar duration (84–90 days). The largest study (n = 169) included was a parallel-arm study conducted by Tice et al. which demonstrated no effect of Promensil compared to placebo. The other parallel-arm study (Van De Weijer and Barentsen, 2002) was small (n = 26) and showed a positive result. Both of the cross-over studies demonstrated positive results and had medium (n = 53) (Hidalgo et al., 2005) to large (n = 109) (Lipovac et al., 2011) study numbers.

There was a lack of homogeneity in the four primary studies. It was determined *a priori* to conduct a sub-group analysis if heterogeneity was found by dividing the studies by clinical design into parallel arm and cross-over sub-groups. Only one of the cross-over studies had a hot flush score (Lipovac et al., 2011) and it demonstrated marked statistical significance (p < 0.00001) between red clover and placebo with a mean reduction of 7.77 hot flushes per day. By stark comparison the two parallel-arm studies showed no statistical significance between red clover and placebo. The difference between these two sub-groups is likely to explain the lack of homogeneity of the studies.

Interestingly, the earlier systematic reviews and meta-analyses (Coon et al., 2007; Howes et al., 2006; Jacobs et al., 2009; Krebs et al., 2004; Nelson et al., 2006) were undertaken solely on parallelarm studies and were not able to include the cross-over studies cited in this paper (Hidalgo et al., 2005; Lipovac et al., 2011). These reviews demonstrated either no effect or a small positive effect for red clover at 40 mg/day that was of uncertain clinical significance.

Cross-over clinical trials in general provide stronger evidence for effectiveness than parallel-arm clinical trials as they allow comparison at the individual rather than the group level (Elbourne et al., 2002). As such, cross-over trials are more precise than parallelarm studies as variation between measurements in a specific individual is generally smaller than variation in a group population. This strong study design enables lower numbers of participants, as each individual is active in both arms of the study group, whereas participants in parallel studies only receive one medication (either active or placebo).

An additional meta-analysis looking at the effect of red clover versus placebo on the Kupperman Index using data from the two cross-over trials also demonstrated marked statistical significance (p < 0.00001) between red clover and placebo with a mean reduction of 21.8 points on a scale with maximum score of 33. These results suggest that red clover may have clinically significant effect on menopausal symptoms in general and not just on hot flushes specifically.

The adverse events within the studies were self-limiting and did not differ between the active and placebo treatments. The supplements were safe over the duration of the studies (3 months).

A strength of this study is that it used mean difference between baseline and the end of treatment. Previous data on analyses from red-clover studies suggest that using final means has less power to demonstrate a result. One review using final values (Krebs et al., 2004) demostrated that red clover had no improvement in hot flush frequency, while another review that used the mean differences from baseline to endpoint (Coon et al., 2007) demostrated a small positive difference. Vickers has demonstrated that final (or post) values maintain consistent power (approximately 70%) in situations where the baselines have both low and high correlation (Vickers, 2001). In principle, an analysis based on changes from baseline will be more efficient and powerful than a simple comparison of final values, as it removes a component of between-person variability from the analysis.

All five of the clinical trials discussed in this paper and this review have been funded, at least in part, by one or the other of two commercial firms using the standardised extract of red clover isoflavones produced by the Swiss manufacturer. Coon et al. note that this illustrates the need for independent replication and also the difficult of obtaining funding from independent sources for clinical trials on complementary medicines (Coon et al., 2007). In suspecting bias it is refereshing to note that not all the commercially funded trials on red clover have produced a positive result, however, a recent Cochrane Review (Lundh et al., 2012) has concluded that sponsorship of drug studies by commercial firms leads to more favorable results and conclusions than sponsorship by other sources. The only method available to minimise commercial bias is to have more money invested from the public purse for research into complementary medicines. A number of commentators from different countries have pointed out that the widespread use of complementary medicines by their populations is not matched

in any way by the government monies allocted for medical research (Bensoussan and Lewith, 2004; Ernst, 1996). Pragmatically, there is currently no alternative to assessing commercially funded research on complementary medicines because little such research currently exists.

In conclusion, this study demostrates statistical and clinically significant benefit for using a specific standardised extract of red clover isoflavones (Promensil) at 80 mg/day for treating hot flushes in menopausal women. Potential benefits beyond the reduction in hot flushes were also found that deserve further investigation. The benefit of using this specific extract at the specified dose are significant enough to warrant independent replication by a group funded from non-commercial sources.

Conflict of interest

SPM has acted as a consultant to Pharmacare Laboratories Pty Ltd on regulatory and research matters. SPM was supported by a grant by Pharmacare Laboratories Pty Ltd to undertake this systematic review and meta-analysis. The sponsor approved the research objective and otherwise had no role in the design or conduct of the study, the collection, management, analysis and interpretation of the data and agreed *a priori* to publication the results indpendent of the findings.

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