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REVIEW

Red clover for treatment of hot flashes and menopausal symptoms: A systematic review and meta-analysis

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This study evaluated the efficacy of red clover to relieve hot flashes and menopausal symptoms in peri/postmenopausal women. Electronic databases (MEDLINE, Scopus and the Cochrane Library) were searched. The mean frequency of hot flashes in red clover groups was lower compared with that in the control groups (close to statistical significance). Difference in means (MD) of hot flashes frequency was − 1.99 (− 4.12–0.139; $p = 0.067$; heterogeneity $P > 0.01$; $I^2 = 94.93\%$; Random effect model). Subjective (vaginal dryness) and objective (maturation value) symptoms of vaginal atrophy showed a significant improvement with 80-mg dose of red clover. Red clover showed less therapeutic effect on psychology status, sexual problems and sleeping disorders. Red clover consumption may decrease frequency of hot flashes, especially in women with severe hot flashes (≥ 5 per day). Red clover may reduce other menopausal symptoms. Further trials are needed to confirm the current systematic review findings.

Keywords: Hot flushes, menopause, meta-analysis, red clover, systematic review

Introduction

As women approach menopause, they experience a variety of symptoms such as vasomotor disorders (hot flushes and night sweats), urogenital atrophy, depression, anxiety, palpitations, sleep difficulties, headaches, tiredness, lethargy and impaired concentration (Laakmann et al. 2012). Hot flush is the most frequent unpleasant symptom experienced in approximately 70% of women and it is mostly attributed to a drop in oestrogen levels (Stearns et al. 2003). For several decades hormone therapy (HT) has been a very effective treatment for menopause symptoms (Umland 2008).

There was confusion in interpreting the initial results from the Women's Health Initiative (WHI) Estrogen-Alone Trial regarding the risk of developing breast cancer using HT. In fact, oestrogen-alone hormone therapy (ET) did not increase the risk of breast cancer in postmenopausal women, as reported in an updated analysis. However, too little attempt was made by media and by published data to clarify that there is no correlation between ET and breast cancer in postmenopausal women (Burger et al. 2012). Due to this point, several patients using ET have discontinued this treatment (Gompel and Santen 2012; Meherishi et al. 2010). Consequently, many menopausal women became interested in phyto-oestrogens as alternative therapy (Chedraui et al. 2011).

Phyto-oestrogens can be divided into three main classes: isoflavones, lignans and coumestans (Baird et al. 1995; Kotsopoulos et al. 2000; Murkies et al. 1998). There is a growing interest of scientific literature in red-clover-extract-derived isoflavones in menopausal women (Lipovac et al. 2011).

Despite several up-to-date systematic reviews and meta-analysis on the frequency of hot flushes (Krebs et al. 2004; Lethaby et al. 2007; Nelson et al. 2006), the effect of red clover on other menopausal symptoms is still lacking.

Therefore, it was worthwhile to conduct a systematic review to assess the effectiveness of red clover on vasomotor symptoms, genital complaints and psychological state in menopausal women.

Materials and methods

Search strategy

The authors searched electronic sources in MEDLINE, Scopus and the Cochrane Central Register of Controlled Trials for studies which investigated the efficacy of red clover on menopausal symptoms. No time limit was imposed on the search and the last search was performed in April 2014; the search strategy keywords were 'menopause' AND ('red clover' OR 'trifolium pratense' OR 'cow clover' OR 'meadow clover' OR 'purple clover' OR 'beebread' OR 'trefoil'). No language filter was applied.

The inclusion criteria were

1. Randomised Controlled Trials (RCTs)
2. Oral monopreparation of red clover as treatment arm and placebo as control group
3. Available information on at least one among the menopause symptoms frequency, vaginal cytology and/or vaginal dryness

Data extraction

The following data were independently extracted by two authors (MG and TKH) according to a pre-defined checklist defined by

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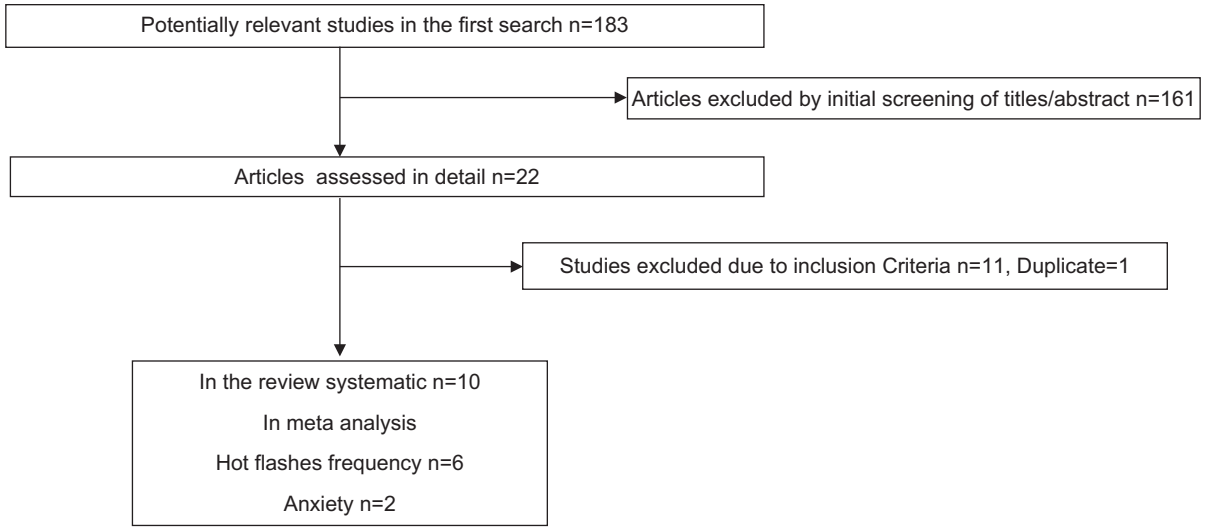

Figure 1. Search strategy of the study.

the review team: population characteristics (participants' menopause status and age), methods (rate of drop out and duration), design study (parallel or crossover), dosage of red clover, sample size in both groups, and quality of RCTs. Disparities were resolved by discussion with a third investigator (RS). The outcomes were mean and standard deviation of post treatment changes in both arms of the included studies.

Quality assessment of the included studies

Trial quality characteristics were assessed by two reviewers using the Oxford Centre for Evidence-based Medicine checklist for therapeutic studies.

Statistical analyses

The main effect size was measured in two ways: difference in means (MD) of quantitative data was calculated for continuous data, while standardised difference in means (SMD) was calculated when studies measured a same outcome in different ways.

We reported the results based on a random effects model (DerSimonian and Laird method) due to potential heterogeneity across included trials in our meta-analysis.

For heterogeneity evaluation, Cochrane Q test ($p < 0.05$ as statistically significant) and I^2 index were used. I^2 index was used to assess whether the variance across studies was real and not due to sampling errors.

We specifically made a funnel plot to assess publication bias (Figure 4). Egger's regression was used to quantify the amount of asymmetry in the funnel plot ($p < 0.05$ was considered statistically significant). All statistical analyses were performed by Comprehensive Meta-analysis Version 2 (Biostat, Englewood, NJ, USA). We only included data from phase of Baber (Baber et al. 1999) and Lipovac trial (Lipovac et al. 2011) to main meta-analysis (Figure 2) in order to avoid the carryover effects.

Results

The selection process of RCTs to include in the systematic review is described in Figure 1. Overall, 10 studies were included in the systematic review. The summarised characteristics of the included studies are shown in Table I. Five trials used Promensil (Baber et al. 1999; Jeri 2002; Knight et al. 1999; Tice et al. 2003; van de Weijer and Barentsen 2002) and only one trial used Rimostil (Tice et al. 2003). Red clover contains genistein, daidzein, formononetin and biochanin (Rock and DeMichele 2003). Rimostil tablets contain a higher proportion of isoflavones daidzin and formononetin. Promensil tablets contain higher proportion of biochanin A and genistein.

Vasomotor symptoms

Vasomotor symptoms include hot flushes, night sweating and palpitations.

The effect red clover on frequency of hot flushes

Six trials (Baber et al. 1999; Jeri 2002; Knight et al. 1999; Lipovac et al. 2011; Tice et al. 2003; van de Weijer and Barentsen 2002) provided sufficient statistical data to be included in the meta-analysis. Meta-analysis of this trials revealed that a daily intake of 40–80 mg of red clover for 4–12 weeks decreased the mean hot flushes frequency compared with the control groups, (close to statistical significance) (MD: -1.99; -4.12 to 0.139; $p = 0.067$; heterogeneity: $p < 0.01$; $I^2 = 94.93\%$; random effect model; 445 women). The funnel plot is shown in Figure 2. However, the heterogeneity of the included trials was high ($I^2 = 94\%$); therefore, a sub-group analysis and sensitivity analysis were performed to assess this heterogeneity.

MD were larger in trials that used red clover at dose of > 40 mg/day ($p = 0.156$), in post-menopausal women ($p = 0.005$) and in patients with ≥ 5/day hot flushes ($p = 0.014$). Results of the subgroup analyses are shown in Table II. The funnel plot and Egger's test ($p = 0.303$) did not demonstrate any asymmetry. The funnel plot is shown in Figure 4.

Night sweating

Two trials (Hidalgo et al. 2005; Lipovac et al. 2011) assessed the effect of red clover on night sweating. In the trial by Hidalgo et al. compared with baseline, the percentage of symptomatic patients reporting night sweating decreased by 66% in the red clover group while it remained almost unchanged in placebo group (3%). The difference between the two groups was statistically significant ($p < 0.05$) (Hidalgo et al. 2005).

In the trial of Lipovac et al. compared with baseline, night sweating daily frequency decreased by 73% in the red clover group ($p = 0.0001$) while it remained close to baseline in the placebo group. The difference between the two groups was statistically significant ($p = 0.0001$) (Lipovac et al. 2011). To sum up, it seems that red clover is effective in relieving night sweating; however, future RCTs are needed to confirm present findings.

Table I. Characteristics of 10 randomised placebo-controlled trials included in our systematic review.

Author, Y	Duration; Wk	Age;Y	Design	Status of menopause	Level of complaints	Outcome	Duration of amenorrhoea/ serum FSH level	Drop out%	Isoflavone/ mg	Type of interventions	Participants Intervention/ control	Randomization technique	Blinding method	ITT	Baseline comparability	Major relevant findings
Jeri, 2002	16	52	Parallel	Post	≥ 5/day hot flashes	Frequency hot flashed	> 12;> 30mIU/mL	0%	40	PRO	15/15	No	No	No	No details	* ↓38.5 %Hot flashes in Pro vs. placebo ($p < 0.001$)
Lipovac, 2011	12 × 2 WK, 1 WK washout	53.5	Crossover	Post	≥ 35/week hot flashes	Frequency hot flashed HADS (1) anxiety (2) depression Total SDS	> 12;> 35 mIU/ml was	4%	80	PRO	59/59	No	Unclear	Yes	Yes	*↓ 65.3% hot flashes in Pro vs. Placebo at first phase ($p = 0.0001$) *↓ 56% the HADS Sub score 'anxiety' in Pro vs. Placebo ($p < 0.001$) *↓54% the HADS Sub score 'depression' in Pro vs Placebo ($p < 0.001$) *↓ 60% total SDS vs. Placebo ($p < 0.001$)
Baber, 1999	12 × 2 Wk, 4 WK washout	54	Crossover	Peri &post	> 3 day hot flashes	frequency hot flash	6>; > 30 mlU/ml	14%	40	PRO	43/43	No	Unclear	N0	Yes	↑ 16% Hot flashes in pro vs. Placebo at first phase No difference between two groups regarding vaginal maturation Index
Knight, 1999	12	P/53 L/54.5 H/56.1	Parallel	Peri&post	≥ 3/day hot flashes	frequency hot flashes Maturation value	≥ 6; > 40 lU/l	5%	l/40 H/160	PRO	L/12 H/13 P/12	Yes	Yes	No	Yes	↑1% hot flash low dose of pro (40 mg) vs. baseline ↑4% hot flash high dose of pro (160 mg) vs. baseline ↓ 33% hot flash placebo vs. baseline ↓ 5% Maturation value low dose of red clover (40 mg) vs. placebo ↑ 2% Maturation value high dose of red clover (160 mg) vs. placebo

(*Continued*)

Table I. (Continued)

Author, Y	Duration; Wk	Age;Y	Design	Status of menopause	Level of complaints	Outcome	Duration of amenorrhoea/ serum FSH level	Drop out%	Isoflavone/ mg	Type of interventions	Participants Intervention/ control	Randomization technique	Blinding method	ITT	Baseline comparability	Major relevant findings
Geller, 2009	12	Red clover/52.4 P/52	Parallel	Peri&post	≥35 vasomotor symptoms/ week	PSQI GCS Subscale 'Anxiety' DXA (1) lumbar spine (2) total hip (3) femoral neck	Amenorrhoea >6 months <10 years duration FSH >40 mIU/mL	Total (10.1%). Red clover (10%)	Clover/120 mg	Red clover	Red clover/14 P/17	Yes	Unclear	Yes	Yes	↑Overall quality of sleep in red clover vs. placebo ($p = 0.10$). ↓ Impact of hot flashes on sleep in red clover vs. Placebo ($p = 0.82$). ↓Greene Anxiety in red clover vs. Placebo ($p = 0.12$). ↑ lumbar spine in red clover vs. Placebo ($p = 0.52$). ↓ total hip in red clover vs. Placebo ($p = 0.51$). ↓femoral neck in red clover vs. Placebo ($p = 0.84$).
van de Weijer, 2002	12	Pro/54 P/52	Parallel	Post	≥5/day hot flashes	Frequency hot flash	>12	14%	Pro/80 mg	PRO	15/11	Yes	Yes	No	Yes	*↓426% frequency hot flashes in red clover vs. Placebo

(*Continued*)

Table I. (Continued)

Author, Y	Duration; Wk	Age;Y	Design	Status of menopause	Level of complaints	Outcome	Duration of amenorrhoea/ serum FSH level	Drop out%	Isoflavone/ mg	Type of interventions	Participants Intervention/ control	Randomization technique	Blinding method	ITT	Baseline comparability	Major relevant findings
Tice, 2003	12	Pro/52 Rio/52 P/52	Parallel	Peri&post	≥35/week	Frequency of hot flash/WK GCS (1) Vasomotor (2) depression (3) anxiety (4) loss of interest in sex	>6; >30 mlU/ml	2%	Pro/82 mg RIO/57	PRO RIO	PRO/84 RIO/83 p/85	Yes	Yes	Yes	Yes	↓40% frequency of hot flash PRO vs. placebo ↓ 33% frequency of hot flash RIO vs. placebo ↑osteocalcin (ng/ml) pro vs. placebo ($p = 0.90$) ↑ osteocalcin (ng/ml) RIO vs. placebo ($p = 0.62$) ↑ urinary N-Tx (nmol BCE/mmol creatinine) pro vs. placebo ($p = 0.23$) ↑ urinary N-Tx (nmol BCE/mmol creatinine) RIO vs. placebo ($p = 0.11$) Subscale of GCS (1) ↓ depression in each PRO ($p = 0.23$) and RIO ($P = 0.79$) vs. placebo (2) ↓anxiety in each pro ($p = 0.33$) and RIO ($P = 0.80$) vs. placebo vs. placebo (4) ↓ loss of interest in sex in each pro ($p = 0.23$) and RIO ($P = 0.66$) vs. placebo
Giorno, 2010	24	53.7	Parallel	Post	Women having menopausal symptoms	Sexual satisfaction	>12; >30 mIU	12%	40	Trifolium pratense) red clover)	50/50	Yes	Unclear	No	Yes	↔ sexual satisfaction in red clover vs. placebo
Ehsanpour, 2012	10 WK	Red clover/52 P/53.92	Parallel	post	K I ≥ 15	MENQOL (2) psych-social domain (3) sexual domain	12>	0%	45 mg	Red clover	28/27	Yes	Yes	No	Yes	↑9% sexual problem in red clover vs. placebo ↓2% psycho-social domain in red clover vs. placebo

(*Continued*)

Table I. (Continued)

Author, Y	Duration; Wk	Age;Y	Design	Status of menopause	Level of complaints	Outcome	Duration of amenorrhoea/ serum FSH level	Drop out%	Isoflavone/ mg	Type of interventions	Participants Intervention/ control	Randomization technique	Blinding method	ITT	Baseline comparability	Major relevant findings
Hidalgo, 2005	12 × 2 weeks, 1 wk washout	51	Crossover	Post	moderate to severe menopausal symptoms (KI ≥ 15)	KI (1) hot flash (2) sleeping disorders (4) depression (5) vaginal dryness dyspareunia decrease of libido Vaginal cytology (1) Vaginal Index, (2) Karyopyknotic index, (3) Cornification index, (4) superficial cell	12>; >30 mlU/ml	12%	80	Red clover isoflavone supplement	53	Yes	Yes	No	Yes	*↓ KI subscale 'hot flash', 'sleeping disorders' 'depression' vs. placebo *↑ Karyopyknotic index, Cornification index and superficial cells vs. placebo Maturation index (*↓ parabasal, *↑ superficial cells, *↑ and intermediate cells) vs. placebo Vaginal cytology indices inversely correlated with the rate of dyspareunia, Vaginal dryness and libido vs. Placebo *↓ 35% percentage of symptomatic patients reporting Vaginal dryness vs. Placebo *↓ 35.2% percentage of symptomatic patients reporting dyspareunia vs. Placebo *↓ 20.8%percentage of symptomatic patients reporting decrease in libido vs. Placebo

L, low dose; H, high dose; PRO, Promensil; Rio, Rimostil; P, Placebo; Post, post menopause; Peri, perimenopausal; KI, Kupperman index; HADS, The Hospital Anxiety and Depression Scale; GCS, Greene Climacteric Scale; LH, Luteinising hormone; SHBG, Sex hormone-binding globulin; MENQOL, menopause-specific quality of life questionnaire; PSQI, Pittsburgh Sleep Questionnaire; SDS, Zung Self-Rating Depression Scale; ITI, intention-to treat reporting; DXA, dual-energy x-ray absorptiometry.
↔no change, *statistically significant

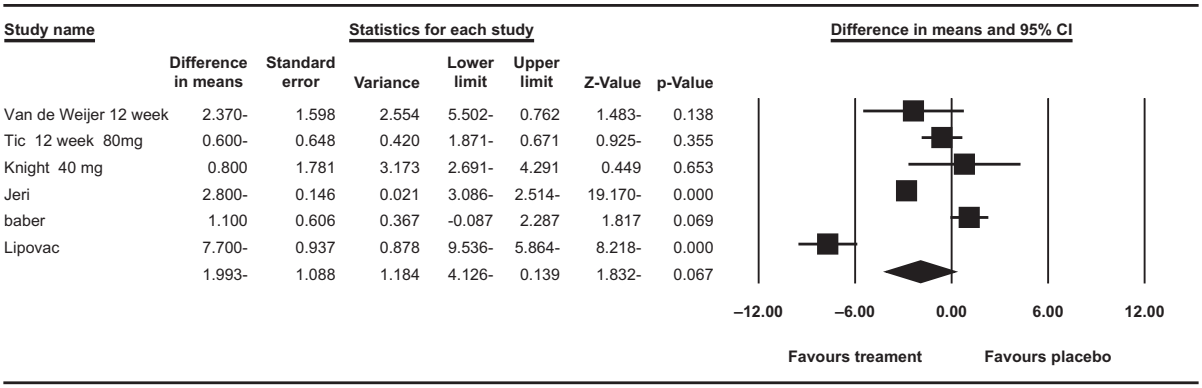

Figure 2. Effects of red clover on frequency of hot flushes, the horizontal lines denote the 95% CI, ■ point estimate (size of the square corresponds to its weight); combined overall effect of treatment.

Palpitations

One trial (Hidalgo et al. 2005) assessed the effect of red clover on palpitations. The percentage of symptomatic patients reporting palpitations decreased from 58.5% to 17% in red clover group ($p < 0.05$) and from 58.5% to 47.2% in placebo group (not significant). The difference between the two groups was statistically significant. Future RCTs are needed to confirm these findings.

Sleeping behaviour

Two trials (Geller et al. 2009; Hidalgo et al. 2005) showed the effect of red clover on sleep behaviour. Geller et al. (Geller et al. 2009) measured the quality of sleep using the Pittsburgh Sleep Quality Index (PSQI) at 12 weeks. This questionnaire also includes an item that assesses the impact of 'hot flushes' on sleep quality. The PSQI questionnaire subscale 'hot impact on sleep' was improved in red clover group compared with placebo, even if this difference was not statistically significant (-0.45, $p = 0.33$). Despite red clover's ability in alleviating hot flushes interference with sleep (although not statistically significant), overall quality of sleep, according to PSQI, was slightly worse in red clover group compared with placebo group, but not statistically significant (0.27, $p = 0.33$). However, the subscale 'hot' is a small part of questionnaire and other confounding factors could have affected the quality of sleep. In another trial by Hidalgo et al. (Hidalgo et al. 2005), the percentage of symptomatic patients reporting 'sleeping disorders' according to the Kupperman index (KI) subscale decreased significantly in red clover arm (from 86.8% to 41.5%) compared with placebo group (from 86.8% to 73.3%). Further trials are needed to assess the effect of red clover on sleeping disorders in more detail.

Vaginal cytology

Three trials (Baber et al. 1999; Hidalgo et al. 2005; Knight et al. 1999) assessed the effect of red clover on vaginal cytology.

One trial by Knight et al. (Knight et al. 1999) compared 3 arms: low dose of red clover isoflavones (40 mg/day), high dose of red clover isoflavones (160 mg/day) and placebo. Low dose of red clover showed greater decline in the maturation value compared with placebo (MD: -2.2; $p = 0.567$), while high dose of red clover showed a slight increase in maturation value compared with placebo group (MD: 1.40; $p = 0.491$). Therefore, only a high dose of red clover can prevent progressive epithelium change.

Another trial by Hidalgo et al. (Hidalgo et al. 2005) measured several indices of oestrogenisation status of the vaginal epithelium including percentage of squamous cells with nucleic pyknosis (karyopyknotic index), percentage of squamous cells with cytoplasmic acidophilia (cornification index), superficial index and the percentage of basal, intermediate and superficial cells (maturation index). The karyopyknotic index and cornification index showed a statistically significant increase in the red clover group compared with placebo group ($p < 0.05$). The maturation index at baseline for both groups was 68.4% for parabasal, 27.4% for intermediate and 4.2% for superficial cells. For both groups, there was a decline in parabasal cells and an increase in superficial and intermediate cells at 12 weeks. Maturation index at the end of the study in the red clover group was 1.9% for parabasal, 39.6% for

Table II. Subgroup analyses of the effects of red clover on the frequency of hot flushes.

Variable	Number of RCTs	Sample size treatment/control)	*P* for heterogeneity	I^2%	*P* value for effect	Random effect model MD (95% CI)
Frequency of hot flashes						
≥ 5/day	4	164/170	> 0.001	94	0.005	− 3.33 (− 5.67 to − 1)
≥ 3/day	2	54/58	0.873	0	0.062	1.06 (− 0.05 to 2.19)
Test for subgroup difference						0.639
Trials that used different soy types						
Post-menopause	3	80/85	> 0.001	92	0.014	− 4.33 (− 7.80 to − 0.87)
Both peri and post	3	138/143	0.154	46	0.611	0.33 (− 0.95 to 1.62)
Test for subgroup difference						0.703
Isoflavone dose (mg/day)						
> 40	3	149/155	> 0.001	94	0.156	− 3.56 (− 8.48 to 1.35)
≤ 40	3	69/73	> 0.001	95	0.783	− 0.45 (− 3.66 to 2.76)
Test for subgroup difference						0.314

MD, difference in means; CI, confidence interval.

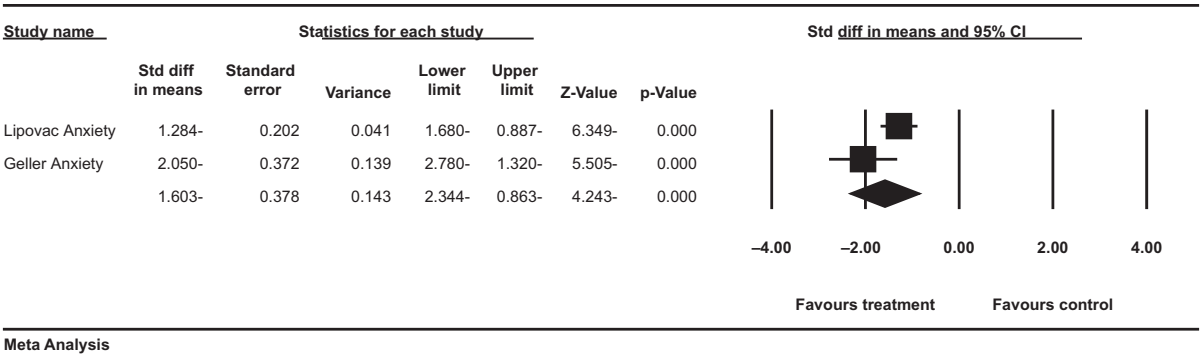

Figure 3. Effects of red clover on anxiety, the horizontal lines denote the 95% CI, ■ point estimate (size of the square corresponds to its weight); combined overall effect of treatment.

intermediate and 57.9% for superficial cells, whereas in the placebo group it was 65.2% for parabasal, 30.2% for intermediate and 4.7% for superficial cells, respectively. The comparison between the red clover and placebo groups revealed that the mean changes were all statistically more prominent in red clover group.

A third trial by Baber et al. (Baber et al. 1999) showed no positive effect on vaginal epithelium maturation index in the red clover group. It seems that red clover acts in a dose-dependent mode on vaginal cytology and low dose of red clover (40 mg/day) could not prevent progressive epithelial change over time (Knight et al. 1999), while an intake of 80 mg/day of red clover showed a significant positive effect on all the indices of oestrogenisation status of the vaginal epithelium (Hidalgo et al. 2005).

Vaginal dryness

Only one trial (Hidalgo et al. 2005) assessed the effect of red clover on vaginal dryness. In this trial, Hidalgo et al. (Hidalgo et al. 2005) reported a significant reduction in the percentage of symptomatic patients with vaginal dryness in the red clover group compared with that in the placebo group (88.7% at baseline, 83% after placebo and 47.2% after supplementation with red clover; $p < 0.05$).

The effect of red clover on psychological state (depression and anxiety)

Depression

Three trials (Hidalgo et al. 2005; Lipovac et al. 2010; Tice et al. 2003) assessed the effectiveness of red clover on depression.

Lipovac et al. (Lipovac et al. 2010) used two different questionnaires to assess depression status: Hospital Anxiety and Depression Scale (HADS) and Zung's Self Rating Depression Scale (SDS). Compared with placebo, red clover showed a statistically significant beneficial effect on depression symptoms as expressed by improvement in the HADS subscale 'depression' ($p < 0.001$) and total SDS ($p < 0.001$).

In the second trial by Hidalgo et al. (Hidalgo et al. 2005), the percentage of symptomatic patients reported in the KI sub-score 'depression' decreased significantly in the red clover group (from 84.9% to 26.4%) compared with placebo group (from 84.9% to 62.3%) ($p < 0.05$).

Finally, in the Tice et al. trial (Tice et al. 2003), the Green subscale 'depression' showed a trend of decrease in Promensil (− 0.7), Rimostil (− 0.4) and placebo (− 0.3) groups. However, difference between placebo and both intervention groups (Promensil and Rimostil) was not statistically significant ($p = 0.23$ and $p = 0.79$, respectively). Compared with the two trials of Lipovac and Hidalgo, Tice et al. trial showed less beneficial effect of red clover. A possible explanation may be the application of different scales for depression assessment. Tice et al. (Tice et al. 2003) used subscale of Green, while the other trials (Lipovac et al. 2010) used SDS. To sum up, it seems that red clover is effective in relieving depression symptoms; however more studies with special scales for depression assessment are needed to support the present findings.

Anxiety

Three trials (Geller et al. 2009; Lipovac et al. 2010; Tice et al. 2003) assessed the effect of red clover on anxiety. Only two trials (Geller et al. 2009; Lipovac et al. 2010) had sufficient information regarding anxiety and were included in the meta-analysis. The pooled SMD of the sub-score 'anxiety' was lower in the red clover group compared with that in the control group (–1.6; − 2.34

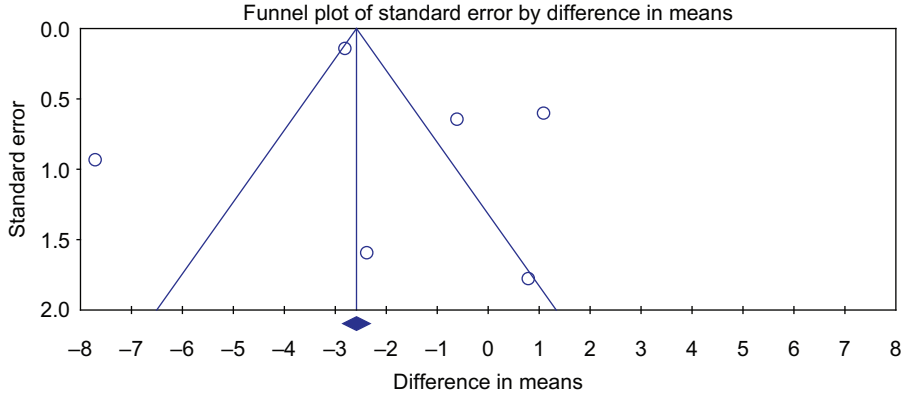

Figure 4. Funnel plot of results from included published studies on the effects of red clover frequency hot flushes.

to − 0.86; $p < 0.0001$; heterogeneity $p = 0.071$; $I^2 = 69.42\%$), the funnel plot is shown in Figure 3. One trial (Tice et al. 2003) was not included in our quantitative analysis due to incomplete reporting. In this trial the Green subscale 'anxiety' decreased by 1.1 and 0.8 points in Promensil and Rimostil groups, respectively, compared with a decrease of 0.7 in placebo group. However, both intervention groups, with Promensil and Rimostil, showed more positive effect compared with placebo, though it was not statistically significant ($p = 0.33$ and $p = 0.80$, respectively).

Overall, it seems that red clover may have psychological benefits. Future studies are still needed to evaluate the effect of red clover with specific depression questionnaire.

Sexual symptoms

Four trials (Chedraui et al. 2006; del Giorno et al. 2010; Ehsanpour et al. 2012; Tice et al. 2003) evaluated the effect of red clover on sexual symptoms. A trial by del Giorno et al. (del Giorno et al. 2010) showed the effect of red clover on sexual satisfaction, as assessed by the Golombok rust inventory of sexual satisfaction – female version (GRISS) scores. GRISS, a 28-item questionnaire, has been developed to estimate the sexual function, sexual satisfaction and also non-sexual relation with the partner. A higher score represents a lower level of sexual satisfaction. There was a comparable decrease from 38.64 ± 16.82 to 34.54 ± 18.18 (10%) in red clover group and from 39.44 ± 16.24 to 34.78 ± 17.94 (11%) in the placebo group, which however was not statistically significant ($p = 0.79$).

A second trial by Ehsanpour et al. evaluated sexual problems using menopause-specific quality of life questionnaire (MENQOL). There were a decline from 14.41 ± 6.16 to 12.70 ± 5.89 (11%) in placebo group with no change in the red clover group. Unexpectedly, placebo group showed more improvement compared with red clover, without a statistically significant difference. Some of the participants in Ehsanpour et al. study did not complete the sexual domain of MENQOL; missing values were obtained using estimate means method from other participants (Ehsanpour et al. 2012). In the same country, Ghazanfarpour et al. showed a poor internal consistency (Cronbach's alpha = 0.3) in sexual domain of MENQOL (Ghazanfarpour et al. 2014). They attributed this to culture and attitude towards sexual matters and also suggested that the Persian version of MENQOL needs to be revised.

In the third trial by Tice et al. (Tice et al. 2003), compared with placebo, both intervention groups Promensil and Rimostil showed more improvement on 'loss of interest in sex,' which however was not statistically significant ($p = 0.23$ and $p = 0.66$, respectively).

In the Chedraui et al. trial (Chedraui et al. 2006), the percentage of symptomatic patients reporting dyspareunia and low libido decreased significantly in the red clover group compared with placebo group ($p < 0.05$).

The effect of red clover on bone

Schult et al. (Schult et al. 2004) measured urinary N-Tx, a marker of bone reabsorption, which increased in both intervention groups, Rimostil (0.61) and Promensil (0.27), while it decreased in control group (− 3.05). Intervention groups showed higher increases compared with placebo, which were not statistically significant ($p = 0.11$ and $p = 0.23$, respectively). Compared with baseline, mean change of concentration of osteocalcin, a marker of bone formation, showed a small decrease in placebo (− 0.27) and Promensil (− 0.18(, but an increase in Rimostil group (0.08). However, neither Rimostil nor Promensil revealed a statistically significant difference compared with placebo ($p = 0.62$ and $p = 0.90$, respectively).

Geller et al. (Geller et al. 2009) measured bone dual-energy x-ray absorptiometry or DXA using three indicators: lumbar spine, total hip and femur. Compared with placebo group, red clover showed slight improvement on lumbar spine (0.081, $p = 0.52$), but it was not able to prevent bone loss over time in total hip (− 0.079, $p = 0.51$) and femoral neck (− 0.033, $p = 0.84$).

Discussion

Agreements and disagreements with previous meta-analyses

Our findings are in agreement with those reported by previous meta-analyses (Krebs et al. 2004; Lethaby et al. 2007; Nelson et al. 2006) which also found some beneficial effects of red clover (although statistically non-significant).

Only one meta-analysis showed statistically significant decrease in hot flushes frequency (weighted mean difference: − 1.5; 95%CI: − 2. to 0.03; $p = 0.05$; 5 trials; fix effects model). A possible explanation of this discrepancy is likely due to differences in methodology, in fact they used fixed effect model which is not methodologically justifiable (Thompson Coon et al. 2007).

We performed sub-group analysis and sensitivity analysis. Our results revealed a statistically significant decrease in hot flushes frequency in RCTs that included only postmenopausal women. The reverse effect was noted in the RCTs that included both peri- and postmenopausal women (Table II). Our results supported Thoapmon's hypothesis (Thompson Coon et al. 2007) that postmenopausal women may receive more beneficial effects. Our study showed that beneficial effects of red clover may be dependent on the level of endogenous oestrogen: our findings showed more beneficial effects in cases with lower oestrogen levels such as in postmenopausal women. Further research is needed to identify possible subgroups (dose of red clover, length and severity of hot flushes) which may increase or decrease influence of red clover on hot flushes (Table II).

It is worth mentioning that red clover consumption showed significant strong effect in alleviating hot flushes frequency in women who experienced ≥ 5 hot flushes per day, while it showed a reverse effect in women with ≥ 3 hot flushes (increased prevalence of hot flushes). Future research is still needed to clarify this issue (Table II).

Sensitivity analysis

We only included the findings of Promensil arm of Tice trial (Tice et al. 2003) and low dose (40 mg/day) of Knight trial (Knight et al. 1999) in our meta-analysis. For sensitivity analyses, the results of high dose of Knight trial (Knight et al. 1999) led to a borderline increase in MD of frequency of hot flushes (MD: − 2.14; 95%CI: − 4.26 to − 0.029, $p = 0.047$; heterogeneity $p < 0.001$; $I^2 = 93.73\%$). Overall effect remained unchanged after including Rimostil arm of Tice trial (Tice et al. 2003) (MD: − 1.85; − 4.02 to 0.311, $p = 0.093$; heterogeneity $p < 0.01$; $I^2 = 94.82\%$).

Clinical treatment satisfaction threshold

Treatment satisfaction threshold was used to identify women who are satisfied with treatment and those who are not. Average reduction of 1.64 hot flushes/day is considered as clinically meaningful treatment threshold (Gracia et al. 2005; Guttuso 2012). Also the FDA and the European Medicines Agency (EMEA) have considered at least 2 hot flushes/day fewer than placebo as clinically meaningful (Colli et al. 2012). Despite marginal statistically significant decrease of frequency of hot flushes, our results revealed a treatment satisfaction (− 1.99 fewer hot flushes per day) which also denotes the effectiveness of red clover in alleviating menopausal symptoms.

A hierarchy of responsiveness to red clover

According to Barbieri's oestrogen threshold hypothesis, tissue response to oestradiol is variable. Moderate concentrations of blood oestrogen are needed to prevent vasomotor symptoms (≥ 40 pg/ml). Higher concentrations are required to prevent the vaginal epithelium atrophy (≥ 60 pg/ml), to affect lipid metabolism (≥ 80 pg/ml.) and synthesis of thyroid-binding globulin or TBG and sex hormone-binding globulin (SHBG) (≥ 100 pg/ml) (Barbieri 1992). Findings of the current systematic review also support the Barbieri's oestrogen threshold hypothesis and suggest a hierarchy of responsiveness to red clover for menopausal symptom relief. A trial of Clifton-Bligh et al. (Clifton-Bligh et al. 2001), which was not included in the current systematic review due to lack of control group, assessed the effect of different doses of red clover on bone metabolism in postmenopausal women. Findings of this trials showed that the bone density of proximal radius and ulna increased from 0.723 ± 0.071 to 0.743 ± 0.066 (2.7%) with 28.5 mg/day of isoflavones, from 0.746 ± 0.059 to 0.779 ± 0.062 (4%) with 57 mg/day and from 0.732 ± 0.070 to 0.754 ± 0.076 (3%) with 85 mg/day. Dose of 57 mg/day of red clover was the most effective to lead to changes in bone mineral of the proximal radius and ulna (Clifton-Bligh et al. 2001), while high dose of 128 mg/day of red clover showed no significant modification in the lumbar spine, total hip and femoral neck (Geller et al. 2009). The effect on osteocalcin and urinary N-Tx, markers of bone turnover, was more prominent with a dose of 57 mg/day compared with that of 82 mg/day (Schult et al. 2004).

It seems that a dose of 40 mg/day of red clover could provide a better effect than a dose of 28.5, 80 or 128 mg/day. Our quantitative analyses showed that 40–80 mg/day of red clover decreased the frequency of hot flushes more efficiently than the placebo (MD: -1.99; -4.12 to 0.139; $p = 0.067$) whereas 160 mg/day of red clover showed no change. Pooled SMD of the hot flushes frequency change was higher with higher doses (>40 vs. ≤ 40 mg/day). Only a moderate dose of 80 mg/day of red clover could stimulate vaginal cytology change (Hidalgo et al. 2005); 40–160 mg/day of red clover also showed a small effect on SHBG.

Selective oestrogen receptor modulators

Evidence indicates that red clover may act as a natural selective oestrogen receptor modulator or SERM. Red clover improves some of the menopause symptoms such as hot flushes (Jeri 2002; Lipovac et al. 2011) and vaginal atrophy symptoms (Hidalgo et al. 2005), while endometrial thickness showed a statistically significant decrease based on trials in post-menopausal women (Imhof et al. 2006). However, the evidence is not based on laboratory data but on clinical findings only. Further specific trials are needed to assess if these effects are clinically relevant or not.

Crossover studies and risk of carryover effect

The risk of carryover effect is a major concern in crossover trials. One month after cessation of the red clover active treatment phase in Baber trial (Baber et al. 1999), participants described significant relief on hot flushes. A significant difference between screening baseline and crossover baseline ($p = 0.003$) was noted in this trial. It seems that a washout period of one month is insufficient to eliminate the carryover effect. We only included the first phase of crossover trials into our systematic review to avoid the possible carryover effect. Future studies should consider a washout period longer than one month to be considered more valid.

Limitations

There are several main limitations which should be taken into account in our systematic review. The presence of significant heterogeneity was one of the main limitations in our study. Heterogeneity might be due to isoflavones' bioavailability, variability between patients, amount of red clover and difference of red clover. Subgroup analyses indicated that only number of hot flushes influenced the effects of red clover on the hot flushes frequency.

Methodological flaws of many included studies were another potential limitation of our systematic review. These methodological flaws can affect the reliability and validity of our findings.

Conclusion

Red clover consumption may decrease frequency of hot flushes especially in women with severe hot flushes (≥ 5 per day) and postmenopausal women. Subjective and objective symptoms of vaginal atrophy showed a significant improvement with a dose of 80 mg/day of red clover. Less positive effect was observed on sleeping disorders, and psychological and sexual problems. Further trials are still needed to confirm the present findings.

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