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Hypericum perforatum with *Vitex agnus-castus* in menopausal symptoms: a randomized, controlled trial

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Abstract

Objective: To evaluate the effectiveness of a phytotherapeutic intervention comprising a combination of *Hypericum perforatum* (St. John's wort) and *Vitex agnus-castus* (Chaste tree/berry) in the management of menopausal symptoms.

Design: A double-blind, randomized, placebo-controlled, parallel trial was performed over 16 weeks in 100 eligible late-perimenopausal or postmenopausal women experiencing hot flushes and other menopausal symptoms. Herbal combination therapy or placebo tablets were administered twice daily. The primary endpoint was hot flush episodes. Secondary endpoints included Greene Climacteric Scale scores, Hamilton Depression Inventory scores, and Utian Quality of Life Scale scores.

Results: Ninety-three women completed the study. Data analysis on an intent-to-treat basis found no significant differences between the two groups for any of the endpoints. Analyses performed at interim data time points revealed no significant differences at week 4, 8, or 12 for daily weighted flushes or scores on the Greene Climacteric Scale or Hamilton Depression Inventory. However, significant improvements across the treatment phase were observed in both the placebo and active treatment groups for these endpoints. No significant change was found for either group on quality of life.

Conclusion: The herbal combination of *H. perforatum* and *V. agnus-castus* was not found to be superior to placebo for the treatment of menopausal symptoms. The herbal combination was well tolerated with no significant adverse events noted in the short term. Robust findings from quality studies such as this are important for informing the community, healthcare providers, and regulatory authorities.

Key Words: Menopause – Hot flushes – Depression – Complementary medicine – *Hypericum perforatum* – *Vitex agnus-castus*.

Despite the incontrovertible efficacy of hormone therapy (HT) for menopausal symptoms, safety concerns generated by reports from large-scale trials[1] resulted in a significant decline in HT use[2] and increased interest in alternatives. In terms of complementary and alternative medicine (CAM), a recent study found that 54% of Australian women transitioning through menopause use some form of treatment or product for the alleviation of menopause-related symptoms.[3]

Of the phytotherapeutic remedies, the phytoestrogen-containing plants have received the most attention in the scientific arena, with relatively little research conducted on other herbs traditionally used for menopausal symptoms.[4] However, concerns have now been raised over the safety of long-term use of some phytoestrogens, specifically on breast and endometrial tissue proliferation.[5,6] The present study, therefore, took a novel approach in that it focused on two nonestrogenic herbs commonly prescribed for menopausal symptoms.[7,8]

Phytotherapeutic menopausal formulations are often found to include the herbs *Hypericum perforatum* (St. John's wort)[7] and *Vitex agnus-castus* (chaste tree/berry),[8] both of which have been demonstrated to act via neurotransmitters and/or opioid receptors.[9-12] Efficacy of central nervous system (CNS)-acting agents for vasomotor symptoms has been demonstrated with pharmaceuticals such as the antidepressant selective serotonin reuptake inhibitors and the anticonvulsant gabapentin,[13] which are believed to exert their

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effects via the complex interaction between hormones and neurotransmitters involved in the physiology of hot flushes.[14] Various CNS mechanisms for *H. perforatum* and *V. agnus-castus* have also been proposed based on in vitro and in vivo models.[9-12,15] For *H. perforatum*, these include inhibition of the uptake of the monoamine neurotransmitters, serotonin, norepinephrine and dopamine, γ-aminobutyric acid, and L-glutamate; down-regulation of β-adrenergic receptors, up-regulation of serotonin 5-HT_2 receptors, and the regulation of genes that control hypothalamic-pituitary-adrenal axis function.[9] It also resulted in differential modulation of the binding properties of 5-HT_{1A}-, 5-HT_{2A}-, and μ-opioid receptors.[10] *V. agnus-castus* extracts have been shown to act as agonists at the κ- and μ-opiate receptors,[12,16] exert a dopaminergic action mediated by D_2 receptor activation,[11] and stimulate melatonin release.[15]

In addition to its well-demonstrated effectiveness for the treatment of mild to moderate depression,[17] evidence has suggested that *H. perforatum* improves vasomotor, psychological, and psychosomatic menopausal symptoms.[18] Several randomized, controlled trials (RCTs) have demonstrated its efficacy when combined with *Cimicifuga racemosa* (black cohosh) for menopausal hot flushes and associated psychological symptoms.[19-22] *V. agnus-castus* was one component of a phytotherapeutic menopause formulation found to be significantly superior to placebo in an RCT on menopausal hot flushes and night sweats[23] and has also shown positive effects for premenstrual syndrome.[24]

The present study investigated the efficacy of a combination of *H. perforatum* and *V. agnus-castus* on the physiological and psychological symptoms of menopause in late perimenopausal and postmenopausal women in a double-blind, randomized, placebo-controlled trial.

METHODS

Approval for the study was obtained from the Human Research Ethics Committee at Royal Melbourne Institute of Technology-University. All participants gave written informed consent before entering the study. Baseline visits were completed in a clinic setting. Follow-up contacts were conducted by telephone.

Women recruited were between ages 40 and 60 years, were postmenopausal (at least 12 months of amenorrhea), or in late perimenopause, defined by an intermenstrual interval of at least 3 months in the previous 12 months,[25] experiencing a minimum of five hot flush/sweating episodes per 24 hours, and scoring 20+ on the Greene Climacteric Scale, consistent with a menopause clinic sample.[26] Hysterectomized women were admitted if they were older than the age of 53 and follicle-stimulating hormone levels were greater than 25 IU/L. Women were ineligible if using formulations that included trial herbs, pharmacological agents known to interact with either herb, or concomitant therapies for menopausal or psychological symptoms (including HT and CAM within the previous 4 weeks, hormone implants within the previous year, and injectables within the previous 6 months, and antidepressant medications within the previous 4-5 wk).

Women with major preexisting illness or a history of mania or substance abuse were excluded. Other exclusion criteria were medically or surgically induced menopause, undiagnosed vaginal bleeding (postmenopausal women), pregnancy or attempting to conceive, or concurrent participation in another clinical trial.

Pretrial symptoms were assessed at baseline using the Greene Climacteric Scale, the self-rated Hamilton Depression Inventory (HDI), the Utian Quality of Life Scale, and a daily hot flush diary for the 2 week run-in period before the treatment phase.

Before inclusion in the trial, medical clearance from a general practitioner was required after a general medical checkup, including heart rate, systolic and diastolic blood pressure, and breast examination. A Pap smear was performed if one had not been performed within the previous 2 years. Participants were asked to maintain their baseline phytoestrogen intake and advised of the relevant foods.

Both the placebo and active treatment were administered in the form of tablets, identical in size, color, coating, weight, and packaging. All tablets were manufactured according to the Code of Good Manufacturing Practice by MediHerb Australia Pty Ltd. Both products are included on the Australia Register of Therapeutic Goods as Listed Medicines and were administered at standard dose levels.

The active treatment was a combination of two herbal extracts, *H. perforatum L.* (Clusiaceae/Guttiferae; St. John's wort) and *V. agnus-castus L.* (Verbenaceae; chaste tree/berry). Each *H. perforatum* tablet contained 300 mg extract equivalent to 1,800 mg dry herb flowering top standardized to contain 990 μg of hypericins, 9 mg of hyperforin, and 18 mg of flavonoid glycosides. A total of three tablets of *H. perforatum* (one in the morning and two later in the day) were given. The hypercin content was consistent with effective doses for mild-moderate depression used in RCTs.[27] Each *V. agnus-castus* tablet contained extract equivalent to 500 mg of dry fruit, two tablets (taken in the morning) or placebo were given daily for 16 weeks. The regimen was determined by referring to previous RCTs of *H. perforatum* in depression[27] and *V. agnus-castus*[15] and is consistent with current usage.[8] Placebos contained the excipients used in the active tablets (see Appendix 1 for further details). At the end of the study, all remaining tablets were returned to the study center and manually counted.

The primary endpoint was the frequency and severity of hot flushes (including night sweats). Secondary endpoints were scores on the Greene Climacteric Scale, the Hamilton Depression Inventory 17-item scale (HDI-17), and the Utian Quality of Life Scale.

Hot flushes were recorded in daily symptom diaries for the 16-week treatment phase and 1 week during posttreatment follow-up (week 24). Participants recorded the number and severity of hot flushes and sweating episodes experienced each day and night, defined as while in bed. These were

TABLE 1. *Baseline characteristics of enrolled participants (n = 100)*

	Placebo (N = 50)	*Hypericum* and *Vitex* (N = 50)
Age at trial start, y	52.5 (3.8)	51.9 (4.3)
Weight, kg	70.1 (13.8)	72.0 (13.1)
Height, cm	163.7 (6.1)	163.8 (6.0)
BMI	26.2 (5.0)	26.8 (4.4)
Perimenopausal, N	16	17
Postmenopausal, N	24	25
Unknown (endometrial ablation), N	10	8
Hysterectomy, N	9	8
Two ovaries retained, N	8	7
One ovary, N	1	1
Months since last period	48.6 (61.9)	48.7 (67.4)
Months since onset of symptoms	57.3 (44.7)	59.2 (50.1)
Previous herbal medicine use, N (no who found it effective)	37 (19)	36 (16)
Previous HT use, N (no. who found it effective)	15 (13)	17 (12)
Vegetarian, N	3	1
Isoflavone intake, mg/wk	76.6 (114.3)[a]	38.4 (69.2)[a]
Lignans in linseeds, g/wk	0.1 (0.2)	0.03 (0.2)
Caffeine: cups of tea/coffee per day	4.2 (2.1)	4.0 (2.4)
Cigarettes per day	2.0 (58)	1.6 (4.7)
Standard alcoholic drinks per week	3.6 (3.7)[a]	5.6 (5.2)[a]
Satisfaction with relationships, N	44	44
No partner, N	11	13
Negative attitude toward menopause, N	27	26
High stressful life events (more than average), N	27	23
Regular stress reduction, N	14	14
Exercise, h/wk	3.7 (3.7)	3.2 (2.6)
Total exercise, d/wk	3.9 (2.6)	3.8 (2.3)
Nulliparity, N	13[b]	5[b]
Employed outside home, N	40	43
Endpoints, unadjusted mean (SD)		
Flushing episodes per day	9.42 (3.31)	9.65 (4.00) (n = 49)
Daily weighted score	16.35 (6.69)	16.53 (9.02)
Greene Climacteric Scale score	22.48 (8.7)	22.00 (6.47)
Hamilton Depression Inventory-17 score	14.30 (4.25)	14.76 (4.42)
Utian Quality of Life Scale score	77.8 (12.06)	79.04 (10.31)

Data are mean (SD).
[a]Significant difference, $P < 0.05$.
[b]Significant difference, $P < 0.05$ using χ^2 test.

subjectively categorized as mild hot flush without perspiration or clamminess), moderate (hot flush associated with perspiration or clamminess), severe (hot flush associated with intense perspiration that required change of clothing).[28]

The Greene Climacteric Scale and the HDI were completed at 4-week intervals throughout the treatment period and at the 8-week follow-up. The Utian Quality of Life Scale was completed at baseline and week 16.

Potential confounding factors were recorded in a weekly lifestyle diary and included phytoestrogen intake recorded on a food frequency questionnaire, caffeine and alcohol consumption, smoking, formal exercise in hours per week, stressful life events recorded on a 5-point Likert scale, concurrent illnesses, and medication. Total lignan and isoflavone intakes were calculated according to the values reported in quantification studies.[29,30] Adverse events were recorded and reported every 4 weeks.

A power calculation was performed to estimate the required sample size. Anticipating a placebo effect of 30% for hot flush symptoms[31-33] based on phytotherapeutic menopause RCTs and 30% for depression,[34] it was calculated that a sample size of 102 would permit sufficient power (0.8) for the detection of moderate effects ($d = 0.5$) in any outcome variables at an α level of 0.05.

The tablets were randomized by MediHerb using a computer-generated random number table and labeled with code numbers. Women who satisfied the inclusion/exclusion criteria were randomly assigned by the principal investigator using block random sampling to ensure both post- and perimenopausal women were evenly assigned to each group. On entry, participants were assigned code numbers sequentially within blocks. The code was concealed from all the investigators and was not broken until the last participant had completed the treatment phase and data had been scored and entered into the database. The success of blinding was evaluated by the principal investigator and participants guessing their allocation retrospectively, before code breaking.

Data were analyzed using Statistical Package for Social Science (SPSS) version 11 with the assistance of a biostatistician. A mixed model, treating group as the between-subject factor and phase as the within-subject factor, was used for the main variables (hot flushes, Greene Climacteric Scale scores, depression, and quality of life). The interaction between group and phase was also examined. Isoflavone intake was included in the model as a covariable where relevant as the groups were not equal at baseline (Table 1). Unadjusted means are included in Appendix 2. Post hoc testing using pairwise comparisons of the estimated marginal means was used for within-group analyses across the trial phases. Hot flush episodes were weighted as 1 for mild, 2 for moderate, and 3 for severe to take into account the severity as well as the frequency. The daily weighted score was calculated by dividing the weekly weighted total by 7. Missing values from the questionnaires were imputed through expectation maximization algorithms in SPSS.

Participants were recruited from January 2004 to May 2005 through newspapers, a radio interview, Web sites at the Royal Melbourne Institute of Technology and the Jean Hailes Foundation for Women's Health, and fliers at community clinics. Participants were followed up at the end of a 2-week nontreatment run-in, at the end of weeks 1, 4, 8, 12, and 16 of the treatment phase, and the end of the 8-week posttreatment follow-up period.

RESULTS

The two groups were similar at baseline on all categorical variables except nulliparity. However a Pearson's correlation found no relationship between nulliparity and hot flushes, depression, or Greene Climacteric Scale scores at baseline for either the completing or enrolled participants.

The two groups were similar at baseline on all continuous variables except isoflavone intake (mean difference = 38.3,

TABLE 2. *Effect of intervention on endpoints*

	Placebo			Herbal combination			
	Baseline (n = 50)	After 16 wk (n = 46)	Mean change	Baseline (n = 50)	After 16 wk (n = 47)	Mean change	Difference between groups at wk 16, *P*
Flushes, daily weighted	16.35 (1.30)	9.32 (1.36)	7.03 (1.89)	16.53 (1.32)[a]	10.86 (1.34)	5.67 (1.89)	0.42
95% CI	13.79-18.92	6.64-12.00	3.32-10.75	13.93-19.12	8.21-13.51	1.96-9.38	
Greene Climacteric Scale total score[b]	22.52 (1.06)	13.35 (1.11)	9.17 (1.53)	21.98 (1.06)	15.73 (1.10)	6.25 (1.52)	0.13
95% CI	20.43-24.61	11.17-15.52	6.16-12.19	19.89-24.06	13.58-17.89	3.25-9.24	
Greene subscales							
Psychological[b]	11.48 (0.71)	6.73 (0.74)	4.75 (1.02)	11.73 (0.71)	8.49 (0.73)	3.24 (1.02)	0.09
95% CI	10.08-12.88	5.28-8.19	2.74-6.76	10.34-13.13	7.06-9.94	1.29-5.24	
Anxiety[b]	6.36 (0.41)	3.71 (0.41)	2.65 (0.57)	6.33 (0.39)	4.60 (0.41)	1.73 (0.57)	0.13
95% CI	5.59-7.14	2.90-4.52	1.53-3.77	5.56-7.11	3.80-5.40	0.62-2.85	
Depression	5.12 (0.37)	3.02 (0.39)	2.10 (0.53)	5.40 (0.37)	3.89 (0.38)	1.51 (0.52)	0.11
95% CI	4.40-5.84	2.27-3.78	1.05-3.14	4.68-6.12	3.15-4.64	0.47-2.55	
Somatic	4.94 (0.35)	2.83 (0.36)	2.11 (0.50)	4.64 (0.35)	3.13 (0.36)	1.51 (0.50)	0.55
95% CI	4.26-5.62	2.12-3.54	1.13-3.10	3.96-5.32	2.43-3.83	0.53-2.49	
Vasomotor	4.28 (0.21)	2.59 (0.22)	1.69 (0.30)	3.92 (0.21)	2.83 (0.22)	1.09 (0.30)	0.43
95% CI	3.87-4.69	2.16-3.02	1.10-2.29	3.51-4.33	2.41-3.25	0.50-1.68	
Sexual	1.72 (0.14)	1.15 (0.15)	0.57 (0.21)	1.74 (0.14)	1.49 (0.15)	0.25 (0.21)	0.11
95% CI	1.44-2.00	0.86-1.45	0.16-0.97	1.46-2.02	1.20-1.78	−0.15-0.65	
Sleep	1.80 (0.13)	1.26 (0.13)	0.54 (0.18)	1.85 (0.13)	1.31 (0.13)	0.54 (0.18)	0.59
95% CI	1.55-2.05	1.00-1.52	0.18-0.90	1.65-2.15	1.11-1.62	0.18-0.90	
Hamilton Depression Inventory	14.30 (0.75)	8.40 (0.78)	5.90 (1.08)	14.76 (0.75)	9.29 (0.77)	5.47 (1.07)	0.42
95% CI	12.83-15.77	6.87-9.93	3.78-8.02	13.29-16.23	7.78-10.80	3.37-7.58	
Utian Quality of Life Scale	77.80 (1.85)	77.22 (1.93)	−0.58 (2.67)	79.04 (1.85)	81.15 (1.93)	2.11 (2.67)	0.15
95% CI	74.15-81.45	73.41-81.02	−5.86-4.69	75.39-82.69	77.35-84·96	−3.16-7.38	

Data are mean (SEM).
[a]n = 49.
[b]Covariates appeared in the model: isoflavone intake

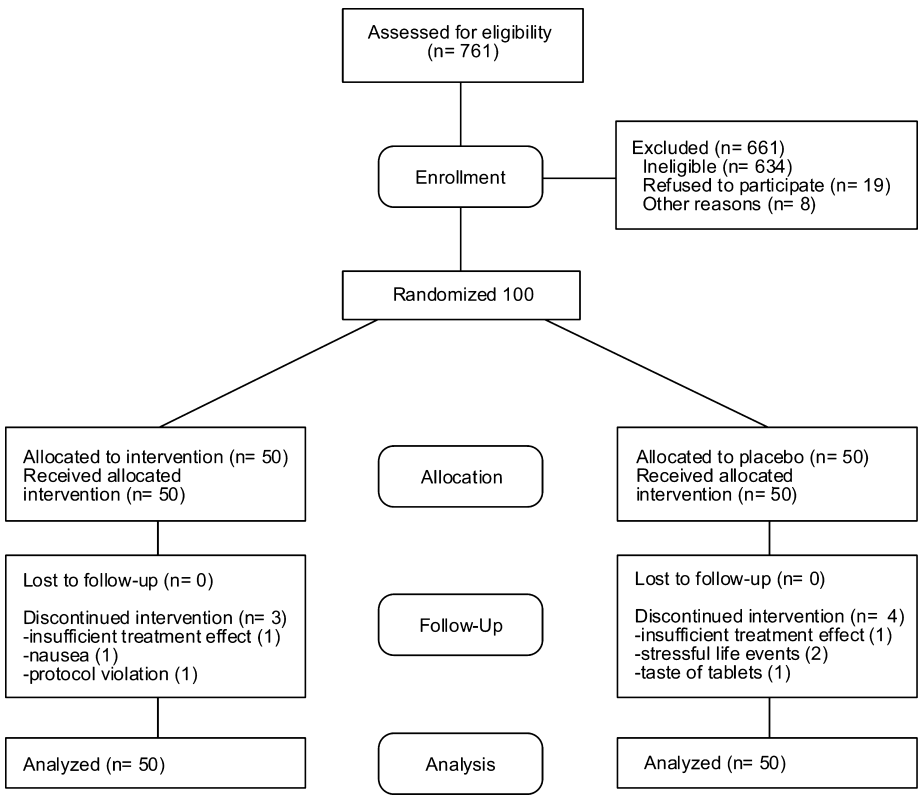

FIG. 1. Participant flow.

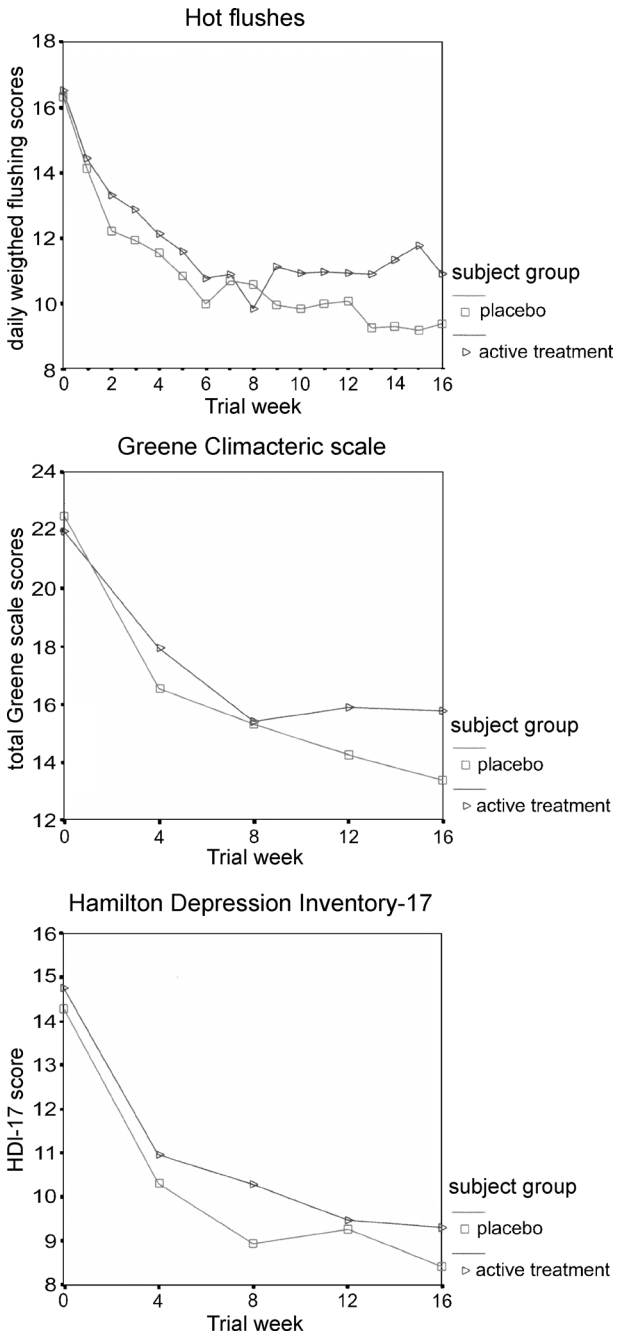

FIG. 2. Effect of intervention on endpoints.

P = 0.042, 95% CI: 1.42-74.97), with the placebo group having the higher intake, and standard drinks per week (mean difference = 1.92, P = 0.035, 95% CI: −3.706 to −0.134), with the active group consuming the greater quantity.

Data were analyzed from 100 participants, 50 in each group, on an intent-to-treat basis.

Of the 100 participants who were randomized, 47 in the active group and 46 in the placebo group completed the study (Fig. 1).

There was no significant difference between the two groups for any of the endpoints at week 16 nor at any time point measured (Table 2): daily weighted hot flush scores (1.54; 95% CI: −2.23 to 5.31, P = 0.42); Greene Climacteric Scale (2.39; 95% CI: −0.68 to 5.45, P = 0.13 [psychological subdomain, 1.76, 95% CI: −0.29 to 3.81, P = 0.09; anxiety, 0.89, 95% CI: −0.25 to 2.03, P = 0.13; depression 0.87; 95% CI, −0.19 to 1.93, P = 0.11; somatic, 0.30, 95% CI: −0.70 to 1.30, P = 0.55; vasomotor, 0.24, 95% CI: −0.36 to 0.85, P = 0.43; sexual, 0.34, 95% CI: −0.07 to 0.75, P = 0.11]; HDI-17 (0.89, 95% CI: −1.27 to 3.04), P = 0.42; and Utian Quality of Life Scale (3.94; 95% CI: −1.45 to 9.32), P = 0.15. Subgroup analysis of the clinically depressed group (HDI-17) did not change the finding.

However, significant improvements were observed for both groups for hot flushes, Greene Climacteric Scale scores, and depression (HDI-17) at week 16 (Fig. 2). The decrease in hot flush scores was significant for the placebo group at $P < 0.001$ and for the active group at $P < 0.01$. Improvements of 50% or more in mean daily weighted hot flush scores were observed in 45.6% of placebo participants and in 43.5% of the active treatment group. Improvements for both groups were significant at $P < 0.001$ on the HDI and Greene Climacteric Scale for overall scores and all subdomains, except for the sexual domain, which was not significant for the active group.

Ancillary analyses

Previous use of phytotherapies modified the effect, with the herb-naive group showing a significantly greater decrease in hot flush scores (−57.67%, 95% CI: 40.49 to 74.86) than the previous users (−32.48%, 95% CI: 19.58 to 45.39, β = −0.215, P = 0.041). Previous positive experience with phytotherapies predicted the overall percentage of improvement in depression (HDI-17) (β = −0.281, P = 0.007), anxiety subscale scores (β = −0.226, P = 0.03), and sleep (Greene Climacteric Scale item 3; β = −0.258, P = 0.02) but did not differ between the active and placebo groups.

Overall compliance with taking the tablets was excellent with a mean of 95% of all tablets taken for both groups. Of the 93 of 100 women completing the trial, only one woman consumed less than 85% of the medications provided (77% medication taken by one placebo group participant).

The main adverse events during the trial were upper respiratory tract infections, not thought to be related to the intervention. There was no significant difference between the two groups for adverse events (χ^2 (1) = 1.10, P = 0.58).

DISCUSSION

No significant difference was found between the herbal combination and placebo in decreasing hot flushes, menopausal symptoms, or depression. This trial reported a high placebo response, which was especially marked for the clinically depressed subgroup. Previous phytotherapy use was associated with a significantly decreased improvement in hot flushes compared with the phytotherapy-naive group. Previous positive experience with phytotherapy predicted the overall percentage of improvement in depression (HDI-17 scores), anxiety, and sleep (Greene Climacteric Scale), but

there was no significant difference between groups for this effect.

The strengths of this study include the robust study design, adequate power, and excellent retention and compliance. It included a homogeneous group of participants with moderately severe overall symptoms. Factors incorporated to control the Hawthorne effect and placebo response include the nontreatment run-in period, trial duration, and a single contact investigator. The intervention trial was produced with high-quality assurance levels and was well-tolerated, and there was an absence of noteworthy adverse events. Potential limitations of the trial include the lack of severity of depression and other psychological symptoms at baseline. Another is the inability to separate the effects of each individual herb in the combination. A criticism of RCTs on fixed formulations in herbal research is that this protocol does not reflect the clinical practice of individualized prescriptions.

There have been no previous published studies of this combination of herbs nor any RCTs of either herb individually in menopause. A 1999 observational study[18] on *H. perforatum* in 111 women over 12 weeks found a 63% decrease in overall menopause symptoms compared with 32% in the present study. That study is not directly comparable to the present study, a robustly designed double-blind, placebo-controlled RCT. Also there was lack of consistency in nomenclature used for describing the different phases of menopause transition and the menopausal rating scales, extracts used, and the administration of a combination in the current study rather than a single herb; the *H. perforatum* extract used in the current study was the phytoequivalent of LI 160 tested in depression studies.

The high placebo response in the current study was consistent with findings from other RCTs of hot flushes or depression.[35,36] Our finding that women with no previous use of phytotherapy were more likely to respond to active treatment or placebo has previously been reported in another study of herbal medicine and menopause.[31] The effect of previous positive experience with phytotherapy in predicting improvements in depression, anxiety, and sleep is a novel finding.

As yet, pharmacology of these two non-estrogenic herbs has not yet been fully elucidated. Their lack of efficacy compared with the prescription nonestrogenic drugs suggests a difference in terms of potency or mechanisms of action. An antagonistic interaction between the two herbs is unlikely based on the current knowledge of their pharmacology. The failure of this study to demonstrate superiority over placebo for depression with *H. perforatum* in combination with *V. agnus-castus* may be due to inadequate symptom severity at baseline, with only 37 women in the clinically depressed subgroup. However, there is an important distinction between depressive syndromes detected by instruments such as the HDI and depressive symptoms, with the latter considered to be more relevant in association with menopause.[37] The other negative findings in the current study are unlikely to be attributable to inadequate symptom severity as baseline hot flushes were as severe as baseline scores from previous positive studies,[23,28] and overall menopausal symptoms measured by the Greene Climacteric Scale were consistent with a menopause clinic sample.[26]

The effect of previous positive experience with phytotherapy in predicting improvements in depression, anxiety, and sleep suggests that these parameters may be more susceptible to the placebo effect and supports the contribution of expectation to the placebo response.[38] Much has been written on ways to control the placebo response in clinical trials, as well as harness the true placebo effect in clinical practice to enhance treatment outcomes. Other factors potentially contributing to the placebo effect in this trial include the natural history of the symptoms, the therapeutic alliance, and experimenter and participant expectations of *H. perforatum*, given its reputation in treating mild to moderate depression. The findings of effect modification of lack of previous experience and positive previous experience with phytotherapy for some parameters warrant further investigation. These could have implications for future research and should potentially be considered when analyzing results of studies.

Although an adverse interaction between the two herbs in the combination seems unlikely, any effect of the individual herbs cannot be established from this trial. Because of synergistic effects, it is not possible to extrapolate from these findings to the effects of formulations combining a greater number of herbs, as is common in clinical practice. A recent example is a trial of the formulation of six herbs including *V. agnus-castus* and *C. racemosa* (black cohosh) that showed a positive effect over placebo for menopausal symptoms.[23] Testing phytotherapies in a way that is both scientifically rigorous and that reflects clinical practice is one of the greatest challenges facing evidence-based CAM. A protocol using three arms has been designed and tested to address this limitation and to reflect the practice of individualized prescribing.[39]

CONCLUSIONS

This is the first RCT of the combination of *H. perforatum* and *V. agnus-castus* for menopausal symptoms. The combination was well tolerated and had a significant effect, although it was not superior to placebo. This was a robust study that further contributes to the growing body of scientific knowledge about complementary therapies from RCTs. Findings from such quality studies are important to inform the community, healthcare providers, and regulatory authorities on the role of CAM.

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APPENDIX 1

Herbal Medicine Intervention (According to Proposed Elaboration of CONSORT Checklist Item 4)

The herbal medicine intervention was a combination of two herbal extracts, *Hypericum perforatum L*. (Clusiaceae/ Guttiferae; St. John's wort) and *Vitex agnus-castus L*, (Verbenaceae; chaste tree/berry) manufactured under the Code of Good Manufacturing Practice by MediHerb Australia Pty Ltd. in their Warwick manufacturing facility. These products are included in the Australian Register of Therapeutic Goods as Listed Medicines.

The extract was obtained from the dry herb flowering top of *H. perforatum* (extraction ratio 6:1 [g/mL]) and dried *V. agnus-castus* fruit (extraction ratio 1:2 [g/mL]; the extraction solvent was 60% ethanol/water. The extract was purchased, identified, and analyzed by MediHerb Quality Assurance Laboratory. Retention samples of raw materials and finished

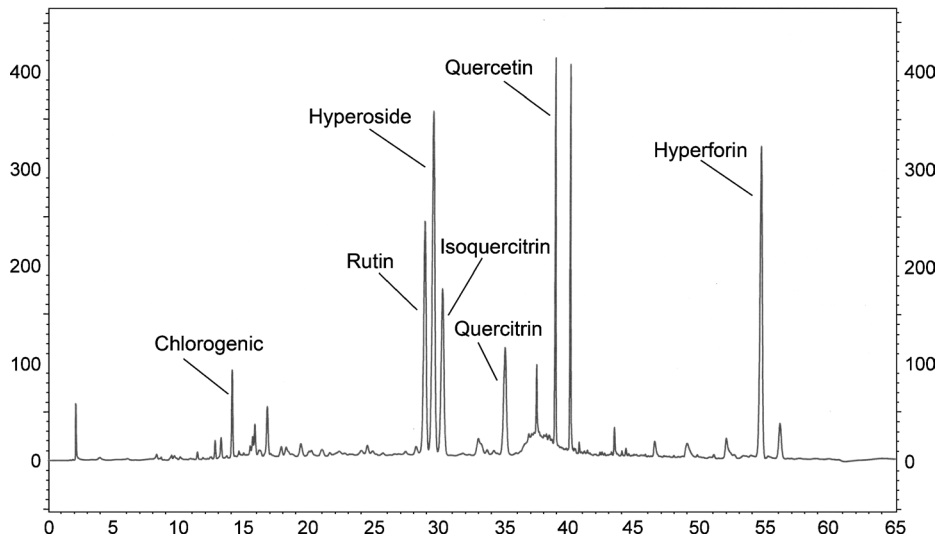

FIG. 3. St. John's wort (Hypericum perforatum) HPLC chromatogram.

tablets were kept at MediHerb; these were validated by chemical fingerprinting against verified botanical samples maintained at the Southern Cross University Herbarium. The St. John's wort tablets were batch 125178 and chaste tree tablets were batch 124324.

The *V. agnus-castus* tablet was not a standardized preparation. The dosage regimen for *H. perforatum* was consitent with a previous study using a daily dose of 900 mg of 6:1 extract.[27]

Qualitative testing

The high-performance liquid chromatography (HPLC) chromatogram for *H. perforatum* can be seen in Figure 3. The method for performing this analysis was as follows: HPLC separation was achieved with a C18, 5-μm column (150 × 4.6 mm) with a gradient elution program using three solvents: methanol (B); 50 mM phosphoric acid in deionized water (C); and acetonitrile (D). The elution program was 0 minutes: B = 0%, C = 85%, D = 15%; 20 minutes: B = 10%, C = 70%, D = 20%; 30 minutes: B = 15%, C = 10%, D = 75%; 55 minutes: B = 15%, C = 5%, D = 80% at a flow rate of 1.0 mL/min. The analysis was done by an individual with more than 20 years of experience in analytical chemistry. Thin-layer chromatography was used for *V. agnus-castus.*

Concentrations of heavy metals were measured by inductively coupled plasma/mass spectrometry.

Standardization

Hypericin was tested using the British Pharmacopoeia UV method. The levels of flavonoid glycosides were determined using a proprietary MediHerb HPLC analytical method, per conditions described above.

The placebo tablets contained the excipients used in the active tablets: modified starch, cellulose, magnesium stearate, and calcium hydrogen phosphate. They were identical to the herbal tablets in size, color, coating, weight, and packaging. All tablets were packaged in amber glass jars with Tamper Tel plastic lids.

The clinician choosing the treatment and dose had 8 years of experience as a chemist and 22 years as a medical herbalist.

As a further check on the validity, the veracity of the code was checked at the conclusion of the trial by Dr. Kerry Penman of MediHerb Australia, using thin-layer chromatography assessment (report available on request).

APPENDIX 2. *Unadjusted scores for Greene Climactic Scale and subscales*

	Placebo			Herbal combination			
	Baseline (n = 50)	After 16 wk (n = 46)	Mean change	Baseline (n = 50)	After 16 wk (n = 47)	Mean change	Difference between groups at wk 16
Greene Climacteric Scale total score	22.48 (1.11)	13.33 (1.11)	9.15	22.00 (1.06)	15.77 (1.09)	6.23	0.12
95% CI	20.40-24.56	11.16-15.50	6.15-12.16	19.92-24.08	13.62-17.91	3.24-9.23	
Greene subscales							
Psychological	11.54 (0.71)	6.76 (0.74)	4.78	11.70 (0.71)	8.45 (0.73)	3.25	0.11
95% CI	10.15-12.93	5.31-8.21	2.77-6.79	10.31-13.09	7.01-9.88	1.25-5.25	
Anxiety	6.42 (0.40)	3.74 (0.41)	2.68	6.30 (0.40)	4.55 (0.41)	1.75	0.16
95% CI	5.64-7.20	2.93-4.55	1.56-3.80	5.52-7.07	3.75-5.35	0.63-2.86	

Data are mean (SEM).