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ORIGINAL ARTICLE

The Effects of a Saffron Extract (affron®) on Menopausal Symptoms in Women during Perimenopause: A Randomised, Double-Blind, Placebo-Controlled Study

Adrian L. Lopresti[1,2], Stephen J. Smith[1,2]

[1]Clinical Research Australia, Perth, Australia, [2]College of Science, Health, Engineering and Education, Murdoch University, Perth, Australia

Objectives: There is preliminary evidence suggesting saffron may effectively treat menopausal symptoms. The aim of this study was to examine the tolerability and efficacy of a standardised saffron extract (affron®) on menopausal complaints in perimenopausal women.

Methods: In this 12-week, parallel-group, double-blind, randomised controlled trial, 86 perimenopausal women experiencing menopausal complaints received either a placebo or 14 mg of a saffron extract (affron®), twice daily. Outcome measures included the Greene Climacteric Scale (GCS), Positive and Negative Affect Schedule (PANAS), and Short Form-36 Health Survey (SF-36).

Results: Based on data collected from 82 participants, saffron was associated with greater improvements in mood and psychological symptoms compared to the placebo. Results from the GCS revealed a significantly greater reduction in the GCS psychological score (P = 0.032), characterised by a 33% reduction in anxiety and a 32% reduction in depression scores from baseline to week 12. There was also a significantly greater reduction in the PANAS negative affect score (P = 0.043) compared to the placebo. However, compared to the placebo, saffron was not associated with greater improvements in vasomotor symptoms, somatic symptoms, or other quality of life measures. Saffron intake was well tolerated with no reported major adverse events.

Conclusions: The saffron extract, affron®, administered for 12 weeks at a dose of 14 mg twice daily was associated with greater improvements in psychological symptoms. Further studies in perimenopausal women presenting with varying severity of menopausal symptoms, using different doses of saffron will be useful to examine in future clinical trials.

Key Words: Anxiety, *Crocus sativus*, Depression, Herbal medicine, Perimenopause

INTRODUCTION

Based on the Staging of Reproductive Aging Workshop (STRAW) criteria, perimenopause is defined as the period between the first major variation in menstrual cycle length (i.e., variations greater than seven days from the individual's normal cycle length) and the completion of 12 consecutive months without any menses [1]. The menopausal transition is associated with changes in sex hormones and reproductive function and is characterised by a range of menopause-specific complaints such as vasomotor symptoms (e.g., hot flushes and cold or night sweats), sleep disturbances, urogenital complaints (e.g., vaginal dryness, painful intercourse, and recurrent urogenital infections), breast pain, joint pain, changes in cognitive function and performance, and mood disturbances including depressive and anxiety-related symptoms [2,3]. The transition into menopause is also associated with an increased risk of osteoporosis [4], metabolic disturbances [5], and cardiovascular disease [6].

Therapeutic options for the management of menopausal symptoms include hormone replacement therapy (HRT), pharmaceutical antidepressants, and

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Address for Correspondence: Adrian L. Lopresti, Clinical Research Australia, 38 Arnisdale Road, Duncraig, WA 6023, Australia
Tel: 61-0894487376, **E-mail:** adrian@clinicalresearch.com.au, **ORCID:** https://orcid.org/0000-0002-6409-7839

physical activity and lifestyle changes [2,7]. Although effective, treatments such as HRT are associated with an increased risk of venous thromboembolism, stroke, cardiovascular disease, gallstones, and breast cancer [2,8-10]. Moreover, despite confirmed therapeutic benefits from antidepressants on depressive symptoms during and after the menopausal transition, it is associated with a high risk of discontinuation due to adverse events [11]. The most common adverse events identified in a meta-analysis by Wu et al. [11] included vomiting, nausea, constipation, lethargy, dry mouth, and headache, with serotonin-norepinephrine reuptake inhibitors (SNRIs) exhibiting a greater adverse effect profile compared to selective serotonin reuptake inhibitors (SSRIs).

Saffron the species is derived from the stigmas of the *Crocus sativus* flower. It has traditionally been used as a treatment for complaints of the eye, skin, respiratory, gastrointestinal, and genitourinary tracts, labour pains, and for its mood-enhancing effects [12,13]. There is also an increasing body of evidence supporting its antidepressant and anxiolytic efficacy in adults with depression and anxiety [14]. In these trials, saffron was well-tolerated with minimal self-reported adverse effects. As a treatment for the alleviation of menopausal symptoms during the menopausal transition, there is preliminary evidence of efficacy. In a 6-week study on post-menopausal women with hot flushes, it was associated with reductions in hot flushes and depressive symptoms [15]. As a component of a multi-herbal formula, saffron was also associated with improvements in physical and mental symptoms in post-menopausal women [16], and an alleviation of physical, psychological, and urogenital symptoms in perimenopausal women [17]. However, despite this preliminary positive evidence, the efficacy and safety of saffron as a standalone treatment on menopausal symptoms during perimenopausal has not been investigated. The aim of this study was to examine the tolerability and efficacy of a standardised saffron extract (affron®) administered for 12 weeks to perimenopausal women experiencing menopausal complaints.

MATERIALS AND METHODS

Study design

This was a two-arm, parallel-group, 12-week, randomised, double-blind, placebo-controlled trial (Fig. 1). The trial protocol was approved by the Human Research Ethics Committee at the National Institute of Integrative Medicine (approval No. 0064E_2020) and was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. ACTRN12620000350921). All participants gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki. An a priori power analysis was undertaken to estimate the required sample size (based on a single outcome variable). In a randomised, controlled study examining the effects of saffron on vasomotor symptoms in postmenopausal women, an effect size of 0.6 compared to the placebo was identified [15]. Assuming a power of 80% and a type one error rate (alpha) of 5%, the number of participants required per group to find an effect on the Greene Climacteric Scale (GCS) total score was estimated as 36. We planned to recruit at least 40 participants per group, which was hypothesised to give suitable power to find an effect compared to the placebo, even after dropouts.

Recruitment and randomisation

Participants were recruited across Australia through social media advertisements in April 2020. Interested participants were directed to a website landing page providing details about the study and a link to complete an initial online screening questionnaire. This online questionnaire screened for current climacteric symptoms, last menstrual cycle, changes in the menstrual cycle, medication use, history of medical/psychiatric disorders, alcohol, nicotine, and other drug use, supplement and vitamin intake, and pregnancy/breastfeeding status. If assessed as likely eligible, volunteers participated in a phone interview with an investigator. The phone interview comprised a structured series of questions to further clarify details pertaining to the eligibility criteria and to obtain further demographic details. Suitable participants were then required to complete online versions of the GCS, Positive and Negative Affect Schedule (PANAS), Short Form-36 Health Survey (SF-36), and an informed consent form. Eligible and consenting participants were randomly assigned to one of two groups (saffron or placebo) using a randomisation calculator (http://www.randomization.com). The randomisation calculator ensured sequence concealment. The randomisation structure comprised 8 randomly permuted blocks, containing 10 participants per block. The participant identification number was allocated according to the order of participant enrolment

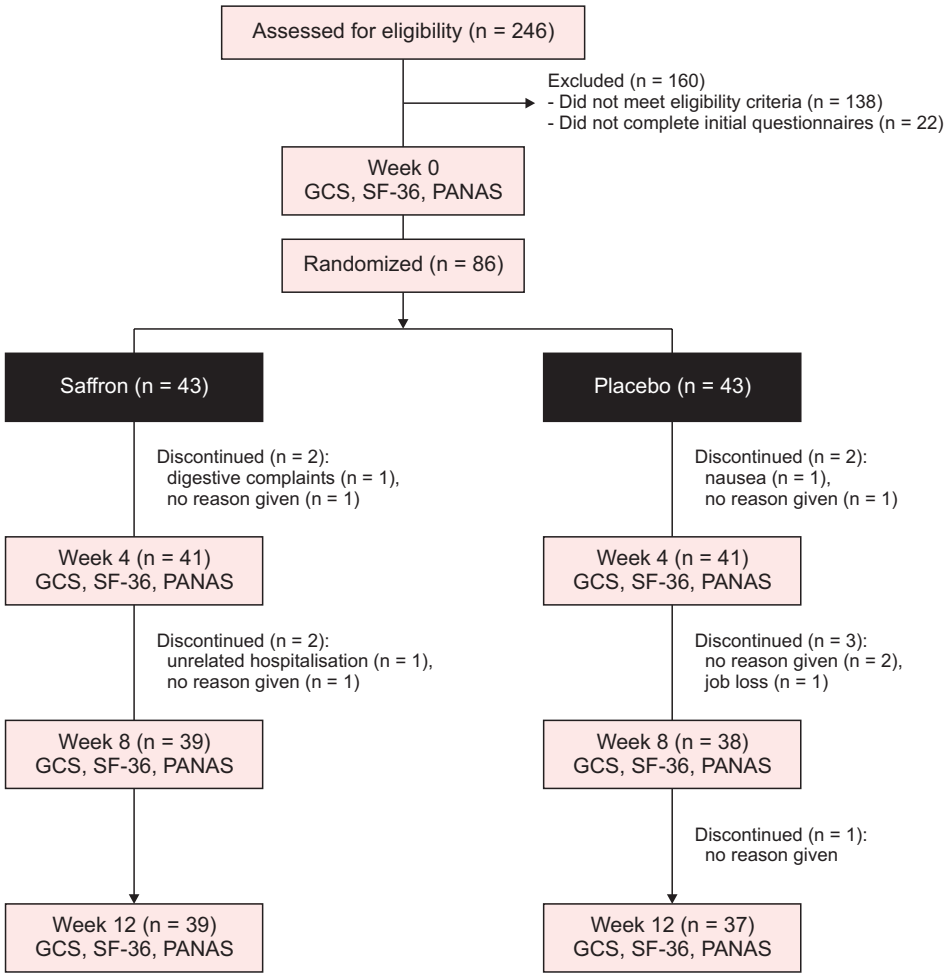

Fig. 1. Systematic illustration of study design. GCS: Greene Climacteric Scale, SF-36: Short Form-36 Health Survey, PANAS: Positive and Negative Affect Schedule.

in the study. All tablets were packed in identical bottles labelled by two intervention codes (held by the study sponsor until all statistical analyses were completed). Participants and study investigators were blind to treatment group allocation until all statistical analyses were completed. No financial compensation was provided to participants for volunteering in this study, although at the end of the study participants allocated to the placebo condition were given a free 12-week supply of saffron tablets.

Participants

Inclusion criteria

Female participants aged between 40 to 60 years with reports of changes in their menstrual cycle for at least 3 months were recruited for this study. Participants needed to have a total score of greater than 16 on the GCS, have an intact uterus and ovaries, a body mass index (BMI) between 18 and 35 kg/m^2, were medication-free for at least 3 months (apart from the contraceptive pill and/or once weekly use of analgesics), were non-smokers, and had no plan to commence new treatments over the study period. Participants were also required to be fluent in English and consented (via an online consent form) to all pertinent aspects of the trial.

Exclusion criteria

Participants who did not have a period in the last 12 months, were consuming more than 14 standard drinks of alcohol per week, had a current or illicit drug abuse within the last 12 months, or were suffering from medical conditions including but not limited to: diabetes, hyper/hypotension, cardiovascular disease, a gastrointestinal disease requiring regular use of medications, gallbladder disease/gallstones/biliary disease, endocrine disease, psychiatric disorder (other than mild-to-moderate anxiety), or neurological disease (Parkinson's

disease, Alzheimer's disease, intracranial haemorrhage, or head or brain injury) were ineligible to participate in the study. Women who had any significant surgeries over the last year, were taking supplements that may affect menopausal symptoms, or were taking saffron supplements were also ineligible to participate in the study.

Interventions

Placebo and saffron tablets were identical in appearance, being matched for colour coating, shape, and size. The active treatment, supplied by Pharmactive Biotech Products, SL, contained 14 mg of a standardised saffron extract (affron®), derived from the stigmas of *C. sativus* L. and standardised to contain > 3.5% Lepticrosalides®, a measure of bioactive compounds present in saffron, including safranal and crocin isomers. The saffron stigmas were cultivated in Alborea (Albacete, Spain) and extracted in the factory of Pharmactive Biotech Products, SL in Madrid (Spain) to produce affron® 3.5% Lepticrosalides®. The placebo tablets contained the same excipients as the active tablet (microcrystalline cellulose and calcium hydrogen phosphate). All tablets were manufactured and packed in an Australian Therapeutic Goods Administration registered plant. All participants were mailed a 12-week supply of tablets and were instructed to take one tablet, twice daily (morning and evening), with or without food for 12 weeks. Medication adherence was measured by tablet count by the participant at week 4, 8, and 12. Efficacy of participant treatment blinding was examined by asking participants to predict group allocation (placebo, saffron, or uncertain) at the end of the study. Directions for use were provided on tablet bottles and participants were also provided with an information sheet about tablet intake and what to do if they missed a dose. This information was also verbally conveyed to participants during their initial telephone interview.

Outcome measures

Primary outcome measure

GCS total score: The GCS is a 21-item, validated, self-report measure designed to assess physical and psychological symptoms associated with the transition into menopause. Each question is rated from zero ("not at all") to three ("extremely") with a maximum total score of 63. The GCS has been demonstrated to have good psychometric properties [18] and is sensitive to treatment for menopausal symptoms [19]. The GCS was completed at baseline, week 4, 8, and 12.

Secondary outcome measures

GCS sub-scale scores: In addition to a total score, the GCS has three sub-scale scores assessing psychological symptoms, somatic/physical symptoms, and vasomotor symptoms. There is also a single question assessing interest in sex. Within the psychological sub-scale, there are 6 questions assessing anxiety symptoms and 5 assessing depressive symptoms.

PANAS: The PANAS is a validated self-report questionnaire that consists of two 10-item scales to measure both positive and negative affect. Each item is rated on a 5-point scale ranging from 1 (not at all) to 5 (very much). Total scores for positive and negative symptoms are calculated. The PANAS has robust psychometric properties in the general population and clinical populations presenting with anxiety, depression, and adjustment disorders [20,21]. The PANAS was completed at baseline, week 4, 8, and 12.

SF-36: The SF-36 is a self-report, quality-of-life measure. Scores are calculated for eight areas including (1) energy/fatigue, (2) physical functioning, (3) bodily pain, (4) general health perceptions, (5) physical role functioning, (6) emotional role functioning, (7) social role functioning, and (8) emotional wellbeing. The SF-36 is a commonly-used outcome measure with strong psychometric properties [22,23]. Scoring for the SF-36 was based on the algorithm developed by RAND Health Care [24]. The SF-36 was completed at baseline, week 4, 8, and 12.

Adverse events: Tolerability and safety of tablet intake by participants were assessed at week 4, 8 and 12 through an online question querying adverse effects that were believed to be associated with tablet intake. Participants were also requested to contact researchers immediately if any adverse effects were experienced.

Statistical analysis

An independent samples *t* test was used to compare demographic variables across the two treatment groups for continuous variables, and Pearson's chi-square was used to compare categorical data. To evaluate study objectives, a repeated-measures ANOVA was used to compare within-group changes over time, and the group (saffron versus placebo) by time interaction effect was used to assess whether changes in outcome scores over time were different between the two groups

(saffron versus placebo). All questionnaire scores were analysed for baseline, week 4, 8, and 12. A Cohen's d was calculated to examine effect sizes. An independent-samples *t* test was also undertaken to examine the percentage change in the GCS total score (primary study objective) from baseline to week 12. A further post-hoc analysis using the independent-samples *t* test was undertaken to examine the percentage change (baseline to week 12) in the GCS psychological, anxiety, and depression sub-scale scores.

The Shapiro–Wilk normality test was conducted to examine the normality of group data. This demonstrated that data were not normally distributed, and this was not corrected by data transformations. However, a repeated-measures ANOVA was considered the most appropriate option for statistical analyses as it is relatively robust to violations of normality [25]. Where necessary, degrees of freedom were adjusted using the

Table 1. Baseline demographic details of participants

Variable		Placebo (n = 43)	Saffron (n = 43)	*P* value
Age (y)		48.63 ± 0.54	49.86 ± 0.49	0.095[a]
Body mass index (kg/m^2)		25.78 ± 0.61	25.34 ± 0.66	0.623[a]
Marital status	Single	8 (18.6)	6 (14.0)	0.559[b]
	Married/de facto	35 (81.4)	37 (86.0)	
Educational status	Secondary	15 (34.9)	17 (39.5)	0.841[b]
	Tertiary	18 (41.9)	18 (41.9)	
	Post-graduate	10 (23.3)	8 (18.6)	
Exercise level	Never/rarely	2 (4.7)	8 (18.6)	0.214[b]
	1–2 times a week	3 (7.0)	4 (9.3)	
	3–5 times a week	18 (41.9)	14 (32.6)	
	≥ 6 times a week	20 (46.5)	17 (39.5)	
Duration in menopausal symptoms	Less than 6 mo	12 (27.9)	8 (18.6)	0.474[b]
	6–12 mo	10 (23.3)	7 (16.3)	
	1–2 y	14 (32.6)	17 (39.5)	
	More than 2 y	7 (16.3)	11 (25.6)	
GCS – total		21.98 ± 1.07	22.84 ± 1.15	0.585[a]
GCS – psychological		12.47 ± 0.71	12.58 ± 0.76	0.911[a]
GCS – somatic		4.67 ± 0.43	5.60 ± 0.44	0.133[a]
GCS – vasomotor		3.21 ± 0.23	2.88 ± 0.24	0.337[a]
PANAS – positive affect		25.81 ± 1.16	25.72 ± 1.15	0.955[a]
PANAS – negative affect		20.42 ± 1.20	20.23 ± 1.19	0.912[a]
SF-36 – physical functioning		88.49 ± 1.66	86.14 ± 2.18	0.394[a]
SF-36 – role limitations due to physical health		69.19 ± 5.63	71.51 ± 5.10	0.760[a]
SF-36 – role limitations due to emotional problems		56.53 ± 6.53	54.26 ± 6.19	0.801[a]
SF-36 – energy/fatigue		39.19 ± 3.30	40.70 ± 2.85	0.730[a]
SF-36 – emotional well-being		61.95 ± 2.79	66.42 ± 2.46	0.234[a]
SF-36 – social functioning		75.16 ± 3.45	79.26 ± 2.93	0.368[a]
SF-36 – pain		70.79 ± 3.19	66.88 ± 2.74	0.355[a]
SF-36 – general health		63.14 ± 2.72	66.40 ± 2.49	0.380[a]

Data are presented as mean ± standard error or n (%).
GCS: Greene Climacteric Scale, PANAS: Positive and Negative Affect Schedule, SF-36: Short Form-36 Health Survey.
[a]By independent samples *t* test. [b]By chi-square test.

Greenhouse–Geisser approach to correct for violations of the sphericity assumption. Data from participants were included in analyses of self-report outcomes if questionnaire data were obtained at week 4 (last observation carried forward from week 4 for missing values). All data were analysed using IBM SPSS Statistics (ver. 26; IBM, Armonk, NY, USA).

RESULTS

Study population

Baseline questionnaire and demographic information

From 246 people who completed the initial online screening questionnaire, 160 individuals were either ineligible (n = 145) or did not complete the initial questionnaires (n = 15). The most common reasons for ineligibility were current medication intake, no menstrual cycle for greater than 1 year, BMI greater than 35 kg/m^2, hysterectomy, or diagnosis of medical conditions included in the exclusion criteria. Seventy-six people completed all study requirements and self-report data from 82 participants who completed at least week-4 questionnaires were used for statistical analyses of self-report outcome measures. Seven participants (placebo [n = 2] and saffron [n = 5]) failed to consume the minimum number of required tablets (i.e., consumed < 80% of tablets). However, data from these participants were included in the statistical analyses as the removal of their results did not significantly influence statistical outcomes. Baseline data of these 86 participants are detailed in Table 1. There were no statistically-significant, between-group differences at baseline. Ten participants withdrew from the study, 4 from the saffron group and 6 from the placebo group. Reasons for withdrawal included no reason given (placebo [n = 4] and saffron [n = 2]), unexpected/unrelated hospitalisation (saffron [n = 1]), digestive complaints (saffron [n = 1]), nausea (placebo [n = 1]), and job loss (placebo [n = 1]).

Outcome measures

GCS total score (primary outcome measure)

Changes in the GCS total score across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's d effect size score are detailed in Table 2 and Figure 2. There was a statistically-significant reduction in the GCS total score over time in both the saffron ($F_{1.8,73}$ = 24.67, P < 0.001) and placebo group ($F_{2.2,87}$ = 6.50, P = 0.002). A between-group analysis revealed there was a non-significant difference in change in the total GCS score between the placebo and saffron group ($F_{2,169}$ = 2.58, P = 0.078, Cohen's d = 0.47). From baseline to week 12, there was a 32% reduction in the total GCS score in the saffron group and a 14% reduction in the placebo group. An independent-samples t test revealed that this difference was statistically significant (T[80] = 2.26, P = 0.027).

GCS sub-scale scores (secondary outcome measure 1)

Changes in the GCS sub-scale scores across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's d effect size score are detailed in Table 2 and Figure 2. A between-group analysis revealed there was a statistically-significant difference in the change in the GCS psychological score between the placebo and saffron groups ($F_{2,163}$ = 3.49, P = 0.032, Cohen's d = 0.59). In both the saffron ($F_{1.9,76}$ = 20.85, P < 0.001) and placebo groups ($F_{1.9,76}$ = 20.85, P = 0.048) there were statistically-significant reductions in the GCS psychological score over time.

In the saffron group, there were statistically-significant reductions in the somatic ($F_{2.0,78}$ = 7.72, P = 0.001) and vasomotor ($F_{2.6,104}$ = 10.74, P < 0.001) scores over time. In addition, there were statistically-significant reductions in the somatic ($F_{2.4,98}$ = 5.68, P = 0.003) and vasomotor ($F_{2.4,96}$ = 5.70, P = 0.003) scores over time in the placebo group. An examination of between-group changes revealed there were no statistically-significant, between-group differences in changes in the somatic ($F_{2.1,175}$ = 0.56, P = 0.589, Cohen's d = 0.09) or vasomotor ($F_{2.5,104}$ = 0.96, P = 0.401, Cohen's d = 0.26) scores over time.

A post-hoc analysis was undertaken to examine changes in the GCS depression and anxiety scores over time. In the saffron group, there was a 33% reduction in the anxiety and a 32% reduction in depression score from baseline to week 12. In the placebo group, there was a 7% increase in the anxiety and a 9% reduction in depression scores from baseline to week 12. An independent samples t test revealed that changes in both the anxiety (T[80] = 2.31, P = 0.023) and depression (T[80] = 2.24, P = 0.028) scores were significantly greater in the saffron group compared to the placebo group.

Table 2. Change in outcome measures

		Placebo (n = 41)					Saffron (n = 41)					Between-group *P* value[b]	Cohen's d effect size
		Week 0	Week 4	Week 8	Week 12	*P* value[a]	Week 0	Week 4	Week 8	Week 12	*P* value[a]		
GCS – total	Mean	21.85	18.15	17.63	17.85	0.002	22.51	16.05	15.24	14.46	< 0.001	0.078	0.47
	SE	1.12	1.29	1.29	1.24		1.16	1.03	1.13	1.00			
GCS – psychological	Mean	12.44	10.78	10.41	10.68	0.048	12.51	8.98	8.51	7.56	< 0.001	0.032	0.59
	SE	0.74	0.75	0.82	0.77		0.76	0.71	0.65	0.58			
GCS – somatic	Mean	4.63	3.41	3.44	3.27	0.001	5.37	3.56	3.46	3.68	0.001	0.589	0.09
	SE	0.45	0.45	0.41	0.39		0.43	0.30	0.42	0.45			
GCS – vasomotor	Mean	3.20	2.34	2.44	2.56	0.003	2.85	2.02	1.80	1.80	< 0.001	0.401	0.26
	SE	0.24	0.31	0.29	0.30		0.25	0.26	0.22	0.22			
PANAS – negative affect	Mean	20.32	19.02	19.29	19.68	0.553	20.51	17.39	16.37	15.85	< 0.001	0.043	0.55
	SE	1.22	1.04	1.06	1.14		1.23	1.07	0.83	0.77			
PANAS – positive affect	Mean	25.88	27.29	27.29	27.32	0.408	25.80	29.44	30.22	29.34	< 0.001	0.169	0.29
	SE	1.21	1.36	1.26	1.38		1.21	1.07	1.11	1.22			
SF-36 – physical functioning	Mean	89.63	90.24	90.85	91.83	0.284	86.44	86.95	86.83	88.29	0.762	0.969	0.03
	SE	1.49	1.68	1.69	1.68		2.28	2.13	2.59	1.85			
SF-36 – role limitations due to physical health	Mean	69.51	82.93	77.44	81.10	0.072	71.34	80.49	84.15	82.93	0.083	0.645	0.01
	SE	5.76	4.66	5.78	4.26		5.28	5.20	4.16	4.66			
SF-36 – role limitations due to emotional problems	Mean	56.85	65.85	61.83	66.66	0.312	54.46	69.93	71.56	83.76	0.001	0.142	0.42
	SE	6.62	6.11	6.65	5.94		6.46	6.14	5.66	5.09			
SF-36 – energy/fatigue	Mean	40.00	45.49	45.24	44.39	0.176	40.73	48.41	50.61	49.63	0.001	0.521	0.22
	SE	3.40	3.62	3.75	3.80		2.99	2.36	2.84	3.11			
SF-36 – emotional well-being	Mean	62.05	64.68	65.66	64.68	0.357	67.22	71.51	75.22	76.00	< 0.001	0.163	0.38
	SE	2.91	2.85	3.15	3.36		2.44	2.27	2.30	2.43			
SF-36 – social functioning	Mean	76.37	78.15	71.17	75.12	0.221	80.98	84.00	81.68	85.56	0.494	0.462	0.28
	SE	3.48	3.34	4.26	3.67		2.72	2.96	3.37	2.88			
SF-36 – pain	Mean	72.83	77.17	81.51	79.95	0.007	67.39	72.73	74.83	73.15	0.079	0.904	0.34
	SE	2.94	2.45	2.39	2.89		2.82	2.87	2.76	3.42			
SF-36 – general health	Mean	64.15	68.05	65.61	65.37	0.172	66.46	70.37	70.12	69.88	0.078	0.631	0.16
	SE	2.72	2.69	2.73	3.24		2.61	2.50	2.75	2.64			

GCS: Greene Climacteric Scale, PANAS: Positive and Negative Affect Schedule, SF-36: Short Form-36 Health Survey, SE: standard error.
[a]By repeated-measures ANOVA, within-group changes. [b]By repeated-measures ANOVA, between-group changes (placebo vs saffron).

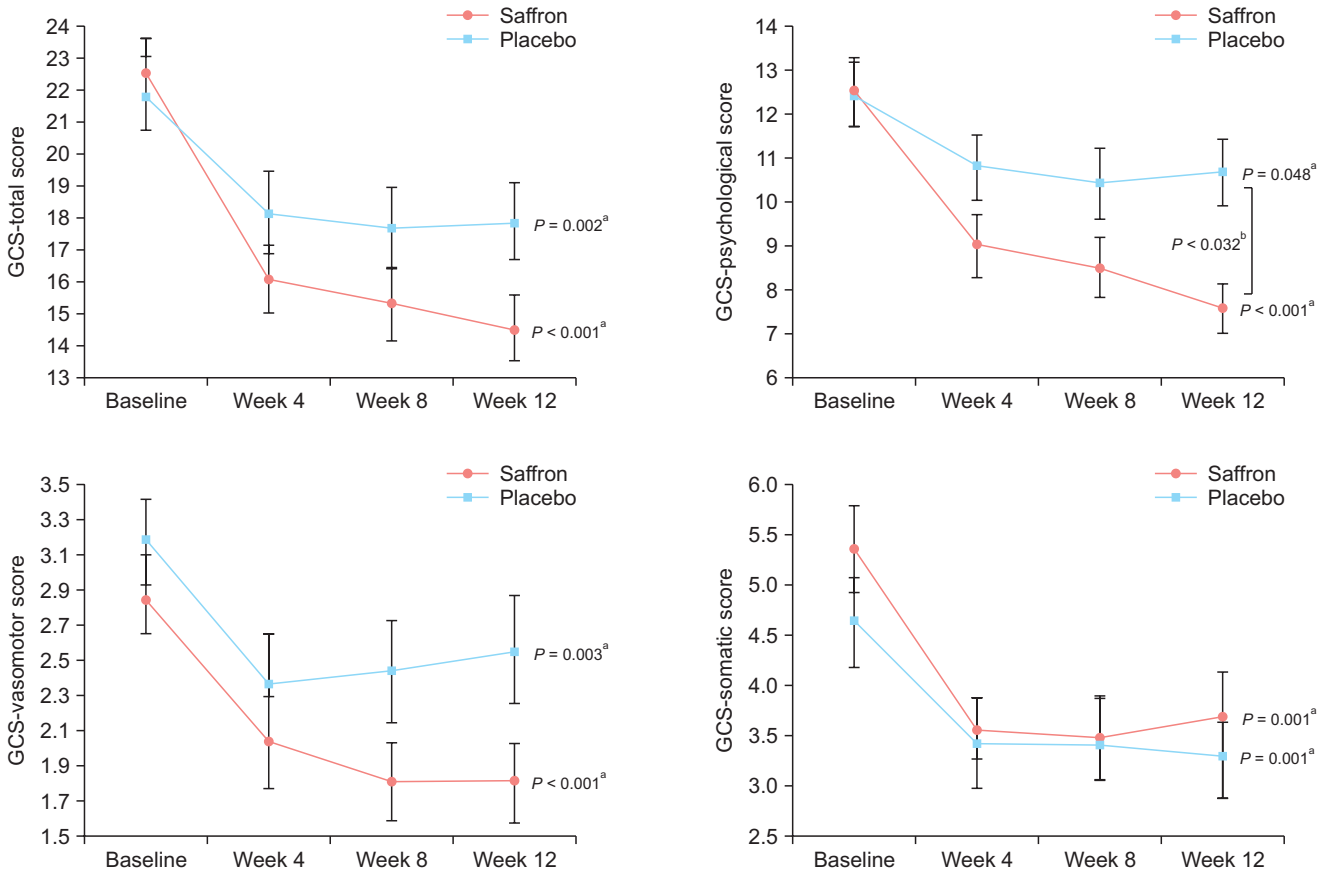

Fig. 2. Change in Greene Climacteric Scale (GCS) scores. [a]*P* value, within group change; [b]*P* value, between-group difference.

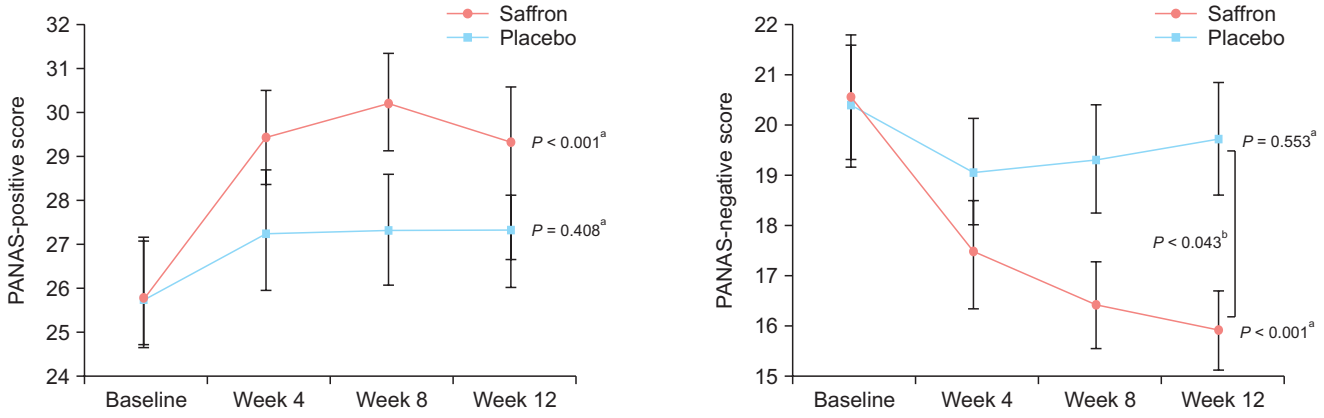

Fig. 3. Change in Positive and Negative Affect Schedule (PANAS) scores. [a]*P* value, within group change; [b]*P* value, between-group difference.

PANAS scores (secondary outcome measure 2)

Changes in the PANAS negative and positive affect scores across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's d effect size score are detailed in Table 2 and Figure 3. A between-group analysis revealed there was a statistically-significant difference in the change in the PANAS negative score ($F_{2.4,194} = 2.99$, $P = 0.043$, Cohen's d = 0.55) but not the PANAS positive score ($F_{2.6,204} = 1.74$, $P = 0.169$, Cohen's d = 0.29) between the placebo and saffron group. In the saffron group, there was a statistically-significant reduction in the PANAS nega-

tive score ($F_{2.3,93}$ = 8.07, P < 0.001) and statistically-significant increase in the positive score ($F_{2.5,101}$ = 9.33, P < 0.001) over time. However, there were no statistically-significant changes in both the PANAS negative ($F_{2.5,99}$ = 0.66, P = 0.553) and positive ($F_{2.5,102}$ = 0.95, P = 0.408) scores in the placebo group over time.

SF-36 subscale scores (secondary outcome measure 3)

Changes in the SF-36 sub-scale scores across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's d effect size score are detailed in Table 2. Between-group analyses revealed there were no statistically-significant differences in the change in any SF-36 sub-scale score between the placebo and saffron groups. However, in the saffron group, there were statistically-significant improvements in the SF-36 role limitation due to emotional problems ($F_{2.3,91}$ = 7.41, P = 0.001), energy/fatigue ($F_{2.3,94}$ = 6.55, P = 0.001), and emotional wellbeing ($F_{2.2,90}$ = 8.11, P < 0.001) sub-scale scores over time. In the placebo group, there was a statistically-significant improvement in only the pain score ($F_{2.5,101}$ = 4.60, P = 0.007) over time.

Intake of supplements

At week 12, participants recorded their quantity of remaining tablets. Ninety percent of participants who completed the study reported taking more than 80% of their tablets.

Efficacy of participant blinding

To evaluate the efficacy of condition concealment over the study, participants were asked at the end of the study to predict group allocation (i.e., placebo, saffron, or uncertain). Efficacy of group concealment was high as only 8% of people in the saffron group and 28% in the placebo group correctly guessed treatment allocation.

Adverse events

No major adverse events were reported by participants although there were two withdrawals from the study due to mild adverse effects. One participant in the saffron group withdrew due to mild digestive complaints/bloating, and one in the placebo group withdrew due to ongoing nausea. The frequency of reported adverse effects is detailed in Table 3, which revealed an overall similar frequency in reported adverse events between the two groups. However, there was a tendency to suggest greater digestive complaints in the saffron group (e.g., flatulence and nausea).

DISCUSSION

In this 12-week, randomised, double-blind, placebo-controlled study, the administration of a saffron extract (affron®) at a dose of 28 mg daily was associated with greater improvements in psychological symptoms in women experiencing perimenopause compared to the placebo. Saffron was also associated with improvements in vasomotor (e.g., hot flushes and night-time sweating) and somatic symptoms, however, changes were not significantly different from the placebo. Saffron intake was well tolerated with no reported major adverse events, although there was a greater number of reports of mild digestive complaints (e.g., flatulence and nausea).

The mood-enhancing effects of saffron have been confirmed in several studies. In a meta-analysis comprising 23 studies, saffron administration had a large positive treatment effect when compared with the placebo on depressive and anxiety symptoms [14]. These studies have been conducted on adults of varying ages, with no trial specifically examining its mood-

Table 3. Frequency of adverse events

	Saffron (n)	Placebo (n)
Flatulence	3	-
Nausea/bloating	2	-
Constipation	-	2
Reflux	1	-
Decreased appetite	-	1
Body odour	-	1
Migraine/headache	2	-
Dry mouth	1	-
Weight gain	-	1
Pressure in head	-	1
Joint pain	1	-
Nightmares	-	1
Occasional hives	1	-
Fatigue	-	1
Increased hot flushes	-	1
Total	11	9

enhancing effects during perimenopause. However, in a study on post-menopausal women with hot flushes, improvements in depressive symptoms were identified after the 6-week administration of a saffron extract [15]. In the current trial, saffron was associated with a 33 and 32 percent reduction in anxiety and depressive symptoms, respectively, suggesting it had a generalised mood-enhancing effect. This is further confirmed by improvements in negative affective symptoms as measured by the PANAS. The PANAS negative affect score is based on ratings associated with the descriptors such as stressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid. There was also a trend to suggest improvements in the PANAS positive symptom score, although changes did no achieve statistical significance compared to the placebo. Most improvements in self-rated mood occurred in the first 4 weeks of the trial, with continued, albeit less pronounced improvements from weeks 4 to 12. Depressive and anxiety symptoms during the menopausal transition are typically treated with SSRIs and SNRIs. However, although effective, they are associated with several adverse effects resulting in high rates of discontinuation [11]. The positive mood-enhancing findings and low frequency of self-reported adverse effects present saffron as a promising natural mood treatment during perimenopause.

The mechanisms associated with the antidepressant and anxiolytic effects of saffron have not yet been determined, although it is postulated to be multifactorial. For example, saffron has been demonstrated to influence neurotransmitter activity, inflammation, hypothalamic-pituitary-adrenal (HPA) axis activity, oxidative stress, mitochondrial activity, and neuroplasticity [26]. Disturbances in these mechanisms have been regularly identified in depression and anxiety [27,28]. Moreover, it is plausible that saffron's mood-enhancing effects during perimenopause may be associated with its influence on sex hormones. In an animal study, the administration of zearalenone (a mycotoxin with potent estrogenic effects) plus saffron to 8-week old female mice was associated with higher serum concentrations in luteinising hormone (LH), follicle-stimulating hormone (FSH), oestradiol, and progesterone compared with zearalenone alone [29]. In a study on adult, female rats, the administration of crocin (an active constituent of saffron) decreased estrogen and progesterone concentrations but did not affect FSH or LH [30]. In a study conducted on female rats treated with the menopause-inducing medication cyclophosphamide, concentrations in oestrogen were altered by saffron administration but only at the highest dose of 2 g/kg/day [31]. Finally, the oral administration of an aqueous saffron extract at a dose of 20 and 80 mg/kg/day for 30 days to adult female rats increased serum concentrations in FSH and progesterone (both doses), and LH and oestrogen (high-dose only) [32]. These animal studies suggest saffron may alter sex hormone concentrations although its effects are influenced by dose, age, and stressor exposure. The applicability of these animal studies during the menopausal transition are also uncertain as validated menopausal animal models were not used [33] and the administered doses of saffron required to have oestrogenic effects were well beyond equivalent human doses used in previous human trials on saffron. Whether the mood-enhancing effects of saffron were due to its influence on sex hormone activity could not be determined in the current study as no biological assessments were undertaken.

Despite previous studies demonstrating the positive effects of saffron, either administered alone or in combination with other herbal ingredients, on vasomotor and somatic symptoms, such benefits were not identified in this study. In a 6-week, randomised, double-blind, placebo-controlled trial, saffron delivered at a dose of 30 mg daily was associated with significant improvements in hot flushes in post-menopausal women experiencing ≥14 hot flushes per week [15]. In a 12-week trial using multiple doses of a mixed herbal combination containing saffron, fennel, and chamomile on peri-menopausal women, there were greater improvements in physical symptoms at the high dose only and greater improvements in psychological and urogenital symptoms (e.g., sexual problems, urinary complains, and vaginal dryness) at the low dose only, compared to the placebo [17]. In another study on post-menopausal women, the herbal combination comprising saffron, tribulus terrestris, zingiber officinale (ginger), and cinnamonum zeylanicum (cinnamon) administered for 4 weeks was associated with greater improvements in physical and mental, but not urogenital symptoms compared to the placebo [16]. The inconsistency in these findings may be due to differences in the population examined (i.e., post-menopausal vs perimenopausal women), and the administration of saffron as a stand-alone compared to a multi-herbal combination. Moreover, the severity and frequency of hot flushes in participants recruited in the study by Kashani et al. [15]

were significantly greater than the levels experienced by the population recruited in the current trial.

Even though the results of this study add to the existing literature, there are several limitations and directions for future research. The assessment of perimenopause was based on self-reports of changes in the menstrual cycle in women aged between 40 and 60 years. Because no formal medical assessment comprising an evaluation of hormone concentrations and a comprehensive examination of confounding medical, lifestyle, and dietary factors was undertaken, it is possible that some women in other reproductive stages were recruited in this study. Validation of these findings in more comprehensively-evaluated perimenopausal women will be useful in future trials.

Even though mood improvements from saffron administration were identified, this was based on the GCS psychological sub-scale score and the PANAS negative affect score. These are validated self-report outcome measures but were not specifically developed for the assessment of depression and anxiety. Using validated, self-report, and clinician-administered anxiety and depression outcome measures will be important to use in future trials. Moreover, in this recruited population, women with severe depressive or anxiety symptoms, or women currently receiving psychological or pharmacological treatment, were excluded from participating in this study. The effects of saffron in women with a formally-diagnosed depression or anxiety-related disorder, and with varying levels of severity, will be useful to examine in future trials.

In this study, saffron was associated with improvements in vasomotor and somatic symptoms; however, changes were not significantly different from those observed in the placebo condition. In the study by Kashani et al. [15], a clearly-defined population of postmenopausal women presenting with severe and frequent hot flushes was recruited. However, participants in this study were recruited based on self-reports of difficulties in overall climacteric symptoms. Concerning vasomotor symptoms, average ratings suggested symptoms were of mild-to-moderate severity and the frequency and severity of hot flushes were much lower than levels experienced by women in the Kashani et al's study [15]. An examination of the effects of saffron in perimenopausal women presenting with more severe, specific climacteric symptoms will be important to examine in future trials. The safety and efficacy of saffron administration in women currently receiving pharmacological treatment for menopausal symptoms also require further investigation. In a previous trial, the adjunct administration of saffron with pharmacological antidepressants was associated with a greater reduction in depressive symptoms [34]. Its co-administration in perimenopausal women currently taking antidepressants and/or on HRT will be important to evaluate in future trials. Saffron used as a component of a multiherbal combination also requires further investigation, particularly as there have been benefits identified in two previous trials [16,17]. Moreover, the efficacy of different saffron extracts should be examined. In this study, the standardised saffron extract, affron®, was used. Given the variability in the quality, purity, and levels of active ingredients in saffron [35], the applicability of these findings to different saffron extracts or as a spice used in cooking is unknown. The efficacy and safety of saffron at different doses and treatment durations will also be helpful to determine whether higher doses or longer treatment periods are required for the alleviation of specific menopausal symptoms. As saffron was only administered for 12 weeks, an examination of its safety as a long-term treatment for climacteric symptoms will also be important. Therefore, studies with longer follow-up are required. Finally, to help understand the mechanisms of action associated with saffron intake, assessment of changes in concentrations of sex hormones, and other pertinent markers such as those associated with inflammation, oxidative stress, HPA-axis activity, and neurotrophic activity will be useful.

The results of this 12-week trial in perimenopausal women provide evidence for the beneficial effects of a standardised saffron extract, affron®, on depressive and anxiety symptoms. However, its influence on vasomotor or other somatic symptoms was not significantly different from the placebo. Given the positive, mood-enhancing findings, further investigations into the benefits of saffron in more clearly-defined populations, presenting with specific menopausal complaints; and using validated self-report, clinician-administered, and biological outcome measures, will be important to conduct in future trials.

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CONFLICT OF INTEREST

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