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An Examination into the Effects of a Saffron Extract (Affron) on Mood and General Wellbeing in Adults Experiencing Low Mood: A Randomized, Double-Blind, Placebo-Controlled Trial

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A B S T R A C T

Background: Saffron, derived from the stigmas of the *Crocus sativus* flower, has been shown in previous trials to have antidepressant effects in clinically diagnosed adults. However, the recruitment of small sample sizes, short treatment periods, and variability in the quality of studies have negatively impacted the strength of conclusions.

Objectives: The purpose of this 2-arm, 12-wk, parallel-group, randomized, double-blind, placebo-controlled trial was to examine the effects of supplementation with a saffron extract (Affron) on mood and sleep in adults experiencing subclinical depressive symptoms.

Methods: Two hundred and two adults aged 18–70 with depressive symptoms were supplemented with 28 mg saffron daily or a placebo. Outcome measures included the Depression, Anxiety, and Stress Scale – 21, Sleep Disturbance and Sleep-Related Impairment Scale, World Health Organization–Five Well-Being Scale, and daily depression, stress, and anxiety ratings.

Results: On the primary outcome measure, compared to the placebo, saffron was associated with greater improvements in the Depression, Anxiety, and Stress scale – 21 depression score (β: –2.92 points; 95% confidence interval: –5.13, –0.71 points; Cohen's d = 0.39), with 72.3% of participants in the saffron group achieving a clinically significant change (a reduction of ≥ 7 points) compared to 54.3% of participants in the placebo group (P = 0.010). However, in the other secondary outcomes, there was no evidence of between-group differences. In exploratory analyses across various strata and assumptions, improvements in sleep disturbances (β: –2.72 points; 95% confidence interval: –4.99, –0.46 points; Cohen's d = 0.44) were identified in a subset of participants with a greater severity of sleep disturbance. There were no serious adverse reactions reported.

Conclusions: This study, the largest conducted to date on saffron, provides evidence supporting the beneficial effects of 3 mo of saffron supplementation on depressive symptoms in adults. Large placebo responses were evident in this study, which require consideration in future trials.

This trial was registered at Australian and New Zealand clinical trials registry as ACTRN12623001358639.

Keywords: saffron, *Crocus sativus*, depression, anxiety, stress, sleep, clinical trial

Introduction

Subclinical, subthreshold, or minor depression (herein referred to as subclinical depression) is defined as the presence of depressive symptoms but without fulfilling all the diagnostic criteria for major depressive disorder [1]. Subclinical depression is typically considered when a person experiences 2–4 depressive symptoms, with 1 of them being either a depressed mood or loss of interest or pleasure, during a 2-wk period [2]. Estimates of prevalence rates of subclinical depression vary widely; however, in a recent review, a point prevalence rate of 11% was identified [3]. Subclinical depression is a significant risk factor for the

Abbreviations: AE, adverse event; CI, confidence interval; CTTES, Clinical Trials Treatment Expectancies Scale; DASS-21, Depression, Anxiety, and Stress Scale - 21; FAS, full analysis set; GLMM, generalized linear mixed model; IP, investigational product; PPS, per-protocol set; PROMIS Sleep, PROMIS Sleep Disturbance and Sleep-Related Impairment Scale; SIMR, single-item mood rating; WHO-5, World Health Organization–Five Well-Being Index.

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progression to major depressive disorder, and compared to nondepressed adults, it is associated with substantially more impairment in physical, social, and occupational function [2–4]. Alarmingly, a meta-analysis indicated that mortality rates in subclinical depression are comparable with those in major depressive disorder [5].

Pharmaceutical antidepressants are often recommended as a primary treatment of major depressive disorder; however, in a meta-analysis, it was concluded that they were unlikely to have clinical advantages over placebo in people with subclinical depression [6]. Interest in plant-based medicines (phytoceuticals) for the treatment of mood disorders is increasing. In guidelines published by the World Federation of Societies of Biological Psychiatry and Canadian Network for Mood and Anxiety Treatments task force, it was concluded that there was supporting grade A evidence and positive directionality for St John's wort, saffron, curcumin, and lavender for the treatment of unipolar depression [7]. However, inconsistency in the quality and standardization of phytoceuticals, trial heterogeneity, and methodological limitations of many studies impacted the strength of recommendations made by the task force. Moreover, the effect of phytoceuticals on people with subclinical depression has received little dedicated investigation.

Saffron stigmas, derived from the *Crocus sativus* flower, have undergone >20 randomized clinical trials for the treatment of major depressive disorder [7,8]. In several meta-analyses, saffron was found to have larger antidepressant effects compared to a placebo [8] and comparable efficacy to pharmaceutical antidepressants [9]. Although mechanisms associated with saffron's mood-enhancing effects require further investigation, it is speculated that it may work through multiple actions such as influencing neurotransmitter activity, moderating the stress response (hypothalamus-pituitary-adrenal axis), and reducing inflammation and oxidative stress [10–12].

Despite these overall positive findings, many saffron trials have recruited small sample sizes, typically ranging from 40 to 80 people, with treatment durations mostly ranging from 4 to 8 wk. Moreover, the populations investigated have typically comprised adults with major depressive disorder [8,10,13], with little investigation on people with subclinical depression. Therefore, in an attempt to add to the body of evidence on the mood-enhancing effects of saffron, the objectives of this study were to investigate the efficacy of a saffron extract (Affron) administered for 12 wk to adults with subclinical depression utilizing a robustly-powered sample size of 202 participants. This makes it the largest study conducted to date on the antidepressant effects of saffron. Based on the existing evidence, it was hypothesized that saffron supplementation in adults experiencing low mood/subclinical depression would be associated with improvements in affective symptoms, particularly in depressive symptoms. Moreover, as saffron has been shown in several trials to support sleep in people with insomnia and sleep disturbances [14–16], changes in sleep outcomes were investigated as secondary outcome measures.

Methods

Study design and procedures

The study received ethics approval in Australia from the National Institute of Integrative Medicine Human Research ethics committee (approval number 0134E_2023), and informed consent was acquired from all participants. This trial was registered prospectively with the Australian and New Zealand clinical trials registry (ACTRN12623001358639).

This was a 12-wk, 2-arm, parallel-group, randomized, double-blind, placebo-controlled trial (Figure 1). Participants were blinded throughout the study, and researchers and the statistician were blind to the treatment allocation until all outcomes were collected and a blind review was completed. The recruitment of volunteers occurred from May to August 2024 through social media advertisements and emails to an in-house database of interested volunteers. Participants completed an online screening questionnaire, where they provided demographic information, details of their health status, medical history, and medication intake, and completed the Depression, Anxiety, and Stress scale – 21 (DASS-21) depression subscale. If they scored ≥10 on the DASS-21 depression subscale and were deemed potentially suitable, based on the eligibility criteria, they were contacted by a researcher for a telephone interview. During this interview, a more comprehensive assessment of the eligibility criteria was undertaken, relevant demographic and sociographic details were obtained, and a full explanation of the study was provided. If eligible and willing to participate in the study, participants were then required to electronically sign the informed consent form. After completion of the consent form, participants were randomly allocated to 1 of 2 groups (saffron or placebo) on a 1:1 ratio using a randomization calculator with the randomization structure consisting of 20 permuted blocks, with 10 participants per block. A participant identification number was designated based on the participant enrolment order, and the randomization sequence was generated by an investigator not directly involved in volunteer recruitment. All tablets were packed in identical containers, and the study sponsor held the bottle codes. Participants were immediately sent, by express freight, a 12-wk supply of the study tablets. Study tablets were typically received 1–4 working days after the telephone interview. Participants were instructed to notify researchers when the tablets were received, after which time they were required to complete all self-report questionnaires (week 0) and to commence their intake of study tablets the following day. All self-report questionnaires were again completed online every 4 wk (i.e., weeks 4, 8, and 12). Moreover, every evening at 8 pm, participants were sent a link on their phone to complete 3 single-item mood ratings (SIMR) and to indicate if study tablets were consumed for the day.

Participants

Inclusion criteria

Inclusion criteria for the trial comprised the following: healthy adults (female or male), 18–70 y; currently experiencing low mood as demonstrated by a score of ≥10 at screening on the DASS-21 depression subscale; nonsmoker; BMI (in kg/m^2) between 18 and 35; had no plan to start a new intervention in the next 3 mo.

Exclusion criteria

The exclusion criteria comprised the following: a diagnosis of a psychiatric disorder by a health professional in the last 12 mo; currently receiving regular psychological therapy/counseling; currently experiencing a severe life stressor (e.g., finances, work, relationship, or health) that significantly influences daily function and activity; in the last 3 mo a diagnosis of, or having an uncontrolled medical disorder comprising hyper/hypotension,

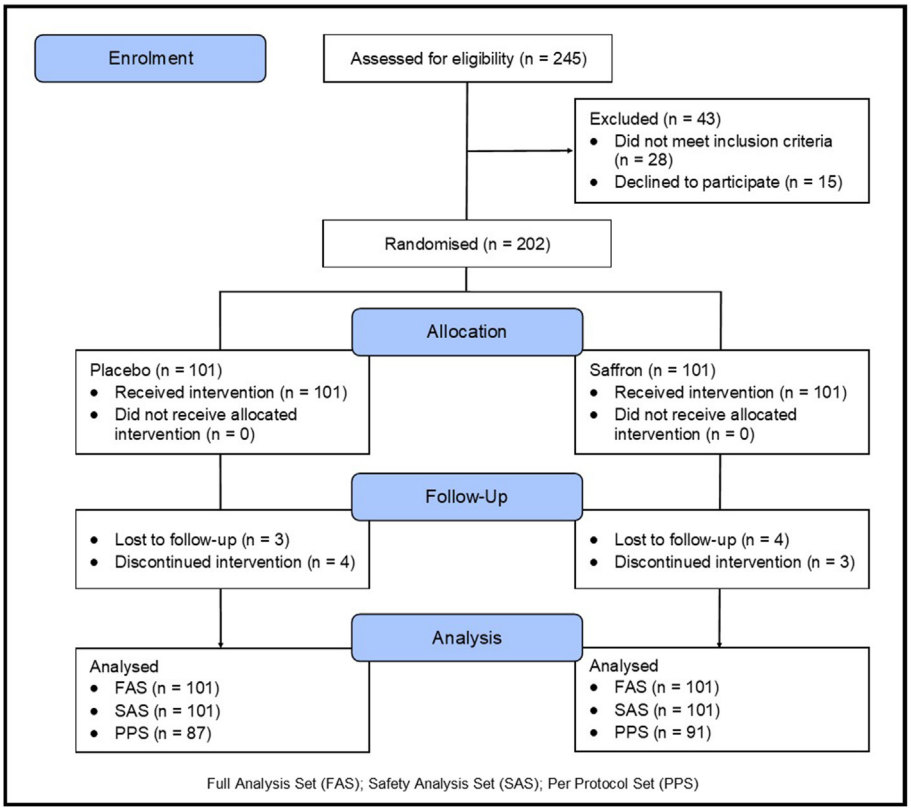

FIGURE 1. Systematic illustration of study design.

gastrointestinal disease, diabetes, cardiovascular disease, autoimmune disease, endocrine disease, or cancer/malignancy; a diagnosis of a neurological disease comprising Parkinson's disease, Alzheimer's disease, multiple sclerosis, stroke, or a brain injury significantly affecting daily function; regular medication intake comprising anticonvulsants, benzodiazepines, opioids, corticosteroids, or immunosuppressants; a change in medication in the last 3 mo or a plan to change during the study duration; in the last 3 mo, commenced or modified the dose of nutritional and/or herbal supplements that may have an effect on the treatment outcomes; a current or 12-mo history of illicit drug use; alcohol consumption of >14 standard drinks per week; pregnant, breastfeeding, or a plan to become pregnant in the next 3 mo; any major surgeries over the past 12 mo that continued to significantly affect daily function; or planned significant lifestyle change in the next 3 mo.

Interventions

The intervention comprised either a saffron extract (Affron) or a placebo (microcrystalline cellulose). Participants were instructed to consume 1 tablet in the morning and 1 tablet in the evening with or without food, with the active intervention delivering 28 mg Affron daily for 12 wk. This is the identical daily dose that has been used in previous studies on Affron [17–19]. The active and placebo tablets were identical in appearance, matched for shape, color, and size, with both tablets containing similar excipients (microcrystalline cellulose, calcium phosphate dihydrate, magnesium stearate, and silicon dioxide). Affron is produced from the stigmas of *Crocus sativus* L. and standardized to contain >3.5% Lepticrosalides, a measure of bioactive compounds contained in saffron, including safranal and crocin isomers. Adherence to tablet intake was assessed by asking participants to provide a count of remaining tablets on week 12. Treatment blinding was evaluated by asking participants to guess their group allocation (placebo, saffron, or unsure) at the end of the study, along with a reason for their prediction.

Outcome measures

Primary outcome measure

DASS-21 depression score. The DASS-21 is a validated self-report questionnaire that assesses symptoms associated with depression, anxiety, and stress over the last 7 d [20]. It consists of 21 items, where 3 subscale scores (depression, anxiety, and stress) are calculated. Scores range from 0 to 42 for each subscale. Because a major criterion for study inclusion was the presence of depressive symptoms, the bulk of clinical trials on saffron have demonstrated positive effects on depression [7], and mechanistic investigations suggest antidepressant activity [10], the DASS-21 depression subscale score was chosen, a priori, as the primary outcome measure. In a study by Ronk et al. [21], a clinically significant change index on the DASS-21 depression score for outpatients with depression was calculated as 6.15. Therefore, a reduction of ≥7 points in the DASS-21 depression score was considered a clinically significant change.

Secondary outcome measures

DASS-21 anxiety and stress scores. The DASS-21 anxiety and stress subscale scores were set as secondary outcome measures.

SIMR. To assess variability in mood over 12 wk, participants completed a daily SIMR for depression, anxiety, and stress. The

rating question was taken from each DASS-21 subscale, which has been shown to be highly correlated with their respective subscale total score [22]. The questions for the SIMR comprised "Today I felt downhearted and blue" (depression), "Today I felt scared without any good reason" (anxiety), and "Today I found it difficult to relax" (stress). The identical rating system as the DASS-21 was used, comprising ratings from 0 (did not apply to me at all) to 3 (applied to me very much, or most of the time).

PROMIS sleep disturbance and sleep-related impairment scale. The PROMIS sleep disturbance and sleep-related impairment scale (PROMIS sleep) is a 16-item self-report inventory that measures sleep quality and sleep-related impairment over the last week. Questions are rated using a 5-point Likert scale, where component scores are calculated for sleep disturbance and sleep-related impairment [23]. Despite having fewer questions than the Pittsburgh Sleep Quality Index and the Epworth sleepiness scale, the PROMIS sleep correlated highly with these measures [24]. The PROMIS sleep is also able to significantly differentiate individuals with and without self-reported sleep disorders and between those with treated and untreated sleep disorders [23].

World Health Organization 5 Well-Being Index (WHO-5). The WHO-5 is a 5-item self-report questionnaire that assesses psychological well-being [25]. Questions are rated based on the last 2-wk using a 5-point Likert scale.

Safety and expectancy measures

The tolerability of tablet intake was assessed monthly using an online question enquiring about the experience of any adverse events (AEs). Moreover, in week 12, participants completed the Global Assessment of Tolerability to Therapy, where they indicated that their tolerability to tablet intake ranged from poor to excellent.

As expectancies can have a significant influence on outcomes in placebo-controlled trials [26], and in line with recent guidelines in nutritional psychiatry [27], the Clinical Trials Treatment Expectancies Scale (CTTES) was completed by participants at baseline. The CTTES, a 6-item questionnaire, is a revision of the Stanford Expectations of Treatment Scale [28], with wording modified to reference clinical trials examining mood-related changes.

Sample size calculations

An a priori power analysis was undertaken to estimate the required sample size (based on a single outcome variable). In previous depression studies on saffron, there has been significant variability in the outcome measures used to detect changes in depression symptoms. In a meta-analysis of randomized, double-blind, placebo-controlled studies, effect sizes to detect significant between-group differences in mean change scores in depression symptoms varied from 0.11 to 3.24 with a pooled effect size of 0.99 [8]. In a study using the DASS-21 depression score as an outcome measure, an effect size of 0.68 to detect a significant between-group difference in the mean change in scores was identified [19]. However, as we recruited adults with subclinical depression, we predicted a smaller effect size of 0.4 to detect between-group differences in mean changes in the primary outcome (DASS-21 depression score). Based on a power of 85% and a type 1 error rate (α) of 5%, the total number of participants required to find an effect was 182. Assuming a 10% dropout rate, a sample size of 202 people was needed to find an effect in the primary outcome measure.

Statistical analysis

Outcome analyses for the self-report questionnaires were conducted on the full analysis set (FAS) using an intention-to-treat analysis and on the per-protocol set (PPS), with all participants retained in originally allocated groups. FAS represents the subset of participants who were randomly assigned, consumed $\geq$1 dose of the investigational product (IP), and had available efficacy data. PPS was defined as the subset of participants who were randomly assigned, who consumed $\geq$1 dose of the trial product, had available efficacy data, and had no major protocol deviations (e.g., withdrew from the study, consumed $<$80% of tablets, started prohibited concomitant medications, had missing data, and/or completed assessments outside proposed time windows). Reasons for exclusions in the PPS are detailed in the Supplemental Table 1.

Generalized linear mixed models (GLMM) were used to assess differences between intervention groups on the DASS-21, PROMIS sleep, and WHO-5 scores. No manual imputation was used for missing data, as GLMM handles missing data using maximum likelihood estimation. Changes in scores from baseline to week 12 were used to examine group differences. To examine within-group changes over time, the GLMM was used, with time points (weeks 0, 4, 8, and 12) included. Random intercepts were utilized in each model, and covariates age, sex, BMI, and CTTES positive and negative expectances scores were included (fixed effects). To examine the clinical significance of the change in the primary outcome measure (DASS-21 depression score), a Pearson χ^2 analysis was undertaken where a reduction of $\geq$7 points from baseline to week 12 was defined as a clinically significant change [21]. Further, post hoc exploratory analyses were undertaken on the DASS-21 stress and anxiety and PROMIS sleep scores in a subset of participants experiencing more severe symptoms at baseline. This comprised analyses of participants scoring outside the normal range on the DASS-21 stress score ($\geq$14), DASS-21 anxiety score ($\geq$8), and with a PROMIS sleep T-score $>$75th percentile (T-score $\geq$56.5) at baseline. For the SIMR scores (depression, anxiety, and stress), mean weekly ratings were calculated using the daily ratings for each week from baseline to endpoint (week 12). If a daily rating was not completed, mean scores were calculated using the available completed ratings for the week. In analyses using GLMM, where applicable, γ (with log link function) and normal (with identity link function) target distributions were used. Appropriate covariance structures were used to model correlations associated with repeated time measurements in all models. Robust estimations were used to handle any violations of model assumptions. Planned contrasts conducted on the SIMR were adjusted using the least significant difference. All data were analyzed using SPSS (version 28; IBM) with a critical P value of $P \leq 0.05$. As there was only 1 a priori primary outcome measure, there was no adjustment to the P value for multiple testing. Moreover, no adjustment to P values was undertaken for the secondary outcome measures. These secondary findings are hoped to help guide planning for future trials, but as this increased risk of type

1 error, statistically significant secondary findings should be considered tentative, requiring validation in future studies.

Results

Study population

A total of 245 people underwent a telephone screening, and 202 people were randomly assigned. The most common reasons for exclusion were withdrawing consent after the telephone interview ($n = 15$) and having a current mental health diagnosis ($n = 11$). Baseline demographic and clinical characteristics are detailed in Table 1. The 2 groups were similarly matched in age, BMI, marital status, educational level, and sex distribution. Baseline scores on outcome measures were also similar between the 2 groups.

Outcome measures

Depressive symptoms

As demonstrated in Table 2 and Figure 2, based on the GLMM, there was a statistically significant greater reduction of 2.92 points [95% confidence interval (CI): 0.71, 5.13 points] in the saffron group compared to the placebo group in the DASS-21 depression score (primary outcome measure) from baseline to week 12 ($P = 0.010$, Cohen's d = 0.39). In the saffron group, the DASS-21 depression score was reduced by a mean of 11.34 points (95% CI: –9.73, –12.95 points), and in the placebo group, it was reduced by 8.42 points (95% CI: –6.79, –10.05 points). After controlling for baseline scores (mean: 21.47), the DASS-21 depression score was reduced by 52.8% in the saffron group, and in the placebo group, it was reduced by 39.2%. Based on a Pearson χ^2 analysis, a larger proportion of participants in the saffron group ($n = 68$; 72.3%) compared to the placebo group ($n = 51$; 54.3%) achieved a clinically significant change in their depression scores from week 0 to week 12 (≥7 points) ($P = 0.010$). An analysis of the PPS (Supplemental Table 2) was consistent with FAS findings, demonstrating significant between-group differences in the change in the DASS-21 depression score (β: –3.07; 95% CI: –0.82, –5.32; d = 0.41, $P = 0.008$). Statistical analyses on the FAS without adjustment for covariates revealed that the crude results were consistent with the adjusted data (Supplemental Table 3)

As demonstrated in Figure 3, based on the GLMM, there was a significant group difference in the overall mean daily depression

TABLE 1
Baseline sociodemographic and clinical characteristics.

		Placebo ($n = 101$)	Saffron ($n = 101$)
Age	Mean (SE)	45.1 (1.31)	43.9 (1.35)
Sex, (%)	Female	73 (72.3)	68 (67.3)
	Male	28 (27.7)	33 (32.7)
Height (m)	Mean (SE)	1.67 (0.01)	1.69 (0.01)
Weight (kg)	Mean (SE)	70.6 (1.42)	72.4 (1.52)
BMI	Mean (SE)	25.3 (0.40)	25.4(0.43)
Marital status (*n* %)	Single	54 (53.5)	45 (44.6)
	Married/defacto	47 (46.5)	56 (55.4)
Educational level (*n* %)	Secondary	41 (40.6)	45 (44.6)
	Tertiary	32 (31.7)	38 (37.6)
	Postgraduate	28 (27.7)	18 (17.8)
International physical activity questionnaire category (*n* %)	Low	53 (52.5)	49 (48.5)
	Moderate	43 (42.6)	46 (45.5)
	High	5 (5.0)	6 (5.9)
Pharmaceutical medication use (medications consumed by ≥5% of participants included)	No medications	77 (76.2)	71 (70.3)
	Antidepressants	6 (6.0)	6 (6.0)
	Hormonal agents	7 (7.0)	9 (9.0)
	Dyslipidemeics	4 (4.0)	5 (5.0)
Occupation (*n* %)	Retired	3 (3.0)	7 (6.9)
	Unemployed	18 (17.8)	11 (10.9)
	Services and sales worker	2 (2.0)	7 (6.9)
	Professional	15 (14.9)	20 (19.8)
	Elementary occupation	8 (7.9)	7 (6.9)
	Plant and machine operators and assemblers	4 (4.0)	0 (0.0)
	Clerical support worker	7 (6.9)	8 (7.9)
	Craft and related trades worker	3 (3.0)	3 (3.0)
	Manager	8 (7.9)	10 (9.9)
	Student	11 (10.9)	12 (11.9)
	Technicians and associated trades	22 (21.8)	16 (15.8)
DASS depression score at screening	Mean (SE)	25.42 (0.56)	25.26 (0.60)
DASS depression score at day 0	Mean (SE)	21.60 (0.84)	21.78 (0.84)
DASS anxiety score at day 0	Mean (SE)	11.21 (0.73)	11.80 (0.80)
DASS stress score at day 0	Mean (SE)	21.15 (0.80)	22.20 (0.84)
WHO-5	Mean (SE)	6.79 (0.33)	7.34 (0.35)
PROMIS sleep - sleep disturbance T-score	Mean (SE)	58.88 (0.77)	59.20 (0.75)
PROMIS sleep - sleep-related impairment T-score	Mean (SE)	60.83 (0.73)	60.49 (0.79)

Abbreviations: DASS-21, depression, anxiety and stress scale – 21; PROMIS sleep, PROMIS sleep disturbance and sleep-related impairment scale; WHO-5, World Health Organization – 5 wellbeing index.

TABLE 2
Change in self-report questionnaires (estimated marginal means) (full analysis set).

		Placebo (n = 101)					Saffron (n = 101)					Difference: Placebo vs. Saffron (β)	P value[1]	Cohen's d
		Week 0	Week 4	Week 8	Week 12	Change from baseline	Week 0	Week 4	Week 8	Week 12	Change from baseline			
DASS-21 depression score	Mean	22.12	13.92	13.58	13.38	–8.42	22.24	13.20	11.00	10.64	–11.34	–2.92	0.010	0.39
	SE	0.88	0.88	0.88	0.90	0.83	0.86	0.88	0.88	0.88	0.82	1.13		
DASS-21 anxiety score	Mean	11.44	6.70	6.18	5.58	–5.68	12.10	6.10	5.94	5.32	–6.52	–0.84	0.223	0.18
	SE	0.60	0.60	0.62	0.62	0.52	0.60	0.60	0.60	0.62	0.50	0.69		
DASS-21 stress score	Mean	21.56	14.54	13.64	12.92	–8.68	22.56	13.92	12.66	11.80	–10.41	–1.72	0.092	0.25
	SE	0.78	0.78	0.78	0.80	0.75	0.78	0.78	0.78	0.78	0.74	1.02		
PROMIS sleep disturbance (T-score)	Mean	59.37	56.95	55.65	55.26	–4.30	59.79	54.90	54.47	53.90	–5.75	–1.45	0.123	0.23
	SE	0.86	0.83	0.81	0.81	0.69	0.86	0.79	0.79	0.78	0.69	0.93		
PROMIS sleep-related impairment (T-score)	Mean	61.48	57.22	55.93	55.93	–5.72	61.25	54.77	54.73	53.22	–7.70	–1.98	0.078	0.26
	SE	0.93	0.87	0.86	0.85	0.83	0.92	0.83	0.84	0.82	0.82	1.12		
WHO-5 Score	Mean	6.55	9.56	10.11	11.24	4.30	7.16	10.94	11.98	12.56	5.46	1.17	0.105	0.24
	SE	0.48	0.48	0.48	0.49	0.53	0.47	0.48	0.48	0.48	0.52	0.72		

Results (estimated means) are generated from generalized mixed-effects models adjusted for age, sex, BMI, and clinical trials treatment expectancies scale positive and negative expectancies score. All within-group changes from weeks 0–12 were statistically significant at $P < 0.001$ with P values generated from repeated measures generalized mixed-effects models adjusted for age, sex, BMI, and clinical trials treatment expectancies scale positive and negative expectancies score (time effects week 0 and week 12).
Abbreviations: DASS-21, depression, anxiety, and stress scale – 21; PROMIS sleep, PROMIS sleep disturbance and sleep-related impairment scale; WHO-5, World Health Organization–5 Well-Being Index.
[1] P values refer to between-group differences in change in values from baseline to week 12 generated from generalized mixed-effects models and adjusted for age, sex, BMI, and clinical trials treatment expectancies scale positive and negative expectancies score, and corresponding baseline values.

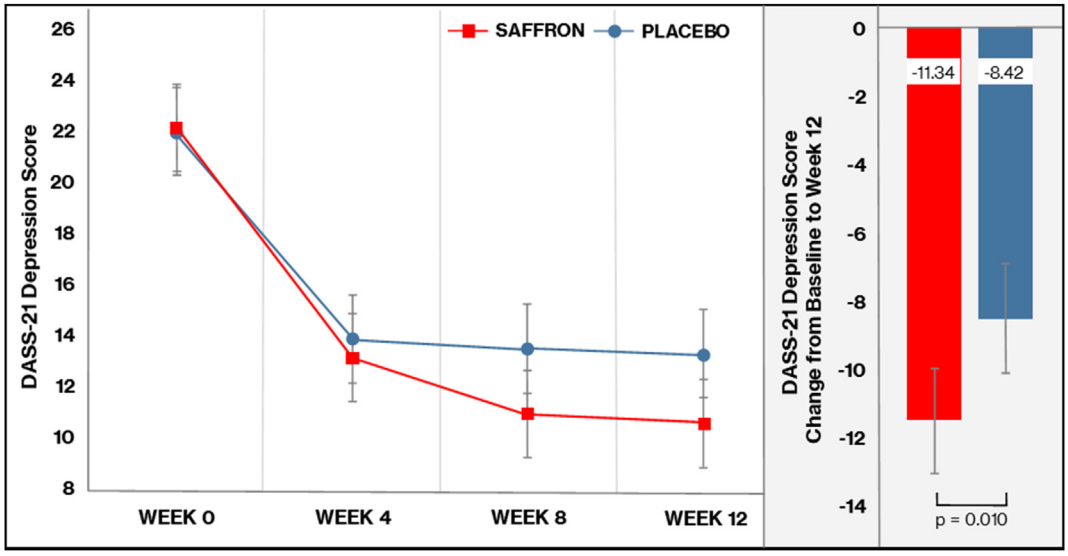

FIGURE 2. Change in DASS-21 depression scores over time (FAS). Error bars represent a 95% confidence interval. Sample size (placebo = 101, saffron = 101). DASS-21, DASS-21, depression, anxiety, and stress scale – 21; FAS, full analysis set.

ratings comprising a mean 0.15 lower rating (95% CI: –0.02, –0.29) in the saffron group compared to the placebo group over the 12-wk intervention ($P = 0.032$).

Anxiety symptoms

As demonstrated in Table 2, based on the GLMM, there was no significant between-group difference in the change in the DASS-21 anxiety score (β: –0.84; 95% CI: 0.51, –2.19; d = 0.18, $P = 0.223$). In the saffron group, the DASS-21 anxiety score was reduced by a mean of 6.52 points (95% CI: –5.54, –7.50 points), and in the placebo group, it was reduced by 5.68 points (95% CI: –4.66, –6.70 points). After controlling for baseline scores (mean: 11.35), the DASS-21 anxiety score was reduced by 57.4% in the saffron group, and in the placebo group, it was reduced by 50.0%. An analysis of the PPS (Supplemental Table 2) was consistent with FAS findings, demonstrating nonsignificant between-group differences in the change in the DASS-21 anxiety score (β: –0.95; 95% CI: 0.44, –2.34; d = 0.21, $P = 0.182$).

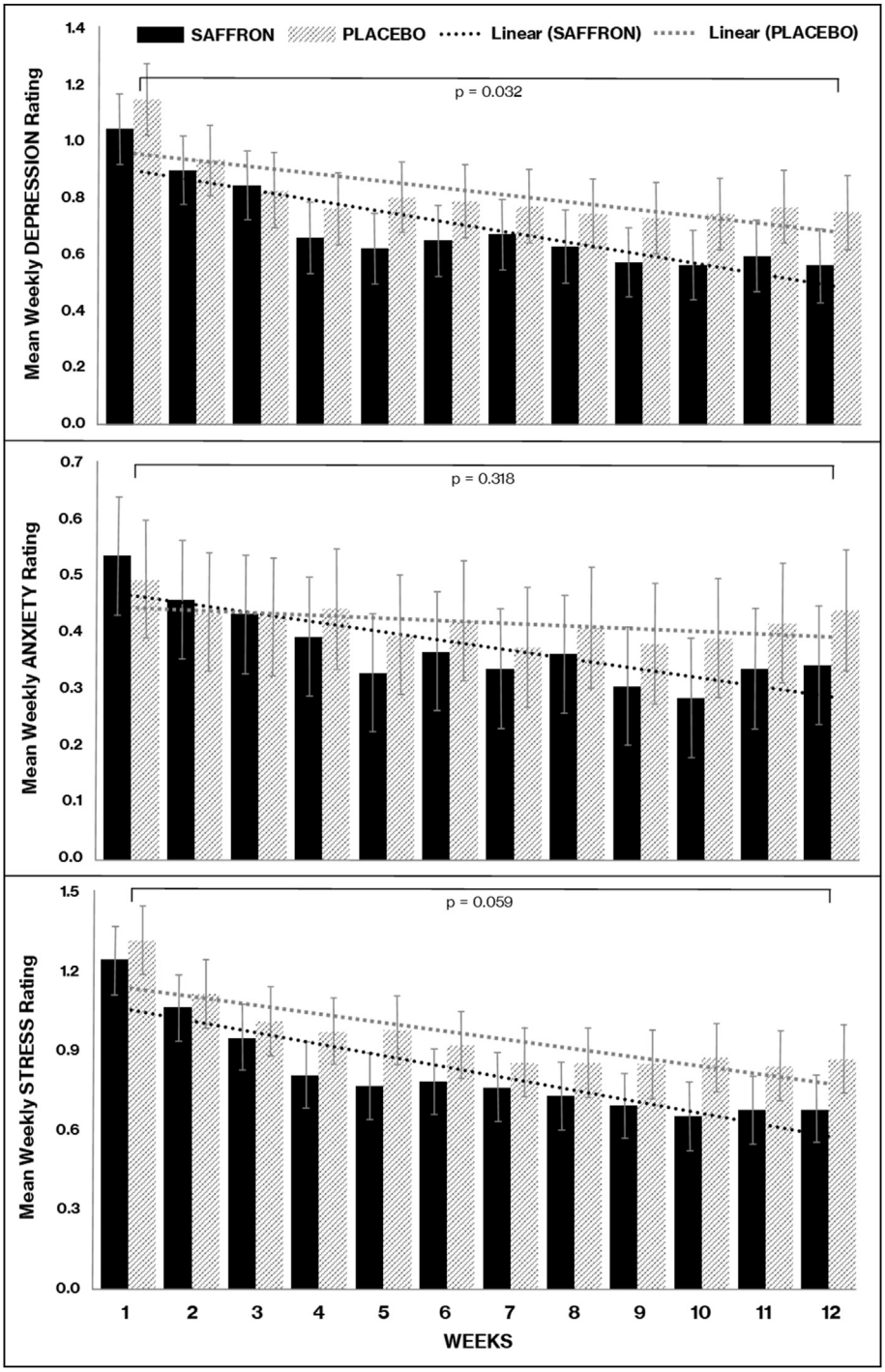

FIGURE 3. Mean weekly SIMR (FAS). Error bars represent a 95% confidence interval. *P* values refer to between-group differences in overall mean daily SIMR collected from baseline to week 12 generated from generalized mixed-effects models and adjusted for age, sex, BMI, and clinical trials treatment expectancies scale positive and negative expectancies scores. Sample size (placebo = 101, saffron = 101). FAS, full analysis set; SIMR, single-item mood rating.

Statistical analyses on the FAS without adjustment for covariates revealed that the crude results were consistent with the adjusted data (Supplemental Table 3). Moreover, as demonstrated in Figure 3, based on the GLMM, there were no significant group differences in mean daily anxiety ratings.

As 33% of participants (n = 67) at baseline had DASS-21 anxiety scores in the normal range (≤7), an exploratory analysis was undertaken to examine if there were any between-group differences in changes in the DASS-21 anxiety score in participants scoring outside the normal range (>7) at baseline. Based on the FAS, this confirmed that in participants who scored >7 at baseline on the DASS-21 anxiety scale, there were no between-group differences in changes in the DASS-21 anxiety score (β: –0.89; 95% CI: 0.99, –2.77; d = 0.17, P = 0.358)

(Supplemental Table 4). From week 0 to week 12, in the saffron group, the DASS-21 anxiety score reduced by 9.14 points (95% CI: –7.77, –10.51 points), and in the placebo group, it reduced by 8.25 points (95% CI: –6.90, –9.60 points). Similar nonsignificant findings were demonstrated in the PPS (Supplemental Table 5).

Stress symptoms

As demonstrated in Table 2, based on the GLMM, there was no significant group difference in the change in the DASS-21 stress score (β: –1.72; 95% CI: 0.28, –3.72; d = 0.25, $P = 0.092$). In the saffron group, the DASS-21 stress score was reduced by a mean of 10.41 points (95% CI: –8.96, –11.86 points), and in the placebo group, it was reduced by 8.68 points (95% CI: –7.21, –10.15 points). After controlling for baseline scores (mean: 21.53), the DASS-21 stress score was reduced by 48.3% in the saffron group, and in the placebo group, it was reduced by 40.3%. An analysis of the PPS (Supplemental Table 2) was consistent with FAS findings, demonstrating nonsignificant between-group differences in the change in the DASS-21 stress score, although a trend was detected (β: –1.97; 95% CI: 0.11, –4.05; d = 0.29, $P = 0.065$). Statistical analyses on the FAS without adjustment for covariates revealed that the crude results were consistent with the adjusted data (Supplemental Table 3). As demonstrated in Figure 3, based on the GLMM, there were no significant group differences in mean daily stress ratings.

As 20% of participants ($n = 41$) at baseline had DASS-21 stress scores in the normal range (≤14), an exploratory analysis was undertaken to examine if there were any between-group differences in changes in the DASS-21 stress score in participants scoring outside the normal range (>14) at baseline. Based on the FAS, this confirmed that in participants who scored >14 at baseline on the DASS-21 scale, there were no between-group differences in changes in the DASS-21 stress score (β: –2.14; 95% CI: 0.23, –4.51; d = 0.30, $P = 0.078$) (Supplemental Table 4). From week 0 to week 12, in the saffron group, the DASS-21 stress score reduced by 12.48 points (95% CI: –10.78, –14.18 points), and in the placebo group, it reduced by 10.34 points (95% CI: –8.60, –12.08 points). However, based on the PPS, between-group differences in changes in the stress score became significant (β: –2.54; 95% CI: –0.09, –4.99; d = 0.35, $P = 0.045$) (Supplemental Table 5). From week 0 to week 12, in the saffron group, the DASS-21 stress score reduced by 12.55 points (95% CI: –10.83, –14.28 points), and in the placebo group, it reduced by 10.02 points (95% CI: –8.19, –11.85 points).

Sleep disturbance

As demonstrated in Table 2, based on the GLMM, there was no significant group difference in the change in the PROMIS sleep disturbance score (β: –1.45; 95% CI: 0.37, –3.27; d = 0.23, $P = 0.123$). After controlling for baseline scores (mean: 59.17), the sleep disturbance score was reduced by 9.7% in the saffron group, and in the placebo group, it was reduced by 7.3%. An analysis of the PPS (Supplemental Table 2) was consistent with FAS findings, demonstrating nonsignificant between-group differences in the change in the PROMIS sleep disturbance score (β: –1.58; 95% CI: 0.32, –3.48; d = 0.25, $P = 0.106$). Statistical analyses on the FAS without adjustment for covariates revealed that the crude results were consistent with the adjusted data (Supplemental Table 3).

A sub-group exploratory analysis of participants having a baseline T-score >75th percentile (≥56.5) revealed a significant group difference (Supplemental Table 4) (β: –2.72; 95% CI: –0.45, –4.99; d = 0.44, $P = 0.020$). For participants in the saffron group who scored above the 75th percentile on the sleep disturbance scale at baseline, the T-score reduced by 7.55 points (95% CI: –2.94, –5.66 points), and in the placebo group, it decreased by 4.83 points (95% CI: –2.94, –5.66 points). After controlling for baseline scores (mean: 63.55), the sleep disturbance score was reduced by 11.9% in the saffron group, and in the placebo group, it was reduced by 7.6%. An analysis of the PPS (Supplemental Table 5) was consistent with FAS findings, demonstrating significant between-group differences in the change in the PROMIS sleep disturbance score (β: –2.79; 95% CI: –0.44, –5.14; d = 0.45, $P = 0.022$).

Sleep-related impairment

As demonstrated in Table 2, based on the GLMM, there was no significant group difference in the change in the PROMIS sleep-related impairment score (β: –1.98; 95% CI: 0.22, –4.18; d = 0.26, $P = 0.078$), although a strong trend was detected. In the saffron group, the PROMIS sleep disturbance score was reduced by a mean of 7.70 points (95% CI: –2.94, –5.66 points), and in the placebo group, it was reduced by 5.72 points (95% CI: –2.94, –5.66 points). After controlling for baseline scores (mean: 60.62), the sleep disturbance score was reduced by 12.7% in the saffron group, and in the placebo group, it was reduced by 9.4%. An analysis of the PPS (Supplemental Table 2) was consistent with FAS findings, demonstrating nonsignificant between-group differences in the change in the PROMIS sleep-related impairment score (β: –2.01; 95% CI: 0.28, –4.30; d = 0.26, $P = 0.087$). Statistical analyses on the FAS without adjustment for covariates revealed that the crude results were consistent with the adjusted data (Supplemental Table 3).

A sub-group exploratory analysis of participants having a baseline T-score >75th percentile (≥56.5) revealed a near significant group difference (Supplemental Table 4) (β: –2.74; 95% CI: –0.04, –5.44; d = 0.37, $P = 0.050$). For participants in the saffron group who scored above the 75th percentile on the sleep-related impairment scale at baseline, the T-score reduced by 9.94 points (95% CI: –2.94, –5.66 points), and in the placebo group, it decreased by 7.19 points (95% CI: –2.94, –5.66 points). After controlling for baseline scores (mean: 64.56), the sleep-related impairment score was reduced by 15.4% in the saffron group, and in the placebo group, it was reduced by 11.1%. An analysis of the PPS (Supplemental Table 5) was consistent with FAS findings, demonstrating nonsignificant between-group differences in the change in the PROMIS sleep-related impairment score, although a strong trend was detected (β: –2.72; 95% CI: 0.10, –5.54; d = 0.36, $P = 0.062$).

General well-being

As demonstrated in Table 2, based on the GLMM, there was no significant group difference in the change in the WHO-5 total score (β: 1.17; 95% CI: –0.24, 2.58; d = 0.24, $P = 0.105$). In the saffron group, the WHO-5 total score was increased by a mean of 5.46 points (95% CI: 4.44, 6.48 points), and in the placebo group, it was increased by 4.30 points (95% CI: 3.26, 5.34 points). After controlling for baseline scores (mean :7.04), the

WHO-5 score was increased by 77.6% in the saffron group, and in the placebo group, it was increased by 61.0%. An analysis of the PPS (Supplemental Table 2) was consistent with FAS findings, demonstrating nonsignificant between-group differences in the change in the WHO-5 score, although a strong trend was detected (β: 1.37; 95% CI: –0.08, 2.82; d = 0.28, $P = 0.066$).

Intake of supplements

IP bottles with remaining tablets were counted by participants on week 12. Based on these details, 95% of participants who completed the study took over 80% of their tablets.

Adverse reactions and treatment discontinuation

Participants reported no serious AEs, and there was a similar frequency of AEs classified as possibly or probably related to the tablet intake (Supplemental Table 6). In the placebo group, 1.0% ($n = 1$) of participants experienced a treatment-related AE, whereas in the saffron group, 2.97% ($n = 3$) of participants experienced a treatment-related AE. In the saffron group, treatment-related AEs included abdominal pain ($n = 1$), headaches ($n = 1$), and skin boils/ itchy skin ($n = 1$). The global assessment of tolerability to therapy results is detailed in Supplemental Table 7, which indicates that in both groups, over 95% of participants reported good or excellent tolerability of tablets.

A total of 14 people discontinued the study, comprising 7 people in each group. In the saffron group, reasons provided for study discontinuation included no reason given ($n = 4$), adverse reaction possibly associated with IP intake ($n = 1$), the reoccurrence of a previous medical condition that was unrelated to IP intake ($n = 1$), and inconsistent tablet intake ($n = 1$). In the placebo group, discontinuation reasons included no reason given ($n = 3$), adverse reaction possibly associated with IP intake ($n = 1$), increased personal stressors ($n = 1$), commencement of antidepressant medication due to a worsening mood ($n = 1$), and an illness occurring before IP commencement ($n = 1$). An analysis of participants discontinuing treatment revealed they had characteristics similar to those of the total sample, as they were similar in sex distribution, age, BMI, DASS-21 depression score at baseline, and CTTES positive and negative expectancies scores.

Efficacy of participant blinding

To assess the effectiveness of condition concealment during the trial, participants predicted their condition allocation (i.e., placebo, saffron, or unsure) at the end of the study. Overall group concealment was high, as 59.1% of participants in the placebo group and 60.0% of participants in the saffron group were unsure or incorrectly guessed treatment allocation. These rates are similar to other trials conducted on saffron and other botanicals for the treatment of mood disturbances [29–31]. For participants in the placebo group who correctly guessed their group allocation, the reasons given for the correct prediction included experiencing no mood improvement ($n = 36$, 38.7%) and there was no saffron taste/odor ($n = 2$, 2.2%). For participants in the saffron group who correctly guessed their group allocation, the reasons given for the correct prediction included experiencing a mood improvement ($n = 33$, 34.7%), there was a saffron taste/odor ($n = 7$, 7.4%), or due to the experience of an adverse reaction ($n = 1$, 1.1%).

Discussion

The results of this 12-wk, randomized, double-blind, placebo-controlled study add to the body of evidence of the antidepressant effects of a saffron extract (Affron) but in a population of generally healthy adults with subclinical depression. Based on the results of the primary outcome measure (DASS-21 depression score), saffron at a dose of 14 mg twice daily was associated with a statistically significant greater reduction in depressive symptoms compared to the placebo. In the saffron group, the DASS-21 depression score was reduced by a mean of 53% from baseline to week 12, compared to a smaller mean reduction of 39% in the placebo group. These results are considered clinically meaningful as 72% of participants in the saffron group, compared to 54% of participants in the placebo group, achieved a clinically meaningful reduction of 7 or more points on the DASS-21 depression score from baseline to week 12. An examination of secondary outcome measures comprising the DASS-21 anxiety and stress scores, WHO-5 total score, and PROMIS sleep scores revealed no significant between-group differences in changes in these scores over time. As not all participants at baseline presented with disturbances in anxiety, stress, or sleep, an exploratory analysis was undertaken on a subset of participants with symptomatic problems in these areas. This revealed that in a subset of participants with sleep problems (represented by a T-score >75th percentile at baseline), there were significant between-group differences comprising a reduction of 12% in the sleep disturbance score in the saffron group (8% reduction in the placebo group), and a 15% reduction in the sleep-related impairment score (11% reduction in the placebo group). However, due to the exploratory nature of these analyses, these findings should be viewed cautiously.

As a monotherapy or adjunctive treatment of depression, saffron has undergone ≥14 randomized, double-blind clinical trials, with the bulk of evidence confirming its antidepressant efficacy [7]. Treatment is typically administered at 28–30 mg daily for 6–8 wk to adults diagnosed with major depressive disorder. In a meta-analysis by Marx et al. [8], an effect size of 0.99 (95% CI: 0.80, 1.19) was calculated based on the results from 14 trials. Twelve of these trials were conducted in Iran and 2 in Australia. In comparison to these trials, a smaller effect size of ~0.4 was identified in this study. The recruitment of a nonclinical, generally healthy population of people with subclinical depression and/or the utilization of the DASS-21 to assess depressive symptomatology could account for this smaller effect size. In the majority of trials, the Beck Depression Inventory and the Hamilton Depression Rating Scale were used as outcome measures. However, as high correlations between the DASS-21 depression score and these outcome measures have been identified [32–34], the discrepancy in effect sizes is unlikely to be primarily attributed to this factor. It is important to note that most of the studies have been conducted in Iran [8]; therefore, cultural variables, including differences in diet, lifestyle, environmental stressors, and even the intestinal microbiome, are plausible factors that require evaluation in future trials [35,36].

Strengths, limitations, and directions for future research

The results of this study provide further confirmation of the antidepressant effects and tolerability of saffron but in adults

with subclinical depression and over a treatment duration of 12 wk. The use of validated self-report measures administered monthly, along with daily mood ratings, provides a clearer understanding of the progression of mood-related changes after saffron administration. Moreover, an analysis of reliable change indices in the DASS-21 depression score demonstrated that for a significant portion of participants supplemented with saffron (72%), clinically meaningful changes were achieved. However, it is important to note that in this study, there were significant placebo responses. For all the administered outcome measures, statistically significant improvements were observed in the placebo group. In fact, clinically meaningful reductions in the DASS-21 depression score occurred in 54% of participants. Placebo responses are common in depression trials and have been observed in previous saffron trials conducted by our research group [18,37,38]. It is important to note that most of the placebo responses occurred in the first 4 wk of treatment, with little improvement occurring thereafter. This may be partly attributed to the population recruited in this trial comprising adults with subclinical depression. Details of participants' history of depressive symptoms and stability of symptoms over time were not examined, which could contribute to the identified significant improvements or regression to the mean changes seen in both the placebo and saffron groups over time. Therefore, utilizing measures to detect treatment effects more sensitively will be important in future trials. This includes utilizing outcome measures that can detect small but meaningful changes, introducing placebo analyzing phases, and extending treatment periods >4 wk, which may result in a waning of the placebo response over time [39,40]. Recruiting participants with a long-term state of subclinical depression will also help confirm the findings identified in this study.

It is important to highlight the eligibility criteria utilized in this study as they are applicable to the generalizability of the findings. Generally, healthy adults with subclinical depression were recruited, and only 6% of participants were taking a pharmaceutical antidepressant. Participants were excluded if they were recently diagnosed with a significant medical condition and/or had an unmanaged medical disease. Moreover, nicotine smokers, significantly under- or overweight participants, people with high alcohol intake, or regular users of illicit drugs were excluded. A population of generally healthy participants was recruited in the study for several reasons. Research has consistently demonstrated that having a comorbid medical condition and the previous-mentioned risk factors can be considerable contributors to depressive symptoms and are associated with treatment-resistant depression [41–45]. In fact, when such comorbidities are present, treatment guidelines often include more comprehensive interventions that target psychological, lifestyle, and dietary factors, along with medical treatments that specifically target physical complications associated with these conditions [46,47]. As a stand-alone intervention, the administration of saffron to participants with such comorbidities would, therefore, not be based on evidence-based best practice. Moreover, in most countries, saffron is classed as a dietary herbal supplement that is available in retail channels without prescription. Accordingly, in most countries, structure, function, or health claims, but not disease-related claims, are only permitted for dietary supplements. Because of these reasons, the results of this study should be considered applicable to generally healthy adults with subclinical depression. Further research into the efficacy and safety of saffron in people presenting with these comorbid conditions is required, ideally administered as an adjunct intervention. Some efficacy has been demonstrated in people with depression and cardiovascular disease, diabetes, and in females undergoing treatment of breast cancer, although further research is advised [48–50].

In this study, several secondary and exploratory analyses were undertaken to help understand the therapeutic efficacy of saffron. These demonstrated between-group differences in changes in sleep outcomes in a subset of participants presenting with sleep-related problems and nonsignificant group differences in changes in anxiety and stress. However, these findings should be viewed cautiously due to an increased risk of type 1 error. Moreover, as participants presenting with stress and anxiety were not specifically recruited, the nonsignificant effects of saffron on these symptoms should also be examined further in future trials. In previous trials on saffron, stress-reducing and anxiolytic effects have been identified [7,51], and several meta-analyses have confirmed sleep improvements in people with insomnia and sleep-related problems [14–16]. Other study limitations included the potential effect of regression to the mean bias associated with natural symptom variation. Moreover, dose-response effects were not examined in this study, which will be important to investigate in future trials.

Finally, a further examination into the mechanisms of action associated with the antidepressant effects of saffron is required. It is speculated that saffron may work through multiple physiological mechanisms. It has been shown to influence the activity of neurotransmitters such as serotonin, dopamine, and glutamate [52,53], modulate the stress response through its effect on hypothalamus-pituitary-adrenal axis activity [54], increase neurotrophins such as brain-derived neurotrophic factor [55,56]; and reduce inflammation and oxidative stress[10–12,57]. However, further research is required to elucidate how saffron works to reduce depressive symptoms.

In summary, this study builds on the World Federation of Societies of Biological Psychiatry and Canadian Network for Mood and Anxiety Treatment guidelines published in 2022, supporting saffron's antidepressant efficacy [7]. It provides additional evidence of safety and efficacy in nonclinical populations over a longer treatment duration of 12 wk and utilizes a robust sample size. Future trials in participants with varying symptomatology and demographic and sociographic characteristics, along with an examination into the mechanistic actions of saffron, will be important to complete in the future.

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Author contributions

The authors' responsibilities were as follows–ALL, SJS, MD-M, MIM-V: conceptualization and methodology; ALL: formal analysis; ALL, SJS: investigation; ALL, SJS: writing—original draft preparation; ALL, SJS, WM, MD-M, MIM-V: writing—review and editing; and all authors: read and approved the final manuscript.

Conflict of interest

ALL is the Managing Director of Clinical Research Australia, a contract research organization that receives research funding from nutraceutical companies. ALL has also received presentation honoraria from nutraceutical companies. SJS is an employee of Clinical Research Australia. WM has received funding and/or attended events funded by Cobram Estate Pty. Ltd. and Bega Dairy and Drinks Pty. Ltd. WM has also received travel funding from the Nutrition Society of Australia, consultancy funding from Nutrition Research Australia and ParachuteBH, and speakers' honoraria from VitaFoods, the Cancer Council Queensland, and the Princess Alexandra Research Foundation. MD-M and MIM-V are employees of the study sponsor, Pharmactive Biotech Products SLU.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2025.05.024.

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