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Original Article

An investigation into an evening intake of a saffron extract (affron®) on sleep quality, cortisol, and melatonin concentrations in adults with poor sleep: a randomised, double-blind, placebo-controlled, multi-dose study

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ABSTRACT

Objective/background: To validate and extend on previous positive findings of the sleep-enhancing effects of saffron supplementation in adults with unsatisfactory sleep.
Patients/methods: In this 28-day, 3-arm, parallel-group, double-blind, randomised controlled trial, 120 adults with unsatisfactory sleep received either a placebo, 14 mg, or 28 mg of a standardised saffron extract (affron®), 1 h before bed. Outcome measures included the Pittsburgh Sleep Diary (with sleep quality ratings as the primary outcome measure), Insomnia Symptom Questionnaire (ISQ), Profile of Mood States, Restorative Sleep Questionnaire, the Functional Outcomes of Sleep Questionnaire, and evening salivary melatonin and cortisol concentrations.
Results: Compared to the placebo, saffron supplementation was associated with greater improvements in sleep quality ratings (primary outcome measure), mood ratings after awakening, the ISQ total score, and ISQ-insomnia classifications. However, there were no significant differences between the saffron and placebo groups in other questionnaire and sleep diary outcome measures. Sleep improvements were similar for the two administered saffron doses. Compared to the placebo, saffron supplementation was associated with increases in evening melatonin concentrations but did not affect evening cortisol. Saffron supplementation was well-tolerated with no reported significant adverse effects.
Conclusions: These results provide further validation of the sleep-enhancing effects of 28-days of saffron supplementation in adults with unsatisfactory sleep. Further research is required to examine the efficacy and safety of saffron supplementation using objective sleep measures, over a longer duration, in people presenting with a diagnosed insomnia disorder and other psychogenic and demographic characteristics, and into its potential sleep-enhancing mechanisms of action.

1. Introduction

Insomnia is characterised by a dissatisfaction with the quantity and/or quality of sleep. It is associated with difficulty falling asleep, frequent night-time awakenings with difficulty in returning to sleep, and/or morning awakenings earlier than desired [1]. Approximately 30–43% of adults report at least one insomnia-related symptom, with research suggesting prevalence rates of insomnia disorder between 5 and 15% [2–4]. As insomnia is a heterogeneous disorder with a multifaceted pathophysiology, its treatment can be difficult. Insomnia is often considered a hyperarousal disorder associated with heightened physiologic, affective, and cognitive activity [5]. This hyperactivity is likely

Abbreviations: ANOVA, Analysis of variance; BMI, Body mass index; FOSQ-10, Functional Outcomes of Sleep Questionnaire – short version; IDO, Indoleamine 2,3-dioxygenase; ISQ, Insomnia Symptom Questionnaire; PHQ-4, Patient Health Questionnaire – 4; PSD, Pittsburgh Sleep Diary; POMS-A, Profile of Mood States - Abbreviated Version; RSQ-W, Restorative Sleep Questionnaire – Weekly Version; SE, Standard Error; TRP, Tryptophan; TDO, Tryptophan-2,3-dioxygenase.

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caused by a range of genetic, environmental, molecular, cellular, and psychological mechanisms. From a molecular perspective, disturbances in neurotransmitters and hormones such as γ-aminobutyric acid, noradrenaline, serotonin, corticotrophin-releasing hormone, cortisol, and melatonin have been identified [6,7]. Consequently, treatments that target these disturbances may prove to be effective agents for the treatment of insomnia. Because of their multiple physiological effects, herbal and plant-based medicines present as potential sleep-enhancing agents [8–10].

Saffron is derived from the stigmas of the Crocus Sativus flower. It is widely used as a natural additive in cooking to enhance the flavour, aroma, and colour of foods. Saffron also has a long history in traditional medicine as a treatment for diseases of the eye, skin, respiratory and gastrointestinal tracts, and for its mood-enhancing effects [11,12]. The majority of human trials have focused on its mental health effects and its efficacy for the treatment of depression and anxiety has been confirmed in several meta-analyses [13–15]. However, in a recent randomised, double-blind, placebo-controlled trial, saffron supplementation for 4 weeks was also associated with sleep-promoting effects in adults presenting with self-reported unsatisfactory sleep [16]. In this trial, compared to the placebo, a saffron extract (affron®) delivered at 14 mg twice daily, improved self-reported sleep quality and restorative sleep. In another trial on adults with a mild to moderate sleep disorder and associated anxiety, the 6-week intake of a saffron extract at 15.5 mg a day was associated with a greater increase in time in bed (based on actigraphy measurements) and self-reported sleep duration and social functioning compared to the placebo [17]. Saffron supplementation at a dose of 100 mg a day for 8 weeks has also improved sleep quality in adults with type 2 diabetes [18]. Due to the positive findings from these trials, the aims of the current study were to further examine and substantiate the efficacy of saffron supplementation using a larger sample size, a single evening dose taken 1 h before bed, and to examine the efficacy of different doses of saffron (14 mg and 28 mg). As there has been no human trial examining the potential mechanisms associated with the effects of saffron on sleep, changes in evening concentration in salivary cortisol and melatonin were also investigated.

2. Materials and methods

2.1. Study design

This was a three-arm, parallel-group, 28-day, 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 number 0073E_2020) and was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. ACTRN12620000973910). Based on a single outcome variable, an *a priori* power analysis was undertaken to estimate the required sample size. In a previous trial examining the sleep-enhancing effects of 28 mg of saffron daily, there was an effect size of 0.6 [16]. 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 of saffron on sleep was estimated as 36. After allowing for an approximate 10% dropout rate, we aimed to recruit 40 participants per group, totalling 120 participants.

2.2. Recruitment and randomisation

Via social media advertisements, participants were recruited throughout Australia between October and November 2020. Interested volunteers were directed to a website page that provided details about the trial and a link to complete an online questionnaire screening for self-reported sleep problems; medication use; history of medical or psychiatric disorders; alcohol, nicotine, and other drug use; supplement and vitamin intake; and pregnancy/breastfeeding status. To assess the severity of depressive and anxiety symptoms, respondents also completed the Patient Health Questionnaire – 4 (PHQ-4). If considered likely eligible, volunteers participated in a telephone interview comprising a structured series of questions to further assess their eligibility and to obtain further demographic details. Suitable participants were then required to complete online versions of the Insomnia Symptom Questionnaire (ISQ), Functional Outcomes of Sleep Questionnaire – short version (FOSQ-10), Restorative Sleep Questionnaire – Weekly Version (RSQ-W), Profile of Mood States - Abbreviated Version (POMS-A), and an informed consent form. Eligible and consenting participants were randomly allocated to one of three groups (saffron 14 mg, saffron 28 mg, or placebo). To ensure sequence concealment, a randomisation calculator (http://www.randomization.com) was used with the randomisation structure comprising 10 randomly permuted blocks, containing 12 participants per block. The participant identification number was assigned based on the order of participant enrolment in the study. All tablets were packed in identical bottles labelled by three intervention codes (held by the tablet manufacturer until final data collection). Participants and study investigators were blind to treatment group allocation until all outcome data were collected. Participants were not financially compensated for volunteering in this study, although at the end of the study, participants allocated to the placebo condition were offered a free 4-week supply of saffron tablets.

2.3. Participants

Inclusion criteria: male and female, physically healthy participants aged 18–70 years, with self-reported unsatisfactory sleep lasting longer than 4 weeks were recruited. Volunteers had a body mass index (BMI) between 20 and 35 and a usual bedtime between 9 pm and 12 a.m. Participants were fluent in English and consented (via an online consent form) to all pertinent aspects of the trial.

Exclusion criteria: Participants were ineligible to participate in the study if they were employed in night shift work, rotational shift work, or were experiencing external factors that may affect their sleep patterns (eg, a child regularly waking, excess noise, snoring partner, or pain condition). People experiencing a sleep disorder other than moderate insomnia (eg, sleep apnoea, periodic limb movement disorder, restless legs syndrome), being diagnosed with a mental health disorder (other than mild depressive or anxiety symptoms as measured by the PHQ-4), consuming more than 3 cups of coffee per day (or equivalent caffeine intake from other caffeinated drinks eg, tea, energy drinks), or an alcohol consumption greater than 14 standard drinks per week were excluded. Participants were also ineligible for the study if they were taking pharmaceutical medications or natural supplements that may affect sleep quality, taking saffron supplements, receiving non-pharmacological treatment for sleep disorders (eg, cognitive behavioural therapy, relaxation therapy), had a current or 12-month history of illicit drug use, or had a recently-diagnosed or unmanaged medical condition 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, neurological disease (Parkinson's, Alzheimer's disease, intracranial haemorrhage, head or brain injury), or acute or chronic pain affecting sleep. Pregnant women, women who were breastfeeding, or women who intended to fall pregnant were also ineligible to participate in the study.

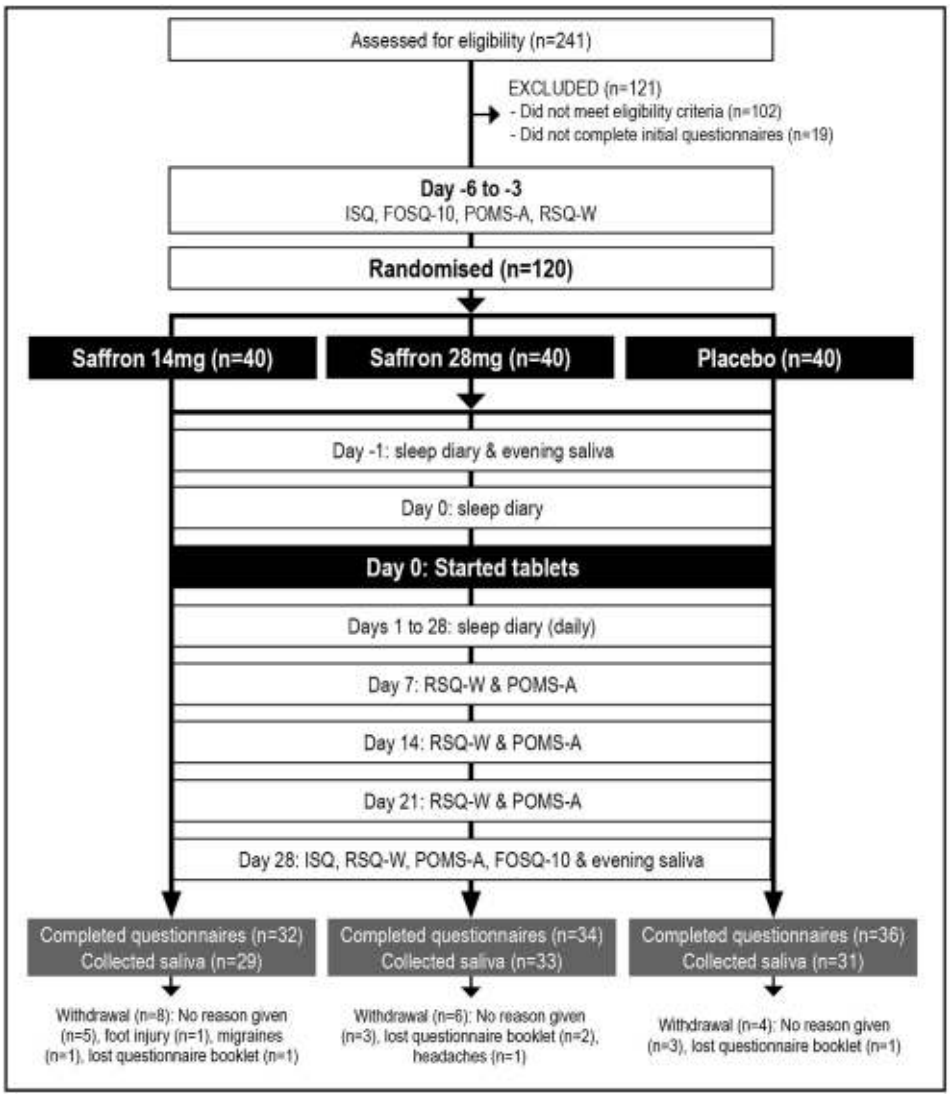

Fig. 1. Systematic Illustration of Study Design. FOSQ-10 = Functional Outcomes of Sleep Questionnaire; ISQ = Insomnia Symptoms Questionnaire; POMS-A = Profile of Mood States, abbreviated version; PSD = Pittsburgh Sleep Diary; RSQ-W – Restorative Sleep Questionnaire, Weekly Version.

2.4. Interventions

Saffron and placebo tablets were identical in appearance, being matched for colour coating, shape, and size. The active ingredient, supplied by Pharmactive Biotech Products SL, comprised a standardised saffron extract, affron®, which is derived from the stigmas of *Crocus sativus* L. The active treatments contained 14 or 28 mg of affron®, which is standardised to contain more than 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). The placebo tablets contained the same excipients as the active tablets (microcrystalline cellulose and calcium hydrogen phosphate). All tablets were manufactured and packed in an Australian Therapeutic Goods Administration– registered plant. All participants were instructed to take 1 tablet daily, 1 h before bedtime, with or without food for 28 days. Tablet adherence was assessed by asking participants to provide a tablet count every 7 days. Treatment blinding was assessed by asking participants to predict group allocation (placebo, saffron, or uncertain) at the end of the study. All tablets were mailed to participants with directions for use provided on tablet bottles. An information sheet about tablet intake and what to do if a dose was missed was also sent to participants. This information was also verbally conveyed to participants during their initial telephone interview.

2.5. Outcome measures

Initial screening questionnaires comprising the Restorative Sleep Questionnaire- Weekly Version (RSQ-W), Insomnia Symptom Questionnaire (ISQ), Functional Outcomes of Sleep Questionnaire-short version (FOSQ-10), and the Profile of Mood States-abbreviated version (POMS-A) were completed online. A response booklet containing copies of the required questionnaires and sleep diaries was then mailed to all participants. The dates for completion of each questionnaire, diary, and saliva collection were recorded in the booklet. Participants were advised to keep their response booklet near their bed and to complete it within 30 min after awakening.

2.5.1. Primary outcome measure

2.5.1.1. Pittsburgh Sleep Diary (PSD), sleep quality rating. The PSD was completed by respondents upon awakening. The PSD shows good retest reliability over a 22-month period. Scores also correlate with circadian type, subjective sleep quality, and objective actigraphy measurements [19]. Respondents rated their sleep quality on a 5-point Likert scale ranging from very bad (1) to very good (5). The PSD was completed daily for 29 days, where days 0 and 1 were recorded before tablet intake (baseline).

2.5.2. Secondary outcome measures: questionnaires and diaries

2.5.2.1. Pittsburgh Sleep Diary (PSD), other sleep quality measures. In addition to sleep quality ratings, PSD scores were calculated for total sleep time (hours), sleep latency (minutes), number of awakenings after sleep onset, mood rating at final awakening [5-point Likert rating ranging from very tense (1) to very calm (5)], and alertness rating at final awakening [5-point Likert rating ranging from very sleepy (1) to very alert (5)].

2.5.2.2. Insomnia Symptom Questionnaire (ISQ). The ISQ is a 13-item self-report instrument designed to assess insomnia [20]. Questions establish the presence of difficulty initiating or maintaining sleep, the feeling of nonrestorative or unrefreshing sleep, the frequency and duration of these complaints, and the severity of sleep problems on daily function. The ISQ has good reliability and correlates with other sleep measures such as sleep diaries, polysomnography, and the Pittsburgh Sleep Quality Index. The ISQ is also able to accurately identify insomnia cases based on the Diagnostic and Statistical Manual of Mental Disorders-IV (DMS-IV) and Research Diagnostic Criteria [20,21]. The ISQ was completed at baseline (3–6 days before tablet intake) and 28 days after tablet intake.

2.5.2.3. Restorative Sleep Questionnaire – Weekly Version (RSQ-W). The RSQ-W is a validated 11-item questionnaire that assesses restorative sleep by asking respondents to rate on a 5-point scale feelings of tiredness, mood, and energy. The RSQ has good psychometric properties and can distinguish between healthy controls, patients with primary insomnia, and insomnia patients with isolated nonrestorative sleep complaints [22]. The RSQ-W was completed at baseline (3–6 days before tablet intake) and 7, 14, 21, and 28 days after tablet intake.

2.5.2.4. Functional Outcomes of Sleep Questionnaire (FOSQ-10). The FOSQ-10 comprises 10 questions evaluating the respondent's quality of life as it relates to disorders of excessive sleepiness. A total score is calculated plus sub-scale scores for the five domains of day-to-day life comprising (1) general productivity, (2) activity levels, (3) vigilance, (4) social outcomes, and (5) intimacy and sexual relationships. The FOSQ-10 has good psychometric properties and similar reliability and validity to the longer version of the original FOSQ [23]. The FOSQ-10 was completed at baseline (3–6 days before tablet intake) and 28 days after tablet intake.

2.5.2.5. Profile of Moods States – Abbreviated Version (POMS-A). The POMS-A is a psychometrically-validated, 40-item self-report questionnaire that assesses a respondent's current mood state [24]. Participants rated on a 4-point scale (not at all to extremely) how they were currently feeling. Scores were calculated for tension, anger, fatigue, depression, esteem-related affect, vigour, confusion, and total mood disturbance. The POMS-A was completed at baseline (3–6 days before tablet intake) and 7, 14, 21, and 28 days after tablet intake.

2.5.2.6. Salivary cortisol and melatonin (evening). To measure salivary concentrations of cortisol and melatonin, participants were provided with small collection tubes and the whole saliva was collected by unstimulated passive drool. These samples were collected in participants' homes 1 day before, and 28 days after tablet intake. Participants were instructed to collect approximately 5 mls of saliva at 10 pm. They were requested to not consume any food or drink for at least 15 min before collecting the sample. If participants went to bed earlier than this time, they were requested to collect the sample before going to bed. However, they were requested to collect the two evening saliva samples at the same time. All saliva samples were immediately frozen at −20 °C until hormone analysis. On the day of analysis, samples were thawed and precipitated mucins and particulate debris were separated by centrifugation at 1,500g for 25 min at room temperature. An aliquot of the supernatant was used for the measurement of cortisol and melatonin and was measured in duplicate using a commercially available competitive enzyme-linked immune sorbent assay kit (Salimetrics, Carlsbad, CA) according to the manufacturer instructions.

2.5.2.7. Adverse events. Tolerability and safety of tablet intake by participants were assessed every 7 days via an online question querying adverse effects that were believed to be associated with tablet intake. Participants were also requested to contact the researchers immediately if any adverse effects were experienced.

2.6. Statistical analysis

For baseline data, a one-way analysis of variance (ANOVA) was used to compare group data for continuous variables, and a Pearson's Chi-square test was used to compare categorical data. Change in scores was calculated for all outcome measures. The PSD scores were analysed for changes from mean baseline (day −1 and 0) to the mean of week 4 (days 22–28), while change scores for the remaining outcome measures (FOSQ-10, ISQ, RSQ-W, POMS-A, and salivary hormones) were calculated from baseline to day 28. A general linear, univariate ANOVA was used to examine between-group differences in changes in the primary outcome measure, PSD sleep quality ratings. Two separate multivariate ANOVAs comprising the remaining PSD scores, FOSQ-10 total score, ISQ total score, and RSQ-W total score; and POMS-A subscale scores, were used to examine between-group differences in change in scores. Helmert contrasts were used to examine the effects of saffron (both doses) versus placebo, and to compare the effects of the two doses of saffron (14 mg versus 28 mg). As an exploratory investigation, univariate analyses on sleep-related outcome measures were undertaken (ie, the remaining PSD scores, FOSQ-10 total score, ISQ total score, and RSQ-W total score). For all outcome measures, a Cohen's d was also calculated (saffron 14 mg and saffron 28 mg compared to the placebo). The normality of residuals was assessed by the visual inspection of Q–Q plots and analysis of skewness and kurtosis. This demonstrated that most data were normally distributed. As salivary hormone concentrations were not normally distributed and transformations or removal of outliers did not normalise data, a non-parametric test (Independent-Samples Mann–Whitney U Test) was used to compare between-group differences in changes in evening cortisol and melatonin concentrations from baseline to day 28, and a Wilcoxon signed-rank test was used to examine within-group changes over time. Two separate between-group analyses were conducted comprising saffron combined versus placebo, and saffron 14 mg versus saffron 28 mg.

Data from all participants who returned their response booklets were included in the analyses. An intention to treat analysis was also conducted on change scores in sleep-related questionnaires and diary measures. All data were analysed using SPSS (version 26; IBM, Armonk, NY). The critical *p*-value was set at $p \leq 0.05$ for all analyses.

3. Results

3.1. Study population

3.1.1. Baseline questionnaire and demographic information

As detailed in Fig. 1, from 241 people who completed the initial online screening questionnaire, 121 individuals did not complete the initial baseline questionnaires (n = 19) or were ineligible (n = 102). One hundred and twenty volunteers participated in the study and questionnaire/diary data from 102 participants who returned questionnaire booklets were used for statistical analyses. Pre and post saliva samples were obtained from 93 participants. Eight participants who returned questionnaire booklets did not collect both pre and post saliva collections due to reports of insufficient saliva flow (n = 4), lost collection tubes (n = 2), or forgetfulness (n = 2). Details of participant demographic information and baseline scores of the total recruited sample are detailed in Table 1. Baseline demographic details and questionnaire responses were equivalent across all groups. Eighteen participants withdrew or did not return response booklets (4 x placebo, 8 x saffron 14 mg, 6 x saffron 28 mg). Reasons for withdrawal included no reason given (n = 11), lost questionnaire booklet (n = 4), migraines/headaches (n = 2), and a foot injury (n = 1).

3.2. Outcome measures

3.2.1. Primary outcome measure: PSD sleep quality ratings

Changes in the PSD sleep quality ratings (baseline and week 4) across the three treatment groups and univariate ANOVA significance levels as detailed in Table 2 and Fig. 2 (Supplementary file Table 1 contains scores for all weekly time points). There was a statistically-significant between-group difference in change in PSD sleep quality ratings between the saffron (combined) and placebo group (p = 0.023), but no significant difference between the two saffron doses (p = 0.949). An ITT analysis revealed a similar statistically significant difference in change in sleep quality ratings between the saffron (combined) and placebo group (p = 0.045). From baseline to week 4, there was a non-significant increase of 8.43% in sleep quality ratings in the placebo group ($F_{1,35} = 3.56$, p = 0.068), and statistically significant increases of 24.60% in the saffron 14 mg ($F_{1,31} = 22.00$, p < 0.001) and 22.26% in the saffron 28 mg groups ($F_{1,33} = 14.48$, p = 0.001). Compared to the placebo, there was a Cohen's d effect size of 0.53 in the saffron 14 mg group and 0.45 in the saffron 28 mg group.

3.2.2. Secondary outcome measures: PSD scores, RSQ-W, and ISQ

Changes in the remaining PSD scores, RSQ-W, and ISQ scores (baseline and week 4) across the three treatment groups and univariate ANOVA significance levels are detailed in Table 2 (Supplementary file Table 1 contains scores for all weekly time points). A multivariate analysis revealed there was a non-significant difference in change in overall scores when all three groups were compared ($F_{16,186} = 1.33$, p = 0.183). However, a univariate analysis revealed there were statistically-significant between-group differences in change in PSD mood ratings ($F_{2,99} = 4.49$, p = 0.014) and change in ISQ scores ($F_{2,99} = 3.18$, p = 0.046). Helmert contrasts between the combined saffron groups and placebo group revealed that there were statistically-significant differences in change in PSD mood ratings (p = 0.009) and ISQ total score (p = 0.013). An ITT analysis revealed similarly statistically significant differences in change in PSD mood ratings (p = 0.011) and ISQ total score (p = 0.024) between the saffron (combined) and placebo group. From baseline to week 4, there was a non-significant decrease of 1.58% in mood upon awakening ratings in the placebo group ($F_{1,35} = 0.24$, p = 0.631), and statistically-significant increases of 7.26% in the saffron 14 mg ($F_{1,31} = 4.68$, p = 0.038) and 14.42% in the saffron 28 mg groups ($F_{1,33} = 9.66$, p = 0.004). In relation to ISQ scores, from baseline to week 4, there was a non-significant increase of 3.86% in the placebo group ($F_{1,35} = 0.92$, p = 0.345), and statistically-significant increases of 15.75% in the saffron 14 mg ($F_{1,31} = 10.17$, p = 0.003) and 16.81% in the saffron 28 mg groups ($F_{1,33} = 27.04$, p < 0.001).

3.2.3. Secondary outcome measures: POMS-A scores

Changes in the POMS-A subscale scores across the three treatment groups and univariate ANOVA significance levels are detailed in Table 2. A multivariate analysis revealed there was a non-significant difference in change in overall scores when all three groups were compared ($F_{14,188} = 0.53$, p = 0.915). Univariate analyses and helmert contrasts also revealed non-significant between-group differences in changes in all POMS-A subscale scores.

3.2.4. Secondary outcome measure: ISQ insomnia classification

Rates of insomnia based on the ISQ insomnia classification are detailed in Table 2. There was a 6%, 22% and 26% reduction in the number of insomnia classifications from baseline to week 4 in the placebo, saffron 14 mg, and saffron 28 mg groups, respectively. A chi-square test revealed changes in insomnia classifications were significantly different between the saffron groups (combined) and the placebo group (p = 0.041).

3.2.5. Secondary outcome measure: salivary hormones

Changes in evening salivary hormones concentrations across the three treatment groups and significance values for the independent-samples Mann–Whitney U Test and Wilcoxon Signed Rank Test are detailed in Table 3. Group comparisons revealed that changes in melatonin concentrations were significantly different in the combined saffron groups compared to the placebo (p = 0.021) (see Fig. 3). A Wilcoxon Signed Rank Test revealed that melatonin concentrations from baseline to day 28 significantly increased in the combined saffron groups (p = 0.036) but there was no statistically-significant change in the placebo (p = 0.526), saffron 14 mg (p = 0.128), or saffron 28 mg (p = 0.268) groups. A statistically-significant decrease in cortisol concentrations from baseline to day 28 was identified in the combined saffron groups (p = 0.040) and saffron 28 mg group (p = 0.032) but these decreases did not differ significantly from changes in the placebo group (see Fig. 3). No other statistically-significant within or between-groups differences in changes in salivary hormone concentrations were identified.

3.2.6. Intake of supplements

On days 7, 14, 21 and 27 participants recorded their quantity of remaining supplements. On day 28, 97% of participants reported taking greater than 80% of their capsules and there were no differences in the frequency of capsule intake among the 3 groups.

Table 1
Baseline demographic details of participants.

		Placebo	Saffron 14 mg	Saffron 28 mg	p-value
		n = 40	n = 40	n = 40	
Age	Mean	52.18	55.03	50.43	0.109[a]
	SE	1.91	0.93	1.63	
Gender	Female (n)	28	27	30	0.754[b]
	Male (n)	12	13	10	
BMI	Mean	25.55	26	25.93	0.873[a]
	SE	0.68	0.64	0.66	
Marital status	Single	14	12	11	0.761[b]
	Married/defacto	26	28	29	
Educational level	Secondary	14	18	13	0.764[b]
	Tertiary	14	10	13	
	Post-graduate	12	12	14	
Exercise level (n)	Never/rarely	8	6	4	0.408[b]
	1 to 2 times a week	3	2	7	
	3 to 5 times a week	10	13	14	
	6+ times a week	19	19	15	
Duration of sleep problems	Less than 6 months	3	2	1	0.919[b]
	6–12 months	5	5	7	
	1–2 years	3	8	5	
	2–5 years	13	13	12	
	5–10 years	15	11	14	
	10 or more years	1	1	1	
ISQ - Insomnia Diagnosis	Yes	25	28	24	0.624[b]
	No	15	12	16	
FOSQ-10	Mean	28.25	27.15	28.68	0.522[a]
	SE	0.92	1	1	
ISQ	Mean	35.63	36.4	34.48	0.553[a]
	SE	1.3	1.17	1.29	
RSQ	Mean	40.69	40.96	43.98	0.608
	SE	2.72	2.29	2.70	
POMS - Tension	Mean	0.63	0.53	0.62	0.743[a]
	SE	0.09	0.09	0.09	
POMS - Anger	Mean	0.38	0.36	0.34	0.944[a]
	SE	0.07	0.09	0.07	
POMS - Fatigue	Mean	1.43	1.43	1.46	0.990[a]
	SE	0.13	0.16	0.14	
POMS - Depression	Mean	0.5	0.43	0.41	0.742[a]
	SE	0.09	0.1	0.08	
POMS - Esteem-related affect	Mean	2.31	2.42	2.48	0.444[a]
	SE	0.1	0.08	0.1	
POMS- Vigour	Mean	0.74	0.83	0.8	0.841[a]
	SE	0.11	0.11	0.12	
POMS - Confusion	Mean	0.73	0.54	0.67	0.254[a]
	SE	0.09	0.07	0.08	
PSD		n = 36	n = 32	n = 34	
Sleep Quality Rating	Mean	2.61	2.48	2.65	0.661[a]
	SE	0.11	0.13	0.15	
Mood rating after waking	Mean	3.17	3.17	3.12	0.949[a]
	SE	0.13	0.15	0.12	
Alertness rating after waking	Mean	2.47	2.81	2.66	0.222[a]
	SE	0.14	0.14	0.14	
Time taken to fall asleep (min)	Mean	39.71	54.75	38.32	0.363[a]
	SE	7.73	10.98	7.86	
Total sleep time (min)	Mean	381.31	332.56	348.69	0.114[a]
	SE	16.47	16.98	16.81	
Number of waking	Mean	2.99	2.81	2.9	0.885[a]
	SE	0.27	0.24	0.23	
Salivary hormones		n = 31	n = 29	n = 33	
Cortisol (ug/dL)	Mean	0.095	0.079	0.084	0.264[a]
	SE	0.014	0.008	0.006	
Melatonin (pg/mL)	Mean	9.58	9.36	7.27	0.368[a]
	SE	1.04	1.31	1.18	

BMI = Body Mass Index; FOSQ-10 = Functional Outcomes of Sleep Questionnaire; POMS-A = Profile of Mood States – Abbreviated Version (POMS-A); PSD = Pittsburgh Sleep Diary; RSQ-W = Restorative Sleep Questionnaire – Weekly Version; SE = standard error.
[a] One-way ANOVA.
[b] Chi-square Test.

Table 2
Change in questionnaire outcome measures.

		Placebo (n = 36)			Saffron 14 mg (n = 32)			Saffron 28 mg (n = 34)			p-value for change scores			Cohen's d (saffron 14 mg vs placebo)	Cohen's d (saffron 28 mg vs placebo)
		Baseline	Week 4	Change (baseline to week 4)	Baseline	Week 4	Change (baseline to week 4)	Baseline	Week 4	Change (baseline to week 4)	All groups	Saffron vs placebo	Saffron 14 mg vs 28 mg		
Sleep Quality Rating	Mean	2.61	2.83	0.22	2.48	3.09	0.61	2.65	3.24	0.59	0.074[a]	0.023[a]	0.949[a]	0.530	0.450
	SE	0.11	0.11	0.12	0.13	0.13	0.13	0.15	0.15	0.16					
Mood rating after waking	Mean	3.17	3.12	−0.05	3.17	3.40	0.23	3.12	3.57	0.45	0.014	0.009	0.199	0.450	0.670
	SE	0.13	0.14	0.11	0.15	0.13	0.10	0.12	0.14	0.14					
Alertness rating after waking	Mean	2.47	2.82	0.35	2.81	3.08	0.27	2.66	3.20	0.54	0.347	0.725	0.161	0.110	0.250
	SE	0.14	0.14	0.10	0.14	0.14	0.15	0.14	0.16	0.15					
Time taken to fall asleep (min)	Mean	39.71	26.15	−13.56	54.75	36.28	−18.47	38.32	22.65	−15.68	0.873	0.663	0.771	0.120	0.060
	SE	7.73	4.13	5.39	10.98	5.43	8.16	7.86	3.47	6.41					
Total sleep time (min)	Mean	381.31	405.90	24.59	332.56	389.16	56.60	348.69	395.87	47.18	0.204	0.086	0.616	0.440	0.270
	SE	16.47	11.63	14.29	16.98	14.93	10.22	16.81	14.27	13.71					
Number of awakenings	Mean	2.99	2.03	−0.95	2.81	1.82	−0.99	2.90	2.01	−0.89	0.940	0.955	0.730	0.030	0.060
	SE	0.27	0.20	0.19	0.24	0.15	0.24	0.23	0.22	0.18					
RSQ	Mean	41.28	49.38	8.10	39.91	53.56	13.65	42.50	57.53	15.03	0.320	0.140	0.782	0.290	0.330
	SE	2.99	2.78	3.41	2.44	2.82	3.24	2.52	3.49	3.72					
ISQ	Mean	34.50	33.17	1.33	36.50	30.75	5.75	34.15	28.41	5.74	0.046	0.013	0.994	0.470	0.590
	SE	1.28	1.28	1.39	1.27	1.96	1.80	1.35	1.63	1.10					
FOSQ	Mean	28.75	30.08	−1.33	27.31	29.81	−2.50	28.62	30.68	−2.06	0.624	0.364	0.721	0.240	0.140
	SE	0.96	0.99	0.87	1.12	1.07	0.78	0.98	0.85	0.90					
POMS-A - Tension	Mean	0.56	0.60	−0.04	0.58	0.42	0.16	0.58	0.38	0.20	0.162	0.060	0.785	0.350	0.460
	SE	0.09	0.09	0.08	0.11	0.09	0.11	0.09	0.07	0.09					
POMS-A - Anger	Mean	0.35	0.36	−0.01	0.41	0.25	0.16	0.29	0.20	0.10	0.395	0.199	0.636	0.290	0.230
	SE	0.07	0.08	0.10	0.11	0.07	0.11	0.07	0.06	0.06					
POMS-A - Fatigue	Mean	1.37	1.32	0.05	1.41	1.22	0.19	1.38	1.13	0.25	0.700	0.425	0.795	0.140	0.190
	SE	0.14	0.17	0.18	0.17	0.19	0.17	0.15	0.18	0.18					
POMS-A - Depression	Mean	0.47	0.29	0.18	0.48	0.28	0.21	0.42	0.25	0.17	0.968	0.951	0.805	0.040	0.020
	SE	0.10	0.07	0.11	0.12	0.07	0.12	0.09	0.07	0.09					
POMS-A - Esteem-related affect	Mean	2.29	2.32	−0.03	2.41	2.51	−0.10	2.46	2.56	−0.10	0.878	0.612	0.965	0.120	0.090
	SE	0.11	0.10	0.12	0.09	0.09	0.09	0.10	0.13	0.12					
POMS-A - Vigour	Mean	0.71	1.16	−0.46	0.84	1.34	−0.51	0.86	1.40	−0.54	0.938	0.739	0.901	0.060	0.080
	SE	0.11	0.13	0.16	0.12	0.15	0.15	0.13	0.18	0.18					
POMS-A - Confusion	Mean	0.67	0.49	0.18	0.58	0.43	0.15	0.63	0.45	0.18	0.971	0.894	0.839	0.050	0.000
	SE	0.09	0.08	0.08	0.09	0.07	0.10	0.08	0.07	0.09					
ISQ - Insomnia criteria met	Yes	58%	53%	6%	75%	53%	22%	59%	32%	27%	–	0.041[b]	–	–	–

[a] Univariate ANOVA.
[b] Chi-square test.

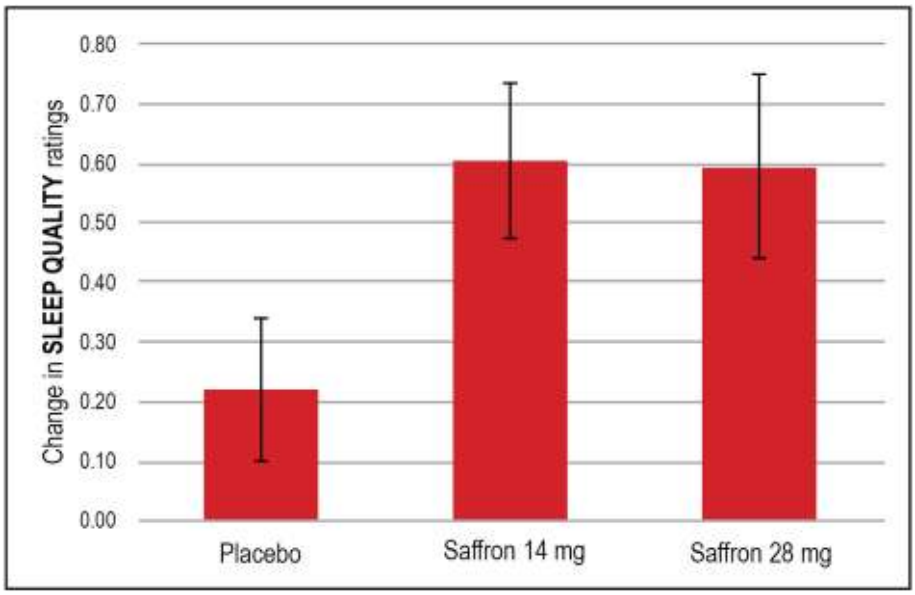

Fig. 2. Changes in sleep quality ratings from baseline to week 4.

3.2.7. 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 condition allocation (ie, placebo, saffron, or uncertain). Efficacy of group concealment was high as 67% of participants either incorrectly guessed treatment allocation or were unsure.

3.2.8. Adverse events

The frequency of self-reported adverse effects is detailed in Table 4. There were no significant differences in the frequency of reported adverse effects between the groups and no serious adverse events were reported by participants. There were no reports of any adverse effects in 82.5% of participants in the placebo and saffron 14 mg groups, and 87.5% of participants in the saffron 28 mg group.

4. Discussion

In this randomised, double-blind, placebo-controlled study, saffron supplementation for 28 days at a dose of 14 and 28 mg, taken 1 h before bedtime was associated with greater improvements in several sleep-related measures. Compared to the placebo, participants supplemented with saffron experienced greater improvements in sleep quality ratings (primary outcome measure), mood ratings after awakening, and the ISQ total score. Insomnia classifications based on the ISQ also revealed that saffron supplementation was associated with greater reductions in rates of insomnia (24% reduction) compared to the placebo (6% reduction). However, there were no significant differences between the saffron and placebo groups in changes in self-reported alertness ratings after awakening, total sleep time, time to sleep onset, number of awakenings after sleep onset, restorative sleep (measured with the RSQ), and quality of life as associated with excessive sleepiness (measured with the FOSQ-10). There were also no between-group differences in mood-related changes as measured by the POMS-A. A comparison of the two doses of saffron (14 mg and 28 mg) demonstrated that sleep-related improvements were similar for the two administered doses. To help elucidate the physiological effects of saffron supplementation, changes in evening concentrations of salivary cortisol and melatonin were measured over time. Compared to the placebo, saffron supplementation was associated with increases in evening melatonin concentrations. Evening cortisol concentrations also significantly decreased after saffron supplementation, but group comparisons indicated that changes did not differ significantly between the saffron and placebo groups. Saffron was well tolerated with no serious adverse effects and similar rates of reported adverse effects in the placebo and saffron groups. However, one participant in each saffron group did report withdrawing from the study due to headaches/migraines. This adverse effect has not been identified previous trials on saffron so will require monitoring in future trials.

The results of this study are consistent with those identified in a previous study on saffron [16]. In this previous trial, saffron was administered at a dose of 14 mg twice daily for 28 days, which contrasts with the current study where supplementation was administered once daily, 1 h before bed. Together, these results suggest that supplementation once or twice daily at total daily doses of either 14 or 28 mg may be effective in improving sleep quality in adults with self-reported poor sleep. A more comprehensive comparison of these two trials revealed that improvements in self-reported mood upon awakening occurred in the current trial only; however, improvements in self-reported restorative sleep occurred in the previous trial only. Discrepancies in these findings could not be elucidated as identical saffron extracts (affron®) were used and recruited populations were similar in age, duration of sleep problems, BMI, and gender distribution. However, RSQ baseline scores were slightly lower in the current trial compared to the previous one and the RSQ weekly version was used in the current trial compared to the monthly version used in the previous trial. Moreover, it is possible that twice-daily supplementation may be required to increase self-reported restorative sleep, although this requires further investigation.

Table 3
Change in salivary hormones concentrations.

		Placebo (n = 31)				Saffron 14 mg (n = 29)				Saffron 28 mg (n = 33)				Combined saffron p-value[a]	p-value[b]	
		Day 0	Day 28	Change from baseline	p-value[a]	Day 0	Day 28	Change from baseline	p-value[a]	Day 0	Day 28	Change from baseline	p-value[a]		Saffron vs placebo	Saffron 14 mg vs 28 mg
Evening Cortisol (ug/dL)	Mean	0.095	0.075	−0.02	0.205	0.079	0.079	0.001	0.428	0.084	0.074	−0.01	0.032	0.040	0.935	0.667
	SE	0.014	0.011	0.018		0.008	0.015	0.018		0.006	0.016	0.014				
Evening Melatonin (pg/mL)	Mean	9.58	8.34	−1.24	0.526	9.36	11.01	1.65	0.128	7.27	8.02	0.75	0.268	0.036	0.021	0.485
	SE	1.04	0.8	0.9		1.31	1.74	1.34		1.18	1.17	0.95				

[a] Wilcoxon Signed Rank Test.
[b] Mann-Whitney U test.

An important observation in this study is the similar sleep-enhancing efficacy of the two doses of saffron. This finding contrasts with a study on the mood-enhancing effects of saffron where greater efficacy was obtained at the higher dose of 28 mg, compared to 22 mg [25]. It is important to note that the current study was underpowered to detect small-to-moderate treatment effects (ie, effect sizes of 0.6 and lower), which may account for the lack of significant differences between the two treatment doses. It is also possible that a longer duration of intake is required to achieve greater sleep-enhancing benefits at a higher dose. Alternatively, the timing of tablet intake (1 h before bed) might have masked a dose-reponse effect. In a pharmacokinetic study on healthy adults volunteers, the administration of saffron tablets at a dose of 56 and 84 mg dose-dependently increased plasma concentrations of crocetins [26]. However, at 1-h post-dose, plasma concentrations of crocetins were similar for the two doses (this is the time participants went to bed in our study). Even though greater plasma crocetin concentrations occurred with the higher dose, the maximum concentration occurred 90 min after intake. Because this would occur 30 min after bedtime, sleep-enhancing effects may not be realised. However, this postulation requires further investigation in future trials.

To help elucidate hormonal changes associated withs saffron supplementation, evening salivary concentrations in cortisol and melatonin were measured. Saffron supplementation was associated with increases in evening melatonin concentrations compared to the placebo. This suggests that one mechanism associated with saffron's sleep-enhancing effects may be via its influence on melatonin. Melatonin is a circadian hormone produced by the pineal gland at night which peaks between 3.00 and 4.00 a.m. Melatonin regulates many biological functions such as sleep, circadian rhythms, immunity, and reproduction [27,28]. Melatonin also has antioxidant and anti-inflammatory effects [29]. In several studies, adults with insomnia had lower concentrations of melatonin compared to regular sleepers [30,31]**.** Even though melatonin in most of these studies was measure via blood, salivary concentrations are reliable markers of indices of serum concentrations [32]. Saffron could influence melatonin in several ways. The amino acid tryptophan (TRP) is the precursor to melatonin synthesis. As TRP can be metabolised by gut microbiota, saffron might influence gut microbiota to increase tryptophan availability [33]. This has not yet been examined, although in an animal trial saffron did alter gut microbiota in rats [34]. For the synthesis of melatonin, TRP is converted into 5-hydroxytryptophan and serotonin with the rate-limiting enzyme tryptophan hydroxylase. Serotonin is then converted into melatonin with the enzyme arylalkylamine-N-acetyltransferase [28]. Whether saffron influences the activity of these enzymes has not yet been investigated. Serotonin, and therefore melatonin production, can be impacted by the activity of other enzymes, such as indoleamine 2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO). These enzymes divert the conversion of tryptophan from serotonin into the kynurenine pathway. IDO activity is increased by inflammation [35,36] and via saffron's anti-inflammatory effects [37], IDO activity may be downregulated by saffron supplementation. Polyphenols have been shown to inhibit IDO activity although there has been no study examining the effects of saffron or its constituents on IDO activity [38]. Moreover, via its anti-inflammatory effects, saffron may increase melatonin concentrations as inflammatory cytokines such as interferon-γ and interleukin-1β can influence melatonin production and secretion [39,40]. Finally, it is possible that saffron can influence the breakdown or deactivation of melatonin. Liver cytochrome P450 enzymes are involved in the breakdown of melatonin and saffron has been observed to have modest effects on cytochrome P450 activity [41,42].

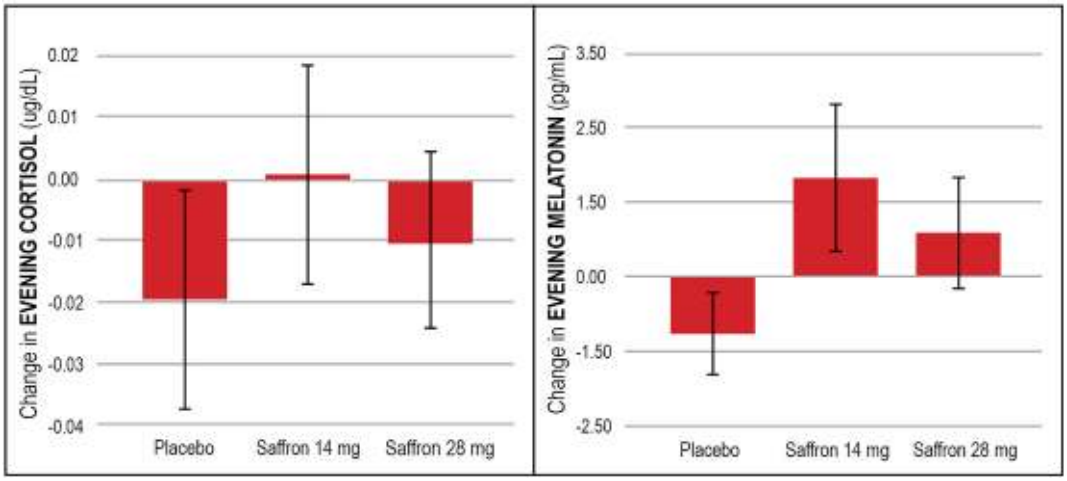

Fig. 3. Changes in salivary hormone concentrations (bars depict standard error bars).

4.1. Limitations and directions for future research

Even though the results of this study are consistent with a previous trial on the sleep-enhancing effects of saffron [16], further research is required to help validate and extend the current findings. In the current trial, self-report questionnaires and diaries were used as outcome measures. Thus, in future trials, it will be important to include objective sleep measures such as polysomnography, actigraphy, or even wrist-worn commercial sleep trackers. The safety and efficacy of longer-term saffron supplementation also require investigation as both sleep studies on saffron were one-month trials. Whether sustained or increased efficacy occurs with longer intake is uncertain. Further adequately powered studies will also be important to help elucidate the efficacy of saffron supplementation based on age, gender, and duration of sleep problems; as well as in people with formally-diagnosed insomnia. Based on the ISQ classification, approximately 57% of people recruited in this trial fulfilled the criteria for insomnia. Even though the ISQ can accurately identify insomnia cases based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, and Research Diagnostic Criteria [20,21], more comprehensive assessments will be helpful to accurately diagnose insomnia disorder. In this trial, 70% of participants were female and the mean age was 52 years. Consequently, many participants were likely peri- or post-menopausal which will influence the generalisability of the findings to other populations. A more accurate assessment of hormonal status in future trials will help to understand its impact on treatment efficacy. Moreover, recruitment via social media advertisements may have biased the population toward social media users with an interest in natural medicine. As saffron has demonstrated efficacy for the treatment of mood disorders, the effects of supplementation in people with comorbid sleep and affective disorders will also be important. In the current trial, only people with mild depression and anxiety symptoms were eligible to participate. Moreover, the effects of saffron as an adjunct treatment to other validated sleep treatments such as cognitive behaviour therapy or other pharmacological interventions will be important to investigate. Further research is required to examine the potential mechanisms associated with saffron's sleep-enhancing effects. Other options include examining its effects on inflammation, oxidative stress, neurological activity, and neurotransmitter production as these have been shown to impact on sleep quality [6,7]. As melatonin concentrations are influenced by light, it will be important in future trials to control for light exposure during collection. Moreover, it will be important to compare salivary melatonin concentrations with healthy sleepers to determine if there are differences and if normalisation in levels is associated with improved sleep patterns. As there are no normative data on melatonin concentrations due to differences in its measurement and other confounding variables, comparisons with healthy sleepers could not be undertaken in this study. Even though saffron had no effect on cortisol in this trial, further investigation into its effects on cortisol and the hypothalamus–pituitary–adrenal axis will be important. This can occur via utilising diurnal cortisol measurements, examining the effects of saffron on the cortisol awakening response [43], using hair cortisol measurements (which provide a chronic cortisol measure) [44], or the use of acute stress models such as the Trier Social Stress Test or the Maastricht Acute Stress Test [45,46]. Finally, the replicability of the current findings needs to be examined using other saffron extracts. In both sleep trials on saffron, a standardised saffron extract (affron®) was used. The quality of saffron extracts can vary significantly and saffron adulteration is common [47,48]. Therefore, before the findings from the current study can be generalised to other saffron extracts, further investigations of those extracts are required.

In summary, the results of this trial provide further confirmation of the sleep-enhancing effects of 28-days of saffron supplementation (affron®) in people with self-reported poor sleep, at doses of 14 and 28 mg, taken 1 h before bed. Saffron was associated with improvements in ratings of sleep quality and mood upon awakening.

Table 4
Frequency of self-reported adverse effects.

	Placebo	Saffron 14 mg	Saffron 28 mg
Irritability	1		
Increased tiredness	1	1	
Headaches/migraine	1	2	2
Vivid dreams	1		
Dry mouth	1		2
Increased urination			1
Yellowing of skin		1	
Nausea			1
Diarrhoea	1		1
Bloating	1		
Dizziness	1		1
Weight gain		1	
Worsened sleep	1		
Chest pain			1
Total number of adverse effects	9	5	9

Moreover, there was an approximately 25% reduction in insomnia diagnoses (based on the ISQ classification) at the end of treatment. However, there were no significant changes in alertness ratings after awakening, total sleep time, time to sleep onset, number of awakenings after sleep onset, restorative sleep, and quality of life. An examination of hormonal changes revealed increases in evening salivary melatonin concentrations with saffron supplementation. Further research will be important to examine the effects of saffron supplementation over longer treatment periods, using diverse populations, different treatment doses, and varied saffron extracts to help clarify the sleep-enhancing effects of saffron. Investigations into other possible mechanisms of action associated with saffron intake will also be important.

Credit author statement

ALL and PDD designed the research; ALL and SJS conducted the research; ALL and PDD analysed the data; and ALL, PDD, and SJS wrote the paper. ALL has primary responsibility for the final content. All authors read and approved the final manuscript.

Author declaration

All authors have seen and approved the manuscript.

Clinical trial registration

This study was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. ACTRN12620000973910). https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=380507.

Funder and role of the funding source

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Conflict of interest

Dr Lopresti is the managing director of Clinical Research Australia, a contract research organisation that has received research funding from nutraceutical companies. Dr Lopresti has also received presentation honoraria from nutraceutical companies. Mr Smith is an employee of Clinical Research Australia and declares no other conflicts of interest. Professor Drummond declares no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2021.08.001.

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