



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

Adrian L. Lopresti<sup>1,2</sup>, Stephen J. Smith<sup>1,2</sup>

<sup>1</sup>Clinical Research Australia, Perth, Australia, <sup>2</sup>College of Science, Health, Engineering and Education, Murdoch University, Perth, Australia

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

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

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

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

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

## INTRODUCTION

Based on the Staging of Reproductive Aging Workshop (STRAW) criteria, perimenopause is defined as the period between the first major variation in menstrual cycle length (i.e., variations greater than seven days from the individual's normal cycle length) and the completion of 12 consecutive months without any menses [1]. The menopausal transition is associated with changes in sex hormones and reproductive function and is characterised by a range of menopause-specific complaints such as vasomotor symptoms (e.g., hot

flushes and cold or night sweats), sleep disturbances, urogenital complaints (e.g., vaginal dryness, painful intercourse, and recurrent urogenital infections), breast pain, joint pain, changes in cognitive function and performance, and mood disturbances including depressive and anxiety-related symptoms [2,3]. The transition into menopause is also associated with an increased risk of osteoporosis [4], metabolic disturbances [5], and cardiovascular disease [6].

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

Received: January 13, 2021 Revised: February 28, 2021 Accepted: April 19, 2021

Address for Correspondence: Adrian L. Lopresti, Clinical Research Australia, 38 Arnisdale Road, Duncraig, WA 6023, Australia

Tel: 61-0894487376, E-mail: [adrian@clinicalresearch.com.au](mailto:adrian@clinicalresearch.com.au), ORCID: <https://orcid.org/0000-0002-6409-7839>

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

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

## MATERIALS AND METHODS

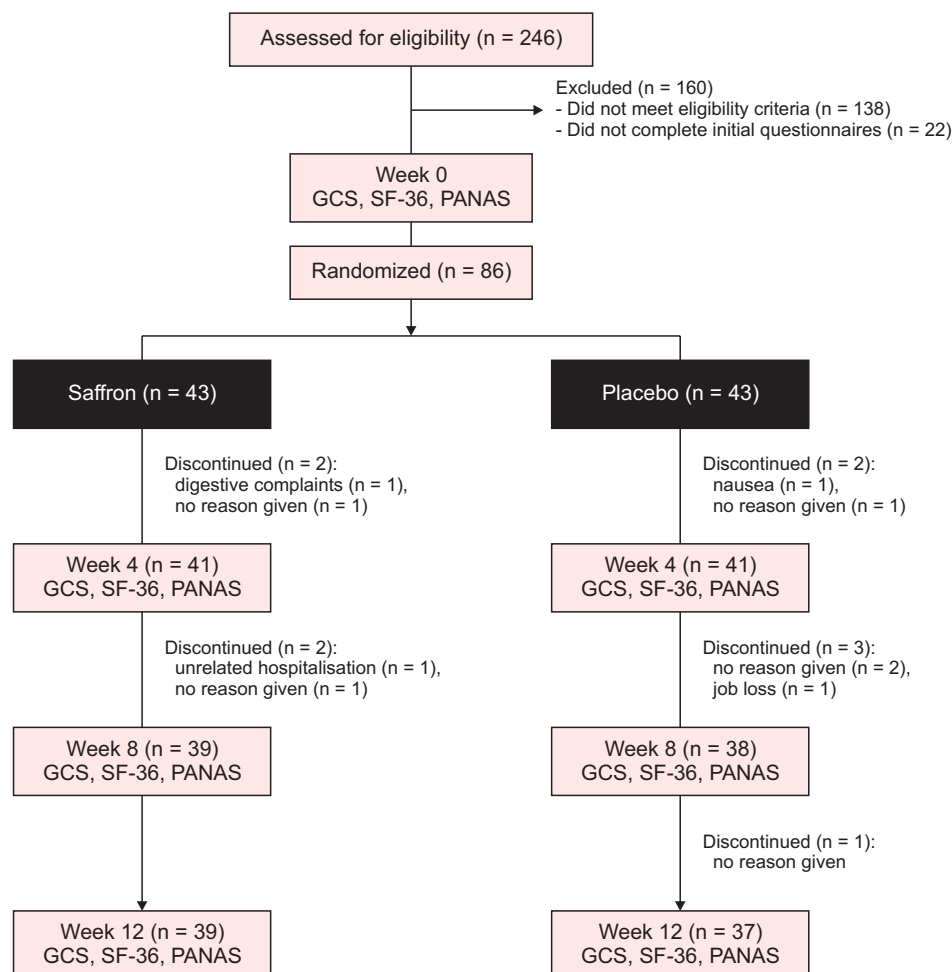
### Study design

This was a two-arm, parallel-group, 12-week, randomised, double-blind, placebo-controlled trial (Fig. 1). The trial protocol was approved by the Human

Research Ethics Committee at the National Institute of Integrative Medicine (approval No. 0064E\_2020) and was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. AC-TRN12620000350921). All participants gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki. An a priori power analysis was undertaken to estimate the required sample size (based on a single outcome variable). In a randomised, controlled study examining the effects of saffron on vasomotor symptoms in postmenopausal women, an effect size of 0.6 compared to the placebo was identified [15]. Assuming a power of 80% and a type one error rate (alpha) of 5%, the number of participants required per group to find an effect on the Greene Climacteric Scale (GCS) total score was estimated as 36. We planned to recruit at least 40 participants per group, which was hypothesised to give suitable power to find an effect compared to the placebo, even after dropouts.

### Recruitment and randomisation

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



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

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

## Participants

### Inclusion criteria

Female participants aged between 40 to 60 years with reports of changes in their menstrual cycle for at least 3 months were recruited for this study. Participants needed to have a total score of greater than 16 on the GCS, have an intact uterus and ovaries, a body mass index

(BMI) between 18 and 35 kg/m<sup>2</sup>, were medication-free for at least 3 months (apart from the contraceptive pill and/or once weekly use of analgesics), were non-smokers, and had no plan to commence new treatments over the study period. Participants were also required to be fluent in English and consented (via an online consent form) to all pertinent aspects of the trial.

### Exclusion criteria

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

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

## Interventions

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

## Outcome measures

### Primary outcome measure

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

ment for menopausal symptoms [19]. The GCS was completed at baseline, week 4, 8, and 12.

### Secondary outcome measures

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

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

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

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

## Statistical analysis

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

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

pression sub-scale scores.

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

**Table 1.** Baseline demographic details of participants

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

Data are presented as mean ± standard error or n (%).

GCS: Greene Climacteric Scale, PANAS: Positive and Negative Affect Schedule, SF-36: Short Form-36 Health Survey.

<sup>a</sup>By independent samples *t* test. <sup>b</sup>By chi-square test.

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

## RESULTS

### Study population

#### *Baseline questionnaire and demographic information*

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

### Outcome measures

#### *GCS total score (primary outcome measure)*

Changes in the GCS total score across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's  $d$  effect size score are detailed in Table 2 and Figure 2. There was a statistically-significant reduction in the GCS total score over

time in both the saffron ( $F_{1,8,73} = 24.67$ ,  $P < 0.001$ ) and placebo group ( $F_{2,2,87} = 6.50$ ,  $P = 0.002$ ). A between-group analysis revealed there was a non-significant difference in change in the total GCS score between the placebo and saffron group ( $F_{2,169} = 2.58$ ,  $P = 0.078$ , Cohen's  $d = 0.47$ ). From baseline to week 12, there was a 32% reduction in the total GCS score in the saffron group and a 14% reduction in the placebo group. An independent-samples  $t$  test revealed that this difference was statistically significant ( $T[80] = 2.26$ ,  $P = 0.027$ ).

#### *GCS sub-scale scores (secondary outcome measure 1)*

Changes in the GCS sub-scale scores across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's  $d$  effect size score are detailed in Table 2 and Figure 2. A between-group analysis revealed there was a statistically-significant difference in the change in the GCS psychological score between the placebo and saffron groups ( $F_{2,163} = 3.49$ ,  $P = 0.032$ , Cohen's  $d = 0.59$ ). In both the saffron ( $F_{1,9,76} = 20.85$ ,  $P < 0.001$ ) and placebo groups ( $F_{1,9,76} = 20.85$ ,  $P = 0.048$ ) there were statistically-significant reductions in the GCS psychological score over time.

In the saffron group, there were statistically-significant reductions in the somatic ( $F_{2,0,78} = 7.72$ ,  $P = 0.001$ ) and vasomotor ( $F_{2,6,104} = 10.74$ ,  $P < 0.001$ ) scores over time. In addition, there were statistically-significant reductions in the somatic ( $F_{2,4,98} = 5.68$ ,  $P = 0.003$ ) and vasomotor ( $F_{2,4,96} = 5.70$ ,  $P = 0.003$ ) scores over time in the placebo group. An examination of between-group changes revealed there were no statistically-significant, between-group differences in changes in the somatic ( $F_{2,1,175} = 0.56$ ,  $P = 0.589$ , Cohen's  $d = 0.09$ ) or vasomotor ( $F_{2,5,104} = 0.96$ ,  $P = 0.401$ , Cohen's  $d = 0.26$ ) scores over time.

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

Table 2. Change in outcome measures

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

GCS: Greene Climacteric Scale, PANAS: Positive and Negative Affect Schedule, SF-36: Short Form-36 Health Survey, SE: standard error.

<sup>a</sup>By repeated-measures ANOVA, within-group changes. <sup>b</sup>By repeated-measures ANOVA, between-group changes (placebo vs saffron).

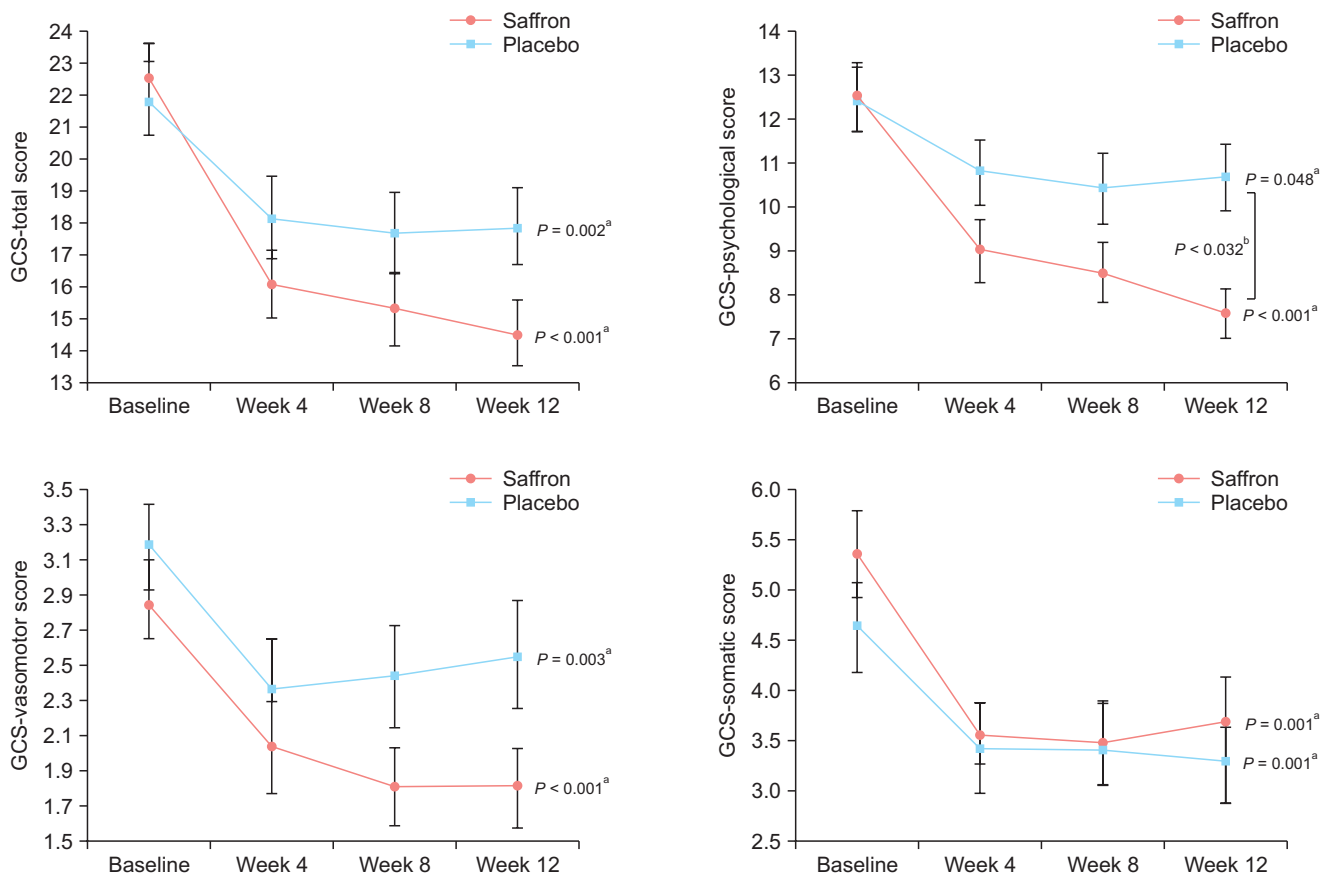


Fig. 2. Change in Greene Climacteric Scale (GCS) scores. <sup>a</sup>*P* value, within group change; <sup>b</sup>*P* value, between-group difference.

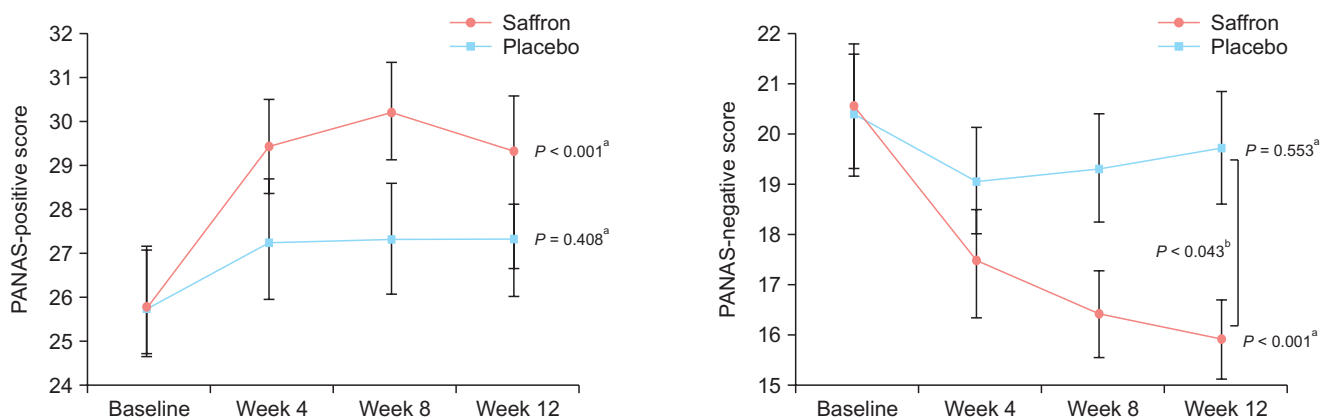


Fig. 3. Change in Positive and Negative Affect Schedule (PANAS) scores. <sup>a</sup>*P* value, within group change; <sup>b</sup>*P* value, between-group difference.

### PANAS scores (secondary outcome measure 2)

Changes in the PANAS negative and positive affect scores across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's *d* effect size score are detailed in Table 2 and Figure 3. A between-group analysis revealed there was

a statistically-significant difference in the change in the PANAS negative score ( $F_{2,4,194} = 2.99$ ,  $P = 0.043$ , Cohen's  $d = 0.55$ ) but not the PANAS positive score ( $F_{2,6,204} = 1.74$ ,  $P = 0.169$ , Cohen's  $d = 0.29$ ) between the placebo and saffron group. In the saffron group, there was a statistically-significant reduction in the PANAS nega-

tive score ( $F_{2,3,93} = 8.07, P < 0.001$ ) and statistically-significant increase in the positive score ( $F_{2,5,101} = 9.33, P < 0.001$ ) over time. However, there were no statistically-significant changes in both the PANAS negative ( $F_{2,5,99} = 0.66, P = 0.553$ ) and positive ( $F_{2,5,102} = 0.95, P = 0.408$ ) scores in the placebo group over time.

### *SF-36 subscale scores (secondary outcome measure 3)*

Changes in the SF-36 sub-scale scores across the placebo and saffron groups over time, repeated measures ANOVA significance levels, and the Cohen's d effect size score are detailed in Table 2. Between-group analyses revealed there were no statistically-significant differences in the change in any SF-36 sub-scale score between the placebo and saffron groups. However, in the saffron group, there were statistically-significant improvements in the SF-36 role limitation due to emotional problems ( $F_{2,3,91} = 7.41, P = 0.001$ ), energy/fatigue ( $F_{2,3,94} = 6.55, P = 0.001$ ), and emotional wellbeing ( $F_{2,2,90} = 8.11, P < 0.001$ ) sub-scale scores over time. In the placebo group, there was a statistically-significant improvement in only the pain score ( $F_{2,5,101} = 4.60, P = 0.007$ ) over time.

### *Intake of supplements*

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

### *Efficacy of participant blinding*

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

### *Adverse events*

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

the two groups. However, there was a tendency to suggest greater digestive complaints in the saffron group (e.g., flatulence and nausea).

## DISCUSSION

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

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

**Table 3.** Frequency of adverse events

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

enhancing effects during perimenopause. However, in a study on post-menopausal women with hot flushes, improvements in depressive symptoms were identified after the 6-week administration of a saffron extract [15]. In the current trial, saffron was associated with a 33 and 32 percent reduction in anxiety and depressive symptoms, respectively, suggesting it had a generalised mood-enhancing effect. This is further confirmed by improvements in negative affective symptoms as measured by the PANAS. The PANAS negative affect score is based on ratings associated with the descriptors such as stressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid. There was also a trend to suggest improvements in the PANAS positive symptom score, although changes did not achieve statistical significance compared to the placebo. Most improvements in self-rated mood occurred in the first 4 weeks of the trial, with continued, albeit less pronounced improvements from weeks 4 to 12. Depressive and anxiety symptoms during the menopausal transition are typically treated with SSRIs and SNRIs. However, although effective, they are associated with several adverse effects resulting in high rates of discontinuation [11]. The positive mood-enhancing findings and low frequency of self-reported adverse effects present saffron as a promising natural mood treatment during perimenopause.

The mechanisms associated with the antidepressant and anxiolytic effects of saffron have not yet been determined, although it is postulated to be multifactorial. For example, saffron has been demonstrated to influence neurotransmitter activity, inflammation, hypothalamic-pituitary-adrenal (HPA) axis activity, oxidative stress, mitochondrial activity, and neuroplasticity [26]. Disturbances in these mechanisms have been regularly identified in depression and anxiety [27,28]. Moreover, it is plausible that saffron's mood-enhancing effects during perimenopause may be associated with its influence on sex hormones. In an animal study, the administration of zearalenone (a mycotoxin with potent estrogenic effects) plus saffron to 8-week old female mice was associated with higher serum concentrations in luteinising hormone (LH), follicle-stimulating hormone (FSH), oestradiol, and progesterone compared with zearalenone alone [29]. In a study on adult, female rats, the administration of crocin (an active constituent of saffron) decreased estrogen and progesterone concentrations but did not affect FSH or LH [30]. In a study conducted on female rats treated with the

menopause-inducing medication cyclophosphamide, concentrations in oestrogen were altered by saffron administration but only at the highest dose of 2 g/kg/day [31]. Finally, the oral administration of an aqueous saffron extract at a dose of 20 and 80 mg/kg/day for 30 days to adult female rats increased serum concentrations in FSH and progesterone (both doses), and LH and oestrogen (high-dose only) [32]. These animal studies suggest saffron may alter sex hormone concentrations although its effects are influenced by dose, age, and stressor exposure. The applicability of these animal studies during the menopausal transition are also uncertain as validated menopausal animal models were not used [33] and the administered doses of saffron required to have oestrogenic effects were well beyond equivalent human doses used in previous human trials on saffron. Whether the mood-enhancing effects of saffron were due to its influence on sex hormone activity could not be determined in the current study as no biological assessments were undertaken.

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

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

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

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

In this study, saffron was associated with improvements in vasomotor and somatic symptoms; however, changes were not significantly different from those observed in the placebo condition. In the study by Kashani et al. [15], a clearly-defined population of postmenopausal women presenting with severe and frequent hot flushes was recruited. However, participants in this study were recruited based on self-reports of difficulties in overall climacteric symptoms. Concerning vasomotor symptoms, average ratings suggested symptoms were of mild-to-moderate severity and the frequency and severity of hot flushes were much lower than levels experienced by women in the Kashani et al's study [15]. An examination of the effects of saffron in perimenopausal women presenting with more severe, specific climacteric symptoms will be important to examine in future trials. The safety and efficacy of saffron administration in women currently receiving phar-

macological treatment for menopausal symptoms also require further investigation. In a previous trial, the adjunct administration of saffron with pharmacological antidepressants was associated with a greater reduction in depressive symptoms [34]. Its co-administration in perimenopausal women currently taking antidepressants and/or on HRT will be important to evaluate in future trials. Saffron used as a component of a multi-herbal combination also requires further investigation, particularly as there have been benefits identified in two previous trials [16,17]. Moreover, the efficacy of different saffron extracts should be examined. In this study, the standardised saffron extract, affron<sup>®</sup>, was used. Given the variability in the quality, purity, and levels of active ingredients in saffron [35], the applicability of these findings to different saffron extracts or as a spice used in cooking is unknown. The efficacy and safety of saffron at different doses and treatment durations will also be helpful to determine whether higher doses or longer treatment periods are required for the alleviation of specific menopausal symptoms. As saffron was only administered for 12 weeks, an examination of its safety as a long-term treatment for climacteric symptoms will also be important. Therefore, studies with longer follow-up are required. Finally, to help understand the mechanisms of action associated with saffron intake, assessment of changes in concentrations of sex hormones, and other pertinent markers such as those associated with inflammation, oxidative stress, HPA-axis activity, and neurotrophic activity will be useful.

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

## ACKNOWLEDGMENTS

The authors gratefully acknowledge Pharmactive Biotech Products, SL for funding the project and supplying the saffron extract, and Lipa Pharmaceutical Pty Ltd.

for manufacturing the tablets used in this study.

## CONFLICT OF INTEREST

This study was funded by Pharmactive Biotech Products, SL. Pharmactive Biotech Products, SL was not involved in the design of the research, analysis of data, or the writing of the report. Dr. Lopresti is the managing director of Clinical Research Australia, a contract research organisation that received funding from Pharmactive Biotech Products, SL for this study. Dr. Lopresti has received either presentation honoraria or clinical trial grants from nutraceutical companies to complete previous clinical trials. Mr. Smith is an employee of Clinical Research Australia and declares no other conflicts of interest.

## REFERENCES

1. Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10 addressing the unfinished agenda of staging reproductive aging. *Menopause* 2012; 19: 387-95.
2. Davis SR, Lambrinoudaki I, Lumsden M, Mishra GD, Pal L, Rees M, et al. Menopause. *Nat Rev Dis Primers* 2015; 1: 15004.
3. Santoro N, Epperson CN, Mathews SB. Menopausal symptoms and their management. *Endocrinol Metab Clin North Am* 2015; 44: 497-515.
4. Ji MX, Yu Q. Primary osteoporosis in postmenopausal women. *Chronic Dis Transl Med* 2015; 1: 9-13.
5. Kozakowski J, Gietka-Czernel M, Leszczyńska D, Majos A. Obesity in menopause - our negligence or an unfortunate inevitability? *Prz Menopauzalny* 2017; 16: 61-5.
6. Rosano GM, Vitale C, Marazzi G, Volterrani M. Menopause and cardiovascular disease: the evidence. *Climacteric* 2007; 10 Suppl 1: 19-24.
7. Martin KA, Manson JE. Approach to the patient with menopausal symptoms. *J Clin Endocrinol Metab* 2008; 93: 4567-75.
8. Eisenberger A, Westhoff C. Hormone replacement therapy and venous thromboembolism. *J Steroid Biochem Mol Biol* 2014; 142: 76-82.
9. Henderson VW, Lobo RA. Hormone therapy and the risk of stroke: perspectives 10 years after the Women's Health Initiative trials. *Climacteric* 2012; 15: 229-34.
10. Fait T. Menopause hormone therapy: latest developments and clinical practice. *Drugs Context* 2019; 8: 212551.
11. Wu CK, Tseng PT, Wu MK, Li DJ, Chen TY, Kuo FC, et al. Antidepressants during and after menopausal transition: a systematic review and meta-analysis. *Sci Rep* 2020; 10: 8026.
12. Javadi B, Sahebkar A, Emami SA. A survey on saffron in major islamic traditional medicine books. *Iran J Basic Med Sci* 2013; 16: 1-11.
13. Hosseinzadeh H, Nassiri-Asl M. Avicenna's (Ibn Sina) the Canon of Medicine and saffron (*Crocus sativus*): a review. *Phytother Res* 2013; 27: 475-83.
14. Marx W, Lane M, Rocks T, Ruusunen A, Loughman A, Lopresti A, et al. Effect of saffron supplementation on symptoms of depression and anxiety: a systematic review and meta-analysis. *Nutr Rev* 2019; 77: 557-71.
15. Kashani L, Esalatmanesh S, Eftekhari F, Salimi S, Foroughifar T, Etesam F, et al. Efficacy of *Crocus sativus* (saffron) in treatment of major depressive disorder associated with post-menopausal hot flashes: a double-blind, randomized, placebo-controlled trial. *Arch Gynecol Obstet* 2018; 297: 717-24.
16. Taavoni S, Ekbatani NN, Haghani H. Effect of *Tribulus terrestris*, ginger, saffron, and *Cinnamomum* on menopausal symptoms: a randomised, placebo-controlled clinical trial. *Prz Menopauzalny* 2017; 16: 19-22.
17. Mahdavian M, Mirzaei Najmabadi K, Hosseinzadeh H, Mirzaeian S, Badiie Aval S, Esmaeili H. Effect of the mixed herbal medicines extract (fennel, chamomile, and saffron) on menopause syndrome: a randomized controlled clinical trial. *J Caring Sci* 2019; 8: 181-9.
18. Greene JG. Constructing a standard climacteric scale. *Maturitas* 1998; 29: 25-31.
19. Moradi F, Jahanian Sadatmahalleh S, Ziaei S. The effect of hormone replacement therapy on cognitive function in postmenopausal women: an RCT. *Int J Reprod Biomed* 2018; 16: 767-74.
20. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol* 1988; 54: 1063-70.
21. Díaz-García A, González-Robles A, Mor S, Mira A, Quero S, García-Palacios A, et al. Positive and Negative Affect Schedule (PANAS): psychometric properties of the online Spanish version in a clinical sample with emotional disorders. *BMC Psychiatry* 2020; 20: 56.
22. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; 30: 473-83.
23. McHorney CA, Ware JE Jr, Raczek AE. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. *Med Care* 1993; 31: 247-63.
24. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-Item Health Survey 1.0. *Health Econ* 1993; 2: 217-27.
25. Tabachnick BG, Fidell LS. Using multivariate statistics. 5th ed. Boston: Pearson Allyn and Bacon; 2007.
26. Lopresti AL, Drummond PD. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of

- underlying antidepressant mechanisms of action. *Hum Psychopharmacol* 2014; 29: 517-27.
27. Anderson G, Berk M, Dean O, Moylan S, Maes M. Role of immune-inflammatory and oxidative and nitrosative stress pathways in the etiology of depression: therapeutic implications. *CNS Drugs* 2014; 28: 1-10.
28. Davis J, Maes M, Andreazza A, McGrath JJ, Tye SJ, Berk M. Towards a classification of biomarkers of neuropsychiatric disease: from encompass to compass. *Mol Psychiatry* 2015; 20: 152-3.
29. Ahmad B, Shrivastava VK, Saleh R, Henkel R, Agarwal A. Protective effects of saffron against zearalenone-induced alterations in reproductive hormones in female mice (*Mus musculus*). *Clin Exp Reprod Med* 2018; 45: 163-9.
30. Zohrabi D, Parivar K, Sanati MH, Hayati Roodbari N. Effects of crocin on the Pituitary-Gonadal axis and hypothalamic Kiss-1 gene expression in female Wistar rats. *Int J Fertil Steril* 2018; 12: 56-60.
31. Vasegh M, Koohpeyma F, Jahromi HK, Bathaee SH, Saberi R, Azhdari S, et al. Investigating effects of hydroalcoholic extract of saffron on sex hormones in female rats undergoing chemotherapy with cyclophosphamide. *Comp Clin Pathol* 2015; 24: 399-402.
32. Ai J, Nekooeian AA, Takhshid MA, Mostafizi N, Mehrabani D. Effect of aqueous extract of *Crocus sativus* L. (saffron) stigma on serum levels of gonadotropins and folliculogenesis in adult rats. *J Appl Anim Res* 2009; 35: 49-52.
33. Koebele SV, Bimonte-Nelson HA. Modeling menopause: the utility of rodents in translational behavioral endocrinology research. *Maturitas* 2016; 87: 5-17.
34. Lopresti AL, Smith SJ, Hood SD, Drummond PD. Efficacy of a standardised saffron extract (affron®) as an add-on to antidepressant medication for the treatment of persistent depressive symptoms in adults: a randomised, double-blind, placebo-controlled study. *J Psychopharmacol* 2019; 33: 1415-27.
35. Khilare V, Tiknaik A, Prakash B, Ughade B, Korhale G, Nalage D, et al. Multiple tests on saffron find new adulterant materials and reveal that 1st grade saffron is rare in the market. *Food Chem* 2019; 272: 635-42.

## SCIENTIFIC INVESTIGATIONS

## Effects of saffron on sleep quality in healthy adults with self-reported poor sleep: a randomized, double-blind, placebo-controlled trial

Adrian L. Lopresti, PhD<sup>1,2</sup>; Stephen J. Smith, MA<sup>1,2</sup>; Alexandra P. Metse, PhD<sup>1</sup>; Peter D. Drummond, PhD<sup>1</sup>

<sup>1</sup>College of Science, Health, Engineering, and Education, Murdoch University, Perth, Western Australia, Australia; <sup>2</sup>Clinical Research Australia, Perth, Western Australia, Australia

**Study Objectives:** Herbal medicines are frequently used by adults with sleep difficulties. However, evidence of their efficacy is limited. Therefore, the goal of this study was to examine the sleep-enhancing effects of a standardized saffron extract (affron).

**Methods:** This was a 28-day, parallel-group, double-blind, randomized controlled trial. Sixty-three healthy adults aged 18–70 with self-reported sleep problems were recruited and randomized to receive either saffron extract (affron; 14 mg twice daily) or a placebo. Outcome measures included the Insomnia Severity Index (ISI; primary outcome measure) collected at baseline and days 7, 14, 21, and 28 and the Restorative Sleep Questionnaire (RSQ) and the Pittsburgh Sleep Diary (PSD) collected on days –1, 0, 3, 7, 14, 27, and 28.

**Results:** Based on data collected from 55 participants, saffron was associated with greater improvements in ISI total score ( $P = .017$ ), RSQ total score ( $P = .029$ ), and PSD sleep quality ratings ( $P = .014$ ) than the placebo. Saffron intake was well tolerated with no reported adverse effects.

**Conclusions:** Saffron intake was associated with improvements in sleep quality in adults with self-reported sleep complaints. Further studies using larger samples sizes, treatment periods, objective outcome measures, and volunteers with varying demographic and psychographic characteristics are required to replicate and extend these findings.

**Clinical Trial Registration:** Registry: Australian New Zealand Clinical Trials Registry; Name: Effects of Saffron on Sleep Quality in Healthy Adults with Self-Reported Unsatisfactory Sleep; URL: <https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=377781>; Identifier: ACTRN12619000863134.

**Keywords:** sleep, insomnia, saffron, herbal

**Citation:** Lopresti AL, Smith SJ, Metse AP, Drummond PD. Effects of saffron on sleep quality in healthy adults with self-reported poor sleep: a randomized, double-blind, placebo-controlled trial. *J Clin Sleep Med*. 2020;16(6):937–947.

### BRIEF SUMMARY

**Current Knowledge/Study Rationale:** Research into the efficacy of herbal medicines as natural sleep-enhancing agents is limited. In this 8-week, randomized, double-blind, placebo-controlled study, the sleep-enhancing effects of a standardized saffron extract (affron) were investigated in adults with self-reported poor sleep.

**Study Impact:** Saffron supplementation for 8 weeks was associated with improvements in insomnia, sleep quality, and restorative sleep. Further research is required to replicate and extend these findings in other populations.

### INTRODUCTION

Results from population-based surveys in Australia and other developed countries indicate that 10–45% of adults report regular difficulty either falling or staying asleep (hereafter, “poor sleep”).<sup>1–3</sup> This has significant health implications as poor sleep quality can have a negative impact on both mental and physical health and can interfere with daily function.<sup>4</sup> In a cohort of almost 5,000 adults, poor sleep was associated with a 29% increased risk of cardiovascular disease.<sup>5</sup> Sleep problems, such as insomnia, are also associated with a 2.6 times greater risk of developing depression.<sup>6</sup> Insomnia is also associated with reduced work productivity and increased work absenteeism.<sup>7,8</sup> In 2010, diagnosed sleep disorders in Australia were estimated to cost \$5.1 billion (Australian dollars), comprising 5% from direct health care costs, 60% from productivity losses, and 13% from informal care and

other indirect costs, resulting from motor vehicle and workplace accidents.<sup>9</sup>

Herbal medicines are one of the most frequently used complementary and alternative treatments for insomnia. In community-dwelling older adults with self-reported sleep problems, use of a sleep product was reported by 35% of respondents, with 22% using over-the-counter sleep aids and 12.5% using herbal or natural aids.<sup>10</sup> In a randomly selected sample of almost 1,000 adults, 18.5% of participants reported using a natural sleep aid over the past 12 months.<sup>11</sup> However, the safety and efficacy of many herbal medicines for the treatment of sleep problems are uncertain. In a systematic review of 14 randomized controlled trials of herbal medicines (comprising valerian, kava, and chamomile) and involving 1,600 participants with insomnia, it was concluded that, although these treatments were generally safe and well tolerated, there was insufficient evidence that they provided any benefit to adults with

insomnia.<sup>12</sup> This indicates that, despite their widespread use, there is a paucity of data on the efficacy of natural herbal products.

Saffron, a spice derived from the stigmas of the *Crocus sativus* flower, has been confirmed in several systematic reviews and meta-analyses to be an effective natural agent for the treatment of mild-to-moderate depression.<sup>13–15</sup> As a sleep-enhancing agent, there is preliminary evidence to suggest it may also be an effective natural sleep aid. In a small pilot study, the 4-week administration of a saffron extract (affron) resulted in greater improvements in sleep quality, sleep latency, and daytime dysfunction in a subset of poor sleepers compared with placebo.<sup>16</sup> As an adjunct to antidepressants in adults with unremitted depression, symptomatic improvements in sleep quality were also noted after saffron administration.<sup>17</sup> In a randomized, double-blind, placebo-controlled, crossover study, the 14-day administration of crocetin (an active constituent in saffron) in adults with mild sleep complaints was associated with increases in electroencephalography (EEG) delta activity and self-reported improvements in sleepiness and refreshment on rising. However, there were no significant changes in other sleep parameters.<sup>18</sup> Improvements in sleep quality have also been reported in adults with type 2 diabetes with comorbid depression and anxiety after an 8-week intake of saffron.<sup>19</sup> Although promising, the robustness of these findings is hindered by small sample sizes, short treatment periods, use of nonstandardized saffron extracts, and investigations in

populations where sleep is not the primary presenting complaint. Therefore, the goal of this study was to investigate the sleep-enhancing effects and safety of a standardized saffron extract (affron) in adults with self-reported poor sleep.

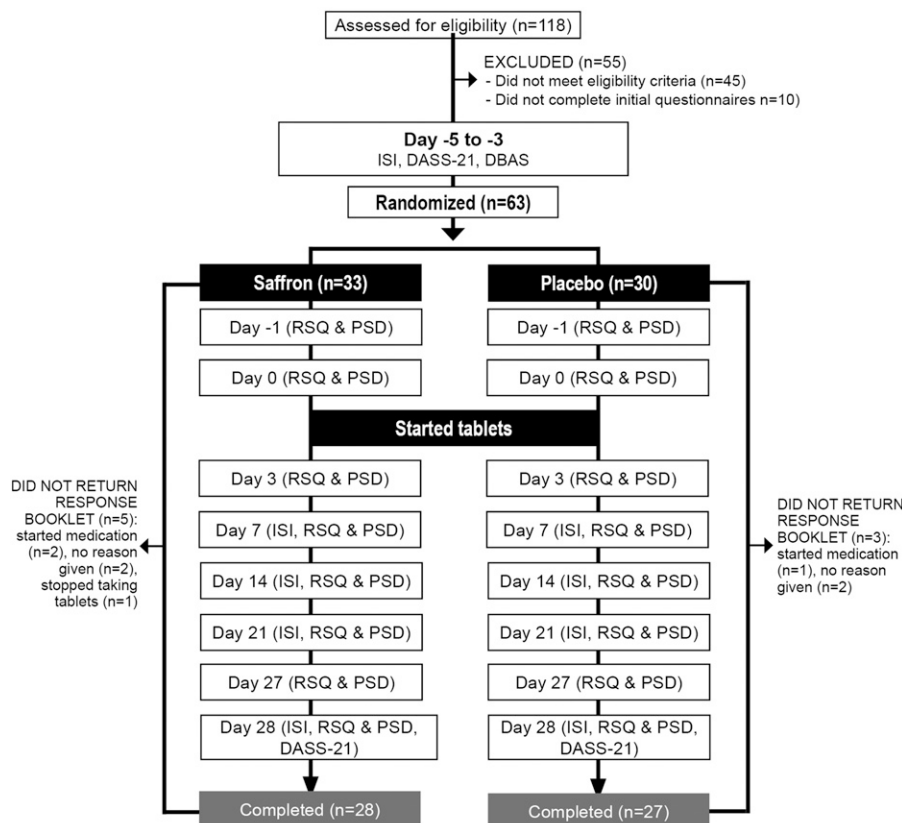
## METHODS

### Study design

This was a 2-arm, parallel-group, 28-day, randomized, double-blind, placebo-controlled trial (Figure 1). The trial protocol was approved by the Human Research Ethics Committee at the National Institute of Integrative Medicine (approval number 0054E\_2019) and was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID: ACTRN12619000863134).

An a priori power analysis was undertaken to estimate the required sample size (based on a single outcome variable). In a recent randomized controlled pilot study, saffron had an effect size of 1.1 in adults with poor sleep compared with the placebo.<sup>16</sup> Sample size calculations were based on a more conservative effect size of 0.8. Assuming a power of 80% and a type 1 error rate ( $\alpha$ ) of 5%, the number of participants required per group to find an effect for the Insomnia Severity Index (ISI) was estimated as 21. After allowing for a 15% drop-out rate, we aimed to recruit at least 25 participants per group.<sup>20</sup>

Figure 1—Systematic illustration of the study design.



DASS-21 = Depression, Anxiety, Stress Scale-21; DBAS = Dysfunctional Beliefs Associated with Sleep Questionnaire; ISI = Insomnia Severity Index; PSD = Pittsburgh Sleep Diary; RSQ = Restorative Sleep Questionnaire.

## Recruitment and randomization

Participants were recruited across Perth, Western Australia, through social media advertisements between July and August 2019. As interest to participate in the study was high, we were able to recruit more than our minimum projected sample size of 50 prior to our projected timeline ( $n = 63$ ). Interested participants were directed to a website landing page providing details about the study and a link to complete an initial online screening questionnaire. This online questionnaire screened for current depressive and/or anxiety symptoms; any medication use; history of medical/psychiatric disorders; alcohol, nicotine, and other drug use; supplement and vitamin intake; and pregnancy/breastfeeding status. If assessed as likely eligible, volunteers participated in a phone interview with an investigator. The phone interview comprised a structured series of questions to further clarify details pertaining to the eligibility criteria (eg, current and past mental health history, alcohol and other drug use, supplement intake, current medical conditions, and medication intake) and to obtain further demographic details. Suitable participants were then required to complete online versions of the ISI, Depression, Anxiety, and Stress Scale–21 (DASS-21), Dysfunctional Beliefs Associated with Sleep Questionnaire (DBAS), and an informed consent form. Eligible and consenting participants were randomly assigned to 1 of 2 groups (saffron or placebo) using a randomization calculator (<http://www.randomization.com>). The randomization calculator ensured sequence concealment. The randomization structure comprised 10 randomly permuted blocks, containing 6 participants per block. The participant identification number was allocated according to the order of participant enrollment in the study. All tablets were packed in identical bottles labeled with 2 intervention codes (held by the study sponsor until final data collection). Participants and study investigators were blinded to treatment group allocation until all outcome data were collected. No financial compensation was provided to participants for volunteering in this study, although at the completion of the study, participants allocated to the placebo condition were offered a free 4-week supply of saffron tablets.

## Participants

### Inclusion criteria

Physically healthy male and female participants aged 18–70 years, with self-reported symptoms of poor sleep lasting more than 4 weeks, were recruited for this study. Participants had an ISI score of between 8 (subthreshold insomnia) and 21 (moderate-severity insomnia). All participants were medication free for at least 4 weeks apart from contraceptive pills and no more than once per week use of pain-relieving medications. Volunteers had a body mass index between 18 and 30 kg/m<sup>2</sup> and typical bedtime between 9 PM and 12 AM. Participants were also required to be fluent in English and to have consented (via an online consent form) to all pertinent aspects of the trial.

### Exclusion criteria

Participants employed in night-shift work or rotational-shift work were ineligible to participate in the study. Individuals experiencing a sleep disorder other than moderate insomnia

(eg, sleep apnea, restless legs syndrome, periodic limb movement disorder), chronic, severe sleep disturbance for more than 1 year, diagnosis of a mental health disorder (other than mild depressive or anxiety symptoms as measured by the DASS-21), coffee intake greater than 3 cups per day (or equivalent caffeine intake from other caffeinated drinks, eg, tea, energy drinks), and alcohol consumption greater than 14 standard drinks per week were also ineligible for the study. Participants were also ineligible for the study if they were experiencing external factors that may affect sleep patterns (eg, infants/children regularly awakening, excessive noise, snoring partner), were currently receiving nonpharmacological treatment for sleep disorders (eg, cognitive behavioral therapy [CBT], relaxation therapy), had a current or 12-month history of illicit drug abuse, were currently taking supplements that may affect sleep, were taking saffron supplements, or had a diagnosed medical condition including but not limited to diabetes, hyper-/hypotension, cardiovascular disease, gastrointestinal disease requiring regular use of medications, gallbladder disease/gallstones/biliary disease, endocrine disease, psychiatric disorder, neurological disease (Parkinson disease, Alzheimer disease, intracranial hemorrhage, head or brain injury), or acute or chronic pain affecting sleep. Pregnant women, women who were breastfeeding, or women who intended to become pregnant were also ineligible to participate in the study.

## Interventions

Placebo and saffron tablets were identical in appearance, being matched for color coating, shape, and size. The active treatment, supplied by Pharmactive Biotech Products SL, contained 14 mg of a standardized saffron extract (affron), derived from the stigmas of *Crocus sativus* L. and standardized 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) to produce affron 3.5% Lepticrosalides. The placebo tablets contained the same excipients as the active tablet (microcrystalline cellulose and calcium hydrogen phosphate). All tablets were manufactured and packed in an Australian Therapeutic Goods Administration–registered plant. All participants were instructed to take 1 tablet, twice daily, with or without food for 28 days. Medication adherence was measured by tablet count by the participant on day 28. Efficacy of participant treatment blinding was examined by asking participants to predict group allocation (placebo, saffron, or uncertain) at the completion of the study. Saffron and placebo tablets were mailed to participants with directions for use provided on tablet bottles. Participants were also provided with an information sheet about tablet intake and what to do if they missed a dose. This information was also verbally conveyed to participants during their initial telephone interview.

## Outcome measures

### Primary outcome measures

**Insomnia Severity Index:** The ISI is a self-report instrument measuring respondents' perception of both nocturnal and

diurnal symptoms of insomnia. The ISI comprises 7 items assessing the perceived severity of difficulties initiating sleep, staying asleep, early morning awakenings, satisfaction with current sleep pattern, interference with daily functioning, noticeability of impairment attributed to the sleep problem, and degree of distress or concern caused by the sleep problem. The ISI has good psychometric properties and is sensitive to treatment in clinical trials.<sup>21</sup> The ISI was completed at baseline (from days -5 to -3) and days 7, 14, 21, and 28.

### Secondary outcome measures

**Restorative Sleep Questionnaire:** The Restorative Sleep Questionnaire (RSQ) 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 is able to distinguish between healthy controls, patients with primary insomnia, and insomnia patients with isolated nonrestorative sleep complaints.<sup>22</sup> The RSQ was completed at days -1, 0, 3, 7, 14, 21, 27, and 28.

**Pittsburgh Sleep Diary:** The Pittsburgh Sleep Diary (PSD) is a 14-item sleep diary that respondents complete upon awakening. The PSD shows good retest reliability over a mean inter-test interval of 22 months. Scores also correlate with circadian type, self-reported sleep quality, and objective actigraphy measurements.<sup>23</sup> Scores are calculated for total sleep time (hours), sleep latency (minutes), number of awakenings after sleep onset, sleep quality rating (5-point Likert rating ranging from very bad [1] to very good [5]), mood rating at final awakening (5-point Likert rating ranging from very calm [1] to very tense [5]), and alertness rating at final awakening (5-point Likert rating ranging from very sleepy [1] to very alert [5]). The PSD was completed at days -1, 0, 3, 7, 14, 21, 27, and 28.

**Depression, Anxiety, and Stress Scale-21:** The DASS-21 is a validated self-report measure assessing symptoms of stress, anxiety, and depression.<sup>24</sup> Twenty-one questions are rated on a 4-point scale (0-3), ranging from never to almost always (lower scores indicate a reduction in symptoms). Subscale scores for depression, anxiety, and stress are calculated. The DASS-21 was completed at baseline (days -5 to -3) and day 28.

### Process measures

**Dysfunctional Beliefs Associated with Sleep Questionnaire:** The DBAS is a 16-item questionnaire that assesses beliefs and attitudes about sleep. The DBAS has good reliability as evidenced by adequate internal consistency and temporal stability over a 2-week period. Scores also correlate with other self-report measures of insomnia severity, anxiety, and depression.<sup>25</sup> The DBAS was completed at baseline to examine the impact of dysfunctional beliefs about sleep on change in sleep quality over time. If significant dysfunctional beliefs about sleep present a potential barrier to successful change, this will help to inform further interventional trials.

### Adverse events

The tolerability and safety of supplement intake by participants was assessed at day 28 through an online question querying adverse effects that were believed to be associated

with supplement intake. Participants were also requested to contact researchers immediately if any adverse effects were experienced.

### Data collection procedures

Initial screening questionnaires comprising the ISI, DASS-21, and DBAS 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 and diary were recorded in the booklet. Participants were also advised to keep their response booklet near their bed and to complete it within 30 minutes after awakening.

### Statistical analysis

An independent-samples *t* test was used to compare demographic variables across the 2 treatment groups for continuous variables, and Pearson's chi-square was used to compare categorical data. To evaluate primary study objectives, a repeated-measures analysis of variance (ANOVA) was used to compare within-group changes over time and group (saffron versus placebo)  $\times$  time interaction effects. The ISI total score was analyzed for baseline and mean score across days 7, 14, 21, and 28. Total scores on the RSQ and item scores on the PSD were analyzed for mean baseline (days -1 and day 0) and mean score across days 3, 7, 14, 21, 27, and 28. Eta-squared ( $\eta^2$ ) was calculated to examine effect sizes.

The Shapiro-Wilk normality test was conducted to examine the normality of group data. This demonstrated that data were not normally distributed, and this was not corrected by data transformations. However, a repeated-measures ANOVA was considered the most appropriate option for statistical analyses as it is relatively robust to violations of normality.<sup>26</sup> Where necessary, degrees of freedom were adjusted using the Greenhouse-Geisser approach to correct for violations of the sphericity assumption. To examine the influence of dysfunctional beliefs about sleep on changes in sleep quality, a Pearson's correlation coefficient was calculated between the baseline DBAS total score and percentage change in ISI score (baseline to average ISI score from days 7 to 28). Data from all participants who returned their response booklets were included in analyses. All data were analyzed using SPSS (version 24; IBM Corporation, Armonk, NY).

## RESULTS

### Study population

#### Baseline questionnaire and demographic information

From 118 individuals who completed the initial online screening questionnaire, 55 individuals were either ineligible ( $n = 45$ ) or did not complete the initial questionnaires ( $n = 10$ ). Sixty-three volunteers participated in the study and data from 55 participants who completed all questionnaires over the 28-day period were used for statistical analyses. Baseline data of these 55 participants are detailed in **Table 1** and baseline demographic details of the total recruited sample are detailed in **Table S1** in the supplemental material. Eight participants

**Table 1**—Baseline demographic characteristics of participants.

	Saffron (n = 28)	Placebo (n = 27)	P
Age, years			
Mean	47.86	52.63	.058 <sup>a</sup>
SE	2.05	1.33	
BMI, kg/m <sup>2</sup>			
Mean	25.24	25.64	.684 <sup>a</sup>
SE	0.73	0.65	
Sex, n (%)			
Male	4 (86)	5 (19)	.671 <sup>b</sup>
Female	24 (14)	22 (81)	
Marital status, n (%)			
Single	25 (89)	23 (85)	.684 <sup>b</sup>
Married	3 (11)	4 (15)	
Educational level, n (%)			
Secondary	39 (32)	8 (30)	.659 <sup>b</sup>
Tertiary	15 (54)	17 (63)	
Postgraduate	4 (14)	2 (7)	
Exercise level, n (%)			
Never/rarely	9 (32)	15 (56)	.131 <sup>b</sup>
1–2 times a week	13 (46)	6 (22)	
3–5 times a week	6 (21)	6 (22)	
Duration of sleep problems, n (%)			
<6 months	5 (18)	1 (4)	.394 <sup>b</sup>
6–12 months	3 (11)	3 (11)	
1–2 years	8 (29)	8 (30)	
≥2 years	12 (43)	15 (56)	
ISI			
Mean	15.75	14.74	.290 <sup>a</sup>
SE	0.65	0.69	
DBAS			
Mean	74.50	78.59	.550 <sup>a</sup>
SE	3.95	5.58	
DASS-21			
Depression			
Mean	4.29	3.26	.527 <sup>a</sup>
SE	1.22	1.05	
Anxiety			
Mean	3.07	3.19	.917 <sup>a</sup>
SE	0.69	0.84	
Stress			
Mean	8.64	8.15	.746 <sup>a</sup>
SE	1.04	1.11	
Total			
Mean	16.00	14.59	.679 <sup>a</sup>
SE	2.39	2.40	

(continued in next column)

**Table 1**—Baseline demographic characteristics of participants. (continued)

	Saffron (n = 28)	Placebo (n = 27)	P
PSD			
Total sleep time, hours			
Mean	7.33	6.92	.307 <sup>a</sup>
SE	0.28	0.28	
Sleep latency, minutes			
Mean	39.38	32.61	.444 <sup>a</sup>
SE	7.16	4.97	
Number of wakings after sleep onset			
Mean	3.23	3.15	.826 <sup>a</sup>
SE	0.26	0.28	
Sleep quality			
Mean	2.46	2.78	.120 <sup>a</sup>
SE	0.11	0.16	
Mood on awakening			
Mean	2.88	2.72	.435 <sup>a</sup>
SE	0.11	0.16	
Alertness on awakening			
Mean	2.77	3.02	.254 <sup>a</sup>
SE	0.14	0.17	
RSQ			
Mean	46.73	52.93	.154 <sup>a</sup>
SE	2.46	3.54	

BMI = body mass index; DASS-21 = Depression, Anxiety, Stress Scale–21; DBAS = Dysfunctional Beliefs Associated with Sleep Questionnaire; ISI = Insomnia Severity Index; PSD = Pittsburgh Sleep Diary; RSQ = Restorative Sleep Questionnaire. <sup>a</sup>Independent-samples *t* test. <sup>b</sup>Chi-square test.

withdrew or did not return the response booklets. There were no significant differences in drop-out rates across groups. Reasons for withdrawal included no reason given (*n* = 4), the commencement of new medication (*n* = 3), and deciding to stop taking tablets (*n* = 1). No participant reported withdrawing from the study due to reported adverse events associated with supplement intake.

## Outcome measures

### Insomnia Severity Index (primary outcome measure)

Changes in ISI total score across the 2 treatment groups and repeated-measures ANOVA significance levels are detailed in **Table 2** and **Figure 2**. Reductions in the ISI score were greater in the saffron group than in the placebo group ( $F_{1,53} = 6.07$ ,  $P = .017$ ,  $\eta^2 = 10.3\%$ ). Saffron was associated with a statistically significant reduction in ISI score over time ( $F_{1,27} = 26.32$ ,  $P < .001$ ). However, no significant change was observed in the placebo group ( $F_{1,26} = 2.65$ ,  $P = .116$ ). Within-group contrasts suggest that the majority of changes in ISI scores occurred in the first 7 days of saffron treatment

**Table 2—Change in sleep measures.**

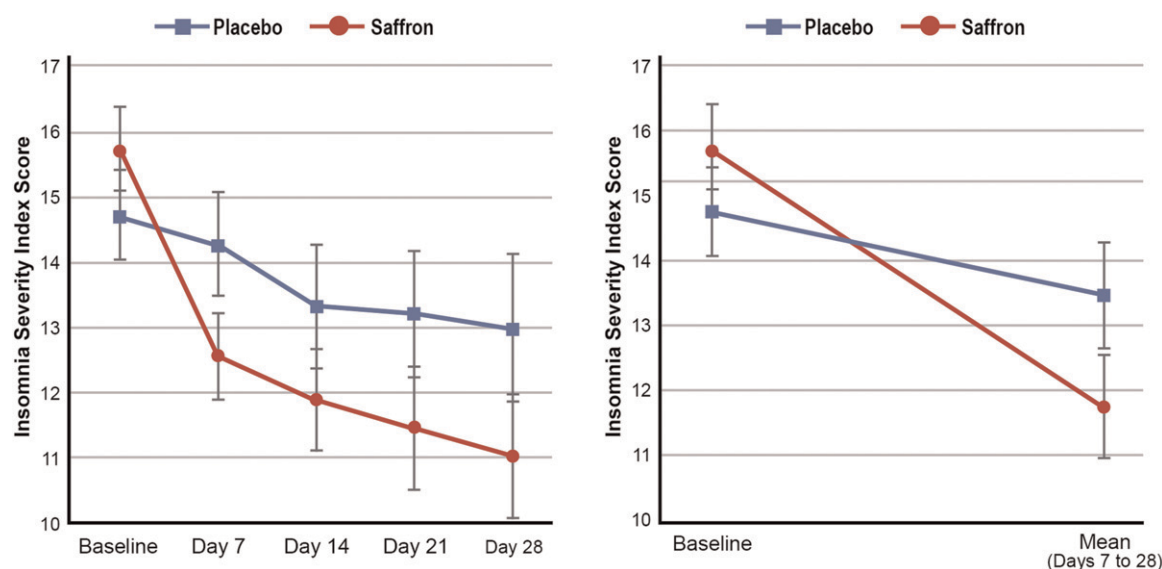
	Baseline	Follow-up	Cohen's <i>d</i> Effect Size	Repeated-Measures ANOVA			
				Time Effects, <i>P</i>	Between-Group Main Effects, <i>P</i>	Time × Group Interaction, <i>P</i>	$\eta^2$ , %
ISI (lower scores indicate improved sleep)							
Saffron (n = 28)							
Mean <sup>a</sup>	15.75	11.74 <sup>b</sup>	1.07	.000	.694	.017	10.3
SE	0.66	0.81					
Placebo (n = 27)							
Mean <sup>a</sup>	14.74	13.46 <sup>b</sup>	0.31	.116			
SE	0.67	0.83					
PSD							
Total sleep time, hours							
Saffron (n = 28)							
Mean <sup>b</sup>	7.33 <sup>a</sup>	7.49 <sup>c</sup>	0.13	.477	.147	.763	0.2
SE	0.28	0.20					
Placebo (n = 27)							
Mean <sup>b</sup>	6.92 <sup>a</sup>	6.99 <sup>c</sup>	0.05	.767			
SE	0.29	0.20					
Sleep latency, minutes							
Saffron (n = 28)							
Mean <sup>b</sup>	39.38 <sup>a</sup>	29.49 <sup>c</sup>	0.32	.149	.702	.298	2.0
SE	6.15	4.73					
Placebo (n = 27)							
Mean <sup>b</sup>	32.61 <sup>a</sup>	31.06 <sup>c</sup>	0.06	.711			
SE	6.26	4.81					
Number of awakenings after sleep onset							
Saffron (n = 28)							
Mean <sup>b</sup>	3.23 <sup>a</sup>	2.51 <sup>c</sup>	0.52	.001	.573	.053	6.9
SE	0.27	0.25					
Placebo (n = 27)							
Mean <sup>b</sup>	3.15 <sup>a</sup>	2.98 <sup>c</sup>	0.13	.443			
SE	0.27	0.25					
Sleep quality (higher scores indicate greater quality)							
Saffron (n = 28)							
Mean <sup>b</sup>	2.46 <sup>a</sup>	2.99 <sup>c</sup>	0.88	.000	.679	.014	10.9
SE	0.14	0.12					
Placebo (n = 27)							
Mean <sup>b</sup>	2.78 <sup>a</sup>	2.81 <sup>c</sup>	0.04	.848			
SE	0.14	0.12					
Mood on awakening (lower scores indicate greater calmness)							
Saffron (n = 28)							
Mean <sup>b</sup>	2.88 <sup>a</sup>	2.84 <sup>c</sup>	0.06	.746	.757	.270	2.3
SE	0.14	0.14					
Placebo (n = 27)							
Mean <sup>b</sup>	2.72 <sup>a</sup>	2.88 <sup>c</sup>	0.20	.257			
SE	0.14	0.14					

(continued on following page)

**Table 2**—Change in sleep measures. (continued)

	Baseline	Follow-up	Cohen's <i>d</i> Effect Size	Repeated-Measures ANOVA			
				Time Effects, <i>P</i>	Between-Group Main Effects, <i>P</i>	Time × Group Interaction, <i>P</i>	$\eta^2$ , %
Alertness on awakening (higher scores indicate greater alertness)							
Saffron (n = 28)							
Mean <sup>b</sup>	2.77 <sup>a</sup>	3.07 <sup>c</sup>	0.46	.037	.763	.061	6.5
SE	0.15	0.12					
Placebo (n = 27)							
Mean <sup>b</sup>	3.02 <sup>a</sup>	2.92 <sup>c</sup>	0.13	.539			
SE	0.16	0.12					
RSQ (high scores indicate greater restorative sleep)							
Saffron (n = 28)							
Mean <sup>b</sup>	46.73 <sup>a</sup>	56.12 <sup>c</sup>	0.72	.000	.526	.029	8.6
SE	3.00	2.69					
Placebo (n = 27)							
Mean <sup>b</sup>	52.93 <sup>a</sup>	54.62 <sup>c</sup>	0.10	.569			
SE	3.06	2.74					

ANOVA = analysis of variance; ISI = Insomnia Severity Index; PSD = Pittsburgh Sleep Diary; RSQ = Restorative Sleep Questionnaire. <sup>a</sup>Mean baseline score from days -1 and 0. <sup>b</sup>Mean score from days 7 to 28. <sup>c</sup>Mean score from days 3 to 28.

**Figure 2**—Change in Insomnia Severity Index scores (error bars depict SEs).

as there were statistically significant improvements from days 0 to 7 ( $F_{1,27} = 21.94$ ,  $P < .001$ ) but no statistically significant changes from days 7 to 14 ( $F_{1,27} = 12.89$ ,  $P = .185$ ), days 14 to 21 ( $F_{1,27} = 5.14$ ,  $P = .352$ ), or days 21 to 28 ( $F_{1,27} = 5.14$ ,  $P = .422$ ).

#### Restorative Sleep Questionnaire (secondary outcome measure 1)

Changes in RSQ total score across the 2 treatment groups and repeated-measures ANOVA significance levels are detailed in **Table 2**. Reductions in the RSQ score were greater in the

saffron group than in the placebo group ( $F_{1,53} = 5.01$ ,  $P = .029$ ,  $\eta^2 = 8.6\%$ ). Saffron was associated with a statistically significant reduction in RSQ score over time ( $F_{1,27} = 25.25$ ,  $P < .001$ ). However, no significant change was observed in the placebo group ( $F_{1,26} = 0.33$ ,  $P = .569$ ).

#### Pittsburgh Sleep Diary (secondary outcome measure 2)

Changes in PSD scores across the 2 treatment groups and repeated-measures ANOVA significance levels are detailed in **Table 2**. Improvements in sleep quality were greater in the

saffron group than in the placebo group ( $F_{1,53} = 6.51$ ,  $P = .014$ ,  $\eta^2 = 10.9\%$ ), and there were near-significant effects in number of awakenings after sleep onset ( $F_{1,53} = 3.92$ ,  $P = .053$ ,  $\eta^2 = 6.9\%$ ) and alertness upon awakening ( $F_{1,53} = 3.66$ ,  $P = .061$ ,  $\eta^2 = 6.5\%$ ). Saffron was associated with statistically significant improvements in the number of awakenings after sleep onset ( $F_{1,27} = 14.65$ ,  $P = .001$ ), sleep quality ( $F_{1,27} = 24.58$ ,  $P < .001$ ), and alertness upon awakening ( $F_{1,27} = 4.82$ ,  $P = .037$ ). No other significant changes were observed in other PSD sleep parameters. In the placebo group, there were no statistically significant changes in any PSD sleep parameters over time.

### Depression, Anxiety, and Stress Scale-21 (secondary outcome measure 3)

Changes in DASS-21 subscores across the 2 treatment groups and repeated-measures ANOVA significance levels are detailed in **Table 3**. All baseline scores were within normal levels and repeated-measures ANOVAs revealed no statistically significant changes in DASS-21 depression, anxiety, or stress scores over time in either the saffron or placebo group.

### Dysfunctional Beliefs Associated with Sleep Questionnaire (process measure)

A statistically significant correlation between baseline DBAS score and percentage change in ISI score was observed

( $r = -.325$ ,  $P = .015$ ), indicating higher DBAS scores were associated with lower change scores.

### Intake of supplements

At day 28, participants recorded their quantity of remaining supplements. All participants reported taking more than 70% of their tablets, although consistency of use over the 28-day period could not be ascertained.

### Efficacy of participant blinding

To evaluate the efficacy of condition concealment over the study, participants were asked at the completion of the study to predict condition allocation (ie, placebo, saffron, or uncertain). Efficacy of group concealment was high as 88% in the saffron group, and 71% in the placebo group either incorrectly guessed treatment allocation or were unsure.

### Adverse events

No significant adverse events were reported by participants and no participant withdrew from the study due to concerns associated with supplement intake. Further confirmation of the tolerability and safety of saffron intake is provided by an examination of satisfaction ratings at the end of the study, which indicated that 4% of participants in the saffron group (compared with 15% in the placebo group) were dissatisfied with their tablet intake.

**Table 3—Change in DASS-21 scores.**

	Baseline	Day 28	Repeated-Measures ANOVA, <i>P</i> Value		
			Time Effects	Between-Group Main Effects	Time × Group Interaction
Depression					
Saffron					
Mean	4.21	4.41	.852	.305	.467
SE	1.11	0.88			
Placebo					
Mean	3.31	2.38	.397		
SE	1.17	0.93			
Anxiety					
Saffron					
Mean	2.97	3.59	.286	.643	.102
SE	0.74	0.69			
Placebo					
Mean	3.31	2.38	.228		
SE	0.79	0.72			
Stress					
Saffron					
Mean	8.41	9.52	.411	.949	.995
SE	1.05	1.31			
Placebo					
Mean	8.38	9.50	.405		
SE	1.11	1.38			

ANOVA = analysis of variance; DASS-21 = Depression, Anxiety, Stress Scale-21.

## DISCUSSION

In this parallel, randomized, double-blind, placebo-controlled trial, the 28-day intake of a standardized saffron extract (affron) was associated with a significant improvement in sleep quality in adults with self-reported poor sleep. Compared with placebo, affron taken at 14 mg twice daily was associated with greater reductions in insomnia severity, as measured by the ISI, and nonrestorative sleep, as measured by the RSQ. Based on sleep diary recordings, saffron also significantly increased ratings of sleep quality, and there were strong trends suggesting improvements in ratings of alertness upon awakening and reductions in the number of awakenings after sleep onset. An examination of changes in ISI scores over time suggests that saffron was associated with relatively rapid improvements in sleep quality as the bulk of sleep improvements occurred in the first 7 days of treatment, with continued, albeit less pronounced, improvements thereafter. It should be noted that, even though saffron was associated with greater improvements in sleep quality compared with the placebo, subthreshold (borderline) insomnia (mean ISI score of 11.7) persisted at the end of the 28-day intervention. An absence of insomnia on the ISI is a score of 7 or less, whereas a score of 15 or more represents clinical (moderate or severe) insomnia.<sup>21</sup>

Saffron intake was well tolerated with no reported adverse events and positive satisfaction ratings. Mood ratings as measured by the DASS-21 also remained constant over the 28-day period, with pre- and postintervention levels remaining within the normal ranges on all these subscales. Interestingly, in this study, dysfunctional beliefs about sleep as measured by the DBAS negatively impacted treatment outcomes in all participants. This suggests that dysfunctional sleep beliefs present as a potential barrier to successful treatment.

The positive results of this study are congruent with previous research examining the sleep-enhancing effects of saffron<sup>16,18,19</sup>; however, this study adds to the body of evidence by using an Australian population with self-reported sleep disturbances and no comorbid medical or psychiatric conditions. Moreover, validated outcome measures were used and the average efficacy of saffron intake over a 28-day period was examined. Unlike many of the previous studies, a standardized saffron extract (affron) was also investigated. Saffron is subject to adulteration,<sup>27</sup> so the quality of extracts can vary significantly. By investigating a standardized extract, the quality and active constituents of saffron used in this study (which undergo chromatographic profiling by the raw ingredient manufacturer prior to commercial release) can be re-examined in future studies.

The mechanisms associated with saffron's potential sleep-enhancing qualities are uncertain. The serotonergic, glutaminergic, and  $\gamma$ -aminobutyric acid (GABA)-ergic systems that are implicated in sleep and insomnia<sup>28–30</sup> are influenced by saffron administration.<sup>31–34</sup> Saffron's anti-inflammatory effects<sup>35</sup> may also be associated with its sleep-enhancing and sleep-restorative effects as insomnia is associated with increased inflammatory markers.<sup>36</sup> In animal models, the individual

saffron constituents, comprising safranal, crocin, and crocetin, are associated with increases in non-rapid eye movement sleep.<sup>37,38</sup> Saffron also modifies EEG activity by increasing delta power.<sup>18</sup> As saffron has positive effects on depressive and anxiety symptoms, its sleep-enhancing effects may also result from its impact on affective symptoms. However, in this study, the recruited population had either no or very mild affective distress and no change was noted in mood-related symptoms, as measured by the DASS-21. While the sleep-enhancing effects of saffron were unlikely to be due to mood-related improvements, they might have been due to distress-related perceptions associated with sleep, which according to the DBAS were associated with reduced treatment efficacy. As participants did not complete a post-evaluation of the DBAS, changes in dysfunctional beliefs about sleep associated with saffron intervention could not be determined. Further research is required to elucidate these findings and the primary mechanisms associated with saffron's sleep-enhancing effects.

## Limitations and directions for future research

Despite positive improvements in sleep quality associated with saffron intake, this study had several limitations. The recruited sample comprised a population with a mild severity of sleep problems (ISI score <21) so the efficacy of saffron in individuals with a greater severity of insomnia is unknown. Most participants also were female (87%) and peri- or postmenopausal. Therefore, the applicability of these findings in males, and younger or older females, requires examination in future trials. Outcome measures comprised validated, self-reported sleep measures; however, no objective measure of sleep change was included. Polysomnography and actigraphy assessments will be important to include in future studies to help validate the results from self-reported assessments. However, it is important to be aware that while self-reported sleep measures do not always closely correspond with objective measures of sleep,<sup>39</sup> they are equally predictive of sleep-related morbidity and mortality.<sup>40,41</sup>

A single dose of 14 mg of affron twice daily was used in the study. The efficacy and safety of using differing doses require further investigation. Dose-escalation studies may also be helpful to examine the impact of dose on the magnitude of treatment effects. As saffron is subject to adulteration and the quality of extracts can vary significantly, the generalizability of these findings to other saffron extracts should also be made cautiously. Therefore, replication using other saffron extracts is essential to assess the generalizability of findings.

In this study, dysfunctional sleep beliefs presented as a barrier to successful treatment. Therefore, modifying dysfunctional beliefs via interventions such as CBT may be associated with greater treatment efficacy. It may be prudent to investigate saffron as an adjunct to CBT compared with a stand-alone intervention in future studies. Moreover, adjunct interventions may increase the likelihood of complete symptom remission as, on average, participants continued to present with borderline insomnia. Finally, the sleep-enhancing effects of saffron in differing populations require further investigation. This includes individuals with comorbid medical and/or psychiatric conditions, chronic or severe sleep disturbances,

and in participants with a range of demographic and psychographic characteristics.

The results from this study indicate that a standardized saffron extract (affron) at a dose of 14 mg twice daily for 28 days improved sleep quality in adults with self-reported poor sleep, with most of these changes occurring in the first 7 days of treatment. Saffron was well tolerated with no reported adverse effects. While positive, these findings require replication using a larger sample size and differing populations.

## ABBREVIATIONS

ANOVA, analysis of variance

CBT, cognitive behavioral therapy

DASS-21, Depression, Anxiety, and Stress Scale–21

DBAS, Dysfunctional Beliefs Associated with Sleep Questionnaire

EEG, electroencephalography

ISI, Insomnia Severity Index

PSD, Pittsburgh Sleep Diary

RSQ, Restorative Sleep Questionnaire

## REFERENCES

1. Buysse DJ, Angst J, Gamma A, Ajdacic V, Eich D, Rossler W. Prevalence, course, and comorbidity of insomnia and depression in young adults. *Sleep*. 2008;31(4):473–480.
2. Schutte-Rodin S, Broch L, Buysse D, Dorsey C, Sateia M. Clinical guideline for the evaluation and management of chronic insomnia in adults. *J Clin Sleep Med*. 2008;4(5):487–504.
3. Adams RJ, Appleton SL, Taylor AW, et al. Sleep health of Australian adults in 2016: results of the 2016 Sleep Health Foundation national survey. *Sleep Health*. 2017;3(1):35–42.
4. Medic G, Wille M, Hemels ME. Short- and long-term health consequences of sleep disruption. *Nat Sci Sleep*. 2017;9:151–161.
5. Bertisch SM, Pollock BD, Mittleman MA, et al. Insomnia with objective short sleep duration and risk of incident cardiovascular disease and all-cause mortality: Sleep Heart Health Study. *Sleep*. 2018;41(6).
6. Baglioni C, Battagliese G, Feige B, et al. Insomnia as a predictor of depression: a meta-analytic evaluation of longitudinal epidemiological studies. *J Affect Disord*. 2011;135(1-3):10–19.
7. Espie CA, Pawlowski B, Waterfield D, Fitton K, Radocchia M, Luik AI. Insomnia symptoms and their association with workplace productivity: cross-sectional and pre-post intervention analyses from a large multinational manufacturing company. *Sleep Health*. 2018;4(3):307–312.
8. Daley D, Morin CM, LeBlanc M, Goggin JP, Savard J, Baillargeon L. Insomnia and its relationship to health-care utilization, work absenteeism, productivity and accidents. *Sleep Med*. 2009;10(4):427–438.
9. Hillman DR, Lack LC. Public health implications of sleep loss: the community burden. *Med J Aust*. 2013;199(8):S7–S10.
10. Maust DT, Solway E, Clark SJ, Kirch M, Singer DC, Malani P. Prescription and nonprescription sleep product use among older adults in the United States. *Am J Geriatr Psychiatry*. 2019;27(1):32–41.
11. Sánchez-Ortuño MM, Belanger L, Ivers H, LeBlanc M, Morin CM. The use of natural products for sleep: a common practice? *Sleep Med*. 2009;10(9):982–987.
12. Leach MJ, Page AT. Herbal medicine for insomnia: a systematic review and meta-analysis. *Sleep Med Rev*. 2015;24:1–12.
13. Marx W, Lane M, Rocks T, et al. The effect of saffron supplementation on symptoms of depression and anxiety: a systematic review and meta-analysis. *Nutr Rev*. 2019;77(8):557–571.
14. Lopresti AL, Drummond PD. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. *Hum Psychopharmacol*. 2014;29(6):517–527.
15. Toth B, Hegyi P, Lantos T, et al. The efficacy of saffron in the treatment of mild to moderate depression: a meta-analysis. *Planta Med*. 2019;85(1):24–31.
16. Nishide A, Fujita T, Nagaregawa Y, et al. Sleep enhancement by saffron extract affron® in randomized control trial. *Jpn Pharmacol Ther*. 2018;46(8):1407–1415.
17. Lopresti AL, Smith SJ, Hood SD, Drummond PD. Efficacy of a standardised saffron extract (affron®) as an add-on to antidepressant medication for the treatment of persistent depressive symptoms in adults: a randomised, double-blind, placebo-controlled study. *J Psychopharmacol*. 2019;33(11):1415–1427.
18. Umigai N, Takeda R, Mori A. Effect of crocetin on quality of sleep: a randomized, double-blind, placebo-controlled, crossover study. *Complement Ther Med*. 2018;41:47–51.
19. Milajerdi A, Jazayeri S, Shirzadi E, et al. The effects of alcoholic extract of saffron (*Crocus sativus* L.) on mild to moderate comorbid depression-anxiety, sleep quality, and life satisfaction in type 2 diabetes mellitus: a double-blind, randomized and placebo-controlled clinical trial. *Complement Ther Med*. 2018;41:196–202.
20. Soper DS. A-priori sample size calculator for Student t-tests [software]. Available at: <http://www.danielsoper.com/statcalc>. Accessed February 11, 2019.
21. Morin CM, Belleville G, Belanger L, Ivers H. The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. *Sleep*. 2011;34(5):601–608.
22. Drake CL, Hays RD, Morlock R, et al. Development and evaluation of a measure to assess restorative sleep. *J Clin Sleep Med*. 2014;10(7):733–741.
23. Monk TH, Reynolds CF 3rd, Kupfer DJ, et al. The Pittsburgh Sleep Diary. *J Sleep Res*. 1994;3(2):111–120.
24. Brown TA, Chorpita BF, Korotitsch W, Barlow DH. Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples. *Behav Res Ther*. 1997;35(1):79–89.
25. Morin CM, Vallières A, Ivers H. Dysfunctional Beliefs and Attitudes about Sleep (DBAS): validation of a brief version (DBAS-16). *Sleep*. 2007;30(11):1547–1554.
26. Tabachnick BG, Fidell LS. *Using multivariate statistics*. Boston: Allyn; 2007.
27. Khilare V, Tiknaik A, Prakash B, et al. Multiple tests on saffron find new adulterant materials and reveal that 1st grade saffron is rare in the market. *Food Chem*. 2019;272:635–642.
28. van Dalsen JH, Markus CR. The involvement of sleep in the relationship between the serotonin transporter gene-linked polymorphic region (5-HTTLPR) and depression: a systematic review. *J Affect Disord*. 2019;256:205–212.
29. Gottesmann C. GABA mechanisms and sleep. *Neuroscience*. 2002;111(2):231–239.
30. Meyerhoff DJ, Mon A, Metzler T, Neylan TC. Cortical gamma-aminobutyric acid and glutamate in posttraumatic stress disorder and their relationships to self-reported sleep quality. *Sleep*. 2014;37(5):893–900.
31. Georgiadou G, Tarantilis PA, Pitsikas N. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of obsessive-compulsive disorder. *Neurosci Lett*. 2012;528(1):27–30.
32. Hosseinzadeh H, Sadeghnia HR. Protective effect of safranin on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. *Phytotherapy*. 2007;14(4):256–262.
33. Sadeghnia HR, Cortez MA, Liu D, Hosseinzadeh H, Snead OC 3rd. Antiabsence effects of safranin in acute experimental seizure models: EEG and autoradiography. *J Pharm Sci*. 2008;11(3):1–14.
34. Berger F, Hensel A, Nieber K. Saffron extract and trans-crocetin inhibit glutamatergic synaptic transmission in rat cortical brain slices. *Neuroscience*. 2011;180:238–247.
35. Zeinali M, Zirak MR, Rezaee SA, Karimi G, Hosseinzadeh H. Immunoregulatory and anti-inflammatory properties of *Crocus sativus* (Saffron) and its main active constituents: a review. *Iran J Basic Med Sci*. 2019;22(4):334–344.
36. Slavish DC, Graham-Engeland JE, Engeland CG, Taylor DJ, Buxton OM. Insomnia symptoms are associated with elevated C-reactive protein in young adults. *Psychol Health*. 2018;33(11):1396–1415.
37. Liu Z, Xu XH, Liu TY, et al. Safranin enhances non-rapid eye movement sleep in pentobarbital-treated mice. *CNS Neurosci Ther*. 2012;18(8):623–630.
38. Masaki M, Aritake K, Tanaka H, Shoyama Y, Huang ZL, Urade Y. Crocin promotes non-rapid eye movement sleep in mice. *Mol Nutr Food Res*. 2012;56(2):304–308.

39. Girschik J, Fritschi L, Heyworth J, Waters F. Validation of self-reported sleep against actigraphy. *J Epidemiol.* 2012;22(5):462–468.
40. Bei B, Milgrom J, Ericksen J, Trinder J. Subjective perception of sleep, but not its objective quality, is associated with immediate postpartum mood disturbances in healthy women. *Sleep.* 2010;33(4):531–538.
41. Kronholm E, Laatikainen T, Peltonen M, Sippola R, Partonen T. Self-reported sleep duration, all-cause mortality, cardiovascular mortality and morbidity in Finland. *Sleep Med.* 2011;12(3):215–221.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge Pharmactive Biotech Products SL for funding the project and supplying affron and LIPA Pharmaceuticals for the preparation of the tablets.

## SUBMISSION & CORRESPONDENCE INFORMATION

**Submitted for publication October 29, 2019**

**Submitted in final revised form February 7, 2020**

**Accepted for publication February 7, 2020**

Address correspondence to: Adrian L. Lopresti, PhD, 38 Arnisdale Rd, Duncraig WA 6023, Australia; Tel: +61 0894487376; Fax: +61 0894478217; Email: a.lopresti@murdoch.edu.au

## DISCLOSURE STATEMENT

All authors have seen and approved the manuscript. This study was funded by Pharmactive Biotech Products SL. Pharmactive Biotech Products was not involved in the design of the research, analysis of data, or in the writing of the report. The authors report no conflicts of interest.



# affron<sup>®</sup> a novel saffron extract (*Crocus sativus* L.) improves mood in healthy adults over 4 weeks in a double-blind, parallel, randomized, placebo-controlled clinical trial



Graham Kell<sup>a</sup>, Amanda Rao<sup>b,\*</sup>, Gavin Beccaria<sup>a</sup>, Paul Clayton<sup>c</sup>, Antonio Manuel Inarejos-García<sup>d</sup>, Marin Prodanov<sup>e</sup>

<sup>a</sup> University of Southern Queensland, School of Psychology and Counselling, Toowoomba, Australia

<sup>b</sup> RDC Clinical, Brisbane, Australia

<sup>c</sup> Institute of Food, Brain and Behaviour, Oxford, UK

<sup>d</sup> Pharmactive Biotech Products S.L. Parque Científico de Madrid, Madrid, Spain

<sup>e</sup> Instituto de Investigación en Ciencias de la Alimentación CIAL (CEI CSIC-UAM), C/Nicolás Cabrera, 9, E-28049 Madrid, Spain

## ARTICLE INFO

### Keywords:

Clinical trial  
Mood disorders  
Saffron extract  
Phytonutrient  
Depression

## ABSTRACT

**Background:** In recent years phytotherapy has been explored as a source for alternative treatments for mood disorders. One potential candidate is saffron (*Crocus sativus* L.), whose main bioactive components are crocins and safranal.

**Objectives:** The aim of this study was to investigate the efficacy of affron<sup>®</sup>, a standardised stigmas extract from *Crocus sativus* L. for improving mood, stress, anxiety and sleep quality in healthy adults.

**Methods:** In this 3 arm study, 128 participants self-reporting low mood but not diagnosed with depression, were given affron<sup>®</sup> at 28 mg/day, 22 mg/day, or a placebo treatment in a randomized, double-blind, placebo-controlled trial for 4 weeks. Mood was measured at baseline and at the end of the study, using the POMS (primary outcome measure) and PANAS questionnaires, and the DASS-21 scale. Sleep was monitored using Sleep Quality Index (PSQI).

**Results:** Analysis indicated a significant decrease in negative mood and symptoms related to stress and anxiety at a 28 mg/day dose (with a significant difference between 28 mg/day and placebo on the POMS Total Mood Disturbance scale,  $p < 0.001$ ,  $d = -1.10$ ), but no treatment effect at the 22 mg/day dose.

**Limitations:** The main weaknesses of this investigation were found in the self-reporting nature of both the screening and the testing.

**Conclusions:** affron<sup>®</sup> increased mood, reduced anxiety and managed stress without side effects, offering a natural alternative to standard treatments.

## 1. Introduction

Mental health disruptions are the leading cause of disability worldwide.<sup>1</sup> In Australia, for example, 45% of the population experienced a mental health condition in their lifetime (at least one of the selected mental disorders: anxiety, mood or substance use disorders), with one million adults suffering from depression, and over two million from anxiety.<sup>2</sup> The two conditions often co-exist; nearly half of those diagnosed with depression are also diagnosed with an anxiety disorder. Published prevalence figures for depression and anxiety only account for diagnosed cases, and true figures are certainly higher.

The number of affected people increases further when including those with subclinical depression, (i.e. those with low mood). These conditions share many symptoms and can be regarded as existing on a spectrum of disorder.

Depression, for example, is a medically defined pathology typically graded from profound down through severe and moderate to mild; whereas low mood describes a temporary emotional state characterized by symptoms usually associated with depression but less severe and/or prolonged,<sup>3–5</sup> with sub-clinical depression occupying a somewhat amorphous intermediate position.

While not considered a pathology, low mood is defined by many of

**Abbreviations:** POMS, Profile of Mood States; DASS-21, depression, anxiety and stress scale (21 question); PANAS, positive and negative affect scale; PSQI, Pittsburgh Sleep Quality Index

\* Corresponding author at: RDC Clinical, PO Box 667, New Farm Qld 4005, Australia.

E-mail address: [amanda@rdcglobal.com.au](mailto:amanda@rdcglobal.com.au) (A. Rao).

<http://dx.doi.org/10.1016/j.ctim.2017.06.001>

Received 3 February 2017; Received in revised form 15 April 2017; Accepted 4 June 2017

Available online 13 June 2017

0965-2299/ © 2017 Elsevier Ltd. All rights reserved.

the same symptoms used to define depression and sub-clinical depression, including sadness, crying, fatigue, pessimism, changes in appetite, changes in sleep patterns, and anhedonia.<sup>6,3,4</sup> Those who report such symptoms often struggle to cope with daily life yet lack the treatments available to those with a diagnosed disorder. Prescription medications are not only inappropriate in these instances, they are often ineffective.<sup>7,8</sup> The rates of remission tend to be low and the risk of relapse high.<sup>9</sup> Additionally, many find the adverse side effects of medications intolerable.<sup>10</sup> The search for alternative treatments has therefore become a high priority in the management of low mood.

Further impetus derives from evidence that both subclinical and chronic mild depression predispose to major clinical depression,<sup>11,12</sup> and low mood is likely also a risk factor.<sup>13,14</sup>

In recent years phytotherapy has been explored as a source for alternative treatments for mood disorders and depression. One potential candidate is saffron (*Crocus sativus* L.), whose main bioactive components, crocins and safranal, are responsible for the spice's aroma and characteristic red color.<sup>15</sup>

There is evidence that crocins act as reuptake inhibitors of dopamine and norepinephrine, while safranal acts primarily on serotonin reuptake.<sup>16–19</sup> The antioxidant properties of saffron derivatives may also be relevant. Mood disorders are associated with elevated oxidative stress and a deficit of exogenous antioxidants,<sup>20,21</sup> affecting immune and inflammatory responses in a way, which may promote neurodegeneration.<sup>22</sup> There is good evidence that the antioxidants in saffron extracts protect against oxidative stress in the central nervous system,<sup>23,24</sup> constituting a second potential mechanism of therapeutic action.

The novel saffron extract affron® is characterized by HPLC–MS/ESI and standardised to safranal and crocins. The aim of this research was to measure the clinical efficacy of affron® for improving mood, reducing the symptoms of anxiety, stress, and improving vigour and sleep quality in healthy participants.

It was hypothesised that a change in mood scores (i.e., a decrease in negative mood scores and an increase in positive mood scores) on the POMS, PANAS, and DASS-21 over four weeks would be significantly greater in the active treatment groups than in the placebo group. It was also hypothesised that a change in these mood scores would be significantly greater in the active treatment groups than in the placebo.

All previous studies of saffron's effect on mood used 30 mg/day for either six or eight weeks, without examining the efficacy of lower dosage rates and shorter intervention periods. The benefits of exploring the minimal effective dose are not only therapeutic (to establish required dosage strength), they are also economic, especially for a spice as expensive as saffron. This study therefore investigated two lower dosage rates (22 mg/day, and 28 mg/day), and a shorter treatment time (four weeks).

## 2. Methods

### 2.1. Saffron extracts

A total of N = 8 batches of affron® samples (*Crocus sativus* L.) obtained from Pharmactive Biotech Products SL were employed for characterization. The samples were packaged in vacuum and stored in darkness at room temperature until analysis.

### 2.2. Reagents

Methanol and acetonitrile were purchased from Sharlau (Barcelona, Spain). All of the solvents were of HPLC degree, and the water used was bi-distilled and purified using a MilliQ Millipore system (Bedford, MA).

Safranal and gallic acid reference substances, sodium carbonate and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (San Luis, USA), and *trans*-crocins-4 from Phytolab (Vestenbergsgreuth, Germany).

### 2.3. HPLC–PAD

High Performance Liquid Chromatography (HPLC) analysis of affron® samples was performed by means of an Agilent Technologies 1220 Infinity series system with photo-diode array detector (PAD), according to Caballero-Ortega et al.<sup>25</sup> The bioactive compounds safranal and crocins were quantified by means of safranal and *trans*-crocins-4 external calibration curves.

### 2.4. HPLC–MS

In order to confirm the identity of each peak, mass spectrometry (MS) was performed by means of Agilent series 1100 (Palo Alto, California, USA), coupled to mass-quadrupole detector (Hewlett-Packard, serie 1100 MSD), with electrospray ionization source (ESI), operated in positive and negative modes, according to Lech et al.<sup>26</sup>

### 2.5. Total phenolic compound content

Total phenolic compound content of affron® was performed by the colorimetric method of Singleton and Rossi,<sup>27</sup> using Folin-Ciocalteu reagent.

Data were expressed as mean value ± standard deviation of three independent measurements.

### 2.6. Clinical trial

The study was conducted in Brisbane, Australia, revised and approved by Queensland Clinical Trials Network Human Research and Ethics Committee, (Application number: HREC2014002). Australia, and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614001053617).

It was conducted in accordance with the Declaration of Helsinki, revised in 1989, and principles of the Australian Regulations on Medical Research involving Human Subjects (National Health and Medical Research Council; Australia).

### 2.7. Participants

A total of 128 healthy adults, aged 18–77 years were recruited from the CRO's subject database and the public media (Table 1). Participants were included for assessment if they were self-reporting low mood, were not diagnosed with depression or another mood disorder, were otherwise healthy (including BMI < 30). Participants were excluded if they had been diagnosed with a mood disorder or had tested positive for depression on the Beck Depression Inventory (BDI > 20). A minimum BDI score was not set, but only those reporting with low mood enrolled in the study.

Key exclusion criteria included: received and/or prescribed Coumadin (Warfarin), Heparin, Dalteparin, Enoxaparin or other anticoagulation therapy; diagnosed with hypertension and receiving and/or prescribed antihypertensive medications, diagnosed with severe renal and/or hepatic insufficiency; had a history of chronic alcohol and/or drug abuse; had participated in any other clinical trial during last 30 days; were currently participating in another clinical trial; diagnosed with a mood disorder (major depressive disorder (MDD), bipolar disorder or substance-induced disorder); had tested positive for moderate to severe depression on the Beck Depression Inventory; suffered from insomnia or had night-shift employment and were unable to have a normal night's sleep; suffered severe Pre-Menstrual Syndrome (PMS) with mood or pain that would change during the study period; suffered from any neurological disorder such as multiple sclerosis; were currently taking supplements (nutrients, including herbs) that would impact mood (St John's Wort, Tryptophan, SAM-E, 5-hydroxytryptophan, Melatonin, GABA); were taking a saffron supplement or could not exclude foods containing saffron or the use of saffron in cooking.

Table 1

**Participant Demographics at Baseline.** Active treatment groups and placebo group evenly matched at baseline in all demographics, with no significant differences between groups.

Demographics	Treatment group			
	Total (N = 121)	28 mg/day (n = 41)	22 mg/day (n = 42)	Placebo (n = 38)
<b>Age</b>				
Mean (SD)	39.1 (13.77)	40.4 (12.71)	36.7 (14.59)	40.38 (13.97)
Range	18–77	21–68	18–77	23–68
<b>Gender (Number, %)</b>				
Female	75 (62.0%)	26 (63.4%)	26 (61.9%)	23 (60.5%)
Male	46 (38.0%)	15 (36.6%)	16 (38.1%)	15 (39.5%)
<b>Status (Number, %)</b>				
Partner	74 (61.2%)	25 (61.0%)	27 (64.3%)	22 (57.9%)
Single	47 (38.8%)	16 (39.0%)	15 (35.7%)	16 (42.1%)
<b>Working (Number, %)</b>				
Employed / student	103 (85.1%)	34 (82.9%)	37 (88.1%)	32 (84.2%)
Unemployed / retired	18 (14.9%)	7 (17.1%)	5 (11.9%)	6 (15.8%)
<b>Weight</b>				
Mean kg (SD)	76.34 (17.22)	75.89 (16.48)	77.54 (18.20)	75.56 (17.39)
<b>BMI</b>				
Mean (SD)	26.42 (6.33)	26.74 (5.90)	27.01 (7.91)	25.38 (4.77)
<b>Smoking (Number, %)</b>				
Yes	17 (14.0%)	8 (19.5%)	6 (14.3%)	3 (7.9%)
No	104 (86.0%)	33 (80.5%)	36 (85.7%)	35 (92.1%)
<b>Alcohol (Number, %)</b>				
3 or less per week	44 (36.4%)	14 (36.1%)	12 (28.6%)	18 (47.4%)
Over 3 per week	77 (63.6%)	27 (65.9%)	30 (71.4%)	20 (52.6%)

Note: No significant differences in demographics between treatments at baseline ( $p > 0.05$ , two-tailed).

## 2.8. Design and intervention

The study was a parallel, double-blind placebo-controlled design. The participants, self-reporting low mood but not diagnosed with depression, were included and randomly assigned to groups receiving the saffron extract (affron<sup>®</sup>, 22 or 28 mg/day), or placebo for 4 weeks.

The active treatment was a TGA listed coated tablet containing either 11 mg or 14 mg of standardised saffron extract (affron<sup>®</sup>), derived from the stigmas of *Crocus sativus* L. and standardised to contain > 3.5% Lepticosalides<sup>®</sup> a measure of bioactive compounds present in saffron, including safranal and crocin. The placebo tablet contained the same excipients as the active tablet (microcrystalline cellulose and calcium hydrogen phosphate). The active and placebo tablets were matched for size shape and coating color. Treatment containers were randomised using Random Allocation Software version 1.0, and labelled with a code. Participants were allocated a corresponding code (e.g., participant 15 received container 15). The randomisation code was maintained by the sponsor to keep the investigators blind and to facilitate code breaking in the case of adverse events. Placebo tablets were identical in appearance to active tablets and contained carrot extract instead of affron<sup>®</sup> (data not shown). The investigator was informed of treatment group allocation post-trial for statistical analyses.

## 2.9. Outcomes

Mood was measured at baseline and at the end of the study, using the following validated questionnaires: Profile of Mood States (POMS; primary outcome), The Positive and Negative Affect Schedule, (PANAS)

and Depression Anxiety Stress States (DASS-21). Sleep was monitored using Pittsburgh Sleep Quality Index (PSQI).

POMS,<sup>28</sup> consists of 65 items, adjectives describing an emotion rated on a five-point scale, where 0 = not at all; 1 = a little; 2 = moderately; 3 = quite a lot; and 4 = extremely (except the items relaxed and efficient, reverse scored). Participants were asked how they felt at that moment, and answers were grouped into six subscales; five negative: Tension (ranged from −36 to 36), Depression (ranged from −60 to 60), Anger (ranged from −48 to 48), Fatigue (ranged from −28 to 28) and Confusion (ranged from −28 to 28) and one positive (Vigour; ranged from −32 to 32). A Total Mood Disturbance (TMD) score was calculated for each participant (Tension + Depression + Anger + Fatigue + Confusion − Vigour) to give an overview of mood state. Change scores from baseline to the end of the study were calculated for each subscale<sup>29</sup> and for TMD, possible scores ranged from −232 to 200.

PANAS,<sup>30</sup> consists of 20 items; 10 positive and 10 negative words. Scoring was on a five-point scale, where 1 = very slightly or not at all; 2 = a little; 3 = moderately; 4 = quite a bit; and 5 = extremely. Participants were asked how they felt over the previous week and answers were grouped into two subscales (Positive Affect, PA; and Negative Affect, NA). The change scores across time (from baseline to the end of the study), for both PA and NA were ranged from −40 to 40.

DASS-21,<sup>31</sup> is designed to measure stress, anxiety, and depression. It consists of 21 self-report items in the form of statements; seven forming the subscale of Depression, seven forming the subscale of Anxiety and seven forming the subscale of Stress. Participants were asked how they felt over the past week and scored each item from 0 to 3, where 0 = never; 1 = sometimes; 2 = often; and 3 = almost always. The variance of the scores across time (from baseline to the end of the study) was calculated for each subscale and each participant.

PSQI,<sup>32</sup> is designed to measure sleep quality using 19 self-rated questions and five questions rated by a person who had close relationship with the participant.

## 2.10. Procedure

Participants were provided with information about the product at the baseline interview, where they gave written informed consent and were advised that they could withdraw at any time. Medical details were then recorded, exclusion criteria checked and demographic data was gathered.

Participants completed the POMS, PANAS, DASS-21, and PSQI at the baseline interview and at the week four interview in the clinic of the investigator (Brisbane). The battery of tests took approximately 30 min per participant.

Once assigned to groups, participants were allocated either 28 mg/day or 22 mg/day of active treatment, or placebo. Each participant was instructed to take two tablets daily for four weeks, one tablet with the morning meal and one tablet with the midday meal. Product containers were returned at the week four interview, and any remaining tablets were recorded. Participants were asked at week 2 and the final interview if there had been any changes to their lifestyle, weight, or if they had noticed any adverse symptoms since starting treatment.

## 2.11. Statistical analyses

A priori power analyses conducted using G\*Power version 3.1.9.2<sup>33</sup> determined a sample size of 93 was required to attain a power of 0.80 for two-tailed tests detecting a large effect size (31 per group). To allow for exclusions and a 30% drop out the aim was to screen 140 participants. Therefore the final sample size of 121 was adequate for the a priori power requirement. In order to control for family wise a conservative Bonferroni corrections was applied to all the data.

Clinical study analyses were completed using IBM Statistical Package for Social Sciences (SPSS) version 23 at an alpha level of 0.05.

Change scores from baseline to week four were calculated for each participant in each mood measure, to reduce within group variance.<sup>29</sup> The group means of these change scores were used to assess the statistical difference between groups by one-way independent ANOVA. Gabriel's pairwise test procedure was used for post-hoc analyses since this was a three arm study with marginally different group sizes (28 mg/day,  $n = 41$ ; 22 mg/day,  $n = 42$ ; placebo,  $n = 38$ ). Gabriel's post hoc test was chosen since it was designed to cope with slightly unequal group sizes.

### 3. Results

#### 3.1. Chemical analysis

The HPLC-PAD/MS analysis of affron<sup>®</sup> identified six crocin isomers, together with picrocrocin, safranal and one kaempferol diglucoside. These results are in a good agreement with those obtained by Lech et al.<sup>26</sup>

As can be observed in Table 3, affron<sup>®</sup> samples showed a minimum content of safranal of 0.03%, whereas total crocin content was over 3.99% on average and a total phenolic compound content of 1.41%.

The sum of the bioactive components safranal and crocin isomers analysed by HPLC, which are also responsible for the main organoleptic properties,<sup>15</sup> herein is referred as Lepticrosalides<sup>®</sup>.<sup>34</sup> The proposed expression of results by HPLC is more objective and is expected to be more reproducible from laboratory to laboratory than the traditional ISO 3632 methodology.<sup>35</sup>

#### 3.2. Clinical study results

After screening 137 potential participants for exclusion criteria, 128 healthy adults aged 18–77 years were randomised into three groups (Table 1). Seven participants were lost to follow-up, leaving 121 participants at completion, allocated to three groups; 28 mg/day, 22 mg/day, and placebo (Fig. 1). The mean age of participants was 39 years. The total sample was 62% female and 38% male. The mean weight at baseline was

**Table 2**  
Mean Change Scores (SD) for Each Scale and Subscale.

Baseline Mean (SD)	Treatment group		
	28 mg/day	22 mg/day	Placebo
POMS Total Mood Disturbance (-32 to 200)	40.2 (38.3)	31.5 (36.2)	38.5 (27.8)
PANAS Positive Affect Score Baseline (10–50)	27.8 (9.3)	27.3 (7.4)	29.8 (10.4)
PANAS Negative Affect Score Baseline (10–50)	20.8 (8.9)	19.3 (6.9)	18.6 (7.1)
DASS21 Depression Score Baseline (0–21)	6.7 (5.8)	6.3 (5.6)	6.4 (6.3)
<b>Change scores per Instrument</b>			
<b>POMS</b>			
Tension	-4.00 (4.65)	-3.10 (4.74)	-1.06 (4.80)
Depression	-8.43 (7.66)	-4.28 (7.43)	-1.33 (6.22)
Anger	-5.05 (5.05)	-3.10 (6.91)	-1.14 (4.80)
Fatigue	-5.00 (5.34)	-2.85 (4.58)	-1.11 (6.31)
Confusion	-4.35 (4.07)	-2.65 (4.07)	-0.83 (3.39)
Vigour	4.00 (5.46)	2.23 (5.71)	-0.39 (6.58)
Total Mood Disturbance	-30.83 (21.56)	-18.36 (27.62)	-5.37 (24.52)
<b>PANAS</b>			
Positive Affect	4.32 (8.04)	3.13 (7.00)	0.91 (6.43)
Negative Affect	-6.63 (5.24)	-3.92 (5.84)	-2.40 (3.65)
<b>DASS</b>			
Depression	-11.22 (7.48)	-5.29 (8.53)	-3.05 (5.86)
Anxiety	-6.44 (6.94)	-4.05 (5.65)	-2.63 (5.58)
Stress	-12.24 (7.74)	-5.62 (7.63)	-3.26 (8.03)
PSQI Global Score	-2.69 (2.61)	-2.27 (3.04)	-0.82 (2.77)

76.3 kg. The mean BMI at baseline was 26. The majority of participants had partners, were working or studying, were non-smokers, consumed over 3 drinks per week, and reported undertaking regular exercise.

No significant differences between groups were observed at baseline in any of the outcomes, and low mood scores for the average participant at baseline were in the mild to moderate range, according to the DASS (average scores; depression = 14.2, anxiety = 8.8, stress = 18.4).

Change scores from baseline to week four were calculated for each participant in each mood measure for use in analyses. This reduced within-group variability that results from individual response specificity. The group means of the change scores were used to assess the statistical difference between groups by one-way independent ANOVA with a Gabriel's post hoc comparison test. Table 2 shows the mean change scores for each scale and subscale.

#### 3.2.1. POMS

As shown in Fig. 2A, all subscales demonstrated a significant improvement as measured by the change scores by week 4 for the group treated with 28 mg/day of affron<sup>®</sup> compared to the rest of groups studied. In order to control for family wise error a conservative Bonferroni corrections was applied where the new alpha was set at ( $p = 0.038$ ).

For the POMS Tension, Depression, and Confusion subscales, a significant treatment over time effect was observed (Tension,  $F(2,113) = 3.82$ ;  $p = 0.025$ ; Depression,  $F(2,113) = 9.46$ ,  $p < 0.001$ ,  $\omega$  (effect size) = 0.36; Confusion,  $F(2,113) = 7.81$ ,  $p = 0.001$ ,  $\omega = 0.32$ ). Gabriel's post hoc test revealed a significant decrease in the above subscales, in the 28 mg/day group compared to the placebo group, (Depression,  $p < 0.001$ ,  $d = -1.02$ ; and Confusion,  $p < 0.001$ ,  $d = -0.94$ ; respectively, indicating a large effect size according to Cohen's conventions).

A significant improvement for the POMS Fatigue subscale was also observed ( $F(2,113) = 4.92$ ,  $p = 0.009$ ,  $\omega = 0.25$ ). Gabriel's post hoc test revealed a significant decrease in fatigue in the group treated with 28 mg/day of affron<sup>®</sup> in comparison with the placebo group, ( $p = 0.007$ ,  $d = -0.67$ ; a medium effect size according to Cohen's conventions).

Furthermore, there was a significant positive improvement for the POMS Vigour subscale ( $F(2,112) = 5.25$ ,  $p = 0.007$ ,  $\omega = 0.26$ ). Gabriel's post hoc test revealed a significant increase in vigour in the 28 mg/day group compared to the placebo group, ( $p = 0.005$ ,  $d = 0.73$ ; a medium effect size according to Cohen's conventions).

Overall, there was a significant treatment effect for the POMS Total Mood Disturbance (TMD) scale ( $F(2,111) = 9.94$ ,  $p < 0.001$ ,  $\omega = 0.37$ ). Gabriel's post hoc test revealed a significant decrease in TMD in the group that consumed 28 mg/day of affron<sup>®</sup> compared to the placebo group, ( $p < 0.001$ ,  $d = -1.10$ ; a large effect size according to Cohen's conventions), (Fig. 2B).

#### 3.2.2. PANAS

Analysis revealed no significant between-group treatment effect on the change scores during the study for Positive Affect ( $F(2,111) = 2.13$ ,  $p = 0.124$ ), but it did show a significant improvement regarding the Negative Affect ( $F(2,111) = 6.97$ ,  $p = 0.001$ ,  $\omega = 0.31$ ). Gabriel's post hoc test revealed a significant decrease in negative affect in the group treated with 28 mg/day of affron<sup>®</sup> compared to the placebo group, ( $p = 0.001$ ,  $d = -0.42$ ) (Fig. 3).

#### 3.2.3. DASS-21

There was a significant treatment effect on the change scores for the DASS Depression subscale,  $F(2,118) = 12.96$ ,  $p < 0.001$ ,  $\omega = 0.41$  (a large effect size). Gabriel's post hoc test revealed that a decrease in depression in the 28 mg/day group was significantly greater than in the 22 mg/day group and the placebo group, ( $p < 0.001$ ,  $d = -1.22$ ;  $p = 0.001$ ,  $d = -0.74$ ) respectively. The 22 mg/day group did not significantly differ in depression from the placebo group ( $p = 0.449$ ).

There was no significant treatment effect on the change scores for the DASS Anxiety subscale,  $F(2,118) = 4.33$ ,  $p = 0.01$ ,  $\omega = 0.23$ . Gabriel's

**Table 3**

HPLC analysis (% dry weight) of safranal and crocin isomers and spectrophotometric quantitative analysis (% dry weight) of total phenolic compound content in affron® samples (N = 8).

Analyte	Mean $\pm$ SD (%)	Range (%)	Proportion (%)				
			P10	P25	P50	P75	P90
safranal	0.04 $\pm$ 0.01	0.03–0.07	0.03	0.03	0.04	0.05	0.07
trans-crocin-4	2.88 $\pm$ 0.59	2.06–3.81	2.31	2.49	2.77	3.29	3.59
Total crocins	5.33 $\pm$ 0.95	3.99–6.86	4.33	4.81	5.18	5.79	6.60
TPCC <sup>a</sup>	1.41 $\pm$ 0.20	1.10 $\pm$ 1.71	1.17	1.28	1.45	1.53	1.63

<sup>a</sup> Total phenolic compound content by Folin-Ciocalteu reagent.

post hoc test revealed that a decrease in anxiety in the 28 mg/day group was significantly greater than in the placebo group,  $p = 0.010$ ,  $d = -0.65$ . The 22 mg/day group did not significantly differ in anxiety from the 28 mg/day group ( $p = 0.149$ ), or the placebo group ( $p = 0.625$ ).

There was a significant treatment effect on the change scores for the DASS Stress subscale,  $F(2,118) = 14.29$ ,  $p < 0.001$ ,  $\omega = 0.42$ . Gabriel's post hoc test revealed that a decrease in stress in the 28 mg/

day group was significantly greater than in the placebo group and 22 mg/day group, ( $p < 0.001$ ,  $d = -1.14$ ;  $p = 0.001$ ,  $d = -0.86$ ) respectively. The 22 mg/day group did not significantly differ in stress from the placebo group ( $p = 0.445$ ). (Fig. 4).

### 3.2.4. PSQI

The effect of the saffron extract on sleep quality was analysed by the

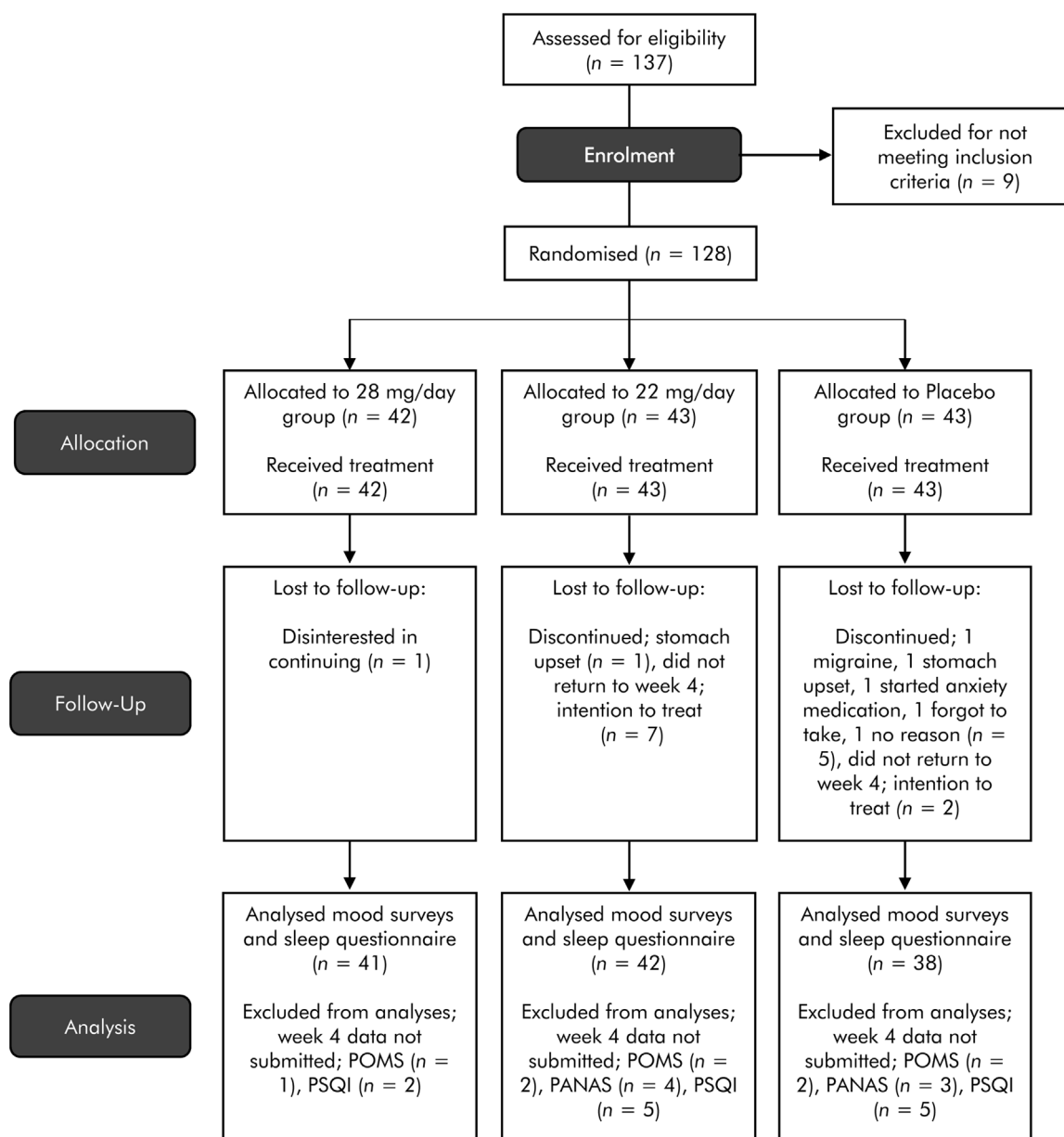


Fig. 1. Participant Flow Chart.

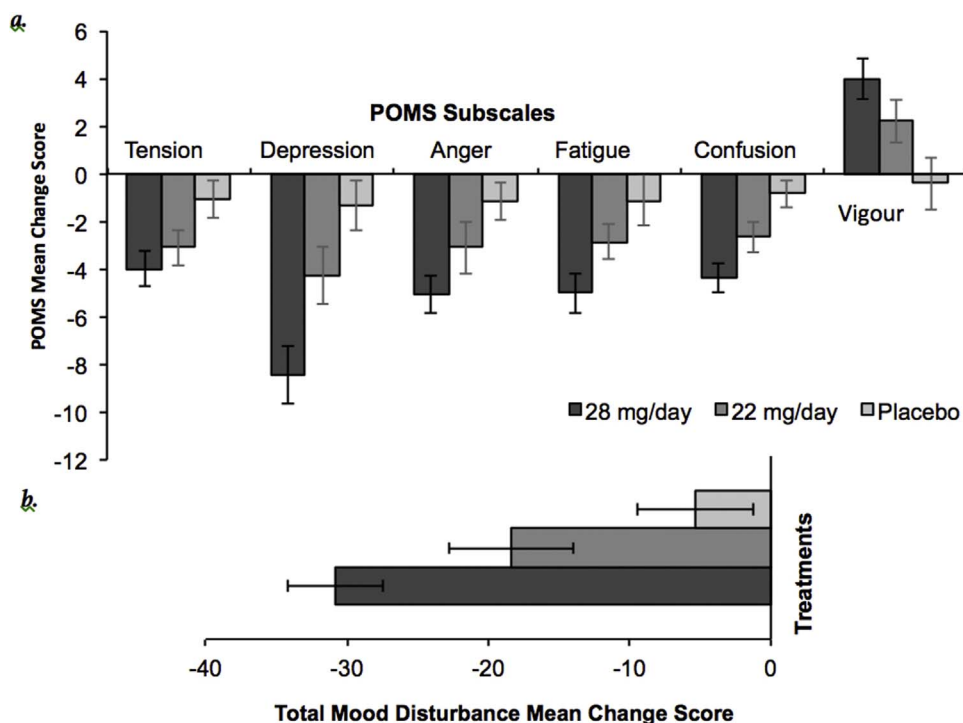


Fig. 2. A) POMS mean change scores, subscales tension, depression, anger, confusion and vigour, and (B) Total Mood Disturbance Mean Change Scores after 4 weeks of treatment with 22 or 28 mg/day affron® or placebo.

PSQ Index. There was no significant improvement in sleep quality in any of the treatment groups (Fig. 5).

### 3.3. Safety and tolerability and compliance

The active treatment was well tolerated. Participants returned unused containers of product at the final interview, and compliance was high and similar between all groups. Participants were monitored for adverse effects at 2 weeks and the final interview. One participant in the placebo group reported a singular event of symptoms of diarrhea.

## 4. Discussion

Results indicated a significant decrease in negative mood and symptoms related to stress and anxiety at a 28 mg/day dose. No significant differences were observed between the group treated with 22 mg/day of the saffron extract and the placebo group. Sleep quality showed a slight improvement at 28 mg/day dose.

The mood elevating and anxiolytic effects of affron® were consistent in both sexes, and achieved without adverse effects on any performance or safety parameters. Our results are consistent with previous studies undertaken on *Crocus sativus* L. that have shown effectiveness in alleviating the symptoms of mild to moderate depression, in some studies as effectively as fluoxetine and imipramine.<sup>16</sup>

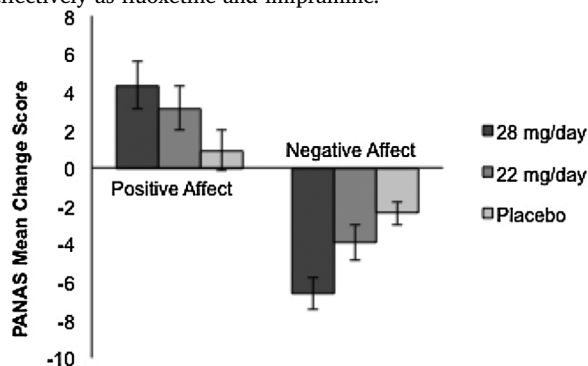


Fig. 3. PANAS Mean change scores, subscales positive affect (PA) and negative affect (NA), after 4 weeks of treatment with 22 or 28 mg/day affron® or placebo.

While we studied a population with self-reported low mood but not diagnosed with depression, our results bring new potential knowledge to the clinical literature, showing that this new standardised saffron extract exerts remarkably consistent positive effects across the POMS-TMD, PANAS and DASS scales. Furthermore, our dosing schedule demonstrated a clear dose-dependent relationship across all scales, making our study the first to identify a clinically appropriate and empirically justified dosage scheme.

To our knowledge, this is the first time that a commercial saffron extract obtained at industrial scale and objectively characterized has been tested on healthy people with a positive effect on overall mood. Given affron®'s excellent safety profile, and data indicating that low mood states may predispose to depressive illness,<sup>13,14</sup> it may be considered a candidate for preventative use in subjects deemed to be at risk of progressing to more severe and eventually clinical manifestations.

### 4.1. Limitations

The effect sizes on the outcomes in this study provided favourable results and demonstrated good internal validity; however, the study was not without its limitations. The main weaknesses of this investigation were found in the self-reporting nature of both the screening and the testing, and the possibility of confounding variables.

First, the measurement of low mood as a construct was an inexact process which relied on self-reporting of low mood at screening. The subjective nature of self-reports may have impacted on the construct

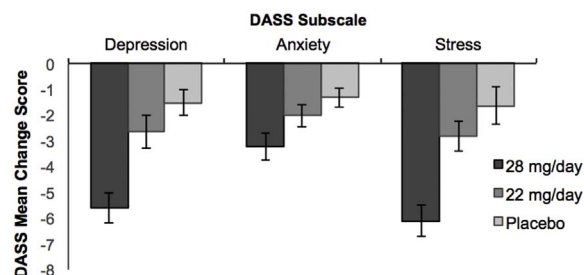


Fig. 4. DASS-21 mean change scores, subscales depression, anxiety and stress, after 4 weeks of treatment with 22 or 28 mg/day affron® or placebo.

validity of the tests by including participants who may have been excluded if a more objective screening process was employed. It is possible that participants with an undiagnosed mood disorder were included in a study that sought to exclude them.

Second, the self-reporting nature of the instruments used may have led to imprecise measures due to the subjective interpretation of items. The possibility of error could be reduced by using blood tests to measure stress hormones.

Third, while the possible confounds of BMI and gender were considered, this study did not control for other variables known to impact the outcome of mood, such as personality.<sup>36</sup>

Finally, to address low mood rather than clinically diagnosed disorders, this study tested a healthy population. It therefore excluded participants with a high BMI, severe PMS, insomnia, and those with a history of drug and alcohol abuse. Since these conditions are often associated with low mood, these exclusions may limit the generalisability of the study. This may be addressed by future research into saffron's efficacy for treating participants whose low mood is comorbid with more severe conditions.

## 5. Conclusion

Overall, the results demonstrated the effectiveness of affron®, a botanical extract from saffron (*Crocus Sativus* L.) on improving low mood, and stress in otherwise healthy participants.

Given the excellent safety profile of this food herb, the well-known issues associated with the tricyclics and SSRI's and the current absence of management tools for low mood, there is now a strong case for using saffron in the long-term and prophylactic management, where appropriate, of low mood states.

## Role of funding source

This research did not receive any specific grant from funding agencies in the public sectors. This study was funded by Pharmactive Biotech Products SL. Pharmactive Biotech Products was not involved in the design of the research, analysis of data, or in the writing of the report.

## Conflict of interest

The authors have declared there is no conflict of interest.

## Authors' contributions

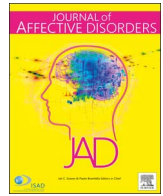
GK, AR, GB PC contributed to the data collection writing, data analyses and data interpretation of the clinical trial that is a part of this manuscript. AI, MG and MP conducted the laboratory analysis of the affron® samples. All the authors read and approved the final draft of the manuscript.

## Acknowledgements

The authors gratefully acknowledge Pharmactive Biotech Products SL Company for funding the project and supplying affron®, LIPA Pharmaceuticals for the preparation of the tablets, and the RDC Clinical for their management of the clinical trial.

## References

- Akhondzadeh BA, Ghoreishi SA, Noorbala AA, Akhondzadeh SH, Rezazadeh SH. Petal and stigma of *Crocus sativus* L. in the treatment of depression: A pilot double-blind randomized trial. *J Med Plants*. 2008;7:29–36.
- Australian Bureau of Statistics. *Australian Social Trends 4102.0*. 2009; 2009:13.
- Keller MC, Nesse RM. Is low mood an adaptation? evidence of subtypes with symptoms that match precipitants. *J Affect Disord*. 2005;86:27–35.
- Nettle D. An evolutionary model of low mood states. *J Theor Biol*. 2009;257:100–103.
- American Psychiatric Association. *Diagnostic and Statistical Manual for Mental Disorders*. 5th ed. 2013; 2013.
- Bolmont B, Abirami JH. State-anxiety and low mood: evidence for a single concept. *Physiol Behav*. 2001;74:421–424.
- Baumeister H, Knecht A, Hutter N. Direct and indirect costs in persons with chronic back pain and comorbid mental disorders—a systematic review. *J Psychosom Res*. 2012;73:79–85.
- Salum GA, Luciano Rassier Isolan LR, Vera Lúcia Bosa VL, et al. The multidimensional evaluation and treatment of anxiety in children and adolescents: rationale, design, methods and preliminary findings. *Rev Bras Psiquiatr*. 2011;33:2.
- Macdonald TM. Treatment of depression: prescription for success? *Primary Care Psychiatry*. 1997;3:7–10.
- Ferguson JM. SSRI antidepressant medication. adverse effects and tolerability. Primary care companion. *J Clin Psychiatry*. 2001;3:1.
- Klein DN, Santiago NG. Dysthymia and chronic depression: introduction, classification, risk factors, and course. *J Clin Psychol*. 2003;59:807–816.
- Cuijpers P, Smit F. Subclinical depression: a clinically relevant condition? *Tijdschr Psychiatr*. 2008;50:519–528.
- Burcusa SL, Iacono WG. Risk for recurrence in depression. *Clin Psychol Rev*. 2007;27:959–985.
- Contreras J, Hare E, Pacheco A, Escamilla M, Raventos H. Is subclinical anxiety an endophenotype for bipolar I patients? A study from a Costa Rican sample. *J Affect Disord*. 2010;122:267–272.
- Ordundi SA, Tsimidou MZ. Saffron quality: effect of agricultural practices, processing and storage. *Prod Pract Qual Assess Food Crops*. 2004;1:209–260.
- Noorbala AA, Akhondzadeh S, Tahmacebi-Pour N, Jamshidi AH. Hydro-alcoholic extract of *Crocus sativus* L. versus fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial. *J Ethnopharmacol*. 2005;97:281–284.
- Hosseinzadeh H, Noraei NB. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents crocin and safranal, in mice. *Phytother Res*. 2009;23:768–774.
- Georgiadou G, Tarantilis PA, Pitsikas N. Effects of the active constituents of *Crocus Sativus* L. crocins, in an animal model of obsessive-compulsive disorder. *Neurosci Lett*. 2012;528:27–30.
- Ettehadhi H, Mojabi SN, Ranjbaran M, et al. Aqueous extract of saffron (*Crocus sativus*) increases brain dopamine and glutamate concentrations in rats. *J Behav Brain Sci*. 2013;3:315–319.
- Maes M, Fišar Z, Medina M, Scapagnini G, Nowak M, Berk M. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress-mitochondrial, antioxidant, and neuroprogressive pathways, and new drug candidates—Nrf2 activators and GSK-3 inhibitors. *Inflammation In Acute And Chronic Neurological And Psychiatric Diseases. Inflammopharmacology*. 2012;20:127–150.
- Lopresti AL, Drummond PD. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of 24 action. *Hum Psychopharmacol*. 2014;29:517–527.
- Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neurosci Biobehav Rev*. 2012;36:764–785.
- Mehri S, Abnous K, Khoei A, Mousavi SH, Shariaty VM, Hosseinzadeh H. Crocin reduced acrylamide-induced neurotoxicity in Wistar rat through inhibition of oxidative stress. *Iran J Basic Med Sci*. 2015;18:902–908.
- Oruc S, Gönül Y, Tunay K, et al. The antioxidant and antiapoptotic effects of crocin pretreatment on global cerebral ischemia reperfusion injury induced by four vessels occlusion in rats. *Life Sci*. 2016;1:79–86.
- Caballero-Ortega H, Pereda-Miranda R, Abdullaev FI. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. *Food Chem*. 2007;100:1126–1131.
- Lech K, Witowska-Jarosz J, Jarosz M. Saffron yellow: characterization of carotenoids by high performance liquid chromatography with electrospray mass spectrometric detection. *J Mass Spectrom*. 2009;44:1661–1667.
- Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965;16:144–158.
- McNair D, Lorr M, Droppleman L. *Profile of Mood States Manual*. San Diego: Educational and Industrial Testing Services; 1971 <http://dx.doi.org/10.1037/h0020742>.
- Edwards RR, Haythornthwaite J. Mood swings: variability in the use of the Profile of Mood States. *J Pain Symptom Manage*. 2004;28:534.
- Watson D, Clark LA. The PANAS-X manual for the positive and negative affect schedule – expanded form. *Unsure*. 1994;277:1–27.
- Lovibond SH, Lovibond PF. *Manual for the Depression Anxiety Stress Scales*. Sydney: Psychology Foundation; 1995.
- Buyssse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatr Res*. 1989;28:193–213.
- Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G\*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods*. 2009;41:1149–1160.
- Lopresti AL, Drummond PD. Efficacy of curcumin, and a saffron/curcumin combination for the treatment of major depression: a randomised, double-blind, placebo-controlled study. *J Affect Disord*. 2016;207:188–196.
- International Standard, Saffron Specification, ISO-3632-1980. Geneva: International Organization for Standardization; 1993.
- Wetherell J, Gatz M, Pedersen N. A longitudinal analysis of anxiety and depressive symptoms. *Psychol Aging*. 2001;16:187–195.



## Research paper

# affron<sup>®</sup>, a standardised extract from saffron (*Crocus sativus* L.) for the treatment of youth anxiety and depressive symptoms: A randomised, double-blind, placebo-controlled study

Adrian L. Lopresti<sup>a,\*</sup>, Peter D. Drummond<sup>a</sup>, Antonio M. Inarejos-García<sup>b</sup>, Marin Prodanov<sup>c</sup>

<sup>a</sup> School of Psychology and Exercise Science, Murdoch University, Perth, Western Australia 6150, Australia

<sup>b</sup> Pharmactive Biotech Products S.L. Parque Científico de Madrid, C/Faraday, 7, 28049 Madrid, Spain

<sup>c</sup> Instituto de Investigación en Ciencias de la Alimentación CIAL (CEI CSIC-UAM), C/ Nicolás Cabrera, 9, 28049 Madrid, Spain

## ARTICLE INFO

## Keywords:

Depression  
Anxiety  
Saffron extract  
Youth  
Teenager  
Clinical trial

## ABSTRACT

**Background:** Saffron has antidepressant and anxiolytic effects in adults with mild-to-moderate depression. However, this is the first study examining its mood-related effects in teenagers.

**Methods:** In this 8-week, randomised, double-blind, placebo-controlled study, youth aged 12–16 years, with mild-to-moderate anxiety or depressive symptoms were given tablets containing placebo or a saffron extract (affron<sup>®</sup>, 14 mg b.i.d). The youth and parent versions of the Revised Child Anxiety and Depression Scale (RCADS) were used as outcome measures.

**Results:** 80 participants were enrolled and 68 completed the study. Based on youth self-reports, affron<sup>®</sup> was associated with greater improvements in overall internalising symptoms ( $p = 0.049$ ), separation anxiety ( $p = 0.003$ ), social phobia ( $p = 0.023$ ), and depression ( $p = 0.016$ ). Total internalising scores decreased by an average of 33% compared to 17% in the placebo group ( $p = 0.029$ ). However, parental reports of improvements were inconsistent as mean improvements in RCADS scores were greater in the saffron group (40% vs 26%) ( $p = 0.026$ ), although no other significant differences were identified. affron<sup>®</sup> was well-tolerated and there was a trend of reduced headaches in participants on the active treatment.

**Limitations:** The use of a self-report instrument, limited study duration, single treatment dose, and non-clinical sample used in this study limit the generalisability of study findings.

**Conclusion:** The administration of a standardised saffron extract (affron<sup>®</sup>) for 8 weeks improved anxiety and depressive symptoms in youth with mild-to-moderate symptoms, at least from the perspective of the adolescent. However, these beneficial effects were inconsistently corroborated by parents.

## 1. Introduction

According to the World Health Organization, psychiatric disorders such as anxiety and depression are among the leading causes of disability worldwide in young people (World Health Organization, 2013). Between 15% and 20% of youth experience an anxiety or depressive disorder before the age of 18. The most common anxiety disorders in youth include separation anxiety disorder (8%), specific phobias (10%), and social phobia (7%). Depression has 1-year prevalence rates of 2.6% in children and 5.7% in adolescents (Beesdo et al., 2009; Costello et al., 2006; Merikangas et al., 2010).

Identifying effective treatments for children and adolescents are important as experiencing a mental health disorder during childhood is associated with a greater risk of suffering a psychiatric disorder during

adulthood (Copeland et al., 2009). Youth mental health disturbances are also associated with poor academic performance (Sijtsma et al., 2014), higher risk of unemployment in adulthood (Egan et al., 2016), increased medical burden (Pape et al., 2012), socialisation difficulties (Zwierzyńska et al., 2013), greater drug and alcohol use (Essau et al., 2014), and increased suicidality (Galaif et al., 2007). Currently, the primary treatments for anxiety and depression in paediatric populations comprise either psychological therapy or pharmaceutical interventions (Cox et al., 2014; James et al., 2015). While these can be effective for many youths, psychological therapy requires significant time commitment and engagement of youth can often be difficult. Pharmaceutical interventions may also be negatively perceived by youth and parents and can be associated with adverse effects (Meredith et al., 2009; Radovic et al., 2014).

\* Correspondence to: A: 38 Arnisdale Rd Duncraig WA 6023, Australia.

E-mail address: [a.lopresti@murdoch.edu.au](mailto:a.lopresti@murdoch.edu.au) (A.L. Lopresti).

Interest in herbal and nutraceutical treatments for mental health disorders is high and could represent a stand-alone or adjunct option for youth suffering from mood-related disturbances. Unfortunately, investigations into these natural agents for youth are limited, characterised by poor study designs (Lopresti, 2015). In adults, some efficacy has been established for omega-3 fatty acids, S-adenosyl-methionine and St John's Wort (Lakhan and Vieira, 2010; Ravindran and da Silva, 2013; Sarris et al., 2011). The latter is commonly used as a natural antidepressant for adults but is hampered by its interactions with many pharmaceutical medications (Soleymani et al., 2017). There is also a strong body of evidence supporting the antidepressant and anxiolytic effects of saffron in adults (Hausenblas et al., 2013; Lopresti and Drummond, 2014) which has the additional benefit of a strong safety and reduced drug interaction profile.

Saffron, a spice derived from the stigmas of the *Crocus sativus* flower, has several pharmacological actions including anti-inflammatory, anticancer, antioxidant, antiplatelet, and neuroprotective properties. It has traditionally been used as an analgesic and sedative, and as a treatment for gastrointestinal, respiratory and infectious diseases (Hossein-zadeh and Nassiri-Asl, 2013). As an antidepressant agent, saffron has been shown through several randomised-controlled trials to be more effective than placebo (Akhondzadeh et al., 2005; Moshiri et al., 2006) and of equivalent efficacy as the antidepressants fluoxetine (Akhondzadeh Basti et al., 2007; Noorbala et al., 2005; Shahmansouri et al., 2014), imipramine (Akhondzadeh et al., 2004), and citalopram (Ghajar et al., 2017) for the treatment of mild-to-moderate depression. Moreover, the antidepressant efficacy of saffron has been confirmed in two meta-analyses and systematic reviews (Hausenblas et al., 2013; Lopresti and Drummond, 2014). However, these studies comprise small populations and have mostly been conducted on Iranian adults. To date, there has also been no study examining the mood-enhancing efficacy of saffron in paediatric populations. Hence, the aim of this study was to examine the efficacy of a standardised saffron extract in youth aged 12–16 years presenting with mild-to-moderate anxiety and/or depressive symptoms. Given the positive findings in adult populations, it was hypothesised that 8-weeks of saffron supplementation would be associated with significant improvements in internalising symptoms (i.e., symptoms of anxiety, depression, and withdrawal).

## 2. Materials and methods

### 2.1. Study design

This was a parallel, 8-week, randomised, double-blind, placebo-controlled trial (Fig. 1). The trial protocol was approved by the Human Research Ethics Committee at Murdoch University, Western Australia, and was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. ACTRN12617000155392). Participants were recruited through social media advertisements and television/ radio interviews between March and June 2017, across Australia.

Participants were randomly and equally allocated into two groups (placebo or affron®) using a randomisation calculator ([www.randomization.com](http://www.randomization.com)). The randomisation structure comprised 8 randomly permuted blocks, containing 10 participants per block. Participant identification number was allocated according to the order of participant enrollment in the study. All capsules were packed in identical containers labelled by two intervention code numbers. Intervention codes were held by the sponsor and a university investigator not directly involved in study recruitment and data collection. Participants and study investigators were not informed of treatment group allocation until all questionnaire data was collected.

An a priori power analysis was undertaken to estimate the required sample size. In a meta-analysis by Hausenblas et al. (2013), an overall effect size of 1.62 was demonstrated in saffron/ placebo-controlled trials on adults with major depressive disorder. However, as there was no study on child populations, we conservatively predicted a smaller

effect size of 0.7. Assuming a power of 80% and a type one error rate (alpha) of 5%, the number of participants per group to find an effect was estimated as 34. After allowing for a 15% drop out rate, we aimed to recruit 40 participants per group.

### 2.2. Participants

**Inclusion criteria:** physically healthy, male and female participants aged 12–16 years, assessed as suffering from mild-to-moderate anxiety or depressive symptoms were included in the study. The severity of symptoms was assessed using the Revised Child Anxiety and Depression Scale (RCADS), youth and adult versions. Participants were included if a total or sub-scale raw score greater than the 60th percentile for respective age and gender was obtained on either the youth or parent measure, based on established normative data (Weiss and Chorpita, 2011). Both parent and youth were required to be fluent in English and to have consented to all pertinent aspects of the trial. Participants were also willing and able to swallow prescribed tablets.

**Exclusion criteria:** youth with a current or 12-month history of any psychiatric disorder other than mild-to-moderate depression or anxiety disorder, or who were currently receiving, or planning to receive a mental health intervention were ineligible to participate in the study. Participants were also excluded if a total or sub-scale raw score on the RCADS (youth or parent score) was greater than the 90th percentile for their respective age and gender, based on established normative data (Weiss and Chorpita, 2011). Youth who were engaging in self-harm behaviours and/or reported thoughts of suicide were also excluded from the study. Participants currently taking any pharmaceutical medication, apart from the occasional use (no more than fortnightly) of analgesics (e.g., ibuprofen, paracetamol), or who were currently taking saffron supplements and/or other herbal supplements were also excluded from the study. A current or history of a clinically significant chronic medical condition including cardiovascular disease, organic brain disorder, seizure, diabetes, use of illicit drugs, or any significant learning disability affecting educational achievement also resulted in exclusion from study participation.

Eligibility was initially assessed via the completion of an online questionnaire that screened for current medication use, suicidal ideation, self-harm behaviours, participation in psychological treatment, history of medical/ psychiatric disorders, and current learning disability. This questionnaire was primarily completed by a parent. If deemed as likely eligible, parents then participated in a phone interview with the primary investigator (a clinical psychologist with 20 years of clinical experience). Youths were also interviewed if uncertainty around psychiatric or medical history or consent to participate in the study remained. The phone interview comprised a structured series of questions examining the eligibility criteria specified above.

### 2.3. Interventions

Placebo and active tablets were identical in appearance, being matched for size, shape and coating colour. The active treatment, supplied by Pharmactive Biotech Products SL., contained 14 mg of a standardised saffron extract (affron®), derived from the stigmas of *Crocus sativus* L. and standardised to contain > 3.5% Lepticrosalides® a measure of bioactive compounds present in saffron, including safranal and crocin isomers.

The saffron stigmas were cultivated in Alborea (Albacete, Spain) and extracted in the factory of Pharmactive Biotech Products SL in Madrid (Spain) to produce affron® 3.5% Lepticrosalides®. The placebo tablets contained the same excipients as the active tablet (micro-crystalline cellulose and calcium hydrogen phosphate). All tablets were manufactured and packed in an Australian Therapeutic Goods Administration registered plant. Details of quantitative analyses of affron® and placebo are included in the supplementary file.

All participants were instructed to take one tablet, twice daily, with

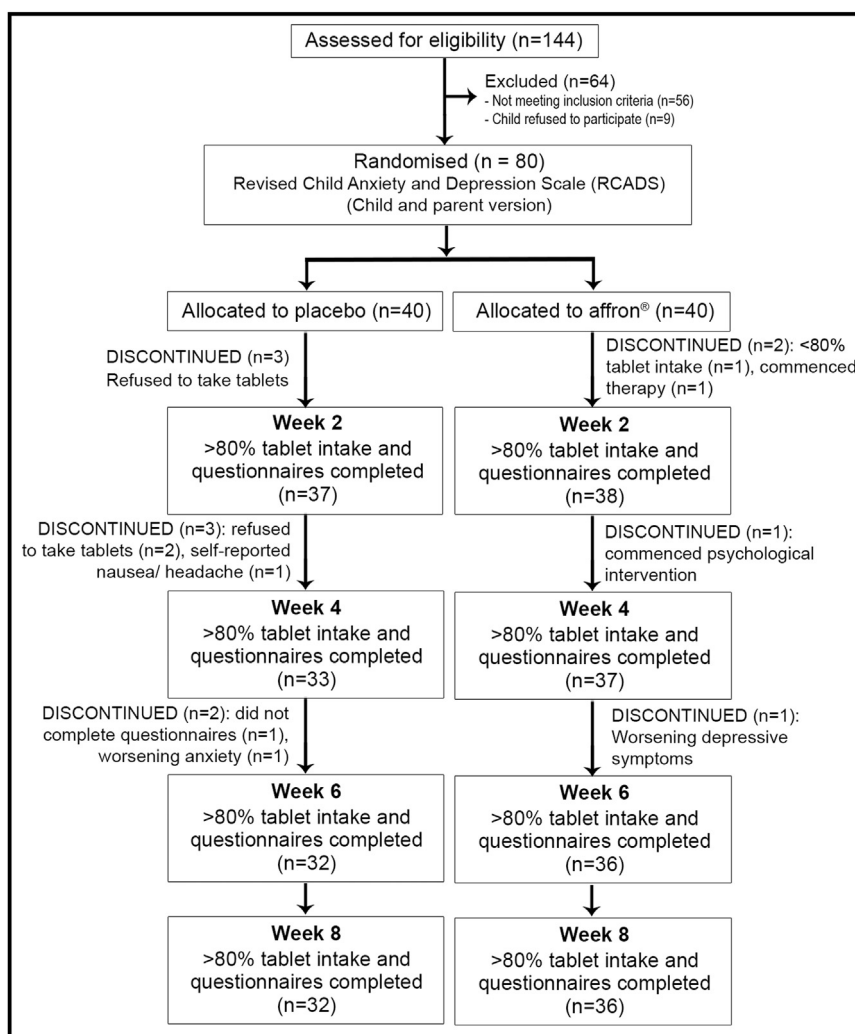


Fig. 1. Systematic illustration of study design.

or without food for 8 weeks. Medication compliance was measured by parent and child-reported pill count at weeks 2, 4, 6 and 8. Efficacy of participant treatment blinding was examined by asking participants and parent to predict group allocation (placebo, saffron or not sure) at the completion of the study.

## 2.4. Outcome measure

### 2.4.1. Revised Child Anxiety and Depression Scale (RCADS), youth and parent versions

The RCADS is a 47-item questionnaire with subscales including separation anxiety, social phobia, generalised anxiety, panic, obsessions/compulsions, and depression. It also yields a Total Anxiety Scale (sum of the 5 anxiety subscales) and a Total Internalising Scale (sum of all 6 subscales). Items are rated on a 4-point Likert-scale from 0 (“never”) to 3 (“always”). The RCADS comprises both a self-report youth version (primary outcome measure) and a parent-report version (secondary outcome measure). Both versions are identical in question content, number, and subscale classification. The RCADS has good psychometric properties with high internal consistency and convergent validity, and has been shown to accurately assess anxiety and depressive symptoms both in clinical and school-based youth (Chorpita et al., 2005; Ebesutani et al., 2010, 2011).

Change in youth scores, rather than parent scores, was selected as the primary outcome measure, as youth scores correlated more highly with other validated child mood measures such as the Child Depression

Inventory and the Revised Children's Manifest Anxiety Scale (Chorpita et al., 2005). This suggests that youth self-reports may provide a better reflection of outcome than the parental-reports, although assessing both was considered appropriate.

## 2.5. Statistical analysis

An independent samples T-test was used to compare demographic variables across the two treatment groups for continuous variables, and Pearson's Chi-square was used to compare categorical data. RCADS subscale scores (parent and youth versions) were analysed for time (baseline, week 2, week 4, week 6, and week 8) and treatment (saffron and placebo) effects using a mixed repeated-measures analysis of variance (ANOVA). To avoid problems of collinearity, total scores for anxiety and internalising symptoms were not included in ANOVA analysis. An independent samples *t*-test was conducted to compare between group change in internalising score over time (week 0 to week 8) and, if a significant multivariate interaction was found, to examine between group differences at varying time points (weeks 2, 4, 6, 8) for all RCADS measures.

There were no significant outliers in data as assessed by the visual inspection of Q-Q plots. Although questionnaire data were not normalised, repeated measures ANOVA was considered appropriate for statistical analyses as it is relatively robust to violations of normality (Tabachnick and Fidell, 2007). Where necessary, degrees of freedom were adjusted using the Greenhouse-Geisser approach to correct for

violations of the sphericity assumption.

To examine the clinical relevance from of the saffron treatment, a further analysis was undertaken to compare percentage of responders across treatment conditions (Snapinn and Jiang, 2007). Based on the most-commonly accepted definition, greater than a 50% reduction in RCADS total internalising score (sum of all subscale measures) was defined as a treatment response and was used for statistical comparisons across treatment conditions (Macher and Crocq, 2004; Nierenberg and DeCecco, 2001). Clinical relevance was also examined by calculating Cohen's d effect size for total and subscale scores of the RCADS. Data from participants were included in analyses if questionnaire data were obtained at week 2 (intention to treat, with last observation carried forward for missing values). For all the tests, statistical significance was set at  $P < 0.05$  (two-tailed). All data were analysed using SPSS (version 24; IBM, Armonk, NY).

### 3. Results

#### 3.1. Study population

##### 3.1.1. Baseline questionnaire and demographic information

144 people were screened for participation in the study and 80 met inclusion/ exclusion criteria and were enrolled to participate. 68 participants complied with all necessary treatment requirements (i.e., consumed  $> 80\%$  of capsules and completed all self-report inventories) over the 8-week trial. Eight dropped out of the placebo condition and 4 dropped out of the active treatment condition. There were no significant differences between the dropout rates across groups. Reasons for withdrawal included inconsistent tablet intake ( $n = 1$ ), refusal to take tablets ( $n = 5$ ), failure to complete questionnaires ( $n = 1$ ), worsening mental health ( $n = 2$ ), and commencement of psychological intervention ( $n = 2$ ). One participant withdrew from the study due to self-reported nausea/headaches believed to arise from tablet intake (placebo condition).

As shown in Table 1, there were no significant differences between the groups on any baseline mood questionnaire scores or demographic variables.

#### 3.2. Outcome measures

##### 3.2.1. RCADS – Youth Scores (Primary Outcome Measure)

Changes in RCADS sub-scale scores (youth version) across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 2 and Fig. 2. The multivariate test confirmed there was a significant time by group interaction ( $F_{24,1002} = 1.532$ ,  $p = 0.049$ ). Significant univariate time  $\times$  group interactions were found for the following sub-scale scores: Separation anxiety ( $F_{2,68,196} = 5.03$ ,  $p = 0.003$ ), social phobia ( $F_{2,92,213} = 3.27$ ,  $p = 0.023$ ), depression ( $F_{2,68,206} = 3.70$ ,  $p = 0.016$ ), and near significance for generalised anxiety ( $F_{2,79,204} = 2.48$ ,  $p = 0.067$ ). An independent samples T-test confirmed significant between group differences at varying time points for generalised anxiety, and obsessions/ compulsions. These are depicted by asterisks in Fig. 2.

As demonstrated in Fig. 2, percentage improvements in RCADS youth scores (from baseline to week 8) were greater in the saffron condition with an average reduction in total internalising symptoms of 33% compared to an average reduction of 17% in the placebo group ( $p = 0.029$ ). A Pearson's Chi-Square analysis also confirmed a greater percentage of treatment responders (defined as greater than 50% reduction in total internalising symptoms) in the saffron group compared to placebo, as evidenced by rates of 37% and 11% respectively ( $\chi^2(1) = 6.96$ ,  $p = 0.014$ , 95% CI [.012, .017], OR = 4.81) (Fig. 3). As depicted in Table 2, Cohen's d effect sizes ranged from a small effect size of 0.26 on the obsessions/compulsions subscale to a moderate effect size of over 0.6 on the total internalising score, and separation anxiety subscale score.

**Table 1**

Mean Baseline & Demographic Details of Participants.

		Placebo	Saffron	p-value
Sample Size (n)		40	40	
Gender	Female	62%	75%	0.228 <sup>a</sup>
	Male	38%	25%	
Age	Mean	13.93	14.08	0.642 <sup>b</sup>
	SE	0.24	0.21	
Weight	Mean	54.30	59.29	0.136 <sup>b</sup>
	SE	2.25	2.39	
YOUTH RCADS Baseline Scores				
Separation Anxiety	Mean	6.08	6.80	0.404 <sup>b</sup>
	SE	0.58	0.64	
Generalised Anxiety	Mean	8.15	8.45	0.674 <sup>b</sup>
	SE	0.50	0.50	
Panic	Mean	9.30	10.18	0.485 <sup>b</sup>
	SE	0.90	0.87	
Social Phobia	Mean	16.48	17.20	0.513 <sup>b</sup>
	SE	0.76	0.80	
Obsessions/Compulsions	Mean	6.10	5.20	0.171 <sup>b</sup>
	SE	0.43	0.49	
Depression	Mean	12.73	13.93	0.289 <sup>b</sup>
	SE	0.61	0.95	
PARENT RCADS Baseline Scores				
Separation Anxiety	Mean	6.65	6.23	0.606 <sup>b</sup>
	SE	0.59	0.57	
Generalised Anxiety	Mean	7.80	6.95	0.180 <sup>b</sup>
	SE	0.42	0.47	
Panic	Mean	6.73	7.63	0.342 <sup>b</sup>
	SE	0.62	0.71	
Social Phobia	Mean	17.08	16.55	0.650 <sup>b</sup>
	SE	0.81	0.82	
Obsessions/Compulsions	Mean	3.70	2.95	0.150 <sup>b</sup>
	SE	0.35	0.38	
Depression	Mean	12.45	12.23	0.817 <sup>b</sup>
	SE	0.54	0.80	

<sup>a</sup> Pearson Chi-Square test.

<sup>b</sup> Independent samples T-Test.

##### 3.2.2. RCADS – Parent Scores (Secondary Outcome Measure)

Changes in RCADS sub-scale scores (parent version) across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 2 and Fig. 2. The multivariate test indicated a non-significant time by group interaction ( $F_{24,1002} = 0.793$ ,  $p = 0.749$ ). However, an independent samples T-test confirmed significant between group differences at varying time points for generalised anxiety, social phobia, and obsessions/ compulsions. These are depicted by asterisks in Fig. 2. Mean improvements in RCADS parent scores were also significantly different in the saffron (40%) and placebo (26%) conditions ( $T_{73} = 2.27$ ;  $p = 0.026$ ). However, a Pearson's Chi-Square analysis revealed no differences in percentage of treatment responders in the saffron and placebo conditions (29% vs 24%) ( $\chi^2(1) = 0.205$ ,  $p = 0.424$ , 95% CI [.787, .802], OR = 1.27). As depicted in Table 2, Cohen's d effect sizes ranged from small effect size of 0.25 on the obsessions/compulsions subscale to a moderate effect size of over 0.57 on the panic subscale score.

##### 3.2.3. Adverse events

The majority of reported adverse events were of minor severity, although one participant in the placebo condition withdrew from the study due to complaints of nausea and stomach pain. There were no significant differences in reported adverse events between placebo and active drug treatment groups, although there was a trend suggesting an increased frequency of headaches in the placebo ( $n = 5$ ) compared to saffron group ( $n = 1$ ).

##### 3.2.4. Efficacy of participant blinding

To evaluate the efficacy of condition concealment over the study, parents and youth were asked at the completion of the study to predict condition allocation (i.e., placebo, saffron or uncertain). Efficacy of

**Table 2**  
Change in Self-Report Scores Over Time, By Treatment Condition.

		Placebo				p-value <sup>a</sup>	Saffron extract				p-value <sup>a</sup>	p-value <sup>b</sup>	Cohen's d effect size	
		Week					Week							
		0	2	4	6		8	0	2	4				6
Youth RCADS Scores														
Separation Anxiety	Mean	6.08	5.62	5.19	4.89	4.78	6.80	4.79	4.66	3.71	3.82	< 0.001	0.003	0.62
	SE	0.58	0.61	0.65	0.59	0.62	0.64	0.62	0.63	0.55	0.61			
Generalised Anxiety	Mean	8.15	7.54	7.51	6.89	6.97	8.45	6.71	5.89	5.89	5.68	< 0.001	0.067	0.44
	SE	0.50	0.59	0.56	0.61	0.66	0.51	0.60	0.55	0.56	0.62			
Panic	Mean	9.30	7.65	7.19	6.41	6.35	10.18	7.24	6.34	6.13	5.50	< 0.001	0.300	0.33
	SE	0.90	0.84	0.85	0.83	0.89	0.87	0.72	0.76	0.66	0.74			
Social Phobia	Mean	16.48	14.57	14.59	12.86	13.57	17.20	13.11	12.45	11.87	11.92	< 0.001	0.023	0.58
	SE	0.76	0.72	0.86	0.76	0.91	0.80	0.85	0.81	0.86	0.89			
Obsessions/ Compulsions	Mean	6.10	5.32	5.11	3.95	4.00	5.20	3.71	2.95	2.71	2.39	< 0.001	0.225	0.26
	SE	0.43	0.45	0.47	0.49	0.52	0.49	0.48	0.60	0.53	0.51			
Depression	Mean	12.73	12.11	12.14	11.92	11.92	13.93	10.97	10.45	10.45	10.55	< 0.001	0.016	0.60
	SE	0.61	0.78	0.83	0.96	0.95	0.95	0.82	0.89	0.91	1.04			
Total Anxiety Score	Mean	45.97	40.70	39.59	35.00	35.68	48.05	35.38	31.84	29.86	28.78	Not assessed <sup>c</sup>	Not assessed <sup>c</sup>	0.58
	SE	2.42	2.21	2.39	2.38	2.49	2.48	2.56	2.54	2.53	2.71			
Total Internalising Score	Mean	58.68	52.81	51.73	46.92	47.59	61.97	46.32	42.24	40.27	39.30	Not assessed <sup>c</sup>	Not assessed <sup>c</sup>	0.61
	SE	2.78	2.69	2.93	3.13	3.19	3.08	3.10	3.20	3.13	3.46			
Parent RCADS Scores														
Separation Anxiety	Mean	6.65	5.78	5.08	4.73	4.84	6.23	4.84	3.55	3.71	3.29	< 0.001	0.285	0.33
	SE	0.59	0.52	0.62	0.60	0.62	0.57	0.57	0.50	0.49	0.49			
Generalised Anxiety	Mean	7.80	6.65	6.14	5.51	6.00	6.95	5.95	4.68	4.79	4.26	< 0.001	0.243	0.30
	SE	0.42	0.38	0.46	0.42	0.47	0.47	0.55	0.48	0.44	0.38			
Panic	Mean	6.73	5.30	4.27	4.27	4.35	7.63	4.97	4.05	3.66	3.00	< 0.001	0.087	0.57
	SE	0.62	0.70	0.68	0.69	0.69	0.71	0.60	0.54	0.57	0.54			
Social Phobia	Mean	17.08	14.24	13.08	12.24	12.62	16.55	12.66	10.63	10.84	10.26	< 0.001	0.226	0.39
	SE	0.81	0.73	0.66	0.64	0.69	0.82	0.83	0.70	0.78	0.69			
Obsessions/ Compulsions	Mean	3.70	2.84	2.54	2.32	2.38	2.95	1.95	1.47	1.24	1.08	< 0.001	0.632	0.25
	SE	0.35	0.30	0.36	0.33	0.37	0.38	0.29	0.26	0.21	0.24			
Depression	Mean	12.45	10.03	9.70	8.70	9.08	12.23	9.55	8.13	7.79	7.63	< 0.001	0.320	0.29
	SE	0.54	0.56	0.63	0.76	0.87	0.80	0.74	0.70	0.81	0.69			
Total Anxiety Score	Mean	41.57	34.81	30.89	29.08	30.19	39.87	30.32	24.37	24.24	21.89	Not assessed <sup>c</sup>	Not assessed <sup>c</sup>	0.46
	SE	2.15	1.84	2.19	2.15	2.27	2.16	2.12	1.80	1.86	1.63			
Total Internalising Score	Mean	53.78	44.84	40.59	37.78	39.03	52.11	39.87	32.34	31.97	29.53	Not assessed <sup>c</sup>	Not assessed <sup>c</sup>	0.43
	SE	2.40	2.08	2.63	2.67	2.97	2.54	2.53	2.19	2.36	1.99			

<sup>a</sup> Repeated measures ANOVA time effects (week 0 to week 8).

<sup>b</sup> Repeated measures ANOVA time × group interaction.

<sup>c</sup> ANOVA not conducted to prevent problems of collinearity.

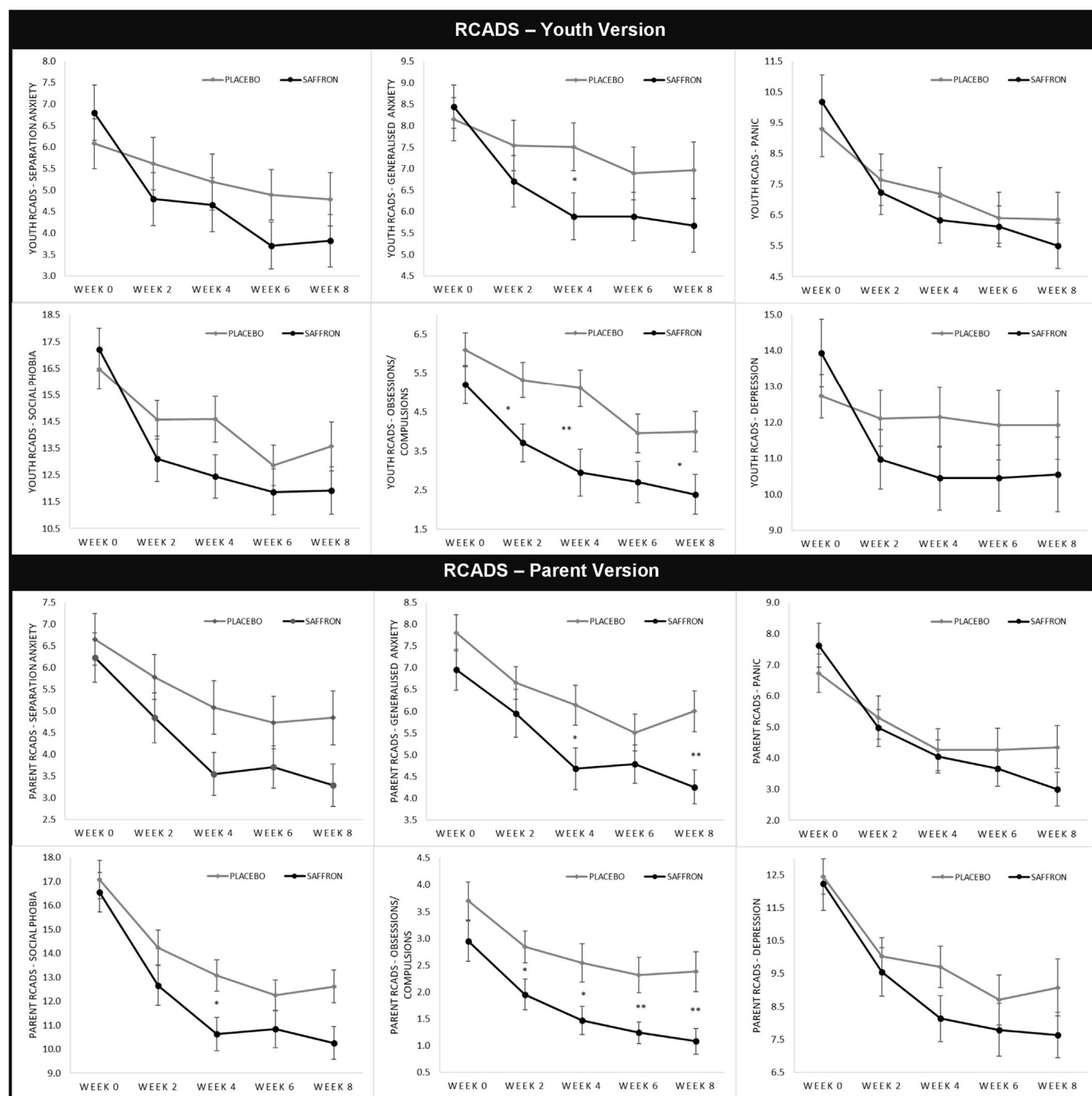


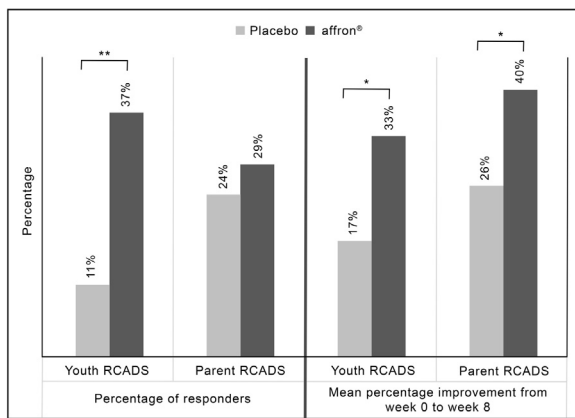
Fig. 2. Change in RCADS Youth & Parent raw scores over 8-week intervention. Vertical bars depict standard errors; Asterisks depict between group difference at specified time point (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

group concealment was high as only 41% of youths and 36% of parents correctly guessed treatment allocation. Approximately 35% of parents and youths were uncertain of treatment allocation, and the remaining incorrectly guessed group allocation.

#### 4. Discussion

The results of this study provide first evidence supporting the beneficial effects of a standardised saffron extract (affron®) for the treatment of anxiety and depressive symptoms in teenage youth. In several randomised-controlled studies, saffron has been shown to be an effective antidepressant and anxiolytic agent in adults with mild-to-moderate depression, with several studies confirming greater efficacy than

placebo (Akhondzadeh et al., 2005; Moshiri et al., 2006) and an equivalent efficacy to the antidepressants fluoxetine (Akhondzadeh Basti et al., 2007; Noorbala et al., 2005; Shahmansouri et al., 2014), imipramine (Akhondzadeh et al., 2004), and citalopram (Ghajar et al., 2017); however, prior to this study, there was no research examining its efficacy in youth (Hausenblas et al., 2013; Lopresti and Drummond, 2014). In this 8-week, randomised, double-blind, placebo-controlled study, saffron was effective in reducing overall internalising symptoms and exhibited greatest benefits on symptoms associated with separation anxiety, depression, and social phobia. However, these positive improvements were primarily reported by youth directly, as inconsistent benefits were noted by parents. Overall, from the adolescents' perspective, saffron treatment was associated with an average 33%



**Fig. 3.** Percentage of treatment responders (i.e., > 50% reduction in total internalising score) and mean percentage improvement in RCADS total internalising score (from baseline to week 8). Asterisks depict between significant group difference at specified time point, based on independent samples T-test (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

reduction in total internalising symptoms, compared to a 17% improvement in the placebo condition. Thirty-seven percent of youth also experienced a response from saffron treatment (defined as at least a 50% reduction in internalising symptoms), compared to only 11% of youth on placebo. From the parent's perspective, there was a statistically significant difference in overall internalising symptoms between the saffron and placebo conditions over time (average improvements of 40% and 26%, respectively); however, no difference in percentage of treatment responders and sub-scale scores were found.

Saffron administration was well-tolerated as there were no significant differences in reported adverse events over the 8-week intervention between saffron and placebo intake. In fact, there were trends to suggest reduced adverse effects in individuals taking saffron\*, particularly in relation to the frequency of headaches. However, this observation requires further investigation through larger-scale studies.

The exact mechanisms behind saffron's antidepressant and anxiolytic efficacy are uncertain, although several options are proposed. In adults, depression and anxiety is associated with several physiological disturbances. These include disturbances in monoaminergic activity particularly associated with serotonin and dopamine; dysregulation in hypothalamus-pituitary-adrenal (HPA) activity; chronic, low-grade inflammation; increased oxidative and nitrosative stress; and neuroprogression (Maes et al., 2011; Miller and Raison, 2015; Moylan et al., 2013). There is evidence to suggest that saffron has a positive effect on several of these mechanisms (Lopresti and Drummond, 2014). For example, saffron and its constituents, crocin, crocetin and safranal, are potent antioxidants and can increase antioxidant activity and lower oxidative stress, as demonstrated via animal and in vitro models (Boskabady and Farkhondeh, 2016; Broadhead et al., 2016; Samarghandian et al., 2017). Saffron also has anti-inflammatory properties (Poma et al., 2012) and may modulate HPA activity in animal stress models by reducing levels of plasma corticosterone (Halataei et al., 2011; Hooshmandi et al., 2011). Finally, there is preliminary evidence to suggest that saffron may also influence monoaminergic activity. Georgiadou et al. (2012) demonstrated that the administration of crocin lowered obsessive-like behaviours in rats exposed to the non-selective serotonin receptor agonist meta-Chlorophenylpiperazine. In another study, the administration of a saffron extract dose-dependently increased brain concentrations of dopamine, and at high doses increased glutamate levels; however, it had no effect on serotonin or norepinephrine concentrations (Ettehadi et al., 2013). The monoaminergic activity of pharmaceutical antidepressants such as serotonin reuptake inhibitors is well recognised; however, recent evidence suggests that they may also have antioxidant and anti-inflammatory effects (Jimenez-Fernandez et al., 2015; Wiedlocha et al., 2017). Saffron as an

adjuvant agent may be particularly pertinent as there are adult studies suggesting that lower premorbid antioxidant levels (Baek et al., 2016), and higher inflammation are associated with increased non-response from antidepressant treatment (Eller et al., 2008).

#### 4.1. Limitations and directions for future research

Youth recruited for this study comprised a population with a mild-to-moderate severity of anxiety and depressive symptoms. As no formal psychiatric assessment was undertaken, the efficacy of saffron in adolescents with a diagnosed mood disorder, or with severe depression or anxiety is unknown. Moreover, our participants were unmedicated and were not receiving any psychiatric intervention so the safety and efficacy of saffron as an adjuvant agent is uncertain. The efficacy of saffron was also only compared to placebo; therefore, its efficacy compared to standard treatments for children and adolescents such as psychological therapy or pharmacotherapy are also unknown and require investigation in future studies.

In this study, we used a saffron extract (saffron\*), derived from the stigmas of *Crocus sativus* L., and standardised to contain > 3.5% Lepticrosalides\* (a measure of bioactive compounds present in saffron, which includes safranal and crocin isomers). This standardisation is important as the compounds in saffron such as crocin, crocetin, and safranal are responsible for its antidepressant effects (Amin et al., 2015; Hosseinzadeh et al., 2004; Talaei et al., 2015; Vahdati Hassani et al., 2014). Moreover, as saffron is the most expensive spice in the world it can be subject to adulteration, further highlighting the importance of standardisation. The quality of saffron extracts may also be influenced by the geographic location it is grown in and cultivation practices used. It is therefore important that the antidepressant and anxiolytic effects of differing saffron extracts be examined for efficacy, safety, and potency.

In this study, we only examined the effects of a fixed 28 mg daily dose of saffron\* standardised by High Performance Liquid Chromatography to 3.5% Lepticrosalides\*. Thus, the influence of varying the initial dose and titrating levels for non-responders also requires investigation. In a recent study on healthy adults, a daily dose of 28 mg was found to have greater mood-enhancing effects than 22 mg over a 4-week period (as measured by the Profile of Mood Scale, Positive and Negative Affect Schedule, and Depression Anxiety Stress Scale) (Kell et al., 2017); however, efficacy in a younger population is uncertain.

The majority of studies investigating the mood-enhancing effects of saffron have been conducted over an 8-week period, although benefits have been identified in as little as 4 weeks (Kell et al., 2017). There is currently no study on the antidepressant and anxiolytic effects of saffron greater than 12 weeks, so the safety and efficacy of saffron over a longer duration requires examination. In one study on adults with Alzheimer's disease, the 12-month administration of 30 mg of saffron daily was well-tolerated (Farokhnia et al., 2014). In another study on adults with anxiety and depression, a higher dosage of 50 mg of saffron daily for 12 weeks was also well tolerated (Mazidi et al., 2016). The effects of both the acute and chronic administration of saffron, at varying doses, will be important to help identify optimal doses and treatment duration.

In this study, the beneficial effects of saffron were reported from youth self-reports. While some positive trends were seen in parental reports as evidenced by an overall greater symptomatic reduction in internalising symptoms over time, improvements from a parental perspective were inconsistent. This might reflect a weakness in the use of self-report questionnaires as a sole measure of treatment efficacy. Validation via clinician-rated measures may, therefore, be prudent in future studies. It is also plausible that the lack of significant findings from parental reports may reflect parent's own mental health. As a strong familial mental health association is common, the lower saffron to placebo differences as noted by parents may reflect a lack of change in parents own mental health, making it difficult for them to accurately

identify a positive change in their child. Moreover, it has been shown that scores on the youth version of the RCADS exhibited higher correlations than the parent version to other validated child mood measures such as the Child Depression Inventory and the Revised Children's Manifest Anxiety Scale (Chorpita et al., 2005). This suggests that youth self-reports may provide a better reflection of outcome than the parental-reports, although this is yet to be adequately investigated. To validate these findings in future studies, the examination of objective outcome measures including physiological markers such as cortisol and peripheral markers of inflammation and oxidative stress may also be important to support outcomes derived from questionnaire and clinician-rated instruments. Collection of these biological markers may also help to decipher saffron's mechanisms of action.

When compared to placebo-controlled studies on adult populations with depression, the magnitude of improvement after saffron intake in this study was substantially lower. Based on the youth version of the RCADS, a Cohen's *d* effect size of 0.61 was found for total internalising symptoms, while a smaller effect size of 0.43 was identified in parental reports. Although positive, the magnitude of these effects compares unfavourably to the mean effect size of 1.62 in the meta-analysis by Hausenblas (2013). In this meta-analysis, data from 5 adult studies on patients with diagnosed major depressive disorder was examined. The discrepancy in findings could be due to saffron having greater effects in adults compared to adolescents, possibly due to differing influences of environmental, psychological, and biological factors. However, it is also possible that larger effects occur in people with clearly defined and diagnosed major depressive disorder, rather than individuals suffering from 'anxiety and depressive symptoms.' The populations used in adult studies were recruited in Iran whereas we recruited an Australian adolescent population. Cultural differences may therefore account for the discrepancy in the magnitude of positive effects. Further studies are required to clarify factors that influence the magnitude of treatment outcomes.

Other study design limitations that need to be noted include the use of self-report pill count as a measure of medication adherence. In future studies, researcher assessment of medication adherence would be preferable. As all study participants were recruited through social media or television/ radio interviews, this may have led to self-referral bias; thus, further examination using alternate recruitment options may be helpful to validate our findings in wider populations. Finally, the participants in this study were aged between 12 and 16 years. This likely includes both pre- and post-pubertal adolescents and the efficacy of saffron may differ across these developmental stages.

In conclusion, this is the first study examining the efficacy of a standardised saffron extract for the treatment of anxiety and depressive symptoms in youth. Findings suggest that saffron extract administration over an 8-week period was beneficial in improving anxiety and depressive symptoms in youth presenting with mild-to-moderate symptoms, at least from the perspective of the adolescent. However, these beneficial effects were inconsistently corroborated by parental observations. Future investigation into the mood-enhancing effects of saffron in youth is therefore important to help substantiate these initial positive findings and overcome the limitations inherent in this current study design.

## Acknowledgements

This study was funded by Pharmactive Biotech Products SL. Pharmactive Biotech Products was not involved in the design of the research, analysis of data, or in the writing of the report.

The authors gratefully acknowledge Pharmactive Biotech Products SL Company for funding the project and supplying affron® and LIPA Pharmaceuticals for the preparation of the tablets.

## Role of the funding source

This study was funded by Pharmactive Biotech Products SL. Pharmactive Biotech Products was not involved in the design of the research, analysis of data, or in the writing of the report.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jad.2018.02.070>.

## References

- Akhondzadeh Basti, A., Moshiri, E., Noorbala, A.A., Jamshidi, A.H., Abbasi, S.H., Akhondzadeh, S., 2007. Comparison of petal of *Crocus sativus* L. and fluoxetine in the treatment of depressed outpatients: a pilot double-blind randomized trial. *Progress. Neuro-Psychopharmacol. Biol. Psychiatry* 31, 439–442.
- Akhondzadeh, S., Fallah-Pour, H., Afkham, K., Jamshidi, A.H., Khalighi-Cigaroudi, F., 2004. Comparison of *Crocus sativus* L. and imipramine in the treatment of mild to moderate depression: a pilot double-blind randomized trial [ISRCTN45683816]. *BMC Complement. Altern. Med.* 4, 12.
- Akhondzadeh, S., Tahmacebi-Pour, N., Noorbala, A.A., Amini, H., Fallah-Pour, H., Jamshidi, A.H., Khani, M., 2005. *Crocus sativus* L. in the treatment of mild to moderate depression: a double-blind, randomized and placebo-controlled trial. *Phytother. Res.* 19, 148–151.
- Amin, B., Nakhsaz, A., Hosseinzadeh, H., 2015. Evaluation of the antidepressant-like effects of acute and sub-acute administration of crocin and crocetin in mice. *Avicenna J. Phytomed.* 5, 458–468.
- Baek, S.E., Lee, G.J., Rhee, C.K., Rho, D.Y., Kim, D.H., Huh, S., Lee, S.K., 2016. Decreased total antioxidant activity in major depressive disorder patients non-responsive to antidepressant treatment. *Psychiatry Investig.* 13, 222–226.
- Beesdo, K., Knappe, S., Pine, D.S., 2009. Anxiety and anxiety disorders in children and adolescents: developmental issues and implications for DSM-V. *Psychiatr. Clin. North Am.* 32, 483–524.
- Boskabady, M.H., Farkhondeh, T., 2016. Antiinflammatory, antioxidant, and immunomodulatory effects of *Crocus sativus* L. and its main constituents. *Phytother. Res.* 30, 1072–1094.
- Broadhead, G.K., Chang, A., Grigg, J., McCluskey, P., 2016. Efficacy and safety of saffron supplementation: current clinical findings. *Crit. Rev. Food Sci. Nutr.* 56, 2767–2776.
- Chorpita, B.F., Moffitt, C.E., Gray, J., 2005. Psychometric properties of the revised child anxiety and depression scale in a clinical sample. *Behav. Res. Ther.* 43, 309–322.
- Copeland, W.E., Shanahan, L., Costello, E.J., Angold, A., 2009. Childhood and adolescent psychiatric disorders as predictors of young adult disorders. *Arch. Gen. Psychiatry* 66, 764–772.
- Costello, E., Erkanli, A., Angold, A., 2006. Is there an epidemic of child or adolescent depression? *J. Child Psychol. Psychiatry* 47, 1263–1271.
- Cox, G.R., Callahan, P., Churchill, R., Hunot, V., Merry, S.N., Parker, A.G., Hetrick, S.E., 2014. Psychological therapies versus antidepressant medication, alone and in combination for depression in children and adolescents. *Cochrane Database Syst. Rev.*
- Ebesutani, C., Bernstein, A., Nakamura, B.J., Chorpita, B.F., Weisz, J.R., Research Network on Youth Mental, H., 2010. A psychometric analysis of the revised child anxiety and depression scale—parent version in a clinical sample. *J. Abnorm. Child Psychol.* 38, 249–260.
- Ebesutani, C., Chorpita, B.F., Higa-McMillan, C.K., Nakamura, B.J., Regan, J., Lynch, R.E., 2011. A psychometric analysis of the revised child anxiety and depression scales—parent version in a school sample. *J. Abnorm. Child Psychol.* 39, 173–185.
- Egan, M., Daly, M., Delaney, L., 2016. Adolescent psychological distress, unemployment, and the great recession: evidence from the National Longitudinal Study of Youth 1997. *Soc. Sci. Med.* 156, 98–105.
- Eller, T., Vasar, V., Shlik, J., Maron, E., 2008. Pro-inflammatory cytokines and treatment response to escitalopram in major depressive disorder. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 32, 445–450.
- Essau, C.A., Lewinsohn, P.M., Olaya, B., Seeley, J.R., 2014. Anxiety disorders in adolescents and psychosocial outcomes at age 30. *J. Affect. Disord.* 163, 125–132.
- Ettehad, H., Mojabi, S.N., Ranjbaran, M., Shams, J., Sahraei, H., Hedayati, M., Asefi, F., 2013. Aqueous extract of saffron (*Crocus sativus*) increases brain dopamine and glutamate concentrations in rats. *J. Behav. Brain Sci.* 3, 315–319.
- Farokhnia, M., Shafiee Sabet, M., Iranpour, N., Gougol, A., Yekehtaz, H., Alimardani, R., Farsad, F., Kamalipour, M., Akhondzadeh, S., 2014. Comparing the efficacy and safety of *Crocus sativus* L. with memantine in patients with moderate to severe Alzheimer's disease: a double-blind randomized clinical trial. *Hum. Psychopharmacol.* 29, 351–359.
- Galaif, E.R., Sussman, S., Newcomb, M.D., Locke, T.F., 2007. Suicidality, depression, and alcohol use among adolescents: a review of empirical findings. *Int. J. Adolesc. Med. Health* 19, 27–35.
- Georgiadou, G., Tarantilis, P.A., Pitsikas, N., 2012. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of obsessive-compulsive disorder. *Neurosci. Lett.* 528, 27–30.
- Ghajar, A., Neishabouri, S.M., Velayati, N., Jahangard, L., Matinnia, N., Haghighi, M., Ghaleiha, A., Afarideh, M., Salimi, S., Meysamie, A., Akhondzadeh, S., 2017. *Crocus sativus* L. versus citalopram in the treatment of major depressive disorder with anxious distress: a double-blind, controlled clinical trial. *Pharmacopsychiatry* 50,

- 152–160.
- Halataei, B.A., Khosravi, M., Arbabian, S., Sahraei, H., Golmanesh, L., Zardooz, H., Jalili, C., Ghoshouni, H., 2011. Saffron (*Crocus sativus*) aqueous extract and its constituent crocin reduces stress-induced anorexia in mice. *Phytother. Res.* 25, 1833–1838.
- Hausenblas, H.A., Saha, D., Dubyak, P.J., Anton, S.D., 2013. Saffron (*Crocus sativus* L.) and major depressive disorder: a meta-analysis of randomized clinical trials. *J. Integr. Med.* 11, 377–383.
- Hooshmandi, Z., Rohani, A.H., Eidi, A., Fatahi, Z., Golmanesh, L., Sahraei, H., 2011. Reduction of metabolic and behavioral signs of acute stress in male Wistar rats by saffron water extract and its constituent safranal. *Pharm. Biol.* 49, 947–954.
- Hosseinizadeh, H., Karimi, G., Niapoor, M., 2004. Antidepressant effect of *Crocus sativus* L. stigma extracts and their constituents, crocin and safranal, in mice. *Acta Hort.* 650, 435–445.
- Hosseinizadeh, H., Nassiri-Asl, M., 2013. Avicenna's (Ibn Sina) the Canon of Medicine and saffron (*Crocus sativus*): a review. *Phytother. Res.* 27, 475–483.
- James, A.C., James, G., Cowdrey, F.A., Soler, A., Choke, A., 2015. Cognitive behavioural therapy for anxiety disorders in children and adolescents. *Cochrane Database Syst. Rev.*
- Jimenez-Fernandez, S., Gurpegui, M., Diaz-Atienza, F., Perez-Costillas, L., Gerstenberg, M., Correll, C.U., 2015. Oxidative stress and antioxidant parameters in patients with major depressive disorder compared to healthy controls before and after antidepressant treatment: results from a meta-analysis. *J. Clin. Psychiatry* 76, 1658–1667.
- Kell, G., Rao, A., Beccaria, G., Clayton, P., Inarejos-Garcia, A.M., Prodanov, M., 2017. affron(R) a novel saffron extract (*Crocus sativus* L.) improves mood in healthy adults over 4 weeks in a double-blind, parallel, randomized, placebo-controlled clinical trial. *Complement Ther. Med.* 33, 58–64.
- Lakhan, S.E., Vieira, K.F., 2010. Nutritional and herbal supplements for anxiety and anxiety-related disorders: systematic review. *Nutr. J.* 9, 42.
- Lopresti, A.L., 2015. A review of nutrient treatments for paediatric depression. *J. Affect. Disord.* 181, 24–32.
- Lopresti, A.L., Drummond, P.D., 2014. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. *Hum. Psychopharmacol.* 29, 517–527.
- Macher, J.P., Crocq, M.A., 2004. Treatment goals: response and nonresponse. *Dialog. Clin. Neurosci.* 6, 83–91.
- Maes, M., Gallecki, P., Chang, Y.S., Berk, M., 2011. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 676–692.
- Mazidi, M., Shemshian, M., Mousavi, S.H., Norouzy, A., Kermani, T., Moghiman, T., Sadeghi, A., Mokhber, N., Ghayour-Mobarhan, M., Ferns, G.A., 2016. A double-blind, randomized and placebo-controlled trial of Saffron (*Crocus sativus* L.) in the treatment of anxiety and depression. *J. Complement Integr. Med.* 13, 195–199.
- Meredith, L.S., Stein, B.D., Paddock, S.M., Jaycox, L.H., Quinn, V.P., Chandra, A., Burnam, A., 2009. Perceived barriers to treatment for adolescent depression. *Med. Care* 47, 677–685.
- Merikangas, K.R., He, J.P., Burstein, M., Swanson, S.A., Avenevoli, S., Cui, L., Benjet, C., Georgiades, K., Swendsen, J., 2010. Lifetime prevalence of mental disorders in U.S. adolescents: results from the National Comorbidity Survey Replication–Adolescent Supplement (NCS-A). *J. Am. Acad. Child Adolesc. Psychiatry* 49, 980–989.
- Miller, A.H., Raison, C.L., 2015. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* 16, 22–34.
- Moshiri, E., Basti, A.A., Noorbala, A.A., Jamshidi, A.H., Hesameddin Abbasi, S., Akhondzadeh, S., 2006. *Crocus sativus* L. (petal) in the treatment of mild-to-moderate depression: a double-blind, randomized and placebo-controlled trial. *Phytomed.: Int. J. Phytother. Phytopharm.* 13, 607–611.
- Moylan, S., Maes, M., Wray, N.R., Berk, M., 2013. The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications. *Mol. Psychiatry* 18, 595–606.
- Nierenberg, A.A., DeCecco, L.M., 2001. Definitions of antidepressant treatment response, remission, nonresponse, partial response, and other relevant outcomes: a focus on treatment-resistant depression. *J. Clin. Psychiatry* 62 (Suppl 16), 5–9.
- Noorbala, A.A., Akhondzadeh, S., Tahmacebi-Pour, N., Jamshidi, A.H., 2005. Hydro-alcoholic extract of *Crocus sativus* L. versus fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial. *J. Ethnopharmacol.* 97, 281–284.
- Pape, K., Bjørngaard, J.H., Holmen, T.L., Krokstad, S., 2012. The welfare burden of adolescent anxiety and depression: a prospective study of 7500 young Norwegians and their families: the HUNT study. *BMJ Open* 2.
- Poma, A., Fontecchio, G., Carlucci, G., Chichirico, G., 2012. Anti-inflammatory properties of drugs from saffron crocus. *anti-Inflamm. Anti-Allergy Agents Med. Chem.* 11, 37–51.
- Radovic, A., Farris, C., Reynolds, K., Reis, E.C., Miller, E., Stein, B.D., 2014. Primary care providers' beliefs about teen and parent barriers to depression care. *J. Dev. Behav. Pediatr.* 35, 534–538.
- Ravindran, A.V., da Silva, T.L., 2013. Complementary and alternative therapies as add-on to pharmacotherapy for mood and anxiety disorders: a systematic review. *J. Affect. Disord.* 150, 707–719.
- Samarghandian, S., Nezhad, M.A., Samini, F., Farkhondeh, T., 2017. The role of saffron in attenuating age-related oxidative damage in rat hippocampus. *Recent Pat. Food Nutr. Agric.*
- Sarris, J., Panossian, A., Schweitzer, I., Stough, C., Scholey, A., 2011. Herbal medicine for depression, anxiety and insomnia: a review of psychopharmacology and clinical evidence. *Eur. Neuropsychopharmacol.: J. Eur. Coll. Neuropsychopharmacol.* 21, 841–860.
- Shahmansouri, N., Farokhnia, M., Abbasi, S.H., Kassaian, S.E., Noorbala Tafti, A.A., Gougol, A., Yekehtaz, H., Forghani, S., Mahmoodian, M., Saroukhani, S., Arjmandi-Beglar, A., Akhondzadeh, S., 2014. A randomized, double-blind, clinical trial comparing the efficacy and safety of *Crocus sativus* L. with fluoxetine for improving mild to moderate depression in post percutaneous coronary intervention patients. *J. Affect. Disord.* 155, 216–222.
- Sijtsema, J.J., Verboom, C.E., Penninx, B.W., Verhulst, F.C., Ormel, J., 2014. Psychopathology and academic performance, social well-being, and social preference at school: the TRAILS study. *Child Psychiatry Hum. Dev.* 45, 273–284.
- Snappin, S.M., Jiang, Q., 2007. Responder analyses and the assessment of a clinically relevant treatment effect. *Trials* 8, 31.
- Soleymani, S., Bahramsoltani, R., Rahimi, R., Abdollahi, M., 2017. Clinical risks of St John's Wort (*Hypericum perforatum*) co-administration. *Expert Opin. Drug Metab. Toxicol.* 1–16.
- Tabachnick, B.G., Fidell, L.S., 2007. *Using multivariate statistics*, 5th ed. Allyn, Boston.
- Talaei, A., Hassanpour Moghadam, M., Sajadi Tabassi, S.A., Mohajeri, S.A., 2015. Crocin, the main active saffron constituent, as an adjunctive treatment in major depressive disorder: a randomized, double-blind, placebo-controlled, pilot clinical trial. *J. Affect. Disord.* 174, 51–56.
- Vahdati Hassani, F., Naseri, V., Razavi, B.M., Mehri, S., Abnous, K., Hosseinizadeh, H., 2014. Antidepressant effects of crocin and its effects on transcript and protein levels of CREB, BDNF, and VGF in rat hippocampus. *Daru* 22, 16.
- Weiss, D.C., Chorpita, B.F., 2011. *Revised Children's Anxiety and Depression Scale: User's Manual*. University of California, Los Angeles.
- Wiedlocha, M., Marcinowicz, P., Krupa, R., Janoska-Jazdzik, M., Janus, M., Debowska, W., Mosiolek, A., Waszkiewicz, N., Szulc, A., 2017. Effect of antidepressant treatment on peripheral inflammation markers - a meta-analysis. *Prog. Neuropsychopharmacol. Biol. Psychiatry*.
- World Health Organization, 2013. *Mental Health Action Plan 2013–2020*. WHO Press, World Health Organization.
- Zwierzyńska, K., Wolke, D., Lereya, T.S., 2013. Peer victimization in childhood and internalizing problems in adolescence: a prospective longitudinal study. *J. Abnorm. Child Psychol.* 41, 309–323.

## Article

# Antianhedonic and Antidepressant Effects of Affron<sup>®</sup>, a Standardized Saffron (*Crocus Sativus* L.) Extract

Laura Orio , Francisco Alen \*, Antonio Ballesta, Raquel Martin and Raquel Gomez de Heras

Department of Psychobiology and Behavioral Sciences Methods, Faculty of Psychology, Complutense University of Madrid, 28223 Madrid, Spain; lorio@psi.ucm.es (L.O.); aj.ballesta@ucm.es (A.B.); rmarti14@ucm.es (R.M.); rgomezhe@psi.ucm.es (R.G.d.H.)

\* Correspondence: falenfar@ucm.es

Academic Editor: Nikolaos Pitsikas

Received: 25 May 2020; Accepted: 7 July 2020; Published: 15 July 2020



**Abstract:** Anxiety and depression have high prevalence in the general population, affecting millions of people worldwide, but there is still a need for effective and safe treatments. Nutritional supplements have recently received a lot of attention, particularly saffron. Thus, several pre-clinical studies support a beneficial role for bioactive compounds, such as saffron, in anxiety and depression. Here we used an animal model of depression based on social isolation to assess the effects of affron<sup>®</sup>, a standardized saffron extract containing  $\geq 3.5\%$  of total bioactive compounds safranal and crocin isomers. Affron<sup>®</sup> was administered both through the oral and the intraperitoneal routes, and several tasks related to anxiety and depression, such as the elevated plus maze, the forced swimming test or the sucrose preference test, were assessed. These tasks model key features of depressive states and anxious states relating to fear, behavioral despair or anhedonia, the lack of motivation and/or pleasure from everyday activities, respectively. Animals receiving oral affron<sup>®</sup> displayed behaviors congruent with improvements in their anxious/depressive state, showing the enhanced consumption of a sweet solution, as well as an increase in certain escape responses in the forced swimming test. Our data support a beneficial role for oral saffron in anxious/depressive states.

**Keywords:** saffron; affron<sup>®</sup>; depression; anxiety; antioxidant

## 1. Introduction

Anxiety and depression are widely acknowledged as psychiatric disorders of global concern that could compromise human welfare [1], thus, the two conditions often co-exist; between 40% and 60% of patients with a common mental health disorder meet criteria for both anxiety and depression [2,3]. According to the World Health Organization data, there is high prevalence for depression and anxiety, affecting more than 300 million people worldwide, and they include the mixed depressive and anxiety disorder in their International Classification of Diseases [4]. These two conditions share common risk factors and many symptoms that can be regarded as existing on a spectrum of the disorder [5].

Mood alterations, including clinical depression, range from non-clinical low mood to major depression [6–8]. This low mood can include many of the symptoms characteristic of depression, such as sadness, crying, fatigue, pessimism, changes in appetite, changes in sleep patterns and anhedonia [6,7,9]. Currently there is no pharmacological treatment for low mood, and prescription medications are not only deemed inappropriate but also ineffective [10,11].

The conventional management of depression and anxiety disorders includes cognitive behavioral therapy, pharmacotherapy or electroconvulsive therapy [8,12]. However, despite the availability of numerous classes of drugs for the treatment, full remission of disease symptoms has remained elusive. Nevertheless, the clinical use of these drugs is limited by their characteristic side effects and poor tolerability profile [13]. Several natural compounds are being considered for their possible role in the

treatment of mood disorders, including saffron, St John's wort, tryptophan and omega-3 fatty acids, among others [14–16].

Saffron dried stigmas from *Crocus sativus* L. are conventionally used as a spice, textile dye or even as a perfume, due to its organoleptic properties. In addition, it is also widely known in traditional medicine for eye problems, headaches, genitourinary complications and other illnesses in different cultures [17–20]. The quality of saffron is determined by its secondary metabolites, such as picrocrocin, which is responsible for the bitter taste, safranal, which is related to saffron aroma, and crocins, which provide the color [21–23]. These compounds, mainly safranal and crocin isomers, as well as their metabolic derivate, crocetin, are related to antioxidant [24,25], anxiolytic [26,27], neuroprotective [28], anti-inflammatory [29–31] antidepressant [32–34] and anti-Alzheimer properties, which have been proven in several clinical trials [35].

As with most psychiatric disorders, the etiopathology of depression appears to be complex and multifactorial, including genetic, social and mood regulation mechanisms, among others. Alterations in neurotransmitter levels, including the abnormal regulation of cholinergic, catecholaminergic (noradrenergic or dopaminergic), glutamatergic and serotonergic (5-HT) neurotransmission have been observed in depressed patients [36]. Neuroendocrine dysregulation may also be a factor, with emphasis on three axes: hypothalamic–pituitary–adrenal and hypothalamic–pituitary–thyroid [37]. A molecular imbalance, characterized by increased levels of oxidative stress and low antioxidant status, has also been observed in patients with depression [38]. This would favor the appearance of immune responses and a pro-inflammatory environment, thus contributing to the pathology of depression [39]. Recently the relation between alterations in neuroplasticity and depression has received considerable attention. The term refers to the ability of the neural system to adapt to the internal and external stimuli and to respond adaptively to future stimuli. Neuroplasticity is of key significance in the brain's adaptation to stress, which may underlie various psychiatric disorders, such as depression, post-traumatic stress disorder, etc., and is the basis of the so-called neuroplasticity theory, which suggests a decrease in neuroplasticity in the hippocampus and prefrontal cortex in depressed patients, as well as a decrease in the concentration of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), in subjects with depression. According to this theory, antidepressants would elevate the concentration of neurotrophic factors and improve neuroplasticity in the hippocampus and PFC [40].

Kell and colleagues found positive effects in subjects self-reporting low mood but not diagnosed with depression or another mood disorder and who were otherwise healthy [33]. Additionally, there is also growing evidence supporting the antidepressant and anxiolytic effects of saffron in humans suffering from depression and anxiety. Thus, saffron extracts can relieve the severity of symptoms of depression and the effect of saffron extracts resemble those of tricyclic (TCA), Selective Serotonin Reuptake Inhibitors (SSRI) and Selective Noradrenaline Reuptake Inhibitors antidepressants in depressed patients [41,42]. Saffron extracts, when administered in combination with pharmacological antidepressants, were also shown to improve some scores related to depression, even in subjects who had been using the antidepressants with no improvement [43]. In this case, the affron<sup>®</sup> dose used was 14 mg b.i.d.

The active principles contained in saffron extracts, which account for the active antidepressants, are basically safranal and crocin isomers [44–46]. However, there is a wide variety of presentations that do not control the content of these bioactive molecules, making it very difficult to compare commercial brands in terms of pharmacological effectiveness. The safety and efficacy of safranal and crocin bioactive components have been described elsewhere, showing an exceptionally low toxicity, with an LD50 for normal cells of 20.7 g/kg [47]. In living animals, doses of the ethanolic extract up to 5 g/kg did not produce any demonstrable acute toxic effects in mice, and thus saffron is considered to have practically no acute toxic effect [48]. In our experiment, affron<sup>®</sup>, a standardized extract from Spanish *C. sativus* stigmas, was used.

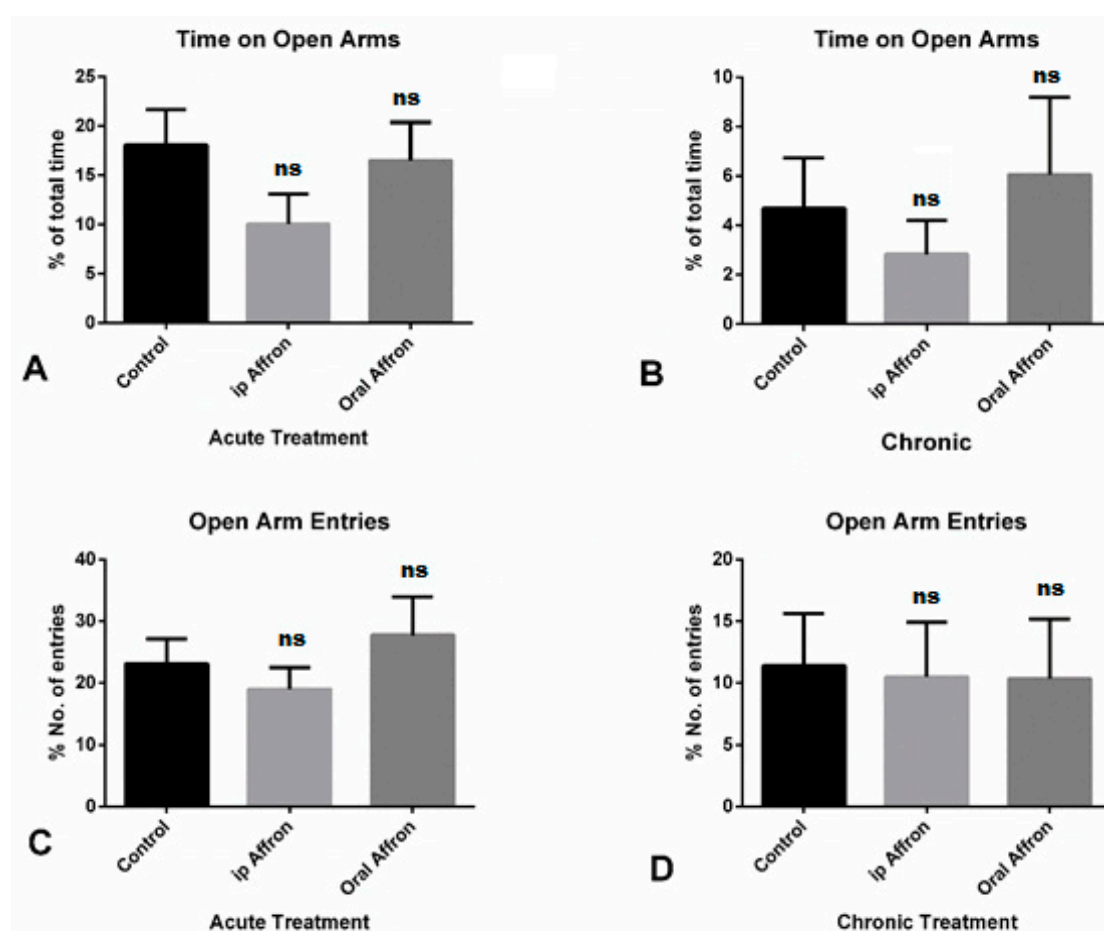
Although there are a number of favorable clinical studies regarding the effects of affron<sup>®</sup> on the modulation of the symptoms of depression and anxiety in humans [43,49–59], it is necessary to observe

its acute and chronic effects in more controlled animal studies to further explore and understand the mechanisms of action of affron® on mood, its safety and the role that the route of administration plays on those effects. The present study is intended to address this scientific need.

## 2. Results and Discussion

### 2.1. Effects of Affron® in the Elevated Plus Maze Test

Affron® was shown to be equally as ineffective either orally administered or by the intraperitoneal route. A one-way ANOVA showed that neither dose of affron® (200 mg/kg p.o. and 50 mg/kg ip) produced changes in any of the anxiety-related parameters (time spent in the open arms and number of entries into open arms of the EPM) ( $p > 0.05$ , not significant (ns)). Bonferroni's post hoc test confirmed that no statistically significant differences existed between treated and placebo groups ( $p > 0.05$ , ns) in acute and chronic treatments. No differences were observed for the time in open arms variable in any of the groups, as shown in Figure 1C,D. Values represent the mean  $\pm$  SEM ( $n = 10$  animals per group).

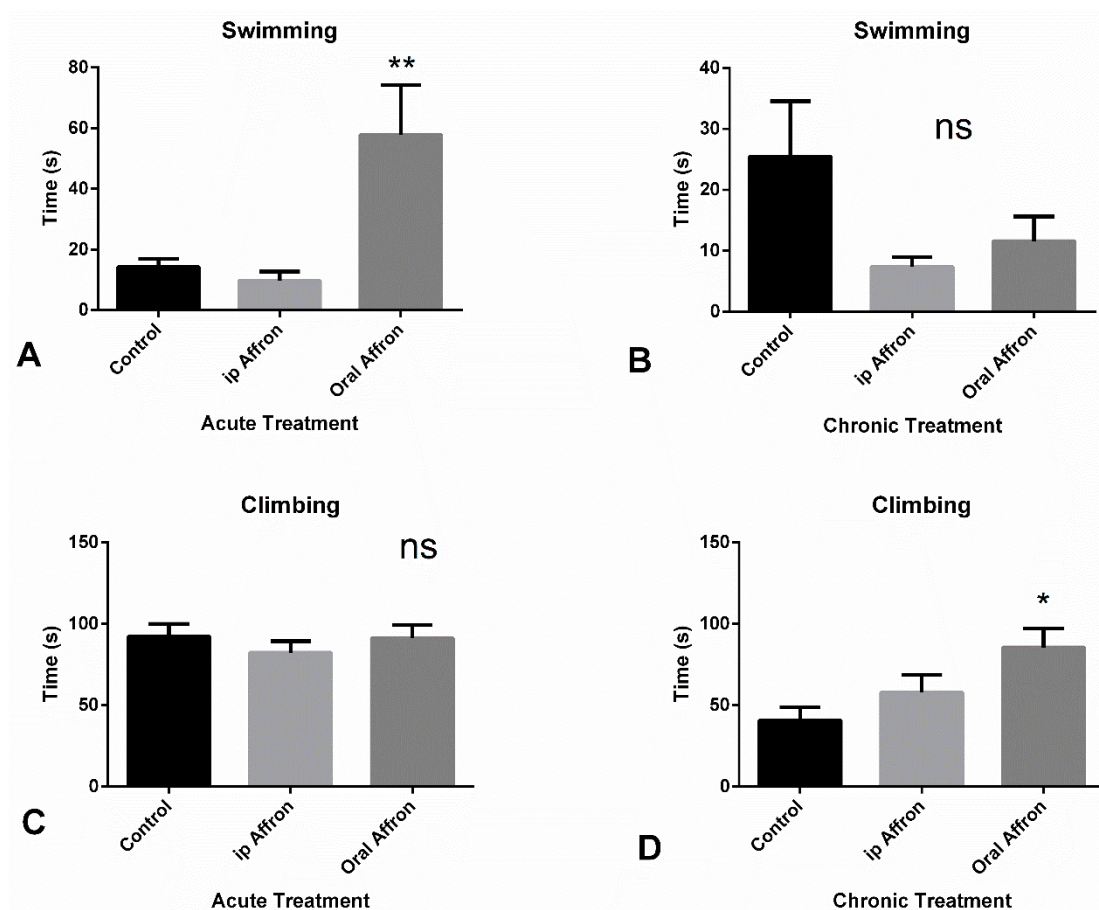


**Figure 1.** Effects of affron in the Elevated Plus Maze. (A,B): Percentage of time spent in open arms over the total in the acute and chronic treatment groups, respectively. (C,D): Percentage of entries in the open arms in the acute and chronic treatment, respectively. No significant effects were found between any of the groups in any condition (ns). The tests were administered on day one of treatment and on day 21 for the chronic group, 30 min after drug administration.

### 2.2. Effects of Affron® in the Forced Swimming Test

A one-way ANOVA revealed that affron® (200 mg/kg, oral) administered 30 min before the FST significantly increased swimming time [ $F(2,27) = 7.37$ ,  $p < 0.01$ ], as shown in Figure 2A] but had no impact on climbing time or immobility time (ns) (data not shown). The post-hoc Bonferroni's test

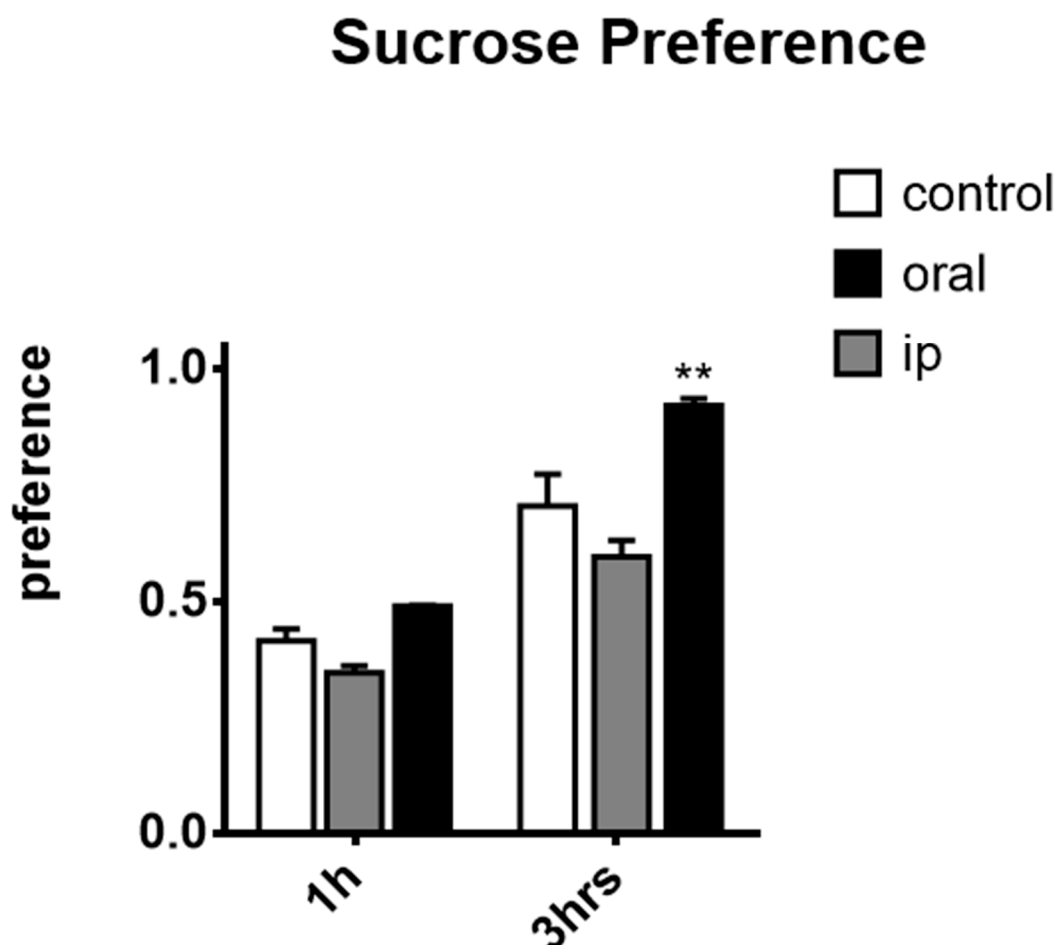
showed that affron<sup>®</sup>, given at a dose of 200 mg/kg significantly increased the time of swimming in the oral group compared to the control and ip groups ( $p < 0.05$  and  $p < 0.01$ , respectively). Concerning the antidepressant-like activity of affron<sup>®</sup>, after 20 days of treatment, an increase in climbing time was observed in the oral group [ $F(2,27) = 4.91$ ,  $p < 0.05$ ], as shown in Figure 2D. A one-way ANOVA did not find any statistically significant difference in the rest of the variables (ns); however, affron<sup>®</sup> treatment, both oral and intraperitoneal, tended to decrease immobility time compared to the control group, which led to a possible antidepressant effect of this compound, as shown in Figure 2D. The post hoc test revealed a significant increase in climbing time in the oral group compared with the control group ( $p < 0.05$ ), but there were no significant differences in the remaining groups (ns).



**Figure 2.** (A,B): Average time spent in immobility or swimming in the Porsolt test in animals receiving either an acute or a chronic treatment with affron through the intraperitoneal (gray column) or the oral (black column) route, respectively (\*\*  $p < 0.01$ ). (C,D): Average time climbing in the acute and chronic treatments, respectively (\*  $p < 0.05$ ; ns = Non Significant). The tests were administered on day one of treatment and on day 21 for the chronic group, 40 min after drug administration.

### 2.3. Effects of affron<sup>®</sup> in the Sucrose Preference Test

Repeated-measures two-way ANOVA found an overall interaction between time and treatments [ $F(6,78) = 12.30$ ,  $p < 0.0001$ ] and main effects of time [ $F(3,78) = 318.7$ ,  $p < 0.0001$ ] and treatment [ $F(2,26) = 19.20$ ,  $p < 0.0001$ ]. Subsequent analysis revealed that, as reflected in Figure 3, after an hour, a significant increase in the sucrose preference was found in the oral group with respect to the intraperitoneal group ( $p < 0.05$ ), but there were no differences between the oral and intraperitoneal groups compared to the control group (ns). Statistically significant differences were observed in the 3 h measure in sucrose consumption between the oral group with respect to the control and intraperitoneal groups ( $p < 0.0001$ ), but there was no difference between the control and the intraperitoneal groups (ns).



**Figure 3.** Sucrose preference test. Sucrose preference was calculated as the quantity of sucrose solution drunk/total fluid intake and is considered an index of the motivational state of the animal, ( $n = 10$  per group) represented as mean  $\pm$  SEM. Repeated-measures two-way ANOVA with Bonferroni post-hoc test: \*\*  $p < 0.001$ ; differences between the oral group and the control group. The tests were administered on day one of treatment and on day 21 for the chronic group, 50 min after drug administration.

The results showed that the oral administration of affron<sup>®</sup> may ameliorate some depressive-like behaviors both acutely and after long-term treatment. Interestingly, no effects were observed with the intraperitoneal administration. Additionally, repeated oral administration reduced anhedonic behavior, assessed in the sucrose preference test—an effect that was not observed under ip administration. Overall, these results are congruent with reports describing the beneficial effects of saffron extract consumption in patients suffering from anxiety or depression [41,42].

Considering the fact that drugs used to treat mental disorders are best studied in models of the disease, we used a rat model of depression, based on isolation [60]. Thus, individual housing has been shown to induce depressive-like behavior [61]. For that, animals were individually housed from arrival at the facilities until the end of the experiments, which allowed us to monitor any possible improvement or amelioration in behaviors related to anxiety, depression or anhedonia after affron<sup>®</sup> supplementation.

Regarding the anxiolytic effects in the elevated plus maze, no significant differences were found between any of the groups in our study. In other studies, higher doses of saffron ip (56 and 80 mg/kg) showed anxiolytic effects in the EPM with a single dose, and a dose-dependent effect that decreased with doses up to 300 mg/kg [62]. It is possible that the lower dose used in our study limited the expression of the anxiolytic potential of affron<sup>®</sup>. It is also possible that differences in the source of plant material could explain the different findings in our study.

Data on the forced-swimming test assessing depressive-like behavior were clearer. On the one hand, affron<sup>®</sup> did not affect immobility time or latency to the immobility period. However, a clear effect in the escape behavior (swimming and climbing) with the oral administration of affron<sup>®</sup> can be observed. Our data are interpreted in line with the presence of antidepressant effect, since the animals clearly show more motivation to fight against the adverse conditions of being exposed to the water environment. These data appear relevant as they highlight the ability of saffron extracts to acutely improve depressive-like behavior.

To our knowledge, the antianhedonic effects of affron<sup>®</sup> in the sucrose preference test have not been previously reported in animal studies. This is the clearest effect found with affron<sup>®</sup> in the study. Oral dose was able to increase the preference for the sweet drink, which is indicative of a positive motivational state. Again, in this situation, oral administration was effective instead of intraperitoneal injection. Interestingly, intraperitoneal administration may even induce the opposite effect (i.e., anhedonia, indicative of desensitization of the brain reward system). It is worth mentioning that the antianhedonic response, measured by sucrose preference, differs in response to antidepressants with different mechanisms of action, such as SSRI and TCA [63].

The fact that the oral administration of affron<sup>®</sup> was the effective route to observe the positive effects of this compound, and not ip administration, may be related to the fact that the active form of crocin isomers from saffron extract is crocetin, which is formed in the intestinal tract by glycosidases of enterocytes [64–66]. Thus, the saffron extract must, apparently, be taken orally to induce its antidepressant effect. In any case, oral administration is the preferred route for any potential nutraceutical since the majority of nutraceuticals are intended for this route of administration.

The neurobiological mechanisms that explain affron's antianhedonic effect and possible antidepressant effect have not been directly assessed yet. Regulation of mood was initially attributed to downregulations in monoamines, such as dopamine, serotonin or noradrenaline [67–69]. Whereas anhedonia is associated with low levels of dopamine, anxiety and depression appear to be more associated with the decreased activity of both serotonin and dopamine. The clear actions of affron<sup>®</sup> in the amelioration of anhedonia suggest a more potent action in the regulation of dopamine than serotonin or other monoamines. Indeed, regarding dopamine modulation, it has been proven that saffron affected monoamine oxidases MAO-A and MAO-B in the brain [68], and the administration of *C. sativus* and its constituents increased brain dopamine levels in a dose-dependent manner [68]. These effects of *C. Sativus* in dopamine, together with the modulation of the excitatory amino acid, glutamate, and interactions with the opioid system have been reported to reduce withdrawal syndrome and may contribute to the amelioration of behavioral symptoms observed here [68].

However, the regulation of mood has been more recently associated with other factors, such as the alteration of neurotrophic factors, dysregulation in the hypothalamus–hypophysis–adrenal axis (HPA), low-grade inflammation or increased oxidative stress [70–72]. Certain evidence suggests that saffron regulates some of these mechanisms [73]. Indeed, saffron and its constituents, crocin and safranal, as well as crocetin, are potent antioxidants that can reduce oxidative stress, as demonstrated in animal models [74–76]. Its anti-inflammatory properties [77,78] and the modulation of the activity HPA in animal models of stress (i.e., by reducing levels of plasma corticosterone [79,80]) have also been proven. Future studies are needed to explore the specific actions of different doses of affron<sup>®</sup> in these processes.

### 3. Material and Methods

#### 3.1. Materials

Affron<sup>®</sup> is a patented compound (ES2573542A1) and has been previously characterized [33]. Samples of saffron stigma extracts marketed under the brand name affron<sup>®</sup> were provided by Pharmactive Biotech Products SL, standardized to ≥3.5% Lepticosalides<sup>®</sup>, a term which characterizes the sum of the bioactive compounds safranal and crocin isomers, analyzed by HPLC [33]. The compound was presented in powder form and stored in darkness until the experiment was performed.

### 3.2. Animals

A total of 30 adult male Wistar rats (ENVIGO, Barcelona, Spain) weighting 300–350 g at the beginning of the experiments were kept under a 12-h light/dark cycle (lights off at 12:00 p.m.) in conditions of constant temperature ( $23 \pm 1$  °C). Standard food and tap water were available ad libitum at the home-cage outside the schedules assigned to experimental manipulation.

All experimental protocols adhered to the guidelines of the Animal Welfare Committee of the Universidad Complutense of Madrid, following European legislation (European Directive 2010/63/UE).

### 3.3. Experimental Design

Animals had an acclimation period of one week, after which they were habituated to manipulation for several days before the experiment started. Upon arrival at the facilities, animals were housed in isolation for the duration of the study, according to a social isolation model of anxious/depressive-like behavior [81]. Animals' weights were recorded every two days during the experiment. Rats were randomly assigned to one of the three of the following experimental groups: Oral affron® (Oral), Intraperitoneally administered affron® (ip) and Vehicle (10 rats per group). In the Oral group, affron® was dissolved in their tap water and the rats were monitored to ensure they consumed an adequate dose during the first hours of the morning (See Figure 4 for a schematic representation of the experimental schedule used).

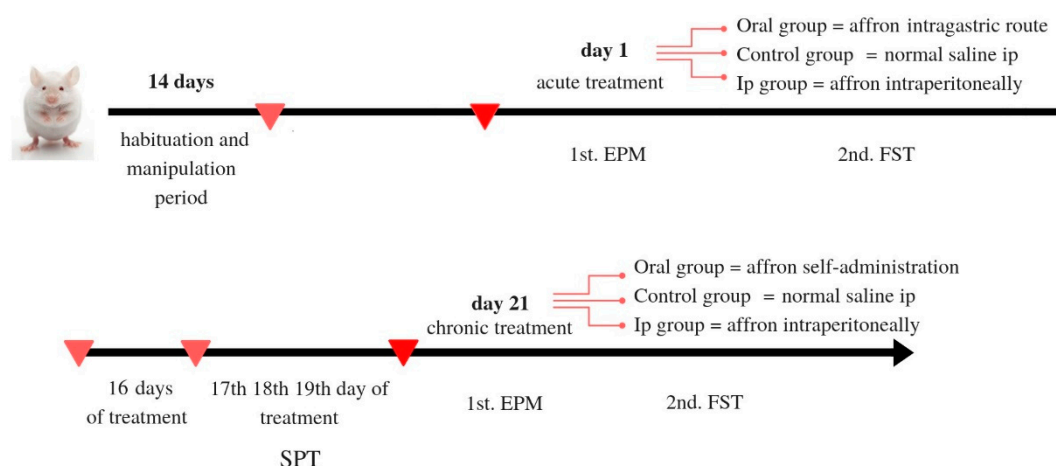


Figure 4. Schematic representation of the treatment schedule.

Behavioral tests were performed on the first day of the experiment in order to assess the acute effects of the treatment, and also after the chronic treatment. The tests used were the elevated plus maze (EPM), the Forced swim test (FST) and the sucrose preference test (SPT).

#### 3.3.1. Treatment

The animals received a single dose of affron® dissolved in distilled water at the beginning of the experiment. Rats in the oral group received 200 mg/kg affron®, which was delivered via the use of an intra-gastric cannula in a volume of 2 mL/kg. The control group was treated with 0.9% saline solution via intraperitoneal injection (ip) in a volume of 2 mL/kg. The ip group received 50 mg/kg affron® dissolved in saline. Thirty minutes after each administration, the animals were tested on the elevated plus maze (EPM) and 30 min later they were assessed in the forced swim test (FST). For the next 20 days, rats in the oral group had free access to 200 mg/kg affron dissolved in their drinking water until they consumed the whole daily dose, which took place in less than 4 h, after which tap water was reintroduced. The rest of the animals received their daily dose via ip. The animals underwent the sucrose preference test on the 17th day of the experiment 30 min after affron® administration. Likewise, on the 21st day of the experiment, the animals were assessed at the elevated plus maze,

and 30 min later, at the forced swimming test. All treatments were prepared and administered daily, and all tests took place between 9:00 and 14:00.

### 3.3.2. Anxiety and Depression-Like Behavior

Several different models were used for the assessment of anxiety- and depression-related behaviors in rodents [82]. The Forced Swim Test and the Sucrose Preference Test were employed to assess any depressive-like behavioral responses, being some of the most commonly used tests to assess this kind of behavior in animal models [83,84]. The elevated plus maze test was chosen to assess anxiety-related behavior. In addition, in the chronic study, sucrose preference was used as a complementary test for anhedonia [63,85]. Anhedonia is defined as the inability to experience pleasure from rewarding or enjoyable activities and constitutes a core symptom of depression in humans [86]. The order of the tests was chosen to minimize any possible interference. Considering the relative distance and difference in environments between the animal facilities and the testing room, 10 min of acclimation were granted prior to the behavioral tests.

#### 3.3.3. Elevated Plus Maze Test

The elevated plus maze (EPM) is based upon the conflict between an innate aversion to open spaces and a tendency to explore new environments (Suo et al., 2013). The apparatus consisted of two open arms (50 × 10 cm), two closed arms (50 × 10 × 20 cm) and a central platform (10 × 10 cm), which raised to a height of 50 cm. The maze was placed in the center of a quiet room and testing was performed under dim light. Each animal was gently placed on the central platform facing one of the closed arms and allowed to explore the maze for 5 min. The light of the test room was adjusted at 350 lux at the center of the maze. Then, the animals were removed and the EPM was cleaned with 30% ethanol between each test to prevent interference resulting from any residual odors of the previous rat. Data were registered automatically using the Mazesoft software. The analysis of exploratory activity in rats included four parameters: number of entries into open arms; number of entries into closed arms; time spent in open arms; time spent in closed arms.

#### 3.3.4. Forced Swim Test (Porsolt Test)

The forced swimming test (FST) was originally introduced in 1977 by Porsolt and has since become a standard for the evaluation of antidepressant drugs [87]. In preparation, 24 h prior to the test, animals were placed in the testing apparatus for 10 min in order to become familiar with the testing environment and to minimize novelty effects [82,88]. Briefly, the rats were individually placed in a transparent methacrylate cylinder (height 50 cm, diameter 30 cm) filled with water (23 ± 1 °C) to a height of 40 cm (a modified version of the FST was used to increase water depth to 40 cm, so the rats were unable to touch the bottom of the tank). Water was replaced for each test. Following each test session, rats were dried using cotton towels and returned to their home cages. All sessions were recorded with a video camera (SONY HDR-CX115, New York, NY, USA). Afterwards, four behavioral categories were quantified using the freeware for behavioral quantification, Raton Time 1.0 (Fixma SL, Valencia, Spain), including immobility latency (latency to immobility), immobility (rat floating in the water with only movements necessary to keep the nose above water), swimming (active horizontal movements around the cylinder) and climbing (upward-directed movement of the forepaws, usually directed against the walls). Animals underwent the test 30 min after the elevated plus maze.

#### 3.3.5. Sucrose Preference Test

The sucrose preference test (SPT) is a reward-related test commonly used as an indicator of anhedonia [89]. No previous food or water deprivation was applied before the test. During the adaptation period, the animals were presented in their home cages with two bottles of the type used for the test, in order to habituate them to testing conditions. The rats were allowed simultaneous access to two identical drinking bottles that contained a 1% sucrose solution for 3 h. The sucrose solution was

prepared daily before the experiment and kept at 4 °C for no longer than 24 h. The position of the drinking bottles was changed after each measurement to exclude the effect of place preference [89,90]. The consumption of water and sucrose solution was calculated by weighing each bottle before, during and after the test. Sucrose preference was calculated as the quantity of sucrose solution drunk/total fluid intake [2]. The sucrose preference test was performed on the 17th day of treatment in order to avoid interferences with the other tests.

### 3.4. Statistical Analyses

The data are expressed as mean  $\pm$  standard error of mean (SEM). Normality data were assessed by D'Agostino and Pearson test. Data were analyzed using GraphPad Prism version 6.0. In order to detect significant differences among the experimental groups, depending on the behavioral test, either one-way analysis of variance with one factor (treatment) or two-way ANOVA comparing two factors—treatment (oral, intraperitoneal, control) and time—was used. Data of the behavioral tests were analyzed as dependent variables, and the Bonferroni post-hoc test for multiple comparisons was used when appropriate. Values of  $p \leq 0.05$  were considered statistically significant.

## 4. Conclusions

In conclusion, this study provides evidence of the antianhedonic, and mild antidepressant actions of a 50 mg/kg acute ip dose and a 200 mg/kg oral dose of affron<sup>®</sup>, a standardized saffron extract, when administered acutely or repeatedly, orally. Future studies are required to ascertain the specific mechanisms of this action. Anyhow, these results open new fields for the possible application of affron<sup>®</sup> to prevent negative emotional states or as a co-adjuvant therapy in the treatment of depression.

**Author Contributions:** L.O., R.G.d.H. and F.A.: Experimental design, supervision and writing. A.B. and R.M.: experimental procedures and data analyses. All authors have read and agree to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** The authors gratefully acknowledge the Pharmactive Biotech Products, SL Company, for funding the project and supplying affron<sup>®</sup>. We also thank Alberto Espinel, Paula Almodóvar and Daniel González Hedström for their help and support.

**Conflicts of Interest:** Other than the funding of this work by Pharmactive Biotech Products SL, the authors declare no conflict of interest.

## References

1. Fajemiroye, J.O.; Galdino, P.M.; Florentino, I.F.; Da Rocha, F.F.; Ghedini, P.C.; Polepally, P.R.; Zjawiony, J.K.; Costa, E.A. Plurality of anxiety and depression alteration mechanism by oleanolic acid. *J. Psychopharmacol.* **2014**, *28*, 923–934. [CrossRef] [PubMed]
2. Kessler, R.C.; Berglund, P.; Demler, O.; Jin, R.; Merikangas, K.R.; Walters, E.E. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* **2005**, *62*, 593–602. [CrossRef] [PubMed]
3. McManus, S.; Meltzer, H.; Brugha, T.; Bebbington, P.; Jenkins, R. *Adult Psychiatric Morbidity in England, 2007. Results of a Household Survey*; The Health and Social Care Information Centre; National Centre for Social Research: Leeds, UK, 2009; p. 12.
4. World Health Organization. International Classification of Diseases for Mortality and Morbidity Statistics (11th Revision). 2018. Available online: <https://icd.who.int/browse11/l-m/en> (accessed on 1 July 2020).
5. Newby, J.M.; McKinnon, A.; Kuyken, W.; Gilbody, S.; Dalgleish, T. Systematic review and meta-analysis of transdiagnostic psychological treatments for anxiety and depressive disorders in adulthood. *Clin. Psychol. Rev.* **2015**, *40*, 91–110. [CrossRef] [PubMed]
6. Keller, M.C.; Nesse, R.M. Is low mood an adaptation? Evidence for subtypes with symptoms that match precipitants. *J. Affect. Disord.* **2005**, *86*, 27–35. [CrossRef] [PubMed]

7. Nettle, D. Understanding of evolution may be improved by thinking about people. *Evol. Psychol.* **2010**, *8*, 205–228. [[CrossRef](#)]
8. Gelenberg, A.J.; Freeman, M.P.; Markowitz, J.C.; Rosenbaum, J.F.; Thase, M.E.; Trivedi, M.H.; Van Rhoads, R.S.; Reus, V.I.; DePaulo, J.R., Jr.; Fawcett, J.A. Practice guideline for the treatment of patients with major depressive disorder third edition. *Am. J. Psychiatry* **2010**, *167*.
9. Bolmont, B.; Abraini, J.H. State-anxiety and low moods: Evidence for a single concept. *Physiol. Behav.* **2001**, *74*, 421–424. [[CrossRef](#)]
10. Baumeister, H.; Knecht, A.; Hutter, N. Direct and indirect costs in persons with chronic back pain and comorbid mental disorders—a systematic review. *J. Psychosom. Res.* **2012**, *73*, 79–85. [[CrossRef](#)]
11. Salum, G.A.; Isolan, L.R.; Bosa, V.L.; Tocchetto, A.G.; Teche, S.P.; Schuch, I.; Costa, J.R.; Costa Mde, A.; Jarros, R.B.; Mansur, M.A.; et al. The multidimensional evaluation and treatment of anxiety in children and adolescents: Rationale, design, methods and preliminary findings. *Rev. Bra. Psiquiatria.* **2011**, *33*, 181–195. [[CrossRef](#)]
12. National Institute for Health and Clinical Excellence. *National Collaborating Centre for Mental Health. The Treatment and Management of Depression in Adults (Updated Edition)*; National Clinical Practice Guideline 90; National Institute for Health and Care Excellence: London, UK, 2010; pp. 466–536.
13. Fajemiroye, J.O.; da Silva, D.M.; de Oliveira, D.R.; Costa, E.A. Treatment of anxiety and depression: Medicinal plants in retrospect. *Fund. Clin. Pharmacol.* **2016**, *30*, 198–215. [[CrossRef](#)]
14. Sarris, J. Herbal medicines in the treatment of psychiatric disorders: 10-year updated review. *Phytother. Res.* **2018**, *32*, 1147–1162. [[CrossRef](#)] [[PubMed](#)]
15. Dome, P.; Tombor, L.; Lazary, J.; Gonda, X.; Rihmer, Z.J.B.R.B. Natural health products, dietary minerals and over-the-counter medications as add-on therapies to antidepressants in the treatment of major depressive disorder: A review. *Brain Res. Bull.* **2019**, *146*, 51–78. [[CrossRef](#)]
16. Mischoulon, D.; Rapaport, M.H.A. Current Role of Herbal and Natural Preparations. *Antidepressants* **2019**, *250*, 225–252.
17. Bathaie, S.Z.; Mousavi, S.Z. Historical uses of saffron: Identifying potential new avenues for modern Research. *Avicenna, J. Phytomed.* **2011**, *1*, 57–66.
18. Fernández, J. Biology, biotechnology and biomedicine of saffron. *Recent Res. Dev. Plant. Sci.* **2004**, *2*, 127–159.
19. Grigg, D.B. *The Agricultural Systems of the World: An. Evolutionary Approach*; Cambridge University Press: Cambridge, UK, 1974.
20. Leone, S.; Recinella, L.; Chiavaroli, A.; Orlando, G.; Ferrante, C.; Leporini, L.; Brunetti, L.; Menghini, L. Phytotherapeutic use of the *Crocus sativus* L. (Saffron) and its potential applications: A brief overview. *Phytother. Res.* **2018**, *32*, 2364–2375. [[CrossRef](#)]
21. Srivastava, R.; Ahmed, H.; Dixit, R.K.; Dharamveer, P.; Saraf, S.A. *Crocus sativus* L.: A comprehensive review. *Pharmacogn. Rev.* **2010**, *4*, 200–208. [[CrossRef](#)]
22. Sampathu, S.; Shivashankar, S.; Lewis, Y.; Wood, A. Saffron (*Crocus sativus* Linn.)—Cultivation, processing, chemistry and standardization. *Crit. Rev. Food Sci. Nutr.* **1984**, *20*, 123–157. [[CrossRef](#)]
23. Kyriakoudi, A.; Ordoudi, S.; Roldan-Medina, M.; Tsimidou, M. Saffron, a Functional Spice. *Austin J. Nutr. Food Sci.* **2015**, *3*, 1051–1059.
24. Farahmand, S.K.; Samini, F.; Samini, M.; Samarghandian, S. Safranal ameliorates antioxidant enzymes and suppresses lipid peroxidation and nitric oxide formation in aged male rat liver. *Biogerontology* **2013**, *14*, 63–71. [[CrossRef](#)]
25. Asdaq, S.M.B.; Inamdar, M.N. Potential of *Crocus sativus* (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. *Appl. Biochem. Biotechnol.* **2010**, *162*, 358–372. [[CrossRef](#)] [[PubMed](#)]
26. Ardebili, D.; Hosseinzadeh, H.; Abnous, K.; Hasani, F.V.; Robati, R.Y.; Razavi, B.M. Involvement of brain-derived neurotrophic factor (BDNF) on malathion induced depressive-like behavior in subacute exposure and protective effects of crocin. *Iran. J. Basic Med. Sci.* **2015**, *18*, 958.
27. Pitsikas, N.; Boultsadakis, A.; Georgiadou, G.; Tarantilis, P.A.; Sakellaris, N. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of anxiety. *Phytomedicine* **2008**, *15*, 1135–1139. [[CrossRef](#)] [[PubMed](#)]
28. Ghasemi, R.; Moosavi, M.; Zarifkar, A.; Rastegar, K. The interplay of Akt and ERK in A $\beta$  toxicity and insulin-mediated protection in primary hippocampal cell culture. *J. Mol. Neurosci.* **2015**, *57*, 325–334. [[CrossRef](#)]

29. Nam, K.N.; Park, Y.M.; Jung, H.J.; Lee, J.Y.; Min, B.D.; Park, S.U.; Jung, W.S.; Cho, K.H.; Park, J.H.; Kang, I.; et al. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. *Eur. J. Pharmacol.* **2010**, *648*, 110–116. [\[CrossRef\]](#)
30. Rathore, B.; Jaggi, K.; Thakur, S.K.; Mathur, A.; Mahdi, F. Anti-inflammatory activity of *Crocus sativus* extract in experimental arthritis. *Int. J. Pharm. Sci. Res.* **2015**, *6*, 1473.
31. Hosseinzadeh, H.; Younesi, H.M. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacol.* **2002**, *2*, 7. [\[CrossRef\]](#)
32. Hosseinzadeh, H.; Parvardeh, S. Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. *Phytomedicine* **2004**, *11*, 56–64. [\[CrossRef\]](#)
33. Kell, G.; Rao, A.; Beccaria, G.; Clayton, P.; Inarejos-Garcia, A.; Prodanov, M. affron((R)) a novel saffron extract (*Crocus sativus* L.) improves mood in healthy adults over 4 weeks in a double-blind, parallel, randomized, placebo-controlled clinical trial. *Complement. Ther. Med.* **2017**, *33*, 58–64. [\[CrossRef\]](#)
34. Abe, K.; Sugiura, M.; Shoyama, Y.; Saito, H. Crocin antagonizes ethanol inhibition of NMDA receptor-mediated responses in rat hippocampal neurons. *Brain Res.* **1998**, *787*, 132–138. [\[CrossRef\]](#)
35. Hosseini, A.; Razavi, B.M.; Hosseinzadeh, H. Pharmacokinetic Properties of Saffron and its Active Components. *Eur. J. Drug Metab. Pharmacokinet.* **2018**, *43*, 383–390. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Chen, M.C.; Gotlib, I.H. 13 Molecular Foundations of the Symptoms of Major Depressive Disorder. *Oxf. Handb. Mol. Psychol.* **2015**, 258.
37. Roy, A.; Roy, R.N. Stress and Major Depression: Neuroendocrine and Biopsychosocial Mechanisms. In *Stress Neuroendocrinology Neurobiology: Handbook of Stress Series 2*; Elsevier: San Diego, CA, USA, 2017; pp. 173–184.
38. Palta, P.; Samuel, L.J.; Miller, E.R.; Szanton, S.L. Depression and oxidative stress: Results from a meta-analysis of observational studies. *Psychosom. Med.* **2014**, *76*, 12–19. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Leonard, B.; Maes, M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neurosci. Biobehav. R.* **2012**, *36*, 764–785. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Jones, K. Review of neuroplasticity and depression: Evidence for the neurotrophic or neuroplasticity theory of depression pathophysiology and systematic review of the neurophysiological implications of long-term antidepressant treatment. 2016. [\[CrossRef\]](#)
41. Marx, W.; Lane, M.; Rocks, T.; Ruusunen, A.; Loughman, A.; Lopresti, A.; Marshall, S.; Berk, M.; Jacka, F.; Dean, O.M. Effect of saffron supplementation on symptoms of depression and anxiety: A systematic review and meta-analysis. *Nutr. Rev.* **2019**. [\[CrossRef\]](#)
42. Tóth, B.; Hegyi, P.; Lantos, T.; Szakács, Z.; Kerémi, B.; Varga, G.; Tenk, J.; Pétervári, E.; Balaskó, M.; Rumbus, Z. The efficacy of saffron in the treatment of mild to moderate depression: A meta-analysis. *Planta Med.* **2019**, *85*, 24–31. [\[CrossRef\]](#)
43. Lopresti, A.L.; Smith, S.J.; Hood, S.D.; Drummond, P.D. Efficacy of a standardised saffron extract (affron(R)) as an add-on to antidepressant medication for the treatment of persistent depressive symptoms in adults: A randomised, double-blind, placebo-controlled study. *J. Psychopharmacol.* **2019**, *33*, 1415–1427. [\[CrossRef\]](#)
44. Hosseinzadeh, H.; Karimi, G.; Niapoor, M. Antidepressant effects of *Crocus sativus* stigma extracts and its constituents, crocin and safranal, in mice. *J. Med. Plants* **2004**, *3*, 48–58.
45. Hassani, F.V.; Naseri, V.; Razavi, B.M.; Mehri, S.; Abnous, K.; Hosseinzadeh, H. Antidepressant effects of crocin and its effects on transcript and protein levels of CREB, BDNF, and VGF in rat hippocampus. *DARU* **2014**, *22*, 16. [\[CrossRef\]](#)
46. Razavi, B.M.; Sadeghi, M.; Abnous, K.; Hasani, F.V.; Hosseinzadeh, H. Study of the role of CREB, BDNF, and VGF neuropeptide in long term antidepressant activity of crocin in the rat cerebellum. *DARU* **2017**, *16*, 1452.
47. Bostan, H.B.; Mehri, S.; Hosseinzadeh, H. Toxicology effects of saffron and its constituents: A review. *Iran. J. Basic Med. Sci.* **2017**, *20*, 110–121. [\[CrossRef\]](#)
48. Lymperopoulou, C.; Lamari, F.J.M.A.P. Saffron safety in humans: Lessons from the animal and clinical studies. *Med. Aromat Plants* **2015**, *4*, e164.
49. Agha-Hosseini, M.; Kashani, L.; Aleyaseen, A.; Ghoreishi, A.; Rahmanpour, H.; Zarrinara, A.R.; Akhondzadeh, S. *Crocus sativus* L. (saffron) in the treatment of premenstrual syndrome: A double-blind, randomised and placebo-controlled trial. *BJOG: Int. J. Obstet. Gy.* **2008**, *115*, 515–519. [\[CrossRef\]](#) [\[PubMed\]](#)

50. Ahmadpanah, M.; Ramezanshams, F.; Ghaleiha, A.; Akhondzadeh, S.; Bahmani, D.S.; Brand, S. Crocus Sativus, L.(saffron) versus sertraline on symptoms of depression among older people with major depressive disorders—a double-blind, randomized intervention study. *Psychiat. Res.* **2019**, *282*, 112–613. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Akhondzadeh, S.; Fallah-Pour, H.; Afkham, K.; Jamshidi, A.-H.; Khalighi-Cigaroudi, F. Comparison of Crocus sativus L. and imipramine in the treatment of mild to moderate depression: A pilot double-blind randomized trial [ISRCTN45683816]. *BMC Complem. Altern. M.* **2004**, *4*, 12. [\[CrossRef\]](#)
52. Basti, A.A.; Moshiri, E.; Noorbala, A.-A.; Jamshidi, A.-H.; Abbasi, S.H.; Akhondzadeh, S. Comparison of petal of Crocus sativus L. and fluoxetine in the treatment of depressed outpatients: A pilot double-blind randomized trial. *Prog. Neuro-Psychoph.* **2007**, *31*, 439–442. [\[CrossRef\]](#)
53. Ghajar, A.; Neishabouri, S.M.; Velayati, N.; Jahangard, L.; Matinnia, N.; Haghighi, M.; Ghaleiha, A.; Afarideh, M.; Salimi, S.; Meysamie, A. Crocus sativus L. versus citalopram in the treatment of major depressive disorder with anxious distress: A double-blind, controlled clinical trial. *Pharmacopsychiatry* **2017**, *50*, 152–160. [\[CrossRef\]](#)
54. Jelodar, G.; Javid, Z.; Sahraian, A.; Jelodar, S. Saffron improved depression and reduced homocysteine level in patients with major depression: A Randomized, double-blind study. *Avicenna, J. Phytomed.* **2018**, *8*, 43.
55. Kashani, L.; Eslatmanesh, S.; Saedi, N.; Niroomand, N.; Ebrahimi, M.; Hosseini, M.; Foroughifar, T.; Salimi, S.; Akhondzadeh, S. Comparison of saffron versus fluoxetine in treatment of mild to moderate postpartum depression: A double-blind, randomized clinical trial. *Pharmacopsychiatry* **2017**, *50*, 64–68. [\[CrossRef\]](#)
56. Noorbala, A.A.; Akhondzadeh, S.H.; Tahmacebi-Pour, N.; Jamshidi, A.H. Hydro-alcoholic extract of Crocus sativus L. versus fluoxetine in the treatment of mild to moderate depression: A double-blind, randomized pilot trial. *J. Ethnopharmacol.* **2005**, *97*, 281–284. [\[CrossRef\]](#)
57. Sahraian, A.; Jelodar, S.; Javid, Z.; Mowla, A.; Ahmadzadeh, L. Study the effects of saffron on depression and lipid profiles: A double blind comparative study. *Asian J. Psychiatr.* **2016**, *22*, 174–176. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Shahmansouri, N.; Farokhnia, M.; Abbasi, S.-H.; Kassaian, S.E.; Tafti, A.-A.N.; Gougol, A.; Yekehtaz, H.; Forghani, S.; Mahmoodian, M.; Saroukhani, S. A randomized, double-blind, clinical trial comparing the efficacy and safety of Crocus sativus L. with fluoxetine for improving mild to moderate depression in post percutaneous coronary intervention patients. *J. Affect. Dis.* **2014**, *155*, 216–222. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Shakiba, M.; Moazen-Zadeh, E.; Noorbala, A.A.; Jafarinia, M.; Divsalar, P.; Kashani, L.; Shahmansouri, N.; Tafakhori, A.; Bayat, H.; Akhondzadeh, S. Saffron (Crocus sativus) versus duloxetine for treatment of patients with fibromyalgia: A randomized double-blind clinical trial. *Avicenna J. Phytomed.* **2018**, *8*, 513.
60. Russell, V.A.; Sagvolden, T.; Johansen, E.B. PMC1180819; Animal models of attention-deficit hyperactivity disorder. *Behav. Brain. Funct.* **2005**, *1*, 9. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Djordjevic, J.; Djordjevic, A.; Adzic, M.; Radojcic, M.B. Effects of chronic social isolation on Wistar rat behavior and brain plasticity markers. *Neuropsychobiology* **2012**, *66*, 112–119. [\[CrossRef\]](#)
62. Hosseinzadeh, H.; Noraei, N.B. Anxiolytic and hypnotic effect of Crocus sativus aqueous extract and its constituents, crocin and safranal, in mice. *Phytother. Res. PTR* **2009**, *23*, 768–774. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Scheggi, S.; De Montis, M.G.; Gambarana, C. PMC6209858; Making Sense of Rodent Models of Anhedonia. *Int. J. Neuropsychopharmacol.* **2018**, *21*, 1049–1065. [\[CrossRef\]](#)
64. Asai, A.; Nakano, T.; Takahashi, M.; Nagao, A. Orally administered crocetin and crocins are absorbed into blood plasma as crocetin and its glucuronide conjugates in mice. *J. Agric. Food. Chem.* **2005**, *53*, 7302–7306. [\[CrossRef\]](#)
65. Xi, L.; Qian, Z.; Du, P.; Fu, J. Pharmacokinetic properties of crocin (crocetin digentiobiose ester) following oral administration in rats. *Phytomedicine* **2007**, *14*, 633–636. [\[CrossRef\]](#)
66. Lautenschlager, M.; Sendker, J.; Huwel, S.; Galla, H.J.; Brandt, S.; Dufer, M.; Riehemann, K.; Hensel, A. Intestinal formation of trans-crocetin from saffron extract (*Crocus sativus* L.) and in vitro permeation through intestinal and blood brain barrier. *Phytomedicine* **2015**, *22*, 36–44. [\[CrossRef\]](#)
67. Delgado, P.L. Depression: The case for a monoamine deficiency. *J. Clin. Psychiatry* **2000**.
68. Khazdair, M.R.; Boskabady, M.H.; Hosseini, M.; Rezaee, R.; Tsatsakis, A. The effects of *Crocus sativus* (saffron) and its constituents on nervous system: A review. *Avicenna, J. Phytomed.* **2015**, *5*, 376.

69. Hosseinzadeh, H.; Sadeghnia, H.R.; Rahimi, A.J.P.m. Effect of safranal on extracellular hippocampal levels of glutamate and aspartate during kainic acid treatment in anesthetized rats. *Planta Med.* **2008**, *74*, 1441–1445. [\[CrossRef\]](#)
70. Maes, M.; Mihaylova, I.; Kubera, M.; Leunis, J.C.; Geffard, M. IgM-mediated autoimmune responses directed against multiple neoepitopes in depression: New pathways that underpin the inflammatory and neuroprogressive pathophysiology. *J. Affect. Dis.* **2011**, *135*, 414–418. [\[CrossRef\]](#)
71. Miller, A.H.; Raison, C.L. Are Anti-inflammatory Therapies Viable Treatments for Psychiatric Disorders?: Where the Rubber Meets the Road. *JAMA Psychiatry* **2015**, *72*, 527–528. [\[CrossRef\]](#)
72. Moylan, S.; Eyre, H.A.; Maes, M.; Baune, B.T.; Jacka, F.N.; Berk, M. Exercising the worry away: How inflammation, oxidative and nitrogen stress mediates the beneficial effect of physical activity on anxiety disorder symptoms and behaviours. *Neurosci. Biobehav. R.* **2013**, *37*, 573–584. [\[CrossRef\]](#)
73. Lopresti, A.L.; Drummond, P.D. Saffron (*Crocus sativus*) for depression: A systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. *Human Psychopharmacol.* **2014**, *29*, 517–527. [\[CrossRef\]](#)
74. Boskabady, M.H.; Farkhondeh, T. Antiinflammatory, Antioxidant, and Immunomodulatory Effects of *Crocus sativus* L. and its Main Constituents. *Phytother. Res. PTR* **2016**, *30*, 1072–1094. [\[CrossRef\]](#)
75. Broadhead, G.K.; Chang, A.; Grigg, J.; McCluskey, P. Efficacy and Safety of Saffron Supplementation: Current Clinical Findings. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 2767–2776. [\[CrossRef\]](#)
76. Samarghandian, S.; Samini, F.; Azimi-Nezhad, M.; Farkhondeh, T. Anti-oxidative effects of safranal on immobilization-induced oxidative damage in rat brain. *Neurosci. Lett.* **2017**, *659*, 26–32. [\[CrossRef\]](#)
77. Poma, A.; Fontecchio, G.; Carlucci, G.; Chichirico, G. Anti-inflammatory properties of drugs from saffron crocus. *Anti-Inflamm. Anti-Allergy Agents Med. Chem.* **2012**, *11*, 37–51. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Wiedlocha, M.; Marcinowicz, P.; Krupa, R.; Janoska-Jazdzik, M.; Janus, M.; Debowska, W.; Mosiolek, A.; Waszkiewicz, N.; Szulc, A. Effect of antidepressant treatment on peripheral inflammation markers—A meta-analysis. *Prog. Neuro-Psychoph* **2018**, *80*, 217–226. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Halataei, B.A.; Khosravi, M.; Arbabian, S.; Sahraei, H.; Golmanesh, L.; Zardooz, H.; Jalili, C.; Ghoshooni, H. Saffron (*Crocus sativus*) aqueous extract and its constituent crocin reduces stress-induced anorexia in mice. *Phytother. Res. PTR* **2011**, *25*, 1833–1838. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Hooshmandi, Z.; Rohani, A.H.; Eidi, A.; Fatahi, Z.; Golmanesh, L.; Sahraei, H. Reduction of metabolic and behavioral signs of acute stress in male Wistar rats by saffron water extract and its constituent safranal. *Pharm. Biol.* **2011**, *49*, 947–954. [\[CrossRef\]](#)
81. Ieraci, A.; Mallei, A.; Popoli, M. Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. *Neural Plast.* **2016**, *33*, 58–64. [\[CrossRef\]](#)
82. Cryan, J.F.; Markou, A.; Lucki, I. Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends Pharmacol. Sci.* **2002**, *23*, 238–245. [\[CrossRef\]](#)
83. Bogdanova, O.V.; Kanekar, S.; D’Anci, K.E.; Renshaw, P.F. Factors influencing behavior in the forced swim test. *Physiol. Behav.* **2013**, *118*, 227–239. [\[CrossRef\]](#)
84. Zhang, J.C.; Wu, J.; Fujita, Y.; Yao, W.; Ren, Q.; Yang, C.; Li, S.X.; Shirayama, Y.; Hashimoto, K. Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in mice after inflammation. *Int. J. Neuropsychopharmacol.* **2014**, *18*. [\[CrossRef\]](#)
85. Eagle, A.L.; Mazei-Robison, M.; Robison, A.J. Sucrose preference test to measure stress-induced anhedonia. *Bio. Protoc.* **2016**, *6*, 1822. [\[CrossRef\]](#)
86. Malhi, G.S.; Mann, J.J. Depression. *Lancet* **2018**, *392*, 2299–2312. [\[CrossRef\]](#)
87. Porsolt, R.D.; Le Pichon, M.; Jalfre, M. Depression: A new animal model sensitive to antidepressant treatments. *Nature* **1977**, *266*, 730–732. [\[CrossRef\]](#)
88. Lucki, I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav. Pharmacol.* **1997**, *8*, 523–532. [\[CrossRef\]](#)
89. Warner-Schmidt, J.L.; Duman, R.S. VEGF as a potential target for therapeutic intervention in depression. *Curr. Opin. Pharmacol.* **2008**, *8*, 14–19. [\[CrossRef\]](#)

90. Pothion, S.; Bizot, J.C.; Trovero, F.; Belzung, C. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. *Behav. Brain Res.* **2004**, *155*, 135–146. [[CrossRef](#)] [[PubMed](#)]

**Sample Availability:** Samples of the compound affron<sup>®</sup> are available from Pharmactive Biotech Products SL.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

# Effects of saffron on sleep quality in healthy adults with self-reported unsatisfactory sleep: a randomised, double-blind, placebo-controlled study

Adrian L Lopresti<sup>1,2</sup>, Stephen J Smith<sup>1,2</sup>, Alexandra P Metse<sup>1</sup>, Peter D Drummond<sup>1</sup>

<sup>1</sup>College of Science, Health, Engineering and Education, Murdoch University, Perth, Western Australia, 6150, Australia

<sup>2</sup>Clinical Research Australia, Perth, Western Australia, 6023, Australia

## Correspondence:

E: [a.lopresti@murdoch.edu.au](mailto:a.lopresti@murdoch.edu.au),

P: +61 0894487376

F: +61 0894478217

A: 38 Arnisdale Rd Duncraig WA 6023

**Keywords:** sleep, insomnia, saffron, herbal

## Funder and Role of the funding source

This study was funded by Pharmactive Biotech Products SL. Pharmactive Biotech Products was not involved in the design of the research, analysis of data, or in the writing of the report.

## Acknowledgements

This study was funded by Pharmactive Biotech Products SL. Pharmactive Biotech Products was not involved in the design of the research, analysis of data, or in the writing of the report.

The authors gratefully acknowledge Pharmactive Biotech Products SL Company for funding the project and supplying affron® and LIPA Pharmaceuticals for the preparation of the tablets.

## Statement of human rights

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

## Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

## Conflict of Interest

The authors AL, SS, AM, and PD declare that they have no conflicts of interest.

# ABSTRACT

## INTRODUCTION

Results from population-based surveys have indicated that 10 to 30% of adults report regular difficulty either getting to or staying asleep (Buysse et al., 2008; Schutte-Rodin et al., 2008). This has significant health implications as poor sleep quality can have a negative impact on both mental and physical health and can interfere with daily function (Medic et al., 2017). In a cohort of 27 studies, comprising more than 70,000 elderly individuals, long and short sleep duration was associated with a 33% increased risk of all-cause mortality (da Silva et al., 2016). Insomnia is also associated with reduced work productivity and increase work absenteeism (Daley et al., 2009; Espie et al., 2018).

Herbal medicines are one of the most frequently used complementary and alternative treatments for insomnia. In community-dwelling older adults with self-reported sleep problems use of a sleep product was reported by 35% of respondents, with 22% using over-the-counter sleep aids, and 12.5% using herbal or natural aids (Maust et al., 2019). In a randomly selected sample of almost 1,000 adults, 18.5% of participants reported using a natural sleep-aid over the last 12 months (Sanchez-Ortuno et al., 2009). However, the safety and efficacy of many herbal medicines for the treatment of sleep problems is uncertain. In a systematic review of 14 randomised-controlled trials, involving 1600 participants with insomnia, it was concluded that there was no statistically significant difference between any herbal medicine and placebo for the treatment of insomnia (Leach and Page, 2015). This indicates that despite their widespread use, there is a paucity of data on the efficacy and safety of natural herbal products.

Saffron, a spice derived from the stigmas of the *Crocus sativus* flower, has been confirmed in several systematic reviews and meta-analyses to be an effective natural agent for the treatment of mild-to-moderate depression (Lopresti and Drummond, 2014; Marx et al., 2019; Toth et al., 2018). As a sleep-enhancing agent, there is preliminary evidence to suggest it may also be an effective natural sleep aid (Milajerdi et al., 2018; Nishide et al., 2018; Umigai et al., 2018). However, further robust clinical trials are required to validate these findings. Therefore, the goal of this study was to investigate the sleep-enhancing effects and safety of a standardised saffron extract (affron®) in adults with self-reported unsatisfactory sleep.

# MATERIALS AND METHODS

## Study design

This was a parallel, 28-day, randomised, double-blind, placebo-controlled trial (Figure 1). The trial protocol was approved by the Human Research Ethics Committee at the National Institute of Integrative Medicine, and was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. ACTRN12619000863134).

An a priori power analysis was undertaken to estimate the required sample size (based on a single outcome variable). In a recent randomised, controlled trial pilot study, saffron had an effect size of 1.1 in adults with poor sleep compared to the placebo (Nishide et al., 2018). Sample size calculations were based on a more conservative effect size of 0.7. 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 for the ISI was estimated as 26. After allowing for a 15% drop out rate, we aimed to recruit 30 participants per group (Soper, 2019).

## Recruitment and randomisation

Participants were recruited across Perth, Western Australia through social media advertisements between July and August 2019. Interested participants were directed to a website landing page providing details about the study and a link to complete an initial online screening questionnaire. This online questionnaire screened for current depressive and/or anxiety symptoms, medication use, history of medical/ psychiatric disorders, alcohol, nicotine, and other drug use, supplement and vitamin intake, and pregnancy/breastfeeding status. If assessed as likely eligible, volunteers participated in a phone interview with an investigator. The phone interview comprised a structured series of questions examining the eligibility criteria specified below.

Eligible and consenting participants completed baseline assessments and were randomly assigned to one of two groups (saffron or placebo) using a randomisation calculator (<http://www.randomization.com>). The randomisation calculator ensured sequence concealment. The randomisation structure comprised 10 randomly permuted blocks, containing 6 participants per block. The participant identification number was allocated according to the order of participant enrolment in the study. All tablets were packed in identical bottles labelled by two intervention codes (held by the study sponsor until final data collection). Participants and study investigators were not informed of treatment group allocation until all questionnaires were completed.

## Participants

*Inclusion criteria:* physically healthy, male and female participants aged 18 to 70 years, with self-reported symptoms of unsatisfactory sleep lasting greater than 4 weeks were recruited for this study. Participants had an ISI score of between 8 (subthreshold insomnia) and 21 (moderate-severity insomnia). All participants were medication-free for at least 4 weeks apart from the contraceptive pill and no more than once a week use of pain-relieving medications. Volunteers had a body mass index (BMI) between 18 and 30 and typical bedtime between 9pm and 12am. Participants were also required to be fluent in English and to have consented (via an online consent form) to all pertinent aspects of the trial.

*Exclusion criteria:* Participants employed in night shift work or rotational shift work were ineligible to participate in the study. People experiencing a sleep disorder other than moderate insomnia (e.g., sleep apnoea, restless legs syndrome, periodic limb movement disorder), chronic, severe sleep disturbance greater than 1 year, diagnosis of a mental health disorder (other than mild depressive or anxiety symptoms as measured by the Depression, Anxiety and Stress Scale-21), coffee intake greater than 3 cups a day (or equivalent caffeine intake from other caffeinated drinks e.g., tea, energy drinks), and alcohol consumption greater than 14 standard drinks per week were also ineligible for the study. Participants were also ineligible for the study if they were experiencing external factors that may affect sleep patterns (e.g., infant/children regularly awakening, excess noise, snoring partner); were currently receiving non-pharmacological treatment for sleep disorders (e.g., cognitive behavioural therapy, relaxation therapy); had a current or 12-month history of illicit drug abuse; were currently taking supplements that may affect sleep; were taking saffron supplements; or had a diagnosed medical condition including but not limited to: diabetes, hyper/hypotension, cardiovascular disease, gastrointestinal disease requiring regular use of medications, gallbladder disease/gallstones/biliary disease, endocrine disease, psychiatric disorder, and 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.

## Interventions

Placebo and saffron tablets were identical in appearance, being matched for colour coating, shape, and size. The active treatment, supplied by Pharmactive Biotech Products SL., contained 14mg of a standardised saffron extract (affron®), derived from the stigmas of *Crocus sativus* L. and standardised to

contain >3.5% Lepticrosalides<sup>®</sup>, a measure of bioactive compounds present in saffron, including safranal and crocin isomers.

The saffron stigmas were cultivated in Alborea (Albacete, Spain) and extracted in the factory of Pharmactive Biotech Products SL in Madrid (Spain) to produce affron<sup>®</sup> 3.5% Lepticrosalides<sup>®</sup>. The placebo tablets contained the same excipients as the active tablet (microcrystalline cellulose and calcium hydrogen phosphate). All tablets were manufactured and packed in an Australian Therapeutic Goods Administration registered plant.

All participants were instructed to take one tablet, twice daily, with or without food for 28 days.

Medication adherence was measured by tablet count by the participant on day 28. Efficacy of participant treatment blinding was examined by asking participants to predict group allocation (placebo, saffron, or uncertain) at the completion of the study.

Saffron and placebo tablets were posted to participants with directions for use provided on tablet bottles. Participants were also provided with an information sheet about tablet intake and what to do if they missed a dose. This information was also verbally conveyed to participants during their initial telephone interview.

## Outcome Measures

### *Primary Outcome Measures:*

*Insomnia Severity Index (ISI):* The ISI is a self-report instrument measuring respondents' perception of both nocturnal and diurnal symptoms of insomnia. The ISI comprises seven items assessing the perceived severity of difficulties initiating sleep, staying asleep, and early morning awakenings, satisfaction with current sleep pattern, interference with daily functioning, noticeability of impairment attributed to the sleep problem, and degree of distress or concern caused by the sleep problem. The ISI has good psychometric properties and is sensitive to treatment in clinical trials (Morin et al., 2011). The ISI was completed at baseline (from days -5 to -3), day 7, 14, 21, and 28.

### *Secondary Outcome Measures:*

*Restorative Sleep Questionnaire (RSQ):* The RSQ 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 had good psychometric properties and is able to distinguish between healthy controls, patients with primary insomnia, and insomnia patients with isolated non-restorative sleep complaints (Drake et al., 2014). The RSQ was completed at day -1, 0, 3, 7, 14, 21, 27 and 28.

*Pittsburgh Sleep Diary (PSD)*: the PSD is a 14-item sleep diary that respondents complete upon awakening. The PSD shows good retest reliability over a mean inter-test interval of 22 months. Scores also correlate with circadian type, subjective sleep quality, and objective actigraphy measurements (Monk et al., 1994). Scores are calculated for total sleep time (hours), sleep latency (minutes), number of awakenings after sleep onset, sleep quality rating [5-point Likert rating ranging from very bad (1) to very good (5)], mood rating at final awakening [5-point Likert rating ranging from very calm (1) to very tense (5)], and alertness rating at final awakening [5-point Likert rating ranging from very sleepy (1) to very alert (5)]. The PSD was completed at day -1, 0, 3, 7, 14, 21, 27 and 28.

*Depression, Anxiety, and Stress Scale – 21 (DASS-21)*: The DASS-21 is a validated self-report measure assessing symptoms of stress, anxiety, and depression (Brown et al., 1997). Twenty-one questions are rated on a 4-point scale (0–3), ranging from never to almost always (lower scores indicate a reduction in symptoms). Sub-scale scores for depression, anxiety, and stress are calculated. The DASS-21 was completed at baseline (days -5 to -3) and day 28.

## *Process Measures*

*Dysfunctional Beliefs About Sleep (DBAS)*: The DBAS is a 16-item questionnaire that assesses beliefs and attitudes about sleep. The DBAS has good reliability as evidenced by adequate internal consistency and temporal stability over a 2-week period. Scores also correlate with other self-report measures of insomnia severity, anxiety, and depression (Morin et al., 2007). The DBAS was completed at baseline to examine the relationship between dysfunctional beliefs about sleep and change in sleep quality over time.

*Adverse events*: Tolerability of supplement intake by participants was assessed at day 28 through an online question querying adverse effects that were believed to be associated with supplement intake. Participants were also requested to contact researchers immediately if any adverse effects were experienced.

Initial screening questionnaires comprising the ISI, DASS-21, and DBAS were completed online. A response booklet containing copies of the required questionnaires and sleep diaries were then posted to all participants. The dates for completion of each questionnaire and diary was recorded in the booklet. Participants were also advised to keep their response booklet near their bed and to complete them within 30 minutes after awakening.

## *Statistical analysis*

An independent samples T-test was used to compare demographic variables across the two treatment groups for continuous variables, and Pearson's Chi-square was used to compare categorical data. A

repeated-measures analysis of variance (ANOVA) was used to compare within-group changes over time and group (saffron versus placebo) x time interaction effects. The ISI total score was analysed for baseline and mean score across days 7, 14, 21, and 28. Total scores on the RSQ and item scores on the PSD were analysed for mean baseline (days -1 and day 0) and mean score across days 3, 7, 14, 21, 27, and 28. Eta-squared ( $\eta^2$ ) was calculated to examine effect sizes.

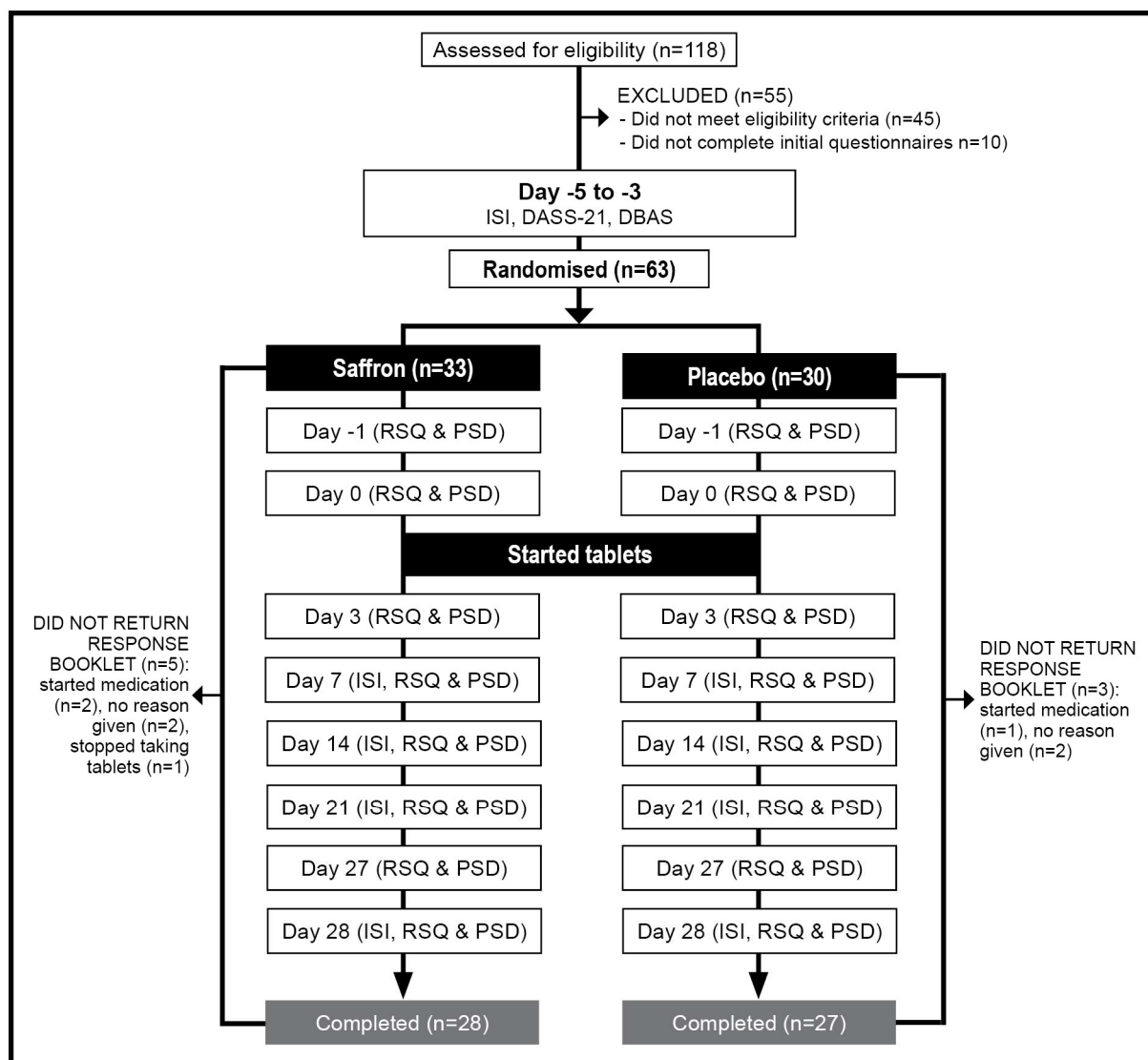
The Shapiro-Wilk normality test was conducted to examine the normality of group data. This demonstrated that data were not normally distributed, and this was not corrected by data transformations. However, a repeated-measures ANOVA was considered the most appropriate option for statistical analyses as it is relatively robust to violations of normality (Tabachnick and Fidell, 2007). Where necessary, degrees of freedom were adjusted using the Greenhouse-Geisser approach to correct for violations of the sphericity assumption.

To examine the influence of dysfunctional beliefs about sleep on changes in sleep quality, a Pearson's correlation coefficient was calculated between the baseline DBAS total score and percentage change in ISI score (baseline to average ISI score from days 7 to 28).

Data from all participants who returned their response booklets were included in analysis. All data were analysed using SPSS (version 24; IBM, Armonk, NY).

## RESULTS

### Study Population



**Figure 1. Systematic Illustration of Study Design**

### *Baseline questionnaire and demographic information*

From 118 people completing the initial online screening questionnaire, 63 volunteers participated in the study. A total of 55 individuals were either ineligible ( $n=45$ ) or did not complete the initial questionnaires ( $n=10$ ). Data from 55 participants who completed all questionnaires over the 28-day period were used for statistical analyses. Eight participants withdrew or did not return response booklets. There were no significant differences in dropout rates across groups. Reasons for withdrawal included no reason given ( $n=4$ ), the commencement of new medication ( $n=3$ ), and deciding to stop taking tablets ( $n=1$ ). No participant reported withdrawing from the study due to reported adverse events associated with supplement intake.

**Table 1. Baseline Demographic Details of Participants**

		Saffron n=33	Placebo n=30	p-value
Age	Mean	48.79	51.10	.354 <sup>a</sup>
	SE	1.87	1.59	
BMI	Mean	24.97	25.55	.541 <sup>a</sup>
	SE	0.69	0.62	
Gender (n)	Male	5	5	.869 <sup>b</sup>
	Female	28	25	
Marital Status (n)	Single	30	25	.367 <sup>b</sup>
	Married	3	5	
Educational Level (n)	Secondary	12	8	.633 <sup>b</sup>
	Tertiary	17	19	
	Post-graduate	4	3	
Exercise Level (n)	Never/Rarely	11	16	.157 <sup>b</sup>
	1 to 2 times a week	15	7	
	3 to 5 times a week	7	7	
Duration of sleep problems (n)	< 6 months	6	1	.312 <sup>a</sup>
	6 to 12 months	3	3	
	1-2 years	10	10	
	2+ years	14	16	
ISI	Mean	15.73	14.57	.197 <sup>a</sup>
	SE	0.61	0.65	
DBAS	Mean	75.27	80.03	.465 <sup>a</sup>
	SE	3.54	5.57	
DASS - Depression	Mean	4.73	3.20	.312 <sup>a</sup>
	SE	1.13	0.95	
DASS - Anxiety	Mean	3.58	2.93	.540 <sup>a</sup>
	SE	0.71	0.77	
DASS - Stress	Mean	9.15	8.27	.556 <sup>a</sup>
	SE	1.10	1.00	
DASS - Total	Mean	17.45	14.40	.356 <sup>a</sup>
	SE	2.43	2.17	
		n=28	n=27	
PSD - Total sleep time (hrs)	Mean	7.33	6.92	.307 <sup>a</sup>
	SE	0.28	0.28	
PSD - Sleep latency (min)	Mean	39.38	32.61	.444 <sup>a</sup>
	SE	7.16	4.97	
PSD - Number of wakings after sleep onset	Mean	3.23	3.15	.826 <sup>a</sup>
	SE	0.26	0.28	
PSD - Sleep quality	Mean	2.46	2.78	.120 <sup>a</sup>
	SE	0.11	0.16	
PSD - Mood on awakening	Mean	2.88	2.72	.435 <sup>a</sup>
	SE	0.11	0.16	
PSD - Alertness on awakening	Mean	2.77	3.02	.254 <sup>a</sup>
	SE	0.14	0.17	
RSQ	Mean	46.73	52.93	.154 <sup>a</sup>
	SE	2.46	3.54	

SE=standard error; a = Independent samples t-test; b = Chi-squareTest.

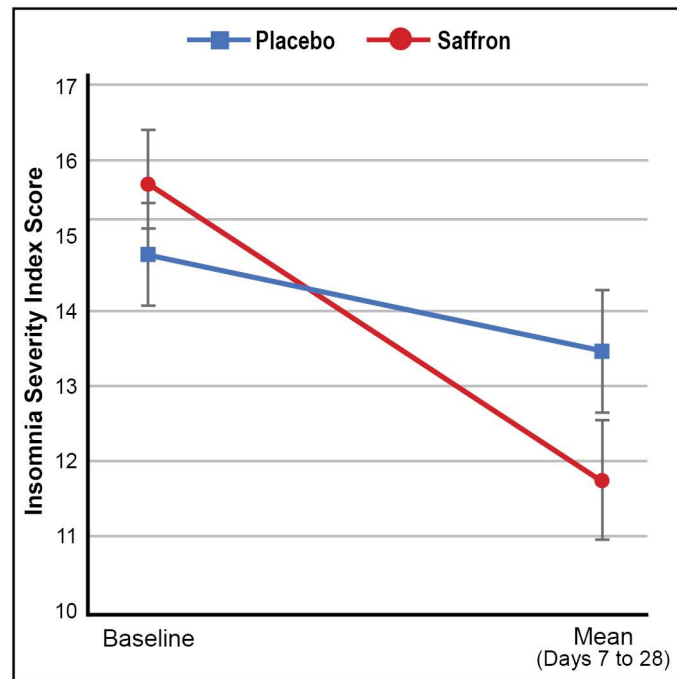
## Outcome Measures

**Table 2. Change in Sleep Measures**

			Baseline	During intervention	Repeated Measures ANOVA		
					p-value time effects	p-value time x group interaction	η <sup>2</sup> (%)
Insomnia Severity Index Total Score (lower scores indicate improved sleep)	Saffron (n=28)	Mean <sup>a</sup>	15.75	11.74 <sup>b</sup>	.000	.017	10.3
		SE	0.66	0.81			
	Placebo (n=27)	Mean <sup>a</sup>	14.74	13.46 <sup>b</sup>	.116		
		SE	0.67	0.83			
PSD - Total sleep time (hrs)	Saffron (n=28)	Mean <sup>b</sup>	7.33 <sup>a</sup>	7.49 <sup>c</sup>	.477	.763	0.2
		SE	0.28	0.20			
	Placebo (n=27)	Mean <sup>b</sup>	6.92 <sup>a</sup>	6.99 <sup>c</sup>	.767		
		SE	0.29	0.20			
PSD - Sleep latency (min)	Saffron (n=28)	Mean <sup>b</sup>	39.38 <sup>a</sup>	29.49 <sup>c</sup>	.149	.298	2.0
		SE	6.15	4.73			
	Placebo (n=27)	Mean <sup>b</sup>	32.61 <sup>a</sup>	31.06 <sup>c</sup>	.711		
		SE	6.26	4.81			
PSD - Number of wakings after sleep onset	Saffron (n=28)	Mean <sup>b</sup>	3.23 <sup>a</sup>	2.51 <sup>c</sup>	.001	.053	6.9
		SE	0.27	0.25			
	Placebo (n=27)	Mean <sup>b</sup>	3.15 <sup>a</sup>	2.98 <sup>c</sup>	.443		
		SE	0.27	0.25			
PSD - Sleep quality (higher scores indicate greater quality)	Saffron (n=28)	Mean <sup>b</sup>	2.46 <sup>a</sup>	2.99 <sup>c</sup>	.000	.014	10.9
		SE	0.14	0.12			
	Placebo (n=27)	Mean <sup>b</sup>	2.78 <sup>a</sup>	2.81 <sup>c</sup>	.848		
		SE	0.14	0.12			
PSD - Mood on awakening (lower scores indicate greater calmness)	Saffron (n=28)	Mean <sup>b</sup>	2.88 <sup>a</sup>	2.84 <sup>c</sup>	.746	.270	2.3
		SE	0.14	0.14			
	Placebo (n=27)	Mean <sup>b</sup>	2.72 <sup>a</sup>	2.88 <sup>c</sup>	.257		
		SE	0.14	0.14			
PSD - Alertness on awakening (higher scores indicate greater alertness)	Saffron (n=28)	Mean <sup>b</sup>	2.77 <sup>a</sup>	3.07 <sup>c</sup>	.037	.061	6.5
		SE	0.15	0.12			
	Placebo (n=27)	Mean <sup>b</sup>	3.02 <sup>a</sup>	2.92 <sup>c</sup>	.539		
		SE	0.16	0.12			
Restorative Sleep Questionnaire (high scores indicate greater restorative sleep)	Saffron (n=28)	Mean <sup>b</sup>	46.73 <sup>a</sup>	56.12 <sup>c</sup>	.000	.029	8.6
		SE	3.00	2.69			
	Placebo (n=27)	Mean <sup>b</sup>	52.93 <sup>a</sup>	54.62 <sup>c</sup>	.569		
		SE	3.06	2.74			

a = mean baseline score from days -1 and 0; b= mean score from days 7 to 28; c = mean score from days 3 to 28

### ISI (Primary Outcome Measure)



**Figure 2: Change in ISI Scores** (error bars depict standard error)

Changes in ISI total score across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 2 and Figure 2. Saffron was associated with a statistically-significant reduction in ISI score over time ( $F_{1,27}=26.32$ ,  $p<.001$ ). However, no significant change was observed in the placebo group ( $F_{1,26}=2.65$ ,  $p=.116$ ). A between-group analysis revealed a statistically-significant time  $\times$  group interaction ( $F_{1,53}=6.07$ ,  $p=.017$ ,  $\eta^2=10.3\%$ ) with greater reductions in ISI score in the saffron group compared to the placebo.

### RSQ (Secondary Outcome Measure 1)

Changes in RSQ total score across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 2. Saffron was associated with a statistically-significant reduction in RSQ score over time ( $F_{1,27}=25.25$ ,  $p<.001$ ). However, no significant change was observed in the placebo group ( $F_{1,26}=0.33$ ,  $p=.569$ ). A between-group analysis revealed a statistically-significant time  $\times$  group interaction ( $F_{1,53}=5.01$ ,  $p=.029$ ,  $\eta^2=8.6\%$ ) with greater improvements in RSQ score in the saffron group compared to the placebo.

## PSD (Secondary Outcome Measure 2)

Changes in PSD scores across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 2. Saffron was associated with statistically-significant improvements in the number of awakenings after sleep onset ( $F_{1,27}=14.65$ ,  $p=.001$ ), sleep quality ( $F_{1,27}=24.58$ ,  $p<.001$ ), and alertness upon awakening ( $F_{1,27}=4.82$ ,  $p=.037$ ). No other significant changes were observed in other PSD sleep parameters. In the placebo group, there were no statistically-significant changes in any PSD sleep parameters over time. Between-group analyses revealed statistically-significant time  $\times$  group interactions in sleep quality ( $F_{1,53}=6.51$ ,  $p=.014$ ,  $\eta^2=10.9\%$ ), and near-significant effects in number of awakenings after sleep onset ( $F_{1,53}=3.92$ ,  $p=.053$ ,  $\eta^2=6.9\%$ ), and alertness upon awakening ( $F_{1,53}=3.66$ ,  $p=.061$ ,  $\eta^2=6.5\%$ ).

## DASS-21 (Secondary Outcome Measure 3)

**Table 3. Change in DASS-21 Scores**

			Baseline	Day 28	Repeated Measure ANOVA	
					p-value time effects	p-value time x group interaction
Depression	Saffron	Mean	4.21	4.41	.852	.467
		SE	1.11	0.88		
	Placebo	Mean	3.31	2.38	.397	
		SE	1.17	0.93		
Anxiety	Saffron	Mean	2.97	3.59	.286	.102
		SE	0.74	0.69		
	Placebo	Mean	3.31	2.38	.228	
		SE	0.79	0.72		
Stress	Saffron	Mean	8.41	9.52	.411	.995
		SE	1.05	1.31		
	Placebo	Mean	8.38	9.50	.405	
		SE	1.11	1.38		

Changes in DASS-21 sub-scores across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 3. A repeated-measures ANOVA revealed there were no statistically-significant changes in DASS-21 depression, anxiety, or stress scores over time in either the saffron or placebo group. Between-group analyses also revealed no statistically-significant time  $\times$  group interactions in depression ( $F_{1,53}=0.54$ ,  $p=.467$ ), anxiety ( $F_{1,53}=2.76$ ,  $p=.102$ ), or stress ( $F_{1,53}=0.00$ ,  $p=.995$ ).

### *DBAS (Process Measure)*

A Pearson's correlation coefficient was calculated to examine the relationship between the baseline DBAS total score and improvement in sleep quality as measured by the percentage change in ISI. A statistically-significant correlation between baseline DBAS score and percentage change in ISI score was observed ( $r=-.325$ ,  $p=.015$ )

### *Intake of supplements*

At day 28, participants recorded their quantity of remaining supplements. All participants reported taking greater than 70% of their tablets.

### *Adverse events*

No significant adverse events were reported by participants, with similar dropout rates across the two conditions. No participant withdrew from the study due to concerns associated with supplement intake. Further confirmation of the tolerability of saffron intake is provided by an examination of satisfaction ratings at the end of the study which indicated that 4% of participants in the saffron group (compared to 15% in the placebo group) were dissatisfied with their tablet intake.

## DISCUSSION

In this parallel, randomised, double-blind, placebo-controlled trial, the 28-day intake of a standardised saffron extract (affron®) was associated with a significant improvement in sleep quality in adults with self-reported unsatisfactory sleep. Compared to the placebo, affron® delivered at 14mg twice daily was associated with greater reductions in insomnia severity, as measured by the ISI, and non-restorative sleep, as measured by the RSQ. Based on sleep diary recordings, saffron also significantly increased ratings in sleep quality, and there were strong trends to suggest improvements in ratings of alertness upon awakening, and reductions in the number of awakenings after sleep onset. Saffron intake was well tolerated with no reported adverse events and positive satisfaction ratings by participants. Mood ratings as measured by the DASS-21 also remained constant over the 28-day period, with pre and post-intervention levels remaining within the normal ranges on all these subscales.

Saffron has an increasing body of evidence supporting its benefits in reducing depressive and anxiety symptoms (Marx et al., 2019) although evidence of its sleep-enhancing benefits is limited. In a small, pilot study, the 4-week administration of the identical saffron extract used in this study (affron®) resulted in greater improvements in sleep quality, sleep latency, and daytime dysfunction in a subset of poor sleepers compared to the placebo (Nishide et al., 2018). As an adjunct to antidepressants in adults

with unremitted depression, symptomatic improvements in sleep quality were also noted after saffron administration (Lopresti et al., 2019). In a randomised, double-blind, placebo-controlled, crossover study, the 14-day administration of crocetin (an active constituent in saffron) in adults with mild sleep complaints was associated with increases in electroencephalography (EEG) delta activity and subjective improvements in sleepiness and refreshment on rising. However, there were no significant changes in other sleep parameters (Umigai et al., 2018). Improvements in sleep quality have also been reported in type-2 diabetic adults with comorbid depression and anxiety after the 8-week intake of saffron (Milajerdi et al., 2018).

The mechanisms associated with saffron's potential sleep-enhancing qualities are uncertain although several possibilities are speculated. The serotonergic, glutaminergic and GABAergic system which are both implicated in sleep and insomnia (Gottesmann, 2002; Meyerhoff et al., 2014; van Dalen and Markus, 2019) have been influenced by saffron administration (Berger et al., 2011; Georgiadou et al., 2012; Hosseinzadeh and Sadeghnia, 2007; Sadeghnia et al., 2008). Saffron's anti-inflammatory effects (Zeinali et al., 2019) may also be associated with its sleep-enhancing and sleep restorative effects as insomnia is also associated with increased inflammatory markers (Slavish et al., 2018). In animal models, the individual saffron constituents comprising safranal, crocin and crocetin, have been associated with increases in non-rapid eye movement sleep (Liu et al., 2012; Masaki et al., 2012). Saffron also modified EEG activity by increasing delta power (Umigai et al., 2018). Further research is required to elucidate these findings and primary mechanisms associated with saffron's sleep-enhancing effects.

## Limitations and Directions for Future Research

Despite positive improvements in sleep quality associated with saffron intake, there are several limitations associated with this study. Outcome measures comprised validated, subjective sleep measures; however, no objective measure of sleep change was included. Polysomnography and actigraphy assessments will be important to include in future studies to help validate the results from subjective assessments. A single dose of 14mg of affron® twice daily was used in the study. The efficacy and safety of using differing doses require further investigation. Dose-escalation studies may also be helpful to examine its impact on the magnitude of treatment effects and in treatment non-responders. As saffron is subject to adulteration and the quality of extracts can vary significantly (Khilare et al., 2019), the generalisability of these findings to other saffron extracts should also be done cautiously. Therefore, replication using other saffron extracts is essential to assess the generalisability of findings. Dysfunctional beliefs about sleep as measured by the DBAS negatively impacted on treatment outcomes in all participants. This suggests that dysfunctional sleep beliefs present as a barrier to successful treatment. Therefore, modifying dysfunctional beliefs via interventions such as cognitive-

behaviour therapy (CBT) may be associated with greater treatment efficacy. Saffron as adjunct to CBT compared to a stand-alone intervention may be prudent to investigate in future studies. Finally, the sleep-enhancing effects of saffron in differing populations require further investigation. This includes individuals with comorbid medical and/or psychiatric conditions, chronic or severe sleep disturbances, and in participants with a range of demographic and psychographic characteristics.

The results from this study have demonstrated that a standardised saffron extract (affron®) at a dose of 14mg, twice daily for 28 days improved sleep quality in adults with self-reported unsatisfactory sleep. Saffron was well tolerated with no reported adverse effects. While positive, these findings require replication using a larger sample size and differing populations.

## References

- Berger, F., Hensel, A., Nieber, K., 2011. Saffron extract and trans-crocin inhibit glutamatergic synaptic transmission in rat cortical brain slices. *Neuroscience* 180, 238-247.
- Brown, T.A., Chorpita, B.F., Korotitsch, W., Barlow, D.H., 1997. Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples. *Behav Res Ther* 35, 79-89.
- Buysse, D.J., Angst, J., Gamma, A., Ajdacic, V., Eich, D., Rossler, W., 2008. Prevalence, course, and comorbidity of insomnia and depression in young adults. *Sleep* 31, 473-480.
- da Silva, A.A., de Mello, R.G., Schaan, C.W., Fuchs, F.D., Redline, S., Fuchs, S.C., 2016. Sleep duration and mortality in the elderly: a systematic review with meta-analysis. *BMJ Open* 6, e008119.
- Daley, M., Morin, C.M., LeBlanc, M., Gregoire, J.P., Savard, J., Baillargeon, L., 2009. Insomnia and its relationship to health-care utilization, work absenteeism, productivity and accidents. *Sleep Med* 10, 427-438.
- Drake, C.L., Hays, R.D., Morlock, R., Wang, F., Shikar, R., Frank, L., Downey, R., Roth, T., 2014. Development and evaluation of a measure to assess restorative sleep. *J Clin Sleep Med* 10, 733-741, 741A-741E.
- Espie, C.A., Pawlecki, B., Waterfield, D., Fitton, K., Radocchia, M., Luik, A.I., 2018. Insomnia symptoms and their association with workplace productivity: cross-sectional and pre-post intervention analyses from a large multinational manufacturing company. *Sleep Health* 4, 307-312.
- Georgiadou, G., Tarantilis, P.A., Pitsikas, N., 2012. Effects of the active constituents of *Crocus Sativus* L., crocins, in an animal model of obsessive-compulsive disorder. *Neuroscience letters* 528, 27-30.
- Gottesmann, C., 2002. GABA mechanisms and sleep. *Neuroscience* 111, 231-239.
- Hosseinzadeh, H., Sadeghnia, H.R., 2007. Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. *Phytomedicine* 14, 256-262.

Khilare, V., Tiknaik, A., Prakash, B., Ughade, B., Korhale, G., Nalage, D., Ahmed, N., Khedkar, C., Khedkar, G., 2019. Multiple tests on saffron find new adulterant materials and reveal that Ist grade saffron is rare in the market. *Food Chem* 272, 635-642.

Leach, M.J., Page, A.T., 2015. Herbal medicine for insomnia: A systematic review and meta-analysis. *Sleep Med Rev* 24, 1-12.

Liu, Z., Xu, X.H., Liu, T.Y., Hong, Z.Y., Urade, Y., Huang, Z.L., Qu, W.M., 2012. Safranal enhances non-rapid eye movement sleep in pentobarbital-treated mice. *CNS Neurosci Ther* 18, 623-630.

Lopresti, A.L., Drummond, P.D., 2014. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. *Hum Psychopharmacol* 29, 517-527.

Lopresti, A.L., Smith, S.J., Hood, S.D., Drummond, P.D., 2019. Efficacy of a standardised saffron extract (affron(R)) as an add-on to antidepressant medication for the treatment of persistent depressive symptoms in adults: A randomised, double-blind, placebo-controlled study. *J Psychopharmacol*, 269881119867703.

Marx, W., Lane, M., Rocks, T., Ruusunen, A., Loughman, A., Lopresti, A.L., Marshall, S., Berk, M., Jacka, F.N., Dean, O.M., 2019. The effect of saffron supplementation on symptoms of depression and anxiety: a systematic review and meta-analysis. *Nutr Rev*.

Masaki, M., Aritake, K., Tanaka, H., Shoyama, Y., Huang, Z.L., Urade, Y., 2012. Crocin promotes non-rapid eye movement sleep in mice. *Mol Nutr Food Res* 56, 304-308.

Maust, D.T., Solway, E., Clark, S.J., Kirch, M., Singer, D.C., Malani, P., 2019. Prescription and Nonprescription Sleep Product Use Among Older Adults in the United States. *Am J Geriatr Psychiatry* 27, 32-41.

Medic, G., Wille, M., Hemels, M.E., 2017. Short- and long-term health consequences of sleep disruption. *Nat Sci Sleep* 9, 151-161.

Meyerhoff, D.J., Mon, A., Metzler, T., Neylan, T.C., 2014. Cortical gamma-aminobutyric acid and glutamate in posttraumatic stress disorder and their relationships to self-reported sleep quality. *Sleep* 37, 893-900.

Milajerdi, A., Jazayeri, S., Shirzadi, E., Hashemzadeh, N., Azizgol, A., Djazayeri, A., Esmailzadeh, A., Akhondzadeh, S., 2018. The effects of alcoholic extract of saffron (*Crocus sativus* L.) on mild to moderate comorbid depression-anxiety, sleep quality, and life satisfaction in type 2 diabetes mellitus: A double-blind, randomized and placebo-controlled clinical trial. *Complement Ther Med* 41, 196-202.

Monk, T.H., Reynolds, C.F., 3rd, Kupfer, D.J., Buysse, D.J., Coble, P.A., Hayes, A.J., Machen, M.A., Petrie, S.R., Ritenour, A.M., 1994. The Pittsburgh Sleep Diary. *J Sleep Res* 3, 111-120.

- Morin, C.M., Belleville, G., Belanger, L., Ivers, H., 2011. The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. *Sleep* 34, 601-608.
- Morin, C.M., Vallieres, A., Ivers, H., 2007. Dysfunctional beliefs and attitudes about sleep (DBAS): validation of a brief version (DBAS-16). *Sleep* 30, 1547-1554.
- Nishide, A., Fujita, T., Nagaregawa, Y., Shoyama, Y., Ohnuki, K., Shimizu, K., Yamamoto, T., Watanabe, T., Ohnuki, K., 2018. Sleep Enhancement by Saffron Extract affron® in Randomized Control Trial. *Jpn Pharmacol Ther* 46, 1407-1415.
- Sadeghnia, H.R., Cortez, M.A., Liu, D., Hosseinzadeh, H., Snead, O.C., 3rd, 2008. Antiabsence effects of safranal in acute experimental seizure models: EEG and autoradiography. *J Pharm Pharm Sci* 11, 1-14.
- Sanchez-Ortuno, M.M., Belanger, L., Ivers, H., LeBlanc, M., Morin, C.M., 2009. The use of natural products for sleep: A common practice? *Sleep Med* 10, 982-987.
- Schutte-Rodin, S., Broch, L., Buysse, D., Dorsey, C., Sateia, M., 2008. Clinical guideline for the evaluation and management of chronic insomnia in adults. *J Clin Sleep Med* 4, 487-504.
- Slavish, D.C., Graham-Engeland, J.E., Engeland, C.G., Taylor, D.J., Buxton, O.M., 2018. Insomnia symptoms are associated with elevated C-reactive protein in young adults. *Psychol Health* 33, 1396-1415.
- Soper, D.S., 2019. A-priori Sample Size Calculator for Student t-Tests [Software].
- Tabachnick, B.G., Fidell, L.S., 2007. Using Multivariate Statistics, 5th edition ed. Allyn, Boston.
- Toth, B., Hegyi, P., Lantos, T., Szakacs, Z., Keremi, B., Varga, G., Tenk, J., Petervari, E., Balasko, M., Rumbus, Z., Rakonczay, Z., Balint, E.R., Kiss, T., Csupor, D., 2018. The Efficacy of Saffron in the Treatment of Mild to Moderate Depression: A Meta-analysis. *Planta Med*.
- Umigai, N., Takeda, R., Mori, A., 2018. Effect of crocetin on quality of sleep: A randomized, double-blind, placebo-controlled, crossover study. *Complement Ther Med* 41, 47-51.
- van Dalen, J.H., Markus, C.R., 2019. The involvement of sleep in the relationship between the serotonin transporter gene-linked polymorphic region (5-HTTLPR) and depression: A systematic review. *J Affect Disord* 256, 205-212.
- Zeinali, M., Zirak, M.R., Rezaee, S.A., Karimi, G., Hosseinzadeh, H., 2019. Immunoregulatory and anti-inflammatory properties of *Crocus sativus* (Saffron) and its main active constituents: A review. *Iran J Basic Med Sci* 22, 334-344.