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Research paper

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

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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®, 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® 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® 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®) 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 (Sijtsema et al., 2014), higher risk of unemployment in adulthood (Egan et al., 2016), increased medical burden (Pape et al., 2012), socialisation difficulties (Zwierzynska 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).

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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-adenosylmethionine 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 (Hosseinzadeh 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). 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 (microcrystalline 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

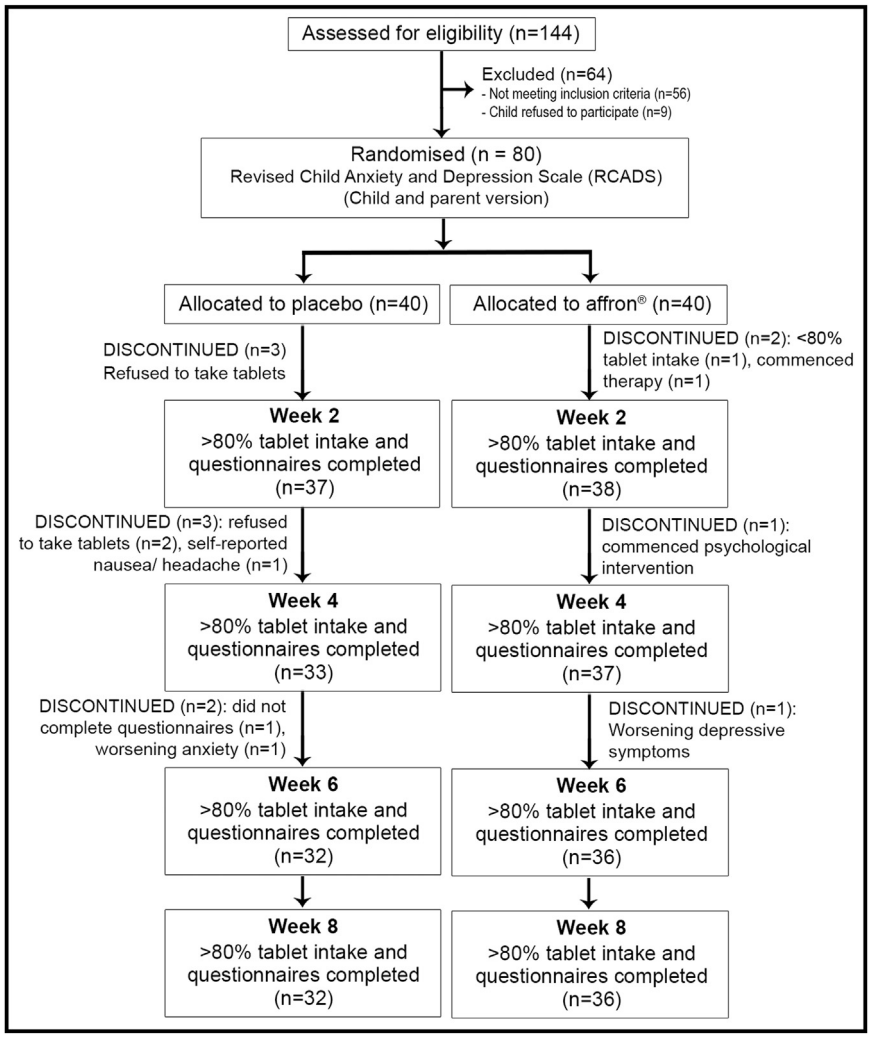

Fig. 1. Systematic illustration of study design.

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 × 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 ($\chi2(1) = 6.96$, $p = 0.014$, 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[a]
	Male	38%	25%	
Age	Mean	13.93	14.08	0.642[b]
	SE	0.24	0.21	
Weight	Mean	54.30	59.29	0.136[b]
	SE	2.25	2.39	
YOUTH RCADS Baseline Scores				
Separation Anxiety	Mean	6.08	6.80	0.404[b]
	SE	0.58	0.64	
Generalised Anxiety	Mean	8.15	8.45	0.674[b]
	SE	0.50	0.50	
Panic	Mean	9.30	10.18	0.485[b]
	SE	0.90	0.87	
Social Phobia	Mean	16.48	17.20	0.513[b]
	SE	0.76	0.80	
Obsessions/Compulsions	Mean	6.10	5.20	0.171[b]
	SE	0.43	0.49	
Depression	Mean	12.73	13.93	0.289[b]
	SE	0.61	0.95	
PARENT RCADS Baseline Scores				
Separation Anxiety	Mean	6.65	6.23	0.606[b]
	SE	0.59	0.57	
Generalised Anxiety	Mean	7.80	6.95	0.180[b]
	SE	0.42	0.47	
Panic	Mean	6.73	7.63	0.342[b]
	SE	0.62	0.71	
Social Phobia	Mean	17.08	16.55	0.650[b]
	SE	0.81	0.82	
Obsessions/Compulsions	Mean	3.70	2.95	0.150[b]
	SE	0.35	0.38	
Depression	Mean	12.45	12.23	0.817[b]
	SE	0.54	0.80	

[a] Pearson Chi-Square test.
[b] 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%) ($\chi2(1) = 0.205$, $p = 0.424$, 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[a]	Saffron extract					p-value[a]	p-value[b]	Cohen's d effect size
		Week 0	Week 2	Week 4	Week 6	Week 8		Week 0	Week 2	Week 4	Week 6	Week 8			
Youth RCADS Scores															
Separation Anxiety	Mean	6.08	5.62	5.19	4.89	4.78	0.018	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	0.093	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	< 0.001	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	< 0.001	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	< 0.001	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	0.602	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	Not assessed[c]	48.05	35.38	31.84	29.86	28.78	Not assessed[c]	Not assessed[c]	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	Not assessed[c]	61.97	46.32	42.24	40.27	39.30	Not assessed[c]	Not assessed[c]	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	< 0.01	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	< 0.001	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	< 0.001	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	< 0.001	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	< 0.001	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	< 0.001	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	Not assessed[c]	39.87	30.32	24.37	24.24	21.89	Not assessed[c]	Not assessed[c]	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	Not assessed[c]	52.11	39.87	32.34	31.97	29.53	Not assessed[c]	Not assessed[c]	0.43
	SE	2.40	2.08	2.63	2.67	2.97		2.54	2.53	2.19	2.36	1.99			

[a] Repeated measures ANOVA time effects (week 0 to week 8).
[b] Repeated measures ANOVA time × group interaction.
[c] ANOVA not conducted to prevent problems of collinearity.

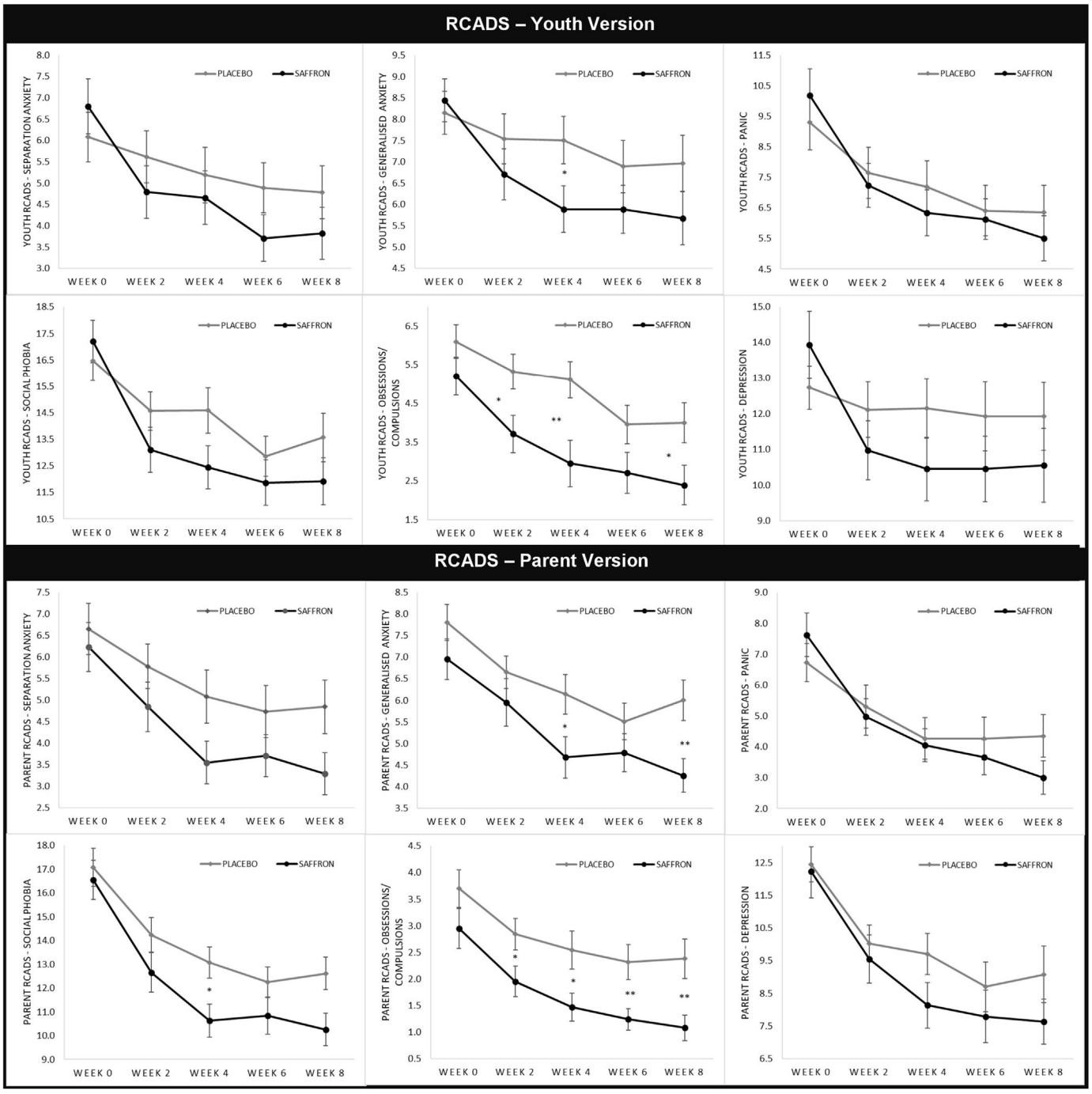

Fig. 2. Change in RCADS Youth & Parent raw scores over 8-week intervention. Vertical bars depict standard errors; Asterisks depict between group difference at specified time point (*p < 0.05; **p < 0.01).

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%

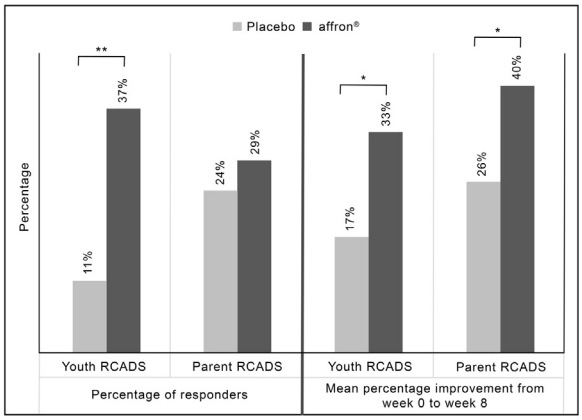

Fig. 3. Percentage of treatment responders (i.e., > 50% reduction in total internalising score) and mean percentage improvement in RCADS total internalising score (from baseline to week 8). Asterisks depict between significant group difference at specified time point, based on independent samples T-test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

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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.

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