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Research paper

A randomised placebo controlled clinical trial on the efficacy of *Caralluma fimbriata* supplement for reducing anxiety and stress in healthy adults over eight weeks

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Background: This study investigated the efficacy of a succulent, *Caralluma fimbriata* extract (CFE) in reducing anxiety and stress in healthy adults.
Methods: An 8 week double-blind randomised clinical trial, in which 97 adults self-reporting mild to moderate anxiety were given 500 mg b.d. CFE (n = 49), or 500 mg b.d. placebo (n = 48). Anxiety and stress were measured at baseline, week 4, and week 8 to investigate the timing of treatment effect using the GAD-7, Perceived Stress Scale (PSS), Positive and Negative Affect Schedule (PANAS), and salivary cortisol. Data were analysed using mixed ANOVAs on SPSS v.24.
Results: Results indicated a significant reduction in anxiety and stress in both groups at week 4 and week 8. The reduction in the CFE group was significantly greater ($p < .05$) than in the placebo group on the GAD-7 and PSS at week 4 and week 8, and in Negative affect at week 4. Improvement in Positive affect was greater in the CFE group than in the placebo group at week 8. Cortisol analysis indicated that CFE may act through the Hypothalamic-Pituitary-Adrenal (HPA) axis, showing statistically significant changes in males, but not in females.
Limitations: Self-reported instruments involve subjective interpretation thus salivary cortisol was employed as a more objective measure. The study would benefit from a larger sample and longer trial, and the inclusion of a wait-list group to allow comparison between treatment and no treatment.
Conclusions: The findings indicate that CFE is superior to placebo in reducing subclinical anxiety and stress over 8 weeks.

1. Introduction

The global prevalence of diagnosed anxiety disorders in Western cultures is greater than 10 percent (Baxter et al., 2013). There are also substantial numbers of people who experience anxiety but have not been diagnosed with a disorder. There is a growing consensus that stress and subclinical (mild to moderate) anxiety also reduce quality of life (Rapaport et al., 2005), and merit treatment (Olatunji et al., 2007).

Pharmaceutical treatments for anxiety come with the risk of adverse effects such as diarrhea and nausea, habituation, dependency and withdrawal, and low to zero efficacy for subclinical presentations (Barrett et al., 2001; Eisenberg et al., 2011; Mathew et al., 2008). Even so, approximately two thirds of Australian prescriptions are prescribed for sub-clinical presentations (Ellis and Smith, 2002; Jureidini and Tonkin, 2006). Alternative treatments have grown in popularity, with an estimated 43 percent of anxiety sufferers using some form of complementary therapy (Eisenberg et al., 1998; Kessler et al., 2001).

The evidence base for herbal alternatives however, is poor (Beaubrun and Gray, 2000; Ernst, 2006), and a need exists for well-documented preventative interventions for subclinical cases, from the alternative sector. *Caralluma fimbriata* extract (CFE) may be a candidate for this gap.

Caralluma is a succulent genus that has been traditionally used in India as an appetite suppressant during famine (Adnan et al., 2014). Pregnane glycosides in CFE are most likely responsible for this effect, through their action on the hypothalamus and the cortisol response (Komarnytsky et al., 2013a,b; Vajha et al., 2014). The majority of CFE research focuses on appetite and weight loss in both males and females aged 25 to 60 years (Arora et al., 2015; Astell et al., 2013; Kamalakkannan et al., 2010; Kumar et al., 2013; Kuriyan et al., 2007).

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One study (Griggs et al., 2015) found CFE effective in the management of Prader-Willi syndrome, reducing not only hyperphagia but also a number of behavioural traits which broadly overlap with anxiety.

Saivasanthi et al. (2011) had previously explored traditional claims of the herb's use as an anti-inflammatory and analgesic, and observed a significant anxiolytic effect of CFE on mice, comparable to Diazepam using the Elevated Plus Maze (EPM). The EPM is used to detect anxiety behaviour in rodents. Greater frequency and time in an open arm of an elevated maze is associated with lower anxiety (Cruz et al., 1994; Espejo, 1997; Lister, 1987).

Rajendran et al. (2014) similarly found treatment with CFE was associated with significant improvements in nootropic and anxiolytic activity for mice, also using the EPM. These findings of enhanced cognition and reduced anxiety raised clinical interest in CFE as a possible alternative to medications such as benzodiazepines, the most prescribed pharmaceutical anxiolytics, which can have adverse effects on cognitive function (Kumar et al., 1987; Lader, 2011). It also highlighted the possible use of CFE for conditions that co-present with anxiety and cognitive decline, such as Alzheimer's Disease.

However, while the EPM has been validated for anxiety behaviours in animals (Lister, 1987), its generalisability to humans has been questioned (Borsini et al., 2002). The present study provides the first human trial of CFE for anxiety, adding the biomarker of cortisol to the trial, which has not been measured in mice models of CFE trials.

While cortisol in mice has not been measured in prior CFE trials, Chen et al. (2016) analysed cortisol and brain-derived neurotropic factor (BDNF) in rodents exhibiting anxiety behaviours. They identified the same hormonal channel in rodents through which CFE's active compound (pregnane glycoside) acts in humans, inhibiting cortisol production (Komarnytsky et al., 2013). This channel, the HPA axis, regulates anxiety through cortisol (de Kloet, Joëls, and Holsboer, 2005; Tsigos and Chrousos, 2002). Therefore, if CFE's biochemical properties include compounds known to lower the cortisol response via the HPA axis, CFE may be an alternative treatment for mild to moderate stress and anxiety. The biomarker of cortisol was therefore added to the present study. Since cortisol responses are mediated by gender, with males typically demonstrating a greater response, gender was accounted for in cortisol analyses (Kirschbaum et al., 1992; Smyth et al., 1998; Tsigos and Chrousos, 2002; Young and Korszun, 1999).

Weight also has a well-documented influence on anxiety in both men and women (Rivenes et al., 2009; Zhao et al., 2009), and may itself be influenced by CFE's anorexigenic effects. It is a potentially confounding variable, so changes in weight and BMI during the intervention were monitored to account for these potential confounders.

It is hypothesised that a reduction in anxiety and stress over eight weeks would be significantly greater in the active treatment group than in the placebo group in all measures.

2. Methods

2.1. Clinical trial

The University of the Sunshine Coast's Human Research Ethics Committee granted ethics approval (S/16/936) to conduct a double-blind randomised placebo controlled clinical trial of *Caralluma fimbriata* supplement. The study was registered as a clinical trial with the Australian New Zealand Clinical Trials Registry (ACTRN: 12616000741482; https://www.anzctr.org.au).

2.2. Participants

Participants were recruited through voluntary sampling using digital advertising (Facebook), a dedicated website (anxietytrial.com.au), and the clinical trial database of RDC Clinical. A psychology clinic and a medical clinic also recommended patients to the trial.

Male and female participants were included for assessment if they were over 18 years, self-reported mild to moderate anxiety on the GAD-7, were not diagnosed with a mood disorder, were generally healthy (BMI < 30), and had given written informed consent.

Once participants were recruited they were mailed the product and three salivary cortisol test kits. Participants were emailed a SurveyMonkey link to the trial survey questionnaire at three time points during the trial: baseline, week 4, and week 8. Reply paid envelopes were provided for saliva sample kits, to be mailed to Australian Clinical Labs in Melbourne by the participants. Saliva integrity remained intact, unrefrigerated, throughout the postage process. Pathology results were sent electronically from Australian Clinical Labs to the investigator within a week of receiving the samples.

After screening interviews (via telephone) with 130 potential participants, 13 were excluded (12 for medical excluding conditions and 1 for being under 18 years), and 117 healthy adults aged 18–70 years were randomised into two groups. Twenty participants were lost to follow-up, leaving 97 active participants at completion (49 in the active group, and 48 in the placebo group). Eight participants (all from the placebo group) were assigned as Intention to Treat (ITT) after completing baseline and week 4 surveys, but not week 8. The “last observation carried forward” method was used for ITT, in which the last observed value was used to fill in a missing value at a later point (Unnebrink and Windeler, 2001). Thus, week 4 values were carried forward to week 8, for the eight participants who left the study between week 4 and week 8. A further eight participants did not submit adequate cortisol samples, so their data were not used for cortisol results.

The mean age of participants was 44 years (Table 1). The total sample was 71% female. The mean weight at baseline was 76.5 kg. The mean BMI at baseline was 26. The majority of participants were non-smokers (91%), consumed 3 or less drinks per week (75%), and reported undertaking regular weekly exercise (55%).

2.3. Materials

2.3.1. Generalized anxiety disorder 7-item (GAD-7)

The GAD-7 (Spitzer et al., 2006) consisted of seven items regarding the previous two weeks (e.g., *I have trouble relaxing*). Scoring was on a four point Likert scale, where 0 = *not at all*; 1 = *several days*; 2 = *more than half the days*; and 3 = *nearly every day*. Cut-off scores for the GAD-7 are 5, 10, and 15 (minimal, mild, moderate, and severe). The internal consistency of the GAD-7 is excellent (Cronbach's $\alpha = 0.92$;

Table 1
Participant demographics at baseline.

Demographics	Total $N = 97$	Group Active Treatment $n = 49$	Placebo $n = 48$
Age (years)			
Mean (SD)	44.2 (14.11)	44.5 (15.22)	44.0 (13.04)
Range	18–79	19–76	18–79
Gender (number, %)			
Female	69 (71.1%)	35 (71.4%)	34 (70.8%)
Male	28 (28.9%)	14 (28.6%)	14 (29.2%)
Weight Mean kg (SD)	76.58 (16.44)	76.36 (14.22)	76.80 (18.58)
BMI Mean (SD)	26.25 (4.39)	26.14 (3.56)	26.37 (5.13)
Smoking; number, (%)			
Yes	6 (6.2%)	3 (6.1%)	3 (6.2%)
No	91 (93.8%)	46 (93.9%)	45 (93.8%)
Alcohol (number per week, %)			
0	43 (44.3%)	21 (42.8%)	22 (45.8%)
3 or less	30 (30.9%)	14 (28.6%)	16 (33.3%)
Over 3	24 (24.8%)	14 (28.6%)	10 (20.9%)
Regular weekly exercise			
Yes (number, %)	54 (55.7%)	29 (59.2%)	25 (52.1%)
No (number, %)	43 (44.3%)	20 (40.8%)	23 (47.9%)

Note. No significant differences between treatments at baseline ($p < .05$, two-tailed).

Spitzer et al., 2006).

2.3.2. Perceived stress scale (PSS)

The PSS (Cohen and Williamson, 1988; Cohen et al., 1983) consisted of ten items regarding the previous month (e.g., "In the past month how often have you felt that things were going your way?"). Scoring was on a five point scale, where 0 = *never*; 1 = *almost never*; 2 = *sometimes*; 3 = *fairly often*; and 4 = *very often*. The PSS reports excellent reliability (Cronbach's α = 0.89; Roberti et al., 2006).

2.3.3. Positive and negative affect schedule (PANAS)

The PANAS (Watson and Clark, 1994) consisted of 20 items; 10 positive words (e.g., *excited*), and 10 negative words (e.g., *irritable*). Scoring was on a five point Likert 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). Both subscales showed strong internal consistency in non-clinical samples of over 1,000 participants (Cronbach's α = 0.89 for PA; α = 0.85 for NA; Crawford and Henry, 2004).

2.3.4. Cortisol

Salivary cortisol (nmol/L) was collected from each participant to gain a biological measure of daily stress (Smyth et al., 1998). The Elecsys Cortisol II (Adamek, 2015) is a validated assay used by Australian Clinical Labs for analysing samples. Although it is an objective measure of stress (Hellhammer et al., 2009; Kahn et al., 1988), cortisol is limited by confounding variables (such as time of day and medical conditions) that could not all be controlled. Participants were asked to provide their samples under the same conditions each time (before breakfast, brushing teeth, and applying makeup). Each outcome was also measured at four weeks to investigate the timing of any potential treatment effect.

2.4. Procedures

2.4.1. Randomisation

Participants were randomised into two groups (active or placebo) through the allocation of numbered treatment bottles. Bottles were randomly allocated a number using the software Sealed Envelope ("Sealed Envelope," 2017) and were labelled identically except for the allocation number. The active treatment and the placebo were identical looking capsules. Participants were allocated containers in numerical sequential order. The randomisation code was maintained independently to keep the investigator blind to treatment allocation, and to facilitate randomisation breaking in the case of an adverse event. The investigator was informed of group allocation post-trial for statistical analyses.

2.4.2. Interviews

Potential participants were provided with information about the product and screened for exclusion criteria at a baseline interview. They were advised they could withdraw at any time. If they wished to proceed, an email was sent with the GAD-7, demographic questions, a consent form, and a Research Project Information Sheet. Participants were included after the consent form was received and the baseline GAD-7 was screened. They were then mailed the saliva sample kits and treatment bottles and emailed the PSS and PANAS to complete before commencing treatment. All measures were resent at week 4 and week 8. If no response was received within 3 days, participants were followed up by telephone call to reduce attrition.

2.4.3. Interventions

Standardisation of CFE primary production was undertaken by Green Chem Bangalore with quality assurance of active compound quantification. Capsules (active and placebo) were produced in Australia by Pharmako Biotechnologies and registered with the Therapeutic Goods Administration, with certificates of analysis provided, ensuring standardisation of bioactive compounds.

Participants were allocated bottles containing either active treatment or placebo. Each participant was instructed to take two capsules, one in the morning and one in the evening 30 min before food for eight weeks. The active treatment contained 500 mg *Caralluma adscendens* var. *fimbriata*, and the placebo contained 500 mg maltodextrin, both in a gelatine capsule. *Caralluma fimbriata* Extract (SLIMALUMA) was supplied by Gencor Pacific Limited, Hong Kong and was manufactured by Green Chem, Bangalore.

2.5. Data analyses

Analyses were completed using IBM Statistical Package for Social Sciences (SPSS) version 24. Data screening confirmed that data were complete and scores were within expected range. A priori power analyses conducted using G*Power version 3.1.9.2 (Buchner et al., 2014) determined a sample size of 86 was required to attain a power of 0.80 for two-tailed tests detecting a medium effect size. This indicated that the initial sample size ($N = 117$) was adequate. The data were screened for univariate outliers (z scores were used at $p < .001$), and all cases were retained. After losing twenty participants to follow-up, the final sample size remained adequate ($N = 97$) based on the a priori power analyses. Data were inspected for normality through visual inspections of histograms, and distribution appeared normal. Shapiro-Wilk statistics indicated a slight positive skewness for GAD-7, the three appetite subscales, and cortisol in the active group, and GAD-7 and cortisol for the placebo group. Data transformations were not applied since skewness was mild and the selected statistic of ANOVA is considered to be robust against mild to moderate violations of normality when those violations are not due to outliers, which they were not (Tabachnick and Fidell, 2014).

Mixed 2 × 3 ANOVAs were conducted for each outcome. The between groups variable (active treatment, placebo) and the repeated measures variable (time of measurement; baseline, week 4, and week 8) were assessed at an alpha level of 0.05. Pairwise comparisons were made to investigate changes in treatment effect over time, using a Bonferroni adjustment. Cohen's d was calculated for each pairwise comparison and small, medium, and large effects were reported (0.20, 0.50, and 0.80 respectively). A paired sample t-test analysed changes in weight and BMI during the intervention to check for variables that may confound anxiety results. Main effects for time and treatment were measured, and eta-squared calculations were reported for small, medium, and large effect sizes (0.02, 0.13, and 0.26 respectively).

Assumptions for homogeneity were assessed for between groups measures using Levene's test, and assumptions of sphericity were checked for repeated measures using Mauchly's test. Homogeneity was not violated in any of the ANOVAs reported, and where violations for sphericity were found, the Greenhouse-Geisser adjustment was applied to the degrees of freedom.

Cortisol data were analysed separately for males and females because prior research indicated that ovarian hormones can modify cortisol levels in the HPA axis (Nolen-Hoeksema, 2001).

3. Results

3.1. Baseline measures

The active treatment group and placebo group were evenly matched at baseline in all demographics, with no significant differences between groups (Table 1). There were also no significant differences between groups at baseline for any of the scales or subscales: Anxiety, $F(1,95) = 0.148$, $p = .701$; Stress, $F(1,95) = 0.005$, $p = .944$; Positive Affect, $F(1,95) = 1.714$, $p = .194$; Negative Affect, $F(1,95) = 0.411$, $p = .523$; Cortisol, $F(1,95) = 0.538$, $p = .465$.

Table 2
Mean weight, BMI, and Standard Deviations (SD) at baseline and week eight.

Measurement	Group Active treatment	Placebo
Mean weight (kg)		
Baseline (SD)	76.4 (14.2)	76.6 (18.6)
Week eight (SD)	76.8 (13.9)	77.2 (18.6)
Mean BMI		
Baseline (SD)	26.1 (3.6)	26.2 (5.1)
Week eight (SD)	26.2 (3.5)	26.5 (5.2)

Note. No significant differences between baseline and week 8 in weight or BMI for either group ($p < .05$, two-tailed).

3.2. Changes in weight and BMI from baseline to week eight

A paired samples *t*-test found changes in weight and BMI from baseline to week 8 in the active group to be non-significant; $t(48) = -0.62$, $p = .541$, and $t(48) = -0.68$, $p = .503$ for weight and BMI respectively. Changes in weight and BMI for the placebo group were also non-significant; $t(47) = -1.51$, $p = .137$, and $t(47) = -1.60$, $p = .121$ for weight and BMI respectively. Table 2 shows mean weight and BMI for both groups.

3.3. Mood measures

Mood measures (GAD-7 for anxiety, PSS for stress, PANAS for positive affect and negative affect, and Cortisol) were recorded at baseline, week 4, and week 8. The mean group scores for each scale are presented in Table 3.

Differences Between Groups in Anxiety (GAD-7). Fig. 1 depicts the mean anxiety scores for both groups at each time point. Mauchly's test indicated that the assumption of sphericity had been violated for the main effect of the anxiety measure, $\chi^2(2) = 8.95$, $p = .011$. Therefore, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.92$). The interaction of the treatment group and time was significant, indicating that anxiety scores were significantly lower in the active group than in the placebo group, $F(1.83,174.18) = 5.08$, $p = .009$, $\eta^2 = 0.051$ (a large effect size). The power of the interaction was strong at 0.79. Pairwise comparisons at week 4 revealed that the active group had significantly lower anxiety than the placebo group, $t(95) = -2.93$, $p = .004$, $d = 0.59$ (a medium effect size). Week 8 comparisons revealed a similar result, with the active group showing significantly lower anxiety than the placebo group, $t(95) = -2.76$, $p = .007$, $d = 0.56$ (a medium effect size).

There were also significant main effects for time, $F(1.83,174.18) = 78.28$, $p < .001$, $\eta^2 = 0.452$ (a large effect size). This indicated that anxiety reduced for both groups at week 4 and week 8 regardless of the treatment. The main effect of treatment was also significant, $F(1,95) = 6.71$, $p = .011$, $\eta^2 = 0.066$ (a medium effect size), showing that the active group overall experienced significantly less anxiety than the placebo group.

Differences Between Groups in Stress (PSS). Fig. 2 depicts the mean stress scores for both groups at baseline, week 4, and week 8. Mauchly's test indicated that the assumption of sphericity had not been violated for the main effect of stress. The interaction of treatment group and time was significant, indicating that stress scores were significantly lower in the active group than in the placebo group, $F(2,190) = 6.19$, $p = .002$, $\eta^2 = 0.061$ (a medium effect size). The power of the interaction was strong at 0.88. Pairwise comparisons at week 4 revealed that the active treatment group had a significantly lower level of stress than the placebo group, $t(95) = -2.43$ $p = .017$, $d = 0.49$ (a medium effect size). Similarly, week 8 pairwise comparisons revealed that the active treatment group reported a significantly lower level of stress than the placebo group, $t(95) = -2.78$, $p = .007$, $d = 0.56$ (a medium effect size).

There were significant main effects for time, $F(2,190) = 70.33$, $p < .001$, $\eta^2 = 0.425$ (a large effect size). This indicated that mean stress levels reduced for both groups regardless of treatment. The main effect for treatment was also significant, $F(1,95) = 4.72$, $p = .032$, $\eta^2 = 0.047$ (a small effect size). This showed that overall, the active group experienced significantly less stress than the placebo group.

Differences Between Groups in Positive Affect (PA). Fig. 3 depicts the mean positive affect scores for both groups at baseline, week 4, and week 8. Mauchly's test indicated that the assumption of sphericity had not been violated. The interaction of treatment group and time was not significant, $F(2,190) = 1.60$, $p = .205$. The power of the interaction was low at 0.34. Pairwise comparisons at week 4 also revealed a non-significant difference between groups, $t(95) = 1.71$, $p = .091$. Week 8 pairwise comparisons however, revealed that the active treatment group reported a significantly higher positive affect than placebo, $t(95) = 2.55$, $p = .012$, $d = 0.51$ (a medium effect size).

There were significant main effects for time, $F(2,190) = 12.67$, $p < .001$, $\eta^2 = 0.118$ (a large effect size) indicating that positive affect increased for both groups. The main effect for treatment was also significant, $F(1,95) = 4.89$, $p = .029$, $\eta^2 = 0.949$ (a large effect size), meaning that overall, the active group experienced significantly more positive affect than the placebo group.

Differences Between Groups in Negative Affect (NA). Fig. 4 depicts the mean negative affect scores for both groups at baseline, week 4, and week 8. Mauchly's test indicated that the assumption of sphericity had not been violated for the main effect of negative affect. The interaction of treatment group and time was not significant, $F(2,190) = 1.37$, $p = .257$. The power of the interaction was low at 0.30. Pairwise comparisons at week 4 revealed that the active treatment group had a significantly lower level of negative affect than the placebo group, $t(95) = -2.04$ $p = .044$, $d = 0.42$ (a medium effect size). By week 8, the pairwise comparisons revealed no significant difference in negative affect between groups, $t(95) = -1.72$, $p = .089$.

There were significant main effects for time, $F(2,190) = 47.84$, $p < .001$, $\eta^2 = 0.335$ (a large effect size) indicating that negative affect decreased for both groups regardless of treatment. However, the main effect for treatment was not significant, $F(1,95) = 3.05$, $p = .084$. This showed that overall, the difference between the active group and the placebo group was not significant.

Table 3
Mean Scores (SD) for each scale and subscale.

Instrument		Group Active treatment Baseline	Week 4	Week 8	Placebo Baseline	Week 4	Week 8
Anxiety	M SD	10.04 (2.88)	5.43*, ** (2.61)	5.18*, ** (3.81)	10.27 (3.00)	7.52* (4.24)	7.40* (4.07)
Stress	M SD	23.57 (4.86)	16.92*, ** (5.40)	15.82*,** (6.55)	23.65 (5.59)	19.85* (6.48)	19.54* (6.67)
Positive affect	M SD	26.92 (6.13)	29.53* (8.28)	31.56*,** (7.88)	25.17 (7.03)	26.75* (7.72)	27.42* (8.47)
Negative affect	M SD	26.86 (6.79)	20.22*, ** (7.04)	20.14* (7.82)	27.77* (7.24)	23.25* (7.54)	22.75 (7.08)
Male cortisol	M SD	12.27 (6.08)	10.91 (5.79)	8.09** (4.66)	11.07 (3.77)	11.57 (4.15)	12.79 (4.47)
Female cortisol	M SD	12.97 (5.53)	12.90 (6.55)	12.48 (6.25)	14.97 (8.28)	13.82 (6.83)	14.67 (6.77)

Note. * = statistically significant difference ($p < .05$) from Baseline; ** = statistically significant difference ($p < .05$) from placebo group at the same time point.

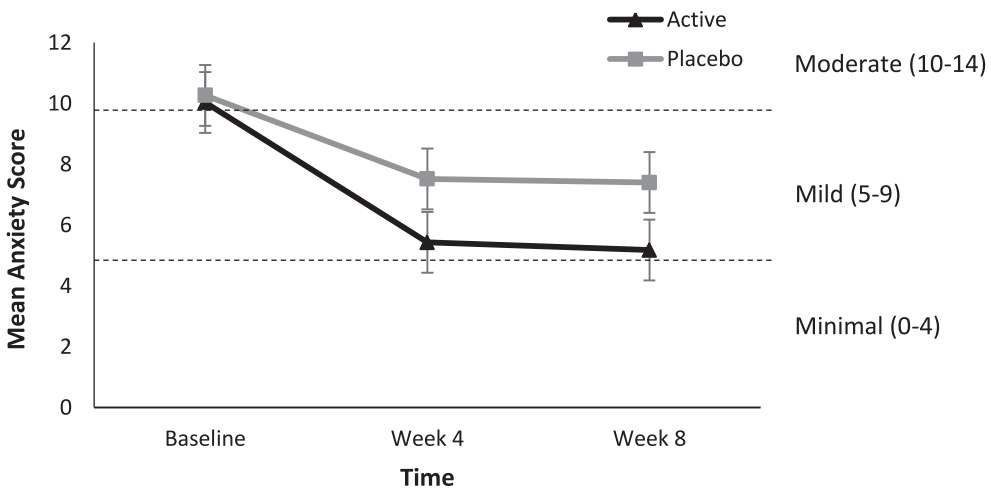


Fig. 1. Mean anxiety scores for active and placebo groups, with severity ranges. Error bars represent standard error of the mean.

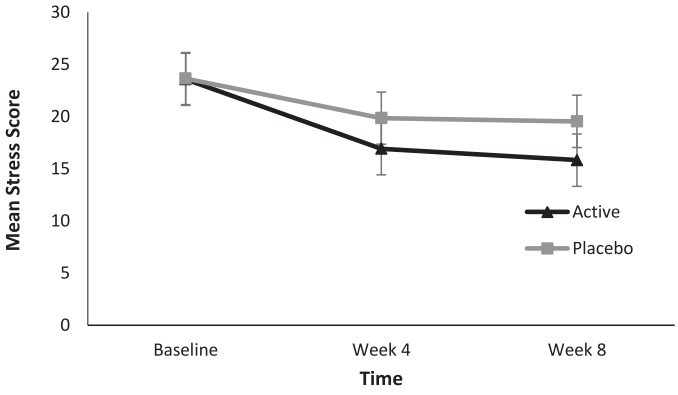


Fig. 2. Mean stress scores at baseline, week 4, and week 8 for the active group and placebo group. Error bars represent standard error of the mean.

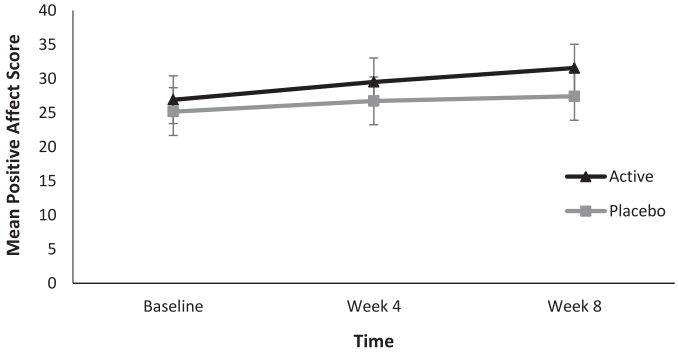


Fig. 3. Mean Positive Affect scores from the PANAS at baseline, week 4, and week 8 for the active group and placebo group. Error bars represent standard error of the mean.

Differences Between Groups in Cortisol. Fig. 5 depicts the mean cortisol readings for males and females in both groups at baseline, week 4, and week 8.

Mauchly's test indicated that the assumption of sphericity had not been violated for the main effect of cortisol in either the male or the female analyses. For males, the interaction of treatment group and time was significant, $F(2,46) = 3.68$, $p = .033$. The power of the interaction was average, at 0.65. Fig. 6 depicts the pairwise comparisons at each time point. Pairwise comparisons at week 4 showed no significant difference between groups, $t(23) = -0.33$, $p = .742$. Pairwise comparisons at week 8 however, revealed that the male active group had a significantly lower cortisol reading than the male placebo group, $t(23) = -2.55$, $p = .018$, $d = 1.03$ (a large effect size). In the male group, there were no significant main effects for time, $F(2,46) = 0.64$, $p = .534$, and no significant main effects for treatment, $F(1,23) = 0.91$, $p = .349$.

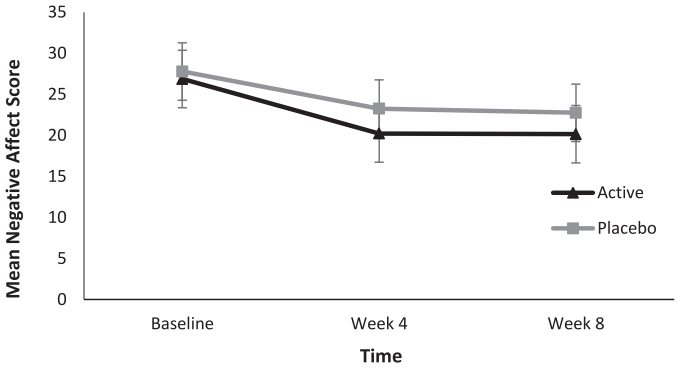


Fig. 4. Mean Negative Affect scores from the PANAS at baseline, week 4, and week 8 for the active treatment group and placebo group. Error bars represent standard error of the mean.

For females, the interaction of treatment group and time was not significant, $F(2,124) = 0.38$, $p = .685$. Pairwise comparisons showed no significant difference between groups at week 4, $t(62) = -0.55$, $p = .587$, or at week 8, $t(62) = -1.30$, $p = .198$. In the female group, there were no significant main effects for time, $F(2,124) = 0.31$, $p = .736$, and no significant effects for treatment, $F(1,62) = 1.37$, $p = .246$.

4. Discussion

The current study is the first analysis of CFE's anxiolytic action on humans, exploring its efficacy in reducing anxiety and stress in healthy adults, using self-report measures and the biomarker of salivary cortisol. The timing of any potential treatment effect was also investigated, with outcomes measured at week 4 and at week 8.

The active treatment group reported a significantly lower anxiety score (GAD-7) and stress score (PSS) than the placebo group at week 4 and at week 8. Clinical relevance was also indicated by the mean anxiety scores in the active treatment group, which declined from the scale's moderate range to mild range during the intervention (Fig. 1). Moreover, the standard deviation of that mean included 14 participants who decreased anxiety by two severity ranges (e.g., from moderate to minimal). This provided a measure of reliable change (i.e., a change in

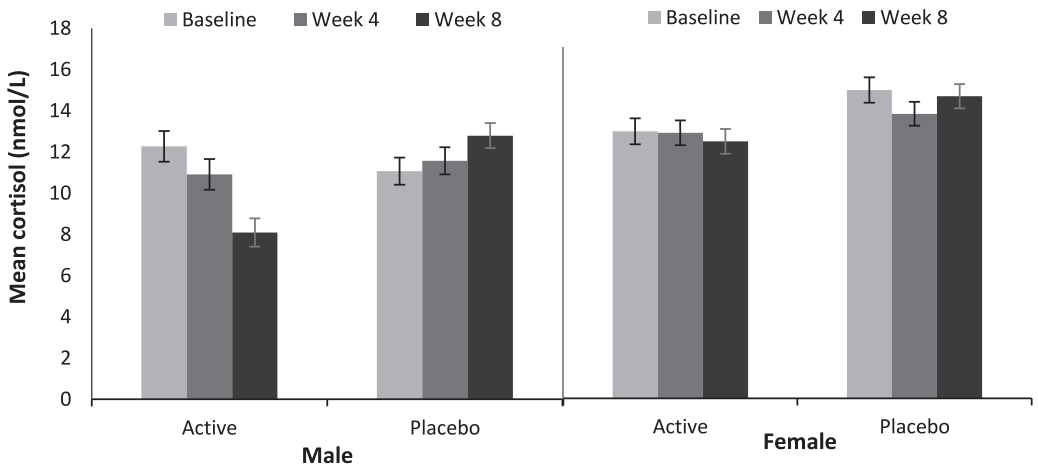


Fig. 5. Mean cortisol readings. Error bars represent standard error of the mean.

score that represents a shift from a dysfunctional to a functional range; Jacobson et al., 1984). Furthermore, these results from CFE treatment were comparable to reliable change results on the GAD-7 from Cognitive Behavioural Therapy (Griffiths and Griffiths, 2015), albeit in a smaller sample. The PSS does not have cut-off scores for severity ranges, so reliable change could not be quantified for stress (Klein et al., 2016).

Pairwise results showed that stress reduction in the active group continued after week 4, whereas it plateaued in the placebo group, suggesting that a placebo effect faded in time while a real effect continued. Furthermore, all Intention To Treat participants were in the placebo group and no participants in the active treatment group stopped taking the product.

The subscales of positive affect and cortisol (in males) approached a significant difference between active and placebo groups at week 4, and reached significance by week 8. This may indicate that positive affect and male cortisol require more time for an effect. The significant cortisol result may also indicate that CFE acts in a deliberate fashion on the HPA axis, although the biochemical mechanism for this action requires further investigaton, and a larger male sample size than this study obtained.

The subscale of negative affect did not show any significant difference between active and placebo groups at week 8, yet there was a difference in week 4. This is explained by the nature of the PANAS, which asked participants how they felt over the previous week. In contrast, the GAD-7 and PSS asked participants how they felt over the previous two weeks and a month, respectively. By considering a longer time frame, the GAD-7 and PSS possibly capture a more stable and hence more accurate measure of affect than the PANAS.

No significant difference between groups was observed for female cortisol, but ovarian hormones can modify the HPA axis and male cortisol responses in anxiety trials are often more pronounced (Nolen-Hoeksema, 2001). This result was therefore anticipated and was likely due to confounding hormonal variables. The non-significant results on the subscales of negative affect and female cortisol then, did not undermine support for the hypothesis.

4.1. Implications

While preliminary, this research carries the significant implication of a possible phytotherapeutic treatment for subclinical anxiety and stress. Considering (a) the adverse side effects of prescription anxiolytics (Mathew et al., 2008); (b) the growing popularity of herbal treatments (Cohen et al., 2005); (c) prior research of CFE's anxiolytic effect in mice (Rajendran et al., 2014); and (d) the evidence that pregnane glycosides active in CFE inhibit cortisol, a key player in the HPA axis's regulation of anxiety in humans (Komarnytsky et al., 2013; Tsigos and Chrousos, 2002), the clinical changes in anxiety indicated in this study favour the therapeutic use of CFE as a herbal anxiolytic.

4.2. Limitations and recommendations

The effect sizes on the primary outcomes suggested good internal validity, but the study has limitations. First, while the decrease in anxiety for the active group was significant, it could be argued that the clinical relevance was marginal. The mean anxiety score of the active group reduced from moderate to mild, but the standard deviation of that mean included 13 participants who remained within the moderate range throughout treatment, so that the reduction in their scores did not necessarily represent a clinical change. A larger sample and longer trial is recommended to test the strength of the apparent treatment effect.

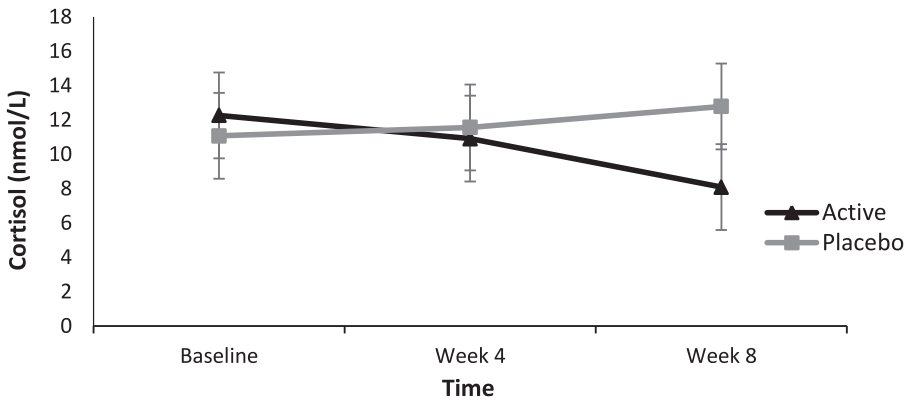


Fig. 6. Mean cortisol reading at each time point for the active and the placebo group. Error bars represent standard error of the mean.

Also, since the PSS did not have cut-off scores for severity ranges, future research could employ an instrument such as the DASS-42 to obtain a measure of stress with cut-off scores to assess clinical significance of stress reduction. The inclusion of a wait-list group is also recommended. This would likely improve results that were approaching significance by allowing comparison between treatment and no treatment.

Second, self-reporting instruments involve the subjective interpretation of items. The more objective measure of salivary cortisol was employed to reduce this error, but this was only meaningful in male participants. The low power of the significant result for males reflects the small sample size of male participants, and this sample size presents a serious limitation to any claim that CFE acts on cortisol with any certainty. A larger sample size tested over a longer period is not only recommended but necessary for future research, and this would reduce the chances of a Type II error.

Third, the possible confounders of BMI and gender were considered (Rivenes et al., 2009; Zhao et al., 2009). BMI did not significantly change in either group throughout the study, suggesting that it would not have confounded results, and gender was accounted for in cortisol testing. However, anxiety and stress can be impacted by variables not accounted for in this study, such as sleep and personality (Thomsen et al., 2003; Wetherell et al., 2001). Future research should account for more covariates that may impact the efficacy of CFE.

Finally, this study analysed mild to moderate anxiety and excluded participants with more severe presentations. This limits the generalisability only to a healthy population. It is therefore recommended that future research include participants with health conditions that are often comorbid with anxiety.

5. Conclusion

Despite the above limitations, this study indicates that 1 g/day of CFE over eight weeks is effective for reducing anxiety and stress in healthy adults. The significant change in male cortisol by week 8 supports the premise that CFE acts via cortisol regulation. The implication of this finding is that CFE could be a phytotherapeutic treatment for subclinical anxiety and stress, and should be the subject of further research, particularly into its affect on hormonal activity in humans.

Conflict of interests

There are no conflicts of interest relating to this submission.

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References

Adamek, J., 2015. Product update: Release of Elecsys Cortisol II. Forrenstrasse, Switzerland.

Adnan, M., Jan, S., Mussarat, S., Tariq, A., Begum, S., Afroz, A., Shinwari, Z.K., 2014. A review on ethnobotany, phytochemistry and pharmacology of plant genus Caralluma R. Br. *J. Pharm. Pharmacol.* 66 (10), 1351–1368. http://doi.org/10.1111/jphp.12265.

Arora, E., Khajuria, V., Tandon, V., Sharma, A., Mahajan, A., Gillani, Z., Choudhary, N., 2015. To evaluate efficacy and safety of Caralluma fimbriata in overweight and obese patients: a randomized, single blinded, placebo control trial. *Perspect. Clin. Res.* 6 (1), 39. http://doi.org/10.4103/2229-3485.148812.

Astell, K.J., Mathai, M.L., McAinch, A.J., Stathis, C.G., Su, X.Q., 2013. A pilot study investigating the effect of Caralluma fimbriata extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial. *Complement. Ther. Med.* 21, 180–189. http://doi.org/10.1016/j.ctim.2013.01.004.

Barrett, J.E., Frank, E., Katon, W., Oxman, T.E., Sengupta, A.S., Sullivan, M., Williams, J., 2001. Treatment of dysthymia and minor depression in primary care: a randomized trial in patients aged 18 to 59 years. *J. Fam. Pract.* 50 (5), 405–412.

Baxter, A.J., Scott, K.M., Vos, T., Whiteford, H.A., 2013. Global prevalence of anxiety disorders: a systematic review and meta-regression. *Psychol. Med.* 43, 897–910. http://doi.org/10.1017/S003329171200147X.

Beaubrun, G., Gray, G.E., 2000. A review of herbal medicines for psychiatric disorders. *Psychiatr. Serv.* 51 (9), 1130–1134. http://doi.org/10.1176/appi.ps.51.9.1130.

Borsini, F., Podhorna, J., Marazziti, D., 2002. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology (Berl.)* 163 (2), 121–141. http://doi.org/10.1007/s00213-002-1155-6.

Buchner, A., Erdfelder, E., Faul, F., & Lang, A. (2014). G*Power. Kiel, Germany: Universitat Kiel.

Chen, J., Wang, Z.Z., Zuo, W., Zhang, S., Chu, S.F., Chen, N.H., 2016. Effects of chronic mild stress on behavioral and neurobiological parameters - role of glucocorticoid. *Horm. Behav.* 78, 150–159. http://doi.org/10.1016/j.yhbeh.2015.11.006.

Cohen, M., Penman, S., Pirotta, M., Da Costa, C., 2005. The integration of complementary therapies in Australian general practice: Results of a national survey. *J. Altern. Complement. Med.* 11 (6), 995–1004. http://doi.org/10.1089/acm.2005.11.995.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. *J. Health Social Behav.* 24, 385–396.

Cohen, S., Williamson, G.M., 1988. Perceived stress in a probability sample of the United States. In: Spacapan, S., Oskamp, S. (Eds.), The Social Psychology of Health. Sage, Newbury Park, CA, pp. 31–67.

Crawford, J., Henry, J., 2004. The positive and negative affect schedule (PANAS): construct validity, measurement properties and normative data in a large non-clinical sample. *Br. J. Clin. Psychol.* 43 (Pt 3), 245–265. http://doi.org/10.1348/0144665031752934.

Cruz, A.P.M., Frei, F., Graeff, F.G., 1994. Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol. Biochem. Behav.* 49 (1), 171–176. http://doi.org/10.1016/0091-3057(94)90472-3.

de Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: From adaptation to disease. *Nat. Rev. Neurosci.* 6 (6), 463–475. http://doi.org/10.1038/nrn1683.

Eisenberg, D., Hunt, J., Speer, N., Zivin, K., 2011. Mental health service utilization among college students in the United States. *J. Nerv. Ment. Dis.* 199 (5), 301–308. http://doi.org/10.1097/NMD.0b013e3182175123.

Eisenberg, D.M., Davis, R.B., Ettner, S.L., Appel, S., Wilkey, S., Van Rompay, M., Kessler, R.C., 1998. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA* 280 (18), 1569–1575. http://doi.org/10.1001/jama.280.18.1569.

Ellis, P., Smith, D., 2002. Treating depression: the beyondblue guidelines for treating depression in primary care: “Not so much what you do but that you keep doing it.”. *Med. J. Aust.* 176 (Supplement) 2002.

Ernst, E., 2006. Herbal remedies for anxiety - a systematic review of controlled clinical trials. *Phytomedicine* 13 (3), 205–208. http://doi.org/10.1016/j.phymed.2004.11.006.

Espejo, E.F., 1997. Structure of the mouse behaviour on the elevated plus-maze test of anxiety. *Behav. Brain Res.* 86 (1), 105–112. http://doi.org/10.1016/S0166-4328(96)02245-0.

Griffiths, C.A., Griffiths, L.J., 2015. Recovery and reliable change rates for patients scoring severe on depression, anxiety or impaired functioning in a psychological therapies service: IAPT. *Ment. Health Rev. J.* 20 (1), 28–35. http://doi.org/http://dx.doi.org/10.1108/MHRJ-06-2014-0022.

Griggs, J.L., Su, X.Q., Mathai, M.L., 2015. Caralluma fimbriata supplementation improves the appetite behavior of children and adolescents with Prader-Willi syndrome. *North Am. J. Med. Sci.* 7 (11), 509.

Hellhammer, D.H., Wüst, S., Kudielka, B.M., 2009. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 34 (2), 163–171. http://doi.org/10.1016/j.psyneuen.2008.10.026.

Jacobson, N.S., Follette, W.C., Revenstorf, D., 1984. Psychotherapy outcome research: Methods for reporting variability and evaluating clinical significance. *Behav. Ther.* 15 (4), 336–352. http://doi.org/doi.org/10.1016/S0005-7894(84)80002-7.

Jureidini, J., Tonkin, A., 2006. Overuse of antidepressant drugs for the treatment of depression. *CNS Drugs* 20 (8), 623–632.

Kahn, J.P., Rubinow, D.R., Davis, C.L., Kling, M., Post, R.M., 1988. Salivary cortisol: a practical method for evaluation of adrenal function. *Biol. Psychiatry* 23 (4), 335–349. http://doi.org/10.1016/0006-3223(88)90284-3.

Kamalakkannan, S., Rajendran, R., Venkatesh, R.V., Clayton, P., Akbarsha, M.A., 2010. Antiobesogenic and antiatherosclerotic properties of caralluma fimbriata extract. *J. Nutr. Metab.* 1–6. *2010*. http://doi.org/10.1155/2010/285301.

Kessler, R.C., Soukup, J., Davis, R.B., Foster, D.F., Wilkey, S.A., Van Rompay, M.I., Eisenberg, D.M., 2001. The use of complementary and alternative therapies to treat anxiety and depression in the United States. *Am. J. Psychiatry* 158 (2), 289–294. http://doi.org/10.1176/appi.ajp.158.2.289.

Kirschbaum, C., Wust, S., Hellhammer, D., 1992. Consistent sex differences in cortisol responses to psychological stress. *Psychosom. Med.* 54 (6), 648–657. http://doi.org/0033-3174/92/5406-0648J0300/0.

Klein, E.M., Brähler, E., Dreier, M., Reinecke, L., Müller, K.W., Schmutzer, G., Beutel, M.E., 2016. The German version of the Perceived Stress Scale – psychometric characteristics in a representative German community sample. *Biomed. Central Psychiatry* 16 (1), 159–169. http://doi.org/10.1186/s12888-016-0875-9.

Komarnytsky, S., Esposito, D., Poulev, A., Raskin, I., 2013a. Pregnane glycosides interfere with steroidogenic enzymes to down-regulate corticosteroid production in human adrenocortical H295R cells. *J. Cell. Physiol.* 228 (5), 1120–1126. http://doi.org/10.1002/jcp.24262.

Komarnytsky, S., Esposito, D., Rathinasabapathy, T., Poulev, A., Raskin, I., 2013b. Effects of pregnane glycosides on food intake depend on stimulation of the melanocortin pathway and BDNF in an animal model. *J. Agric. Food Chem.* 61 (8), 1841–1849. http://doi.org/10.1021/jf3033649.

Kumar, P., Sharma, A., Varshney, P., Venkateswara Rao, C., 2013. Antidiabetogenic and antioxidant effects of Caralluma attenuata extract on streptozotocin induced diabetes in rats. *J. Pharm. Res.* 7 (3), 257–262. http://doi.org/10.1016/j.jopr.2013.03.021.

Kumar, R., Mac, D.S., Gabrielli, W.F., Goodwin, D.W., 1987. Anxiolytics and memory: a comparison of lorazepam and alprazolam. *J. Clin. Psychiatry* 48 (4), 158–160.

Kuriyan, R., Raj, T., Srinivas, S.K., Vaz, M., Rajendran, R., Kurpad, A.V., 2007. Effect of Caralluma Fimbriata extract on appetite, food intake and anthropometry in adult Indian men and women. *Appetite* 48 (3), 338–344. http://doi.org/10.1016/j.appet.2006.09.013.

Lader, M., 2011. Benzodiazepines revisited-will we ever learn? *Addiction* 106 (12), 2086–2109. http://doi.org/10.1111/j.1360-0443.2011.03563.x.

Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl.)* 92 (2), 180–185. http://doi.org/10.1007/BF00177912.

Mathew, S.J., Garakani, A., Reinhard, J.F., Oshana, S., Donahue, S., 2008. Short-term tolerability of a nonazapirone selective serotonin 1A agonist in adults with generalized anxiety disorder: a 28-day, open-label study. *Clin. Ther.* 30 (9), 1658–1666. http://doi.org/10.1016/j.clinthera.2008.09.006.

Nolen-Hoeksema, S., 2001. Gender differences in depression. *Curr. Dir. Psychol. Sci.* 10 (5), 173–176. http://doi.org/10.3109/09540261.2010.492391.

Olatunji, B.O., Cisler, J.M., Tolin, D.F., 2007. Quality of life in the anxiety disorders: a meta-analytic review. *Clin. Psychol. Rev.* 27 (5), 572–581. http://doi.org/10.1016/j.cpr.2007.01.015.

Rajendran, R., Ambikar, D.B., Khandare, R.A., Sannapuri, V.D., Vyawahare, N.S., Clayton, P., 2014. Nootropic activity of Caralluma fimbriata extract in mice. *Food Nutr. Sci.* 05 (02), 147–152. http://doi.org/10.4236/fns.2014.52019.

Rapaport, M.H., Clary, C., Fayyad, R., Endicott, J., 2005. Quality-of-life impairments in depressive and anxiety disorders. *Am. J. Psychiatry* 162 (6), 1171–1178. http://doi.org/10.1176/appi.ajp.162.6.1171.

Rivenes, A.C., Harvey, S.B., Mykletun, A., 2009. The relationship between abdominal fat, obesity, and common mental disorders: results from the HUNT Study. *J. Psychosom. Res.* 66 (4), 269–275. http://doi.org/10.1016/j.jpsychores.2008.07.012.

Roberti, J.W., Harrington, L.N., Storch, E.A., 2006. Further psychometric support for the 10-item version of the Perceived Stress Scale. *J. Coll. Couns.* 9, 135–148.

Saivasanthi, V., Gowthamigoud, Swathi, K., Aakruthi, Sowmya, R., Gupta, A., Rao, A.S., 2011. Evaluation of Caralluma Fimbrita for analgesic, anti inflammatory and anxiolytic activities. *Int. J. Pharm.* 1 (1), 40–45.

Sealed Envelope. (2017). London.

Smyth, J., Ockenfels, M.C., Porter, L., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1998. Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology* 23 (4), 353–370. http://doi.org/10.1016/S0306-4530(98)00008-0.

Spitzer, R.L., Kroenke, K., Williams, J.B.W., Lowe, B., 2006. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch. Intern. Med. J.* 166 (10), 1092–1097.

Tabachnick, B.G., Fidell, L.S., 2014. Using Multivariate Outliers, 6th ed. Harlow Pearson Education Limited, Boston.

Thomsen, D.K., Yung Mehlsen, M., Christensen, S., Zachariae, R., 2003. Rumination-relationship with negative mood and sleep quality. *Personal. Individ. Differ.* 34 (7), 1293–1301. http://doi.org/10.1016/S0191-8869(02)00120-4.

Tsigos, C., Chrousos, G.P., 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J. Psychosom. Res.* 53 (4), 865–871. http://doi.org/10.1016/S0022-3999(02)00429-4.

Unnebrink, K., Windeler, J., 2001. Intention-to-treat: methods for dealing with missing values in clinical trials of progressively deteriorating diseases. *Stat. Med.* 20 (24), 3931–3946. http://doi.org/10.1002/sim.1149.

Vajha, M., Audipudi, A., Murthy, K., Krishna, C., 2014. Evaluation of antiadipogenesis activities of selected species of Caralluma R. Br., Boucerosia wight & arn. and pregnane steroid on cell lines. *Int. J. Pharm. Sci. Res.* 5 (5), 1919–1923. http://doi.org/10.13040/IJPSR.0975-8232.5(5).1919-23.

Watson, D., Clark, L.A., 1994. THE PANAS-X Manual for the Positive and Negative Affect Schedule - Expanded Form.

Wetherell, J.L., Gatz, M., Pedersen, N.L., 2001. A longitudinal analysis of anxiety and depressive symptoms. *Psychol. Aging* 16 (2), 187–195. http://doi.org/10.1037//0882-7974.16.2.187.

Young, E., Korszun, A., 1999. Women, stress, and depression: sex differences in hypothalamic-pituitary-adrenal axis regulation. In: Leibenluft, E. (Ed.), Gender Differences in Mood and Anxiety disorders: From bench to Bedside. American Psychiatric Press, Washington DC, pp. 31–52.

Zhao, G., Ford, E.S., Dhingra, S., Li, C., Strine, T.W., Mokdad, A.H., 2009. Depression and anxiety among US adults: associations with body mass index. *Int. J. Obes.* 33 (2), 257–266. http://doi.org/10.1038/ijo.2008.268.