The chronic effects of a combination of herbal extracts (Euphytose®) on psychological mood state and response to a laboratory stressor: A randomised, placebo-controlled, double blind study in healthy humans

Fiona Dodd[1,*], David Kennedy[1], Emma Wightman[2], Julie Khan[1], Michael Patan[1], Rian Elcoate[1] and Philippa Jackson[1]

[1]Brain, Performance, Nutrition Research Centre, Department of Psychology, Northumbria University, UK

[2] Nutrition Trials At Northumbria (NUTRAN; NUTRAN AT NORTHUMBRIA UNIVERSITY) Northumbria University, UK

***Corresponding Author:** Fiona Dodd, Brain, Performance, Nutrition Research Centre, Department of Psychology, Northumbria University, Newcastle upon Tyne, NE1 8ST, UK.

Email: f.dodd@northumbria.ac.uk

Abstract

Background: Global lifetime prevalence of anxiety disorders has been estimated at approximately 16.6%, with subclinical prevalence likely much higher. Herbal approaches to reduce anxiety may be as effective as pharmacological treatments and are less likely to be associated with adverse side effects. The herbal species valerian, passionflower, hawthorn and ballota have a long history of use as anxiolytics in traditional medicine, further supported by recent pre-clinical and clinical trials.

Aims: To assess the effects of chronic (14 days) supplementation with a multi-herb extract preparation (Euphytose®) on psychological state and psychological and physiological stress responses during a laboratory stressor.

Methods: In this crossover study, 31 healthy participants (aged 19 – 58 years) received a multi-herb extract preparation and placebo for 14 days with a 28-day washout. Anxiety (State-Trait Anxiety Inventory), mood, and physiological measures of stress (heart rate, galvanic skin response, salivary α-amylase and cortisol levels) were measured before and after an Observed Multitasking Stressor. Cognitive performance was also assessed.

Results: Multi-herb extract preparation was associated with reduced tension-anxiety (p = .038), with participants showing an attenuated response to the observed multitasking psychosocial stressor following multi-herb extract preparation, evidenced by lower salivary α-amylase (p = .041) and galvanic skin response (p = .004).

Conclusions: The combination of herbal extracts contained within the multi-herb extract preparation reduced subjective anxiety in a healthy population and lowered electrodermal skin conductance and concentration of salivary α-amylase in response to a psychosocial stressor, compared to placebo. The study was registered on clinicaltrials.gov (identifier: NCT03909906).

Declaration of interest/funding: This work was sponsored by Bayer Healthcare.

Keywords

Herbal extract, valerian, passionflower, hawthorn, ballota, stress, anxiety, mood, cognition

Introduction

The global lifetime prevalence of anxiety disorders has been estimated at approximately 16.6% (Remes et al., 2016), with 8.1% of individuals within the UK having reported suffering from an anxiety disorder including Generalised Anxiety Disorder (GAD), Obsessive-Compulsive Disorder, panic disorder, phobias (McManus et al., 2016). Worryingly, subclinical prevalence is likely much higher (Haller et al., 2014), with substantial increases in GAD observed in younger people in recent years (Slee et al., 2021). Of those that reported suffering from anxiety, 49.9% reported also seeking treatment, 44% of which reported taking medication (McManus et al., 2016). Importantly, mental health in non-clinical populations can be affected by stressors and hassles encountered in daily life. For example, daily hassles in college student populations are shown to be significantly related to anxiety and depression (D'Angelo and Wiekzbicki, 2003), and individual differences in reactivity to daily stressors can predict depressive symptoms (Parrish et al., 2011). Furthermore stressful and adverse life events have been shown to play a role in the onset of anxiety and depressive symptoms (Zou et al., 2018) as well as anxiety disorders (Miloyan et al., 2018). Therefore, appropriately managing daily stress may be important for long-term mental health and for the prevention of mood disorders.

Herbal approaches to reduce anxiety may be as effective as pharmacological treatments (Andreatini et al., 2002; Murphy et al., 2010) and are less likely to be associated with adverse side effects (Alramadhan et al., 2012; Savage et al., 2018). Several herbal species including *Valeriana Officinalis* (valerian), *Passiflora incarnata L.* (passionflower) and *Ballota nigra L.* (ballota) have a long history of use as anxiolytics in traditional medicine (Dhawan et al., 2001a; Shinjyo et al., 2020), further supported by recent pre-clinical and clinical trials. For example, *in vitro* studies suggest that certain constituents of valerian can bind to and influence the activity of

$GABA_A$ sites (Benke et al., 2009), the same sites influenced by benzodiazepines commonly used as prescribed anxiolytics. Valerian extract has also been found to influence the transport of GABA itself (Santos et al., 1994). Whilst modulation of GABA receptors is thought to be one of the leading mechanisms of action of the plant (Orhan, 2021), the extract has also demonstrated partial agonist activity at serotonin receptors (Dietz et al., 2005) as well as adenosine A_1 receptor signalling (Shinjyo et al., 2020). *In vivo*, valerian has potent anxiolytic effects in rodents, with those administered valerian root extract showing significantly lower levels of anxiety than those administered a control substance (Murphy et al., 2010). Valerian, in combination with *Melissa officinalis* (lemon balm), led to significantly lower levels of anxiety during laboratory-induced stress in humans. Here, individuals given a 600 mg dose reported significantly lower levels of anxiety than those given placebo or a higher 1800 mg dose (Kennedy et al., 2006). A similar dose in isolation (530 mg) significantly reduced state anxiety (as measure by the State Trait Anxiety Inventory) following 1 month's supplementation in haemodialysis patients (Tammadon et al., 2021). Anxiolytic effects have also been demonstrated following lower doses. Individuals administered with a 100 mg dose of valerian within a clinical setting, reported feeling subjectively calmer and less anxious compared to controls when receiving dental surgery (Pinheiro et al., 2014). Similarly, 100 mg of valerian provided comfort and relaxation (in the absence of sedating effects) during molar extraction in anxious patients (Farah et al., 2019). Valerian has also led to increases in frontal alpha activity as measured by EEG following a 300 mg daily dose for 1 month, a finding correlated with anxiolysis (Roh et al., 2019).

Passiflora incarnata (passionflower) is a herbal substance that has been seen to provide similar anxiolytic properties as the commonly prescribed benzodiazepine midazolam within dental patients, at a dose of 260

mg (Dantas et al., 2017) and 500 mg (da Cunha et al., 2021). Drops of the extract (equivalent to approximately 500 – 600 mg) also led to reduced anxiety in patients prior to undergoing periodontal treatment (Kaviani et al., 2013). Additionally, 500 mg passionflower significantly reduced levels of subjective anxiety when compared to controls in individuals receiving surgery (Movafegh et al., 2008), with similar results found in individuals who underwent spinal anaesthesia following 700 mg passionflower (Aslanargun et al., 2012). Following chronic administration, passionflower has shown similar anxiolytic potency to oxazepam (Akhondzadeh et al., 2001). Within the few studies that investigate the anxiolytic mechanisms of action of passionflower, research has found that passionflower (*Passiflora coerulea*) acts as a partial agonist on benzodiazepine receptors (Wolfman et al., 1994; Appel et al., 2011). Similarly, *Ballota nigra* (ballota), contains several phenylpropanoids, precursors to flavonoids, which are compounds able to bind to benzodiazepine, dopaminergic and morphinic receptors in rodents; possibly explaining the neuro-sedative properties of the plant (Daels-Rakotoarison et al., 2000). Likewise, *Crataegus sp*. (hawthorn), are a species rich in polyphenols including flavonoids and procyanidins. Hawthorn preparations are effective in the treatment of cardiovascular and ischemic heart disease, with hypotensive effects often reported (Tassell et al., 2010). A small pilot study (N = 36) assessing the effects of 10 weeks' administration of 500 mg hawthorn extract alone or in combination with magnesium in mildly hypertensive adults has provided initial evidence of the anxiolytic effects of this extract. Trends for reduced blood pressure and reduced anxiety in those administered the hawthorn extract were observed, both with hawthorn extract alone and in combination with magnesium, (Walker et al., 2002).

The multi-herb extract preparation (MHEP) Euphytose® contains extracts of the four aforementioned herbal plants, albeit in smaller doses (50mg Valeriana officinalis L. (from the roots), 40mg Passiflora incarnate L.

(aerial parts), 10mg Crataegus sp. (from the leaf and flower), 10mg Ballota nigra L. (from the flowering tops). Evidence has shown that this MHEP combination is able to interact with benzodiazepine receptors, which may underpin the anxiolytic effects (Valli et al., 1991). In outpatients with adjustment disorder and anxious mood, Euphytose® plus *Cola nitida* and *Paullinia cupana* has previously reduced scores on the Hamilton Anxiety Rating Scale, compared to placebo, after 28 days' treatment (Bourin et al., 1997). Currently, evidence to suggest that this specific MHEP is an effective anxiolytic in healthy, sub-clinical populations does not exist within the literature. With the high prevalence of sub-clinical GAD within the general population (Haller et al., 2014), the potential anxiolytic benefits of MHEPs present significant scope for use within this population and warrants further investigation with randomised controlled trials. Previous research has shown that moderate physiological and psychological anxiety and stress responses can be effectively induced in a laboratory context. The Observed Multitasking Stressor (OMS) requires participants to engage with a computerised tracking task and conduct verbal arithmetic while being monitored by a panel of two researchers. The OMS has been shown previously to invoke a physiological and a psychological stress response, demonstrated by an increase in levels of subjective anxiety as measured by the use of the State-Trait Anxiety Inventory, state subscale a validated, widely used measure for fluctuating levels of anxiety (Kennedy et al., 2020; Jackson et al., 2020).

Therefore, the aim of the present study was to assess the effects of chronic (14 days) supplementation with a MHEP (Euphytose®) on psychological state with regards perceived stress and overall mood as well as psychological and physiological stress responses during a laboratory stressor, in a sample of healthy, sub-clinical participants.

Methods

Study design

A randomised, placebo-controlled, double-blind, crossover design was utilised. Participants attended the Brain, Performance, Nutrition Research Centre (BPNRC) laboratory at Northumbria University and were assessed after 14 days supplementation with MHEP, and a matched placebo. The study was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki (1996). The trial was conducted in compliance with protocol/GCP/applicable regulatory requirements and commenced only when a favourable ethical opinion was obtained from the University of Northumbria Department of Psychology Ethics Committee, UK, approval number 13339.

Determination of sample size

The power calculation was made with reference to the medium effect size (Cohen's d = .56) reported in Meier et al. (2018) for the effect of a combination product containing valerian, passion flower and lemon balm on anxiety as measured using the State-trait anxiety inventory, state subscale, administered before and at several time points after a psychological stressor. Therefore, with a mixed design study involving the within subjects factors of treatment and assessment, and between subjects factor of treatment order on the primary outcome measure (state anxiety-STAI), a total sample size of 28 participants was required to meet the conventionally accepted 80% power to detect a significant difference ($\alpha = 0.05$) between treatments.

Study population

A total of 31 healthy adults were randomised, of which 1 withdrew, and 3 participants were withdrawn due to major protocol violations, as they did not fully engage with the tasks (identified in each case by numerous statistical outliers and deviations). The remaining 27 participants (19 female), aged 19 - 58 years (mean = 33.74, standard deviation [SD] = 11.19) self-reported being in good health and were free from any relevant medical condition or disease including psychiatric and neurodevelopmental disorders. Blood pressure (BP) was taken at screening and participants were enrolled into the study if it measured <159 mmHg systolic and <99 mmHg diastolic. Participants confirmed they were not currently taking any relevant pharmaceuticals and had not taken any antibiotics within 4 weeks of screening. They also confirmed they had not taken part in another clinical trial within 30 days and had not experienced an event (personal or professional) likely to have impacted their emotional and/or psychological state within the week prior to starting the study and that they did not have an event planned (personal or professional) likely to affect their emotional, psychological or hormonal state during the course of the study. A full list of the inclusion and exclusion criteria can be found in **Supplemental File 1**. Written, informed consent was obtained from participants prior to any research related procedures being performed. Participants were recruited via an opportunity sample from Northumbria University students and staff and the general population.

Treatment

Participants received MHEP (50 mg *Valeriana officinalis L.*, 40mg *Passiflora incarnate L.*, 10 mg *Crataegus sp.,* 10 mg *Ballota nigra L.*) and a matched placebo in a counterbalanced order. The full composition of the active treatment and placebo is listed in **Supplemental File 2**. Treatments were delivered from the manufacturer

(Bayer Healthcare, Basel, Switzerland) in boxes labelled as placebo and verum. The bottles for each treatment arm were identical. An independent 3rd party who had no further involvement with the trial procedures created a fully counterbalanced computer-generated randomisation schedule (www.randomization.com) and assigned the treatment codes A and B to the treatments. Bottles were labelled with a randomisation number according to the counterbalancing schedule by the lead researcher; randomisation numbers were issued to participants sequentially at visit 1.

Participants were directed to take two tablets with breakfast, lunch, and dinner for a period of 14 days. This was followed by a 28-day washout period, before participants commenced their second treatment period. See **Figure 1** for visual representation of treatment schedule. Compliance was assessed at testing visits 2 and 4 by treatment counts and treatment diaries.

Psychological measures

State-trait anxiety inventory (STAI). The STAI 'State' subscale is a widely used instrument for measuring fluctuating levels of anxiety. The subscale contains 20 statements (e.g. 'I am calm') each with a 4-point Likert scale. Participants rate how much they feel like each statement at the time of making the response. Scores on the STAI range from 20 to 80, with higher scores representing higher levels of anxiety. The Trait subscale also consists of 20 statements, but refers to how participants generally feel (Speilberger et al., 1969). STAI-State was the primary outcome measure.

General health questionnaire (GHQ-12). The General Health Questionnaire is a screening instrument used for assessing general psychological health both in clinical settings and in non-clinical research settings requiring

repeated measurements over time. The GHQ-12 consists of 12 items, each assessing the severity of a mental problem over the past few weeks using a 4-point scale (0–3) with higher scores indicating worse conditions (Goldberg and Williams, 1988).

Perceived stress scale (PSS). The PSS is a 10-item questionnaire that assesses the degree to which situations in one's life are appraised as stressful using a 5-point scale (0–4). It is a widely used research instrument and its validity has been established within a number of populations (Golden-Kreutz et al., 2004; Mimura and Griffiths, 2004; Froelicher et al., 2004).

Profile of mood states (POMS). The POMS is a well-established, factor-analytically derived measure of psychological distress for which high levels of reliability and validity have been documented (Heuchert and McNair, 2012). The POMS consists of 65 adjectives rated on a 0–4 scale that can be consolidated into depression-dejection, tension-anxiety, anger-hostility, confusion-bewilderment, vigour-activity, and fatigue-inertia, subscales. The latter two subscales can be interpreted as measures of fatigue and have been validated as separate factors in a number of studies. Norms have been published for a variety of patient and non-patient groups.

Visual analogue mood scales (VAMS). Participants completed a series of visual analogue scales anchored by 27 antonyms relating to mood and psychological state. Participants moved a marker along the line to describe how they currently feel. Each line was scored as % along the line towards the more positive antonym. Factor analysis of the original 27 items revealed three factors incorporating 18 items (unpublished data). The factors were labelled Alertness (11 items: alert, inattentive; lethargic, energetic; clumsy, co-ordinated; lively, sluggish; quick-witted, slow-witted; sharp, dull; exhausted, refreshed; bored, engaged; focused, unfocused; drowsy,

awake; motivated, unmotivated), Stress (4 items: tense, relaxed; fearful, fearless; stressed, carefree; peaceful, troubled), and Tranquillity (3 items: tranquil, agitated; contented, discontented; friendly, hostile).

Observed multitasking stressor (OMS). The OMS incorporates two elements that have previously been shown to engender a stress response in laboratory studies; extended multitasking and social evaluation. The OMS has previously been shown to provoke a psychological stress response across repeated administrations (Kennedy et al., 2020). Briefly, the OMS comprised verbal completion of three serial subtraction tasks (3's, 7's and 17's) for 4 minutes each (12 minutes in total). Participants were instructed to count backwards from a given, randomly generated, number between 800 and 999 aloud, as quickly as possible. Performance of the task was scored for the total number of correct and incorrect subtractions. In the case of incorrect responses, subsequent responses were scored as correct if they were correct in relation to the new number. During the serial subtraction tasks, participants also completed a computerised tracking task, in which they were required to use the mouse to move a cursor to attempt to track an asterisk that followed a smooth, random, on-screen path; participants were instructed to keep the cursor as close to the asterisk as possible. These tasks were performed in a separate 'interview' room, in front of a panel of three 'judges' who maintained a neutral demeanour throughout the assessment. The computer screen, showing the tracking task, was projected onto a screen to give the impression that the panel was closely monitoring progress. In the lab, before entering the interview room and once back in the lab after completing the OMS, mood was assessed with the STAI (state) and computer delivered visual analogues scales (VAMS) indicating the participants' current level of stress, anxiety, relaxation and calmness (see above). These measures of mood were also repeated every 30 minutes

after completion of the stressor, up to 90 minutes post-stressor, as per **Figure 2**. A full description of the OMS can be found in **Supplemental File 3**.

Physiological measures

Heart rate and galvanic skin response. Heart rate and Galvanic skin response was measured throughout performance of the OMS. Galvanic Skin Response (GSR) and Heart Rate (HR) were measured on testing visits using the Vilistus Digital Sampling Unit (DSU; Durham Systems Management Limited). The GSR sensors, which measured relative changes in skin conductance, were attached to the middle and forth fingertips on the participant's non-dominant hand using Velcro straps. The HR sensor clip, which measured blood volume pulse, was placed on the tip of the index finger or thumb on the non-dominant hand. These sensors were attached at least 1 minute prior to the commencement of recording to allow for stabilisation of the readings. The unit measured 32 and 128 samples per second for GSR and HR respectively.

Salivary cortisol and salivary α-amylase. Saliva samples were obtained throughout the protocol at various time points (baseline, pre-OMS, post-OMS and 15, 30, 60 and 90 minutes post-OMS) using salivettes to measure salivary cortisol response (Poll et al., 2007) and salivary α-amylase response (Justino et al., 2017) (Sarstedt Ltd). Once collected, samples were spun down at 1000 g for 2 minutes. Samples were transferred into Eppendorfs and frozen at -80ºC. Before assaying, the samples were thawed and the cortisol and α-amylase levels in the saliva samples were measured using ELISA (Salimetrics Ltd).

Cognim^{app} smartphone measures

Cognim[app] (www.cognimapp.com) allows for at home assessment of participants on a range of cognitive and mood measures throughout the course of the intervention period. To capture response to treatment for both morning sleep inertia and 'post lunch dip' periods of the day, as well as on-going effects of treatment on subjective stress and any potential sedative effects of the intervention, the Cognim[app] assessment (15 minutes in total) was completed before breakfast and after lunch. A pre-treatment Cognim[app] assessment took place on Days -7 and 36 and then again on days 7 and 14 of each treatment period (i.e. Day 7, 14, 49, 56; see **Figure 1**). Full descriptions of all cognitive tasks are provided in **Supplemental file 4**.

Procedure

Participants attended the Brain, Performance, Nutrition Research Centre laboratory (Northumbria University, UK) on five separate occasions. The first was an introductory visit where informed written consent was obtained. Following the introductory visit, participants attended the laboratory at a pre-arranged time in the afternoon on four separate occasions (visits 1 – 4). The first and third visits comprised the baseline assessments. Visits 2 and 4 were chronic assessments and occurred 15 days (+/- 3 days) after visits 1 and 3, respectively. Each visit was identical, except for the intervention consumed between visits 1 and 2 and visits 3 and 4. See **Figure 1** for a schematic depicting the timeline of study.

Upon arrival at visits 1 – 4, participants were screened for continued eligibility and provided 5 minute baseline GSR and HR readings and a baseline saliva sample. Questionnaires were completed to assess psychological mood/state. After a short (~15 minute) break, participants were taken to an 'interview' room where they underwent the OMS for 15 minutes in front of a panel of two observers whilst also being video recorded and having their GSR and HR readings measured throughout. The, STAI-State, and VAMS were completed in the

lab immediately prior to and after the OMS and at 30, 60 and 90 minutes post-OMS. Seven saliva samples were collected in total (see **Figure 2** for a schematic depicting the procedure during testing visits 1 – 4). Before leaving on testing visits 1 and 3, participants were provided with their treatment. Participants were also instructed to complete the Cognim^{app} assessment battery just before breakfast and after lunch on days 7 and 14 in each treatment period following their baseline Cognim^{app} assessments on Day -7 and Day 36. See **Figure 1** for schematic depicting the study timeline (which also comprises the Cognim^{app} assessments). A full description of the procedure is provided in **Supplemental file 5**.

[Insert Figure 1.]

Figure 1. Timeline of study incorporating mood and observed multitasking stressor (OMS) assessment.

[Insert Figure 2.]

Figure 2. Mood and cbserved multitasking stress (OMS) assessment across study day.

Statistics

For the data collected during the study visits, the general statistical approach comprised the analysis of data collected following each treatment period (i.e. visits 2 and 4), including data collected at the pre-intervention assessment (i.e. visit 1 and 3) as a covariate. The MIXED procedure in SPSS (version 26.0, IBM corp., Armonk,

NY, US) was used for all analyses. For each model, restricted maximum likelihood estimation methods were used and covariance matrix structure was chosen based on the structure that produced the lowest Schwarz's Bayesian Criterion (BIC), an indication of the best fitting model (Drton and Plummer, 2017). Subject was included as a random factor where appropriate. Sidak adjustments were made for multiple comparisons where appropriate. To interrogate the chronic effects of treatment irrespective of the OMS stressor, data collected on arrival at the laboratory, -45 minutes prior to completing the OMS, were analysed including treatment as a fixed factor and pre-intervention values as a covariate. Outcomes included those derived from the POMS, GHQ, PSS, STAI-Trait, STAI-State and VAMS, as well as GSR, BVP and salivary cortisol and salivary α-amylase.

To investigate the effect of treatment on the direct psychological and physiological response to the OMS, data collected at all other time points during the testing visit were analysed in a separate analysis. Outcomes included STAI-State, VAMS, GSR, BVP and salivary cortisol and salivary α-amylase. These were analysed as above, including treatment and assessment as fixed factors, and pre-intervention values as a covariate. For the dual tasking performance outcomes, task was included as an additional factor.

The Cognimapp data were analysed as above, including the fixed factors treatment, visit, and time of day.

In order to assess the stress response elicited by the OMS procedure itself, the VAMS mood, STAI-State and saliva analyte outcomes collected at visits 1 and 3 in the absence of treatment were analysed as above including assessment and visit as fixed factors.

Missing data were left as empty cells as the linear mixed model approach that was applied to the data uses maximum likelihood to estimate the missing values.

Results

Thirty-one participants were randomised to receive treatment. See **Figure 3**. One participant withdrew post-randomisation following testing visit 1 and one following testing visit 2 (this data was included in the analysis). Please see **supplementary tables** for data from all measures.

[Insert Figure 3.]

Figure 3. Flow diagram of disposition of subjects throughout the study.

Handling of missing data

One participant only completed the first phase of the trial including visits 1 and 2. However, these data were included in the analysis therefore 27 datasets were eligible for analysis.

Demographic and other baseline characteristics

Participant demographics and baseline characteristics are summarised in Table 1 below.

Table 1. Baseline characteristics (N=27).

Measure	Mean	SD
Sex ratio (male/female)	0.42	
Age (years)	33.74	11.19

Race (frequency N)		
White	21	
Asian	3	
Black	1	
Mixed race	2	
Education (years)	17.52	2.78
Dietary restrictions (frequency N)		
None	22	
Vegetarian	1	
Vegan	1	
Pescetarian	3	
Fruit & veg consumption (portions/day)	4.02	1.66
Alcohol consumption (units/day)	0.66	0.74
Caffeine consumption (mg/day)	187.30	106.62
Systolic BP (mm/Hg)	118.31	10.86
Diastolic BP (mm/Hg)	78.42	7.06
Heart Rate (beats per minute)	73.43	9.49
BMI (kg/m^2)	24.70	3.49

1 BMI, body mass index; BP, blood pressure; SD, standard deviation.

2

Compliance and treatment guessing

Compliance was at 97.2% during the placebo phase and 98.3% during the MHEP phase of the study. Compliance was based on (returned) treatment counts. Participants responses to the treatment guess questionnaire, completed on the final visit, were analysed via a Chi-square test and revealed that there was no significant difference between the ability to correctly detect the active treatment or the placebo [χ^2 (1) = .619, p = .431].

Baseline comparisons

Pre-intervention visit data (i.e. visits 1 and 3) were analysed for treatment group effects and treatment group x visit interactions to confirm an absence of baseline differences between the groups, or carryover effects from the first treatment period.

No baseline differences were observed for any of the outcomes included in the chronic effects analysis or any of the Cognim^{app} outcomes.

With regards to the analysis of OMS-associated effects for data that were collected between -15 minutes pre-OMS until 90 minutes post-OMS, a significant effect of treatment group was observed for state anxiety [F (1, 222.98) = 8.43, p = .004]. Participants assigned to MHEP reported lower anxiety (30.81) than placebo (32.97) before treatment commenced. A significant effect of treatment was also observed for the OMS dual task speed [F (1, 282.1) = 7.30, p = .007] and accuracy [F (1, 280.3) = 14.79, p < .001] measures (z scores). Participants assigned to MHEP were faster (0.14) and more accurate (0.18) than those assigned to placebo (-0.12, -0.20, respectively) before treatment commenced.

Effect of the OMS (in the absence of treatment)

A significant effect of assessment was observed for state anxiety [F (4, 167.91) = 38.71, p < .001], stress [F (4, 229.06) = 13.99, p < .001] and tranquillity [F (4, 229.05) = 8.14, p < .001]. Post hoc comparisons revealed that the assessment completed immediately after the OMS was significantly higher (state anxiety, stress) or lower (tranquillity) compared to all the other assessments **(Figure 4)**.

An effect of visit was detected for state anxiety [F (1, 55.33) = 9.26, p = .004], with lower anxiety reported at visit 3 (30.24) compared to visit 1 (33.40). Similarly, an effect of visit was also observed for alertness [F (1, 229.38) = 9.15, p = .003], with higher alertness reported at visit 3 (65.08) compared to visit 1 (62.64).

Together these findings indicate that completion of the OMS had the anticipated effect on psychological mood state. The effect of visit suggests mild habituation to the protocol, but this did not interact with assessment on any of the outcomes.

[Insert Figure 4.]

Figure 4. Estimated marginal means for state anxiety (top), stress (middle) and tranquillity (bottom). State anxiety was derived from the State-Trait Anxiety Inventory (STAI), stress and tranquillity were derived from

visual analogue mood scales. Data collected at visits 1 and 3 are presented by assessment time across the study visit. Small letters indicate significant ($p < .05$) post hoc comparisons: a, -15 minutes; c, 30 minutes; d, 60 minutes; e, 90 minutes. OMS, Observed Multitasking Stressor.

A significant effect of assessment was also observed for salivary α-amylase [F (5, 202.78) = 3.83, p = .002]. Post hoc comparisons revealed that the value of the sample collected immediately following the OMS (240.69) was significantly higher than the sample collected immediately prior to the OMS (191.74; p = .003). A significant effect of assessment was also observed for salivary cortisol [F (5, 206.18) = 10.29, p < .001]. However, the pattern of response here was more anticipatory; post hoc comparisons revealed that cortisol concentration was elevated from -15 minutes pre-OMS and only began to decline 60 minutes post OMS **(Figure 5)**.

[Insert Figure 5.]

Figure 5. Estimated marginal means for salivary cortisol (left) and salivary α-amylase (right). Data collected at visits 1 and 3 are presented by assessment time across the study visit. Small letters indicate significant ($p < .05$) post hoc comparisons: a, -15 minutes; b, 0 minutes; c, 15 minutes; d, 30 minutes; e, 60 minutes; f, 90 minutes. OMS, Observed Multitasking Stressor.

Chronic effects analysis in the presence of treatment (MHEP)

A significant main effect of treatment was identified for tension-anxiety on the POMS questionnaire [F (1, 22.13) = 4.84, p = .038], with post hoc pairwise comparisons revealing MHEP resulted in significantly lower tension-anxiety (6.33) than placebo (7.75) **(Figure 6)**.

[Insert Figure 6.]

Figure 6. Estimated marginal means and standard errors (±SE) for post intervention tension-anxiety by treatment group. PLA, placebo; MHEP, multi-herb extract preparation.

Psychological and physiological response to the OMS

Salivary cortisol and salivary α-amylase

A significant main effect of treatment was identified for salivary α-amylase [F (1, 268.32) = 4.20, p = .041], with participants having lower salivary α-amylase following MHEP (209.51) compared to placebo (232.21) overall during the OMS assessment (**Figure 7)**.

[Insert Figure 7.]

Figure 7. Estimated marginal means and standard errors (±SE) for salivary α-amylase. A treatment effect revealed that α-amylase was significantly lower following MHEP, compared to placebo overall during the OMS assessment. PLA, placebo; MHEP, multi-herb extract preparation.

Galvanic skin response (GSR)

A significant main effect of treatment was identified for GSR [F (1, 119.20) = 8.63, p = .004], with participants having a lower galvanic skin response following MHEP (7.59) than placebo (8.43) overall during the OMS assessment **(Figure 8)**.

[Insert Figure 8.]

Figure 8. Estimated marginal means and standard errors (±SE) for GSR µSiemens by treatment group. PLA, placebo; MHEP, multi-herb extract preparation.

Cognim^{app} smartphone measures

A significant interaction between treatment x time of day was identified for digit vigilance false alarms [F (1, 127.61) = 4.13, p = .044]. However, post hoc pairwise comparisons revealed no significant differences between the groups.

A significant main effect of treatment was identified for RVIP false alarms [F (1, 132.86) = 4.27, p = .041], with post hoc pairwise comparisons revealing that MHEP made significantly less false alarms (2.07) than placebo (2.67) **(Figure 9)**.

[Insert Figure 9.]

Figure 9. Estimated marginal means and standard errors (±SE) for post intervention RVIP false alarms by treatment group. PLA, placebo; MHEP, multi-herb extract preparation.

A significant interaction between treatment x visit x time of day was identified for digit vigilance reaction time [F (2, 123.28) = 3.42, p = .036], with post hoc pairwise comparisons revealing that placebo had significantly faster reaction times (494.16 msec) than MHEP (509.80 msec) but only in the +7 day morning assessment (p = .026) **(Figure 10)**.

[Insert Figure 10.]

Figure 10. Estimated marginal means and standard errors (±SE) for post intervention digit vigilance reaction time for treatment x time of day x visit**.** PLA, placebo; MHEP, multi-herb extract preparation.

Discussion

In the current study, 14 days' supplementation with MHEP was associated with reduced tension-anxiety. In addition, participants showed an attenuated response to the OMS psychosocial stressor following MHEP, evidenced by lower salivary α-amylase and galvanic skin response. With regards cognitive performance assessed at home via Cognim^{app}, MHEP led to significantly fewer false alarms on the RVIP task compared with placebo. A significant reduction in speed of performance on the digit vigilance task following MHEP was also observed. However, this isolated negative effect was only observed during the morning assessment on Day 7 and appears to contradict the pattern of response for the other assessments where performance was numerically faster following MHEP.

Concerning mood, tension-anxiety was significantly lower following MHEP, compared to placebo. The POMS questionnaire from which this measure is derived, was completed prior to the start of the study day and therefore represents a reduction in tension-anxiety following 14 days' treatment. Of the species contained within the extract, both passionflower and valerian have demonstrated subjective anxiolytic properties within the literature following an acute, albeit larger, dose of the individual extracts (Aslanargun et al., 2012; Movafegh et al., 2008; Pinheiro et al., 2014; Farah et al., 2019). Since the quantities of valerian and passionflower contained within the MHEP are lower than those previously observed to have anxiolytic effects, the improvement in subjective anxiety seen here may represent the cumulative effect of a smaller dose of each extract. In terms of mechanisms, the sesquiterpene valerenic acid contained within valerian (when extracted from the underground organs of the *V. officinalis* species as in MHEP) has been shown to increase central GABA levels. This leads to a reduction in central nervous system (CNS) activity (Houghton, 1999), which

may have contributed to the reduction in tension-anxiety observed here following MHEP. It has been demonstrated that the ratio of valerenic acid to acetoxy valerenic acid contained within the extract is of importance in this regard, with extracts containing higher levels of valerenic acid leading to more pronounced anxiolytic effects (Becker et al., 2014; Felgentreff et al., 2012; Trauner et al., 2008). With regards the *Passiflora* species, despite a long history of use as an anxiolytic, its mechanism of action is not well understood. A role of the flavonoid chrysin in the agonism of benzodiazepine receptors has been proposed (Wolfman et al., 1994; Appel et al., 2011), however, consensus here is lacking (Movafegh et al., 2008). Interestingly, the anxiolytic activity profile of the *P. incarnata* extract is reportedly determined by the parts of the plant used, with the roots shown to be devoid of anxiolytic effects (Dhawan et al., 2001b) and the leaves said to contain maximum concentrations of bioactive constituents (Dhawan et al., 2004). Importantly, the *P. incarnata* extract contained within MHEP is obtained from the aerial parts of the plant. It should be noted that this was an isolated effect on mood, and there was no evidence of a chronic effect of treatment on state or trait anxiety—the primary outcome measure—or any other of the mood measures. This positive effect, albeit in the expected direction, should therefore be interpreted with caution. One consideration here is the context in which the pre-dose mood and well-being questionnaires were administered. Participants completed these questionnaires and mood scales in full knowledge that they were going to complete the OMS, and this may have influenced their responses on these questionnaires. Subjective well-being has been shown to correlate with current mood (Yardley and Rice, 1991) and is also affected by experimental manipulation (Yap et al., 2017). It may be that that anticipation of the OMS masked any chronic effect of treatment on state anxiety or indeed any of the other subjective measures.

With regards the physiological measures collected during the OMS procedure, an increase in the electrodermal skin conductance response (measured in uSiemens), is recognised as a good indicator of activation of the sympathetic nervous system (Dawson et al., 2017). The observed attenuation of this response during performance of the OMS following MHEP compared to placebo is therefore indicative of a beneficial effect of the treatment. Similarly, a reduction in salivary α-amylase was also observed across the study day following MHEP. Salivary α-amylase is considered a valid measure of autonomic nervous system activation (Nater and Rohleder, 2009), a reduction of which would also indicate an attenuation of the stress response. Euphytose® has been shown to interact with benzodiazepine receptors, which has been proposed as the potential mechanism for its anxiolytic effects (Valli et al., 1991). Previously valerian has been shown to reduce HR during a mentally stressful cognitive task following 7 days administration (Cropley et al., 2002), a finding not replicated in the present study following 14 days administration. However, this was following a considerably larger dose of 600 mg, as compared to the 50 mg dose contained within the MHEP. Similarly, an acute 260 mg dose of passionflower was observed to have the same effect on HR as the drug Midazolam, when administered prior to tooth extraction surgery (Dantas et al., 2017), but, again, this is a larger dose than the 40 mg administered here within the MHEP. Taking into consideration the quantities of each extract contained within MHEP, it is possible that skin conductance and salivary α-amylase are more sensitive to the effects of the lower doses administered here.

Although it could be expected that the active treatment would have a beneficial effect across all the physiological parameters, it should be noted that inconsistencies in these measures are also found in the literature. Cortisol, a steroid hormone, is a reliable measure of the response to acute stress (Hellhammer et

al., 2009). A-amylase, an enzyme found in saliva and involved in digestion, is considered to be a good indicator of autonomic nervous system (ANS) activation, although debate exists over whether levels obtained during stressful situations represent sympathetic or parasympathetic activity, or a combination of both (Ali and Nater, 2020). It is of note here that where laboratory-induced psychological stress paradigms have been adopted previously (including the Trier Social Stress Test), a correlation of salivary α-amylase and cortisol levels was not observed (Chatterton et al., 1996; Nater et al., 2005), leading to the suggestion that these two measures react as a consequence of different, albeit linked, stress systems (Nater et al., 2005). Furthermore, studies that have compared the α-amylase and cortisol response to behavioural stress-reduction interventions, have reported a reduction in α-amylase levels in the absence of a change in cortisol levels (Ali and Nater, 2020). In the present study, analysis of the pre-intervention study visit data showed that cortisol was already elevated at the -15 minute pre-OMS time point—indicative of an anticipatory response to the protocol—which may have also contributed to the null effects on this measure. As described above this anticipatory response to the stressor was also reflected in an absence of findings on the STAI state subscale following treatment. Although mild habituation to the OMS at day 14 may also provide some explain for the absence of effects (on cortisol and the state anxiety), previous research has demonstrated that the OMS is capable of provoking a psychological response following repeated administrations even on the same day (Kennedy et al., 2020).

Considering the Cognim^{app} cognitive performance outcomes, MHEP led to significantly fewer false alarms on the RVIP task compared with placebo. However, the findings here do not appear to represent a consistent pattern of effects for either treatment, rendering interpretation difficult. Specifically, digit vigilance reaction

time was significantly slower following MHEP compared to placebo in the +7 day morning assessment. The number of dependent variables should also be acknowledged; the small effects seen here may not have been detected if the number of analyses conducted were adjusted for. Importantly, despite these minimal and contradictory effects, the null findings overall provide evidence of an absence of consistent adverse effects on performance observed either during the study visit or on the Cognim^{app} assessments as a result of the active treatment. Furthermore, we also observed no effect of MHEP on subjective alertness or on the KSS, a reliable measure of subjective drowsiness. Of the extracts contained within MHEP, those understood to have sedating properties include valerian and ballota. The ability of valerian to bind to adenosine receptors has been reported within animal studies and proposed as one of the mechanisms by which the sedating effects may occur (Murphy et al., 2010). The flowered aerial parts of the *Ballota nigra L.* species (also contained within MHEP) have been used traditionally for their sedative properties, amongst others (Al-Snafi, 2015; Gruenwald et al., 2000). Although there is little evidence within the literature for its efficacy in humans (Morteza-Semnani and Ghanbarimasir, 2019), animal studies have demonstrated the ability of phenylpropanoids within the extract bind to benzodiazepine, dopaminergic and opioid receptors which may explain, in part, its neuro-sedative properties (Daels-Rakotoarison et al., 2000). Therefore, despite the reported sedating effects of some of the extracts contained within the treatment, MHEP was not associated with any changes in subjective arousal, or any consistent negative effects on cognitive performance.

A potential limitation of the current design was the timing of the mood questionnaires and their proximity to the OMS. It could be argued that completing the mood questionnaires immediately prior to the OMS would allow interrogation of the effect of MHEP on anticipatory responses to the stressor; however, it is possible

that their completion within the lab on the same day as the testing visit may have masked any chronic effect of treatment on subjective mood, which is what they were intended to measure. In future, to determine the effect of treatment on general subjective mood (in the absence of an acute stressor) it is recommended that chronic assessments of mood should be completed in a more neutral setting, on a different day to the OMS in order to capture any potentially subtle effects of treatment.

Cognim^{app} is a valuable assessment tool with the ability to capture cognitive performance and mood measures in any setting, but inevitably this comes with some practical limitations. A lab setting provides a quiet environment, free from daily distractions where engagement can be monitored by a study team. Although guidance is provided to the participant to complete the Cognim^{app} assessments with these principles in mind, it is not always practicable when fitting the assessments into their daily lives. Without the ability to monitor participants there is also the possibility that assessments will not be completed within the appropriate timeframe. In order to monitor time of day effects, including the impact of morning sleep inertia and the post-lunch dip, participants were required to complete the assessments before breakfast and 1 hour (2 hours maximum) after finishing their lunch. It was evident from the raw data that not all participants adhered to this period and/or consumed breakfast and lunch at irregular times of the day. However, it could be argued that 'real life' environments provide the ideal setting within which to assess cognitive performance since any findings determined as a result, either positive or negative, would potentially be even more valid. It is likely that a larger data set with this measure would tease out many of these nuances and individual differences to reveal a clearer pattern of effects.

The findings of the present study demonstrate that 14 days' supplementation with a combination of the herbal extracts valerian, passionflower, ballot and hawthorn reduces subjective anxiety in a healthy population and lowers electrodermal skin conductance and concentration of salivary α-amylase in response to a psychosocial stressor, compared to placebo. Future studies may benefit from conducting mood and well-being assessments in the absence of the OMS to remove any anticipatory effects of this measure and/or assessing all physiological and mood outcomes over a longer pre- and post-OMS time-frame in order to ascertain what the extent of the effect of the preparatory response is in this environment.

Acknowledgements

The authors would like to thank Lucy Keeler for her assistance with the saliva analysis, Joanne Forster for her assistance with running study sessions and Bethany Spittlehouse, Amy Ferguson, Lucy Keeler, Julie Khan and Jenny Webster who formed the panel of judges during the observed multitasking stressor.

Declaration of Conflicting Interests

The authors declare that there are no conflicts of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was sponsored by Bayer Healthcare – Consumer Care Basel, Switzerland. Bayer HealthCare provided the study interventions. The sponsor had no involvement in the design of the study, collection, analysis, and interpretation of data, or in writing the manuscript.

Data Availability

The data used to support the findings of this study are included within the supplementary material

References

Akhondzadeh S, Naghavi HR, Vazirian M, et al. (2001) Passionflower in the treatment of generalized anxiety: a pilot double-blind randomized controlled trial with oxazepam. *J Clin Pharm Ther* 26(5): 363-367.

Al-Snafi A (2015) The Pharmacological Importance Of Ballota Nigra –A Review. *Ind J of Pharm Sci & Res* 5: 249-256.

Ali N and Nater UM (2020) Salivary Alpha-Amylase as a Biomarker of Stress in Behavioral Medicine. *International Journal of Behavioral Medicine*. 1-6.

Alramadhan E, Hanna MS, Hanna MS, et al. (2012) Dietary and botanical anxiolytics. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research* 18(4): RA40-RA48.

Andreatini R, Sartori VA, Seabra ML, et al. (2002) Effect of valepotriates (valerian extract) in generalized anxiety disorder: a randomized placebo-controlled pilot study. *Phytotherapy Research* 16(7): 650-654.

Appel K, Rose T, Fiebich B, et al. (2011) Modulation of the γ-aminobutyric acid (GABA) system by Passiflora incarnata L. *Phytother Res* 25(6): 838-843.

Aslanargun P, Cuvas O, Dikmen B, et al. (2012) Passiflora incarnata Linneaus as an anxiolytic before spinal anesthesia. *J Anesth* 26(1): 39-44.

Becker A, Felgentreff F, Schröder H, et al. (2014) The anxiolytic effects of a Valerian extract is based on valerenic acid. *BMC complementary and alternative medicine* 14(1): 267.

Benke D, Barberis A, Kopp S, et al. (2009) GABA A receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. *Neuropharmacology* 56(1): 174-181.

Bourin M, Bougerol T, Guitton B, et al. (1997) A combination of plant extracts in the treatment of outpatients with adjustment disorder with anxious mood: controlled study versus placebo. *Fundamental and Clinical Pharmacology* 11(2): 127-132.

Chatterton RT, Jr., Vogelsong KM, Lu YC, et al. (1996) Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 16(4): 433-448.

Cropley M, Cave Z, Ellis J, et al. (2002) Effect of kava and valerian on human physiological and psychological responses to mental stress assessed under laboratory conditions. *Phytother Res* 16(1): 23-27.

D'Angelo B and Wiekzbicki M (2003) Relations of daily hassles with both anxious and depressed mood in students. *Psychological Reports* 92(2): 416-418.

da Cunha RS, Amorim KS, Gercina AC, et al. (2021) Herbal medicines as anxiolytics prior to third molar surgical extraction. A randomized controlled clinical trial. *Clin Oral Investig* 25(3): 1579-1586.

Daels-Rakotoarison DA, Seidel V, Gressier B, et al. (2000) Neurosedative and antioxidant activities of phenylpropanoids from ballota nigra. *Arzneimittelforschung* 50(1): 16-23.

Dantas LP, de Oliveira-Ribeiro A, de Almeida-Souza LM, et al. (2017) Effects of passiflora incarnata and midazolam for control of anxiety in patients undergoing dental extraction. *Med Oral Patol Oral Cir Bucal* 22(1): e95-e101.

Dawson ME, Schell AM and Filion DL (2017) The electrodermal system. *Handbook of psychophysiology, 4th ed.* New York, NY, US: Cambridge University Press, pp.217-243.

Dhawan K, Dhawan S and Sharma A (2004) Passiflora: a review update. *J Ethnopharmacol* 94(1): 1-23.

Dhawan K, Kumar S and Sharma A (2001a) Anti-anxiety studies on extracts of Passiflora incarnata Linneaus. *Journal of Ethnopharmacology* 78(2-3): 165-170.

Dhawan K, Kumar S and Sharma A (2001b) Anxiolytic activity of aerial and underground parts of Passiflora incarnata. *Fitoterapia* 72(8): 922-926.

Dietz BM, Mahady GB, Pauli GF, et al. (2005) Valerian extract and valerenic acid are partial agonists of the 5-HT5a receptor in vitro. *Brain Res Mol Brain Res* 138(2): 191-197.

Drton M and Plummer M (2017) A Bayesian information criterion for singular models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* 79(2): 323-380.

Farah GJ, Ferreira GZ, Danieletto-Zanna CF, et al. (2019) Assessment of Valeriana officinalis l. (Valerian) for Conscious Sedation of Patients During the Extraction of Impacted Mandibular Third Molars: A Randomized, Split-Mouth, Double-Blind, Crossover Study. *J Oral Maxillofac Surg* 77(9): 1796.e1791-1796.e1798.

Felgentreff F, Becker A, Meier B, et al. (2012) Valerian extract characterized by high valerenic acid and low acetoxy valerenic acid contents demonstrates anxiolytic activity. *Phytomedicine* 19(13): 1216-1222.

Froelicher ES, Li WW, Mahrer-Imhof R, et al. (2004) Women's initiative for non-smoking (WINS) VI: reliability and validity of health and psychosocial measures in women smokers with cardiovascular disease. *Heart and Lung* 33(3): 162-175.

Goldberg DP and Williams P (1988) *The User's Guide to the General Health Questionnaire.* Windsor: NFER-Nelson.

Golden-Kreutz DM, Browne MW, Frierson GM, et al. (2004) Assessing Stress in Cancer Patients: A Second-Order Factor Analysis Model for the Perceived Stress Scale. *Assessment* 11(3): 216-223.

Gruenwald J, Brendler T and Jaenicke C (2000) *PDR for Herbal Medicines.* Montvale, NJ: Medical Economics Company.

Haller H, Cramer H, Lauche R, et al. (2014) The prevalence and burden of subthreshold generalized anxiety disorder: a systematic review. *BMC Psychiatry* 14: 128.

Hellhammer DH, Wüst S and Kudielka BM (2009) Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 34(2): 163-171.

Heuchert JP and McNair DM (2012) *Profile of Mood States Second Edition Manual.* Toronto, Canada: Multi-Health Systems, Inc.

Houghton PJ (1999) The scientific basis for the reputed activity of Valerian. *Journal of Pharmacy and Pharmacology* 51(5): 505-512.

Jackson PA, Forster J, Khan J, et al. (2020) Effects of Saffron Extract Supplementation on Mood, Well-Being, and Response to a Psychosocial Stressor in Healthy Adults: A Randomized, Double-Blind, Parallel Group, Clinical Trial. *Front Nutr* 7: 606124.

Justino AB, Teixeira RR, Peixoto LG, et al. (2017) Effect of saliva collection methods and oral hygiene on salivary biomarkers. *Scand J Clin Lab Invest* 77(6): 415-422.

Kaviani N, Tavakoli M, Tabanmehr M, et al. (2013) The efficacy of passiflora incarnata linnaeus in reducing dental anxiety in patients undergoing periodontal treatment. *J Dent (Shiraz)* 14(2): 68-72.

Kennedy DO, Bonnländer B, Lang SC, et al. (2020) Acute and Chronic Effects of Green Oat (Avena sativa) Extract on Cognitive Function and Mood during a Laboratory Stressor in Healthy Adults: A Randomised, Double-Blind, Placebo-Controlled Study in Healthy Humans. *Nutrients* 12(6).

Kennedy DO, Little W, Haskell CF, et al. (2006) Anxiolytic effects of a combination of Melissa officinalis and Valeriana officinalis during laboratory induced stress. *Phytother Res* 20(2): 96-102.

McManus S, Bebbington P, Jenkins R, et al. (2016) Mental health and wellbeing in England: Adult Psychiatric Morbidity Survey 2014. A survey carried out for NHS Digital by NatCen Social Research and the Department of Health Sciences, University of Leicester.

Meier S, Haschke M, Zahner C, et al. (2018) Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress setting in healthy men - An explorative randomized placebo-controlled double-blind study. *Phytomedicine* 39: 85-92.

Miloyan B, Joseph Bienvenu O, Brilot B, et al. (2018) Adverse life events and the onset of anxiety disorders. *Psychiatry Res* 259: 488-492.

Mimura C and Griffiths P (2004) A Japanese version of the perceived stress scale: translation and preliminary test. *International Journal of Nursing Studies* 41(4): 379-385.

Morteza-Semnani K and Ghanbarimasir Z (2019) A review on traditional uses, phytochemistry and pharmacological activities of the genus Ballota. *J Ethnopharmacol* 233: 197-217.

Movafegh A, Alizadeh R, Hajimohamadi F, et al. (2008) Preoperative oral Passiflora incarnata reduces anxiety in ambulatory surgery patients: a double-blind, placebo-controlled study. *Anesth Analg* 106(6): 1728-1732.

Murphy K, Kubin ZJ, Shepherd JN, et al. (2010) Valeriana officinalis root extracts have potent anxiolytic effects in laboratory rats. *Phytomedicine* 17(8-9): 674-678.

Nater UM and Rohleder N (2009) Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* 34(4): 486-496.

Nater UM, Rohleder N, Gaab J, et al. (2005) Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol* 55(3): 333-342.

Orhan IE (2021) A Review Focused on Molecular Mechanisms of Anxiolytic Effect of Valerina officinalis L. in Connection with Its Phytochemistry through in vitro/in vivo Studies. *Curr Pharm Des* 27(28): 3084-3090.

Parrish BP, Cohen LH and Laijrenceaij JP (2011) Prospective relationship between negative affective reactivity to daily stress and depressive symptoms. *Journal of Social and Clinical Psychology* 30(3): 270-296.

Pinheiro ML, Alcantara CE, de Moraes M, et al. (2014) Valeriana officinalis L. for conscious sedation of patients submitted to impacted lower third molar surgery: A randomized, double-blind, placebo-controlled split-mouth study. *J Pharm Bioallied Sci* 6(2): 109-114.

Poll EM, Kreitschmann-Andermahr I, Langejuergen Y, et al. (2007) Saliva collection method affects predictability of serum cortisol. *Clin Chim Acta* 382(1-2): 15-19.

Remes O, Brayne C, van der Linde R, et al. (2016) A systematic review of reviews on the prevalence of anxiety disorders in adult populations. *Brain Behav* 6(7): e00497.

Roh D, Jung JH, Yoon KH, et al. (2019) Valerian extract alters functional brain connectivity: A randomized double-blind placebo-controlled trial. *Phytother Res* 33(4): 939-948.

Santos MS, Ferreira F, Cunha AP, et al. (1994) An aqueous extract of valerian influences the transport of GABA in synaptosomes. *Planta Med* 60(3): 278-279.

Savage K, Firth J, Stough C, et al. (2018) GABA-modulating phytomedicines for anxiety: A systematic review of preclinical and clinical evidence. *Phytother Res* 32(1): 3-18.

Shinjyo N, Waddell G and Green J (2020) Valerian Root in Treating Sleep Problems and Associated Disorders-A Systematic Review and Meta-Analysis. *J Evid Based Integr Med* 25: 2515690x20967323.

Slee A, Nazareth I, Freemantle N, et al. (2021) Trends in generalised anxiety disorders and symptoms in primary care: UK population-based cohort study. *Br J Psychiatry* 218(3): 158-164.

Speilberger CD, Gorsuch RL and Lushene RE (1969) *The State Trait Anxiety Inventory Manual.* Palo Alto: Consulting Psychologists Press.

Tammadon MR, Nobahar M, Hydarinia-Naieni Z, et al. (2021) The Effects of Valerian on Sleep Quality, Depression, and State Anxiety in Hemodialysis Patients: A Randomized, Double-blind, Crossover Clinical Trial. *Oman Med J* 36(2): e255.

Tassell MC, Kingston R, Gilroy D, et al. (2010) Hawthorn (Crataegus spp.) in the treatment of cardiovascular disease. *Pharmacognosy Reviews* 4(7): 32-41.

Trauner G, Khom S, Baburin I, et al. (2008) Modulation of GABAA receptors by valerian extracts is related to the content of valerenic acid. *Planta medica* 74(01): 19-24.

Valli M, Paubert-Braquet M, Picot S, et al. (1991) Euphytose®, an association of plant extracts with anxiolytic activity: investigation of its mechanism of action by an in vitro binding study. *Phytother Res* 5(6): 241-244.

Walker AF, Marakis G, Morris AP, et al. (2002) Promising hypotensive effect of hawthorn extract: a randomized double-blind pilot study of mild, essential hypertension. *Phytother Res* 16(1): 48-54.

Wolfman C, Viola H, Paladini A, et al. (1994) Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from Passiflora coerulea. *Pharmacol Biochem Behav* 47(1): 1-4.

Yap SCY, Wortman J, Anusic I, et al. (2017) The effect of mood on judgments of subjective well-being: Nine tests of the judgment model. *Journal of Personality and Social Psychology* 113(6): 939-961.

Yardley JK and Rice RW (1991) THE RELATIONSHIP BETWEEN MOOD AND SUBJECTIVE WELL-BEING. *Social Indicators Research* 24(1): 101-111.

Zou P, Sun L, Yang W, et al. (2018) Associations between negative life events and anxiety, depressive, and stress symptoms: A cross-sectional study among Chinese male senior college students. *Psychiatry Res* 270: 26-33.