Date: 2nd July 2018

Study code: 57BV1

Study title: Acute and chronic (28 days) effects of *Avena sativa* extract on physiological and psychological responses to stress, and mood and cognitive function

Ethics code: 10084

Study protocol

DESIGN

The study will follow a randomised, double-blind, placebo-controlled, parallel groups design.

PROCEDURE

Testing will take place in a suite of testing facilities with participants visually isolated from each other. Participants will attend the laboratory on 3 separate occasions, an introductory visit between 1 and 14 days before the first day of treatment, and two testing days (Day 1 and Day 29).

The Introductory visit to the laboratory will comprise: briefing on requirements of the study, obtaining of informed consent, health screening, completion of the Caffeine Consumption Questionnaire (CCQ) and State-Trait Anxiety Inventory (STAI) trait subscale, training on the cognitive and mood measures and collection of demographic data. Participants will also complete the baseline Cognim^{app} assessment of mood (GAD-7, Bond-Lader, VAS).

For the two ensuing laboratory-based testing sessions (Day 1, Day 29) participants will attend the laboratory before 8.00 am having consumed a standardised breakfast of cereal and/ or toast at home no later than an hour before arrival. They must have refrained from alcohol for 24 hours and caffeine for 18 hours. The methodology during these visits will be identical. On arrival on each day participants will complete the GHQ-12 (mental health) and POMS (Mood/depression/arousal). Each subsequent assessment will comprise a short set of cognitive tasks (Corsi Blocks, Stroop, RVIP, Numeric Working Memory – 12 minutes) and completion of the Observed Multitasking Stressor (OMS) test session. This 15 minute OMS takes place in front of a panel of three observers, and comprises provision of a saliva sample, completion of the Perceived Stress Scale (PSS) (before the stressor only) followed by STAI-state, Bond-Lader Mood Scales and visual analogue scales (VAS) which are completed before and after the stressor. The stressor comprises the performance of three separate verbal serial subtraction tasks (Serial 3s, Serial 7s, Serial 17s – 4 minutes per task) whilst concomitantly performing a computerised tracking task. Finally a further saliva sample is taken. Heart rate and galvanic skin response (GSR) are recorded throughout.

After the first cognitive/OMS assessment participants will take their treatment for the day and will undergo identical Cognitive/OMS assessments commencing 120 and 240 minutes post-dose. Following completion of the final assessment at 240 min post-dose participants will complete the GHQ-12 and POMS (to assess any general effects on anxiety, depression, and mood).

Additionally, an interim mood assessment (GAD-7, Bond-Lader, VAS) in the participant's own home, delivered using Cognim^{app} software by mobile phone or the internet, will be included at weekly intervals (Day 8, 15, 22) between laboratory visits with a final assessment during Day 29.

Figures 1 depicts the running order of the OMS and Figures 2 and 3 depict the laboratory-based testing session timeline and chronic study overview respectively.

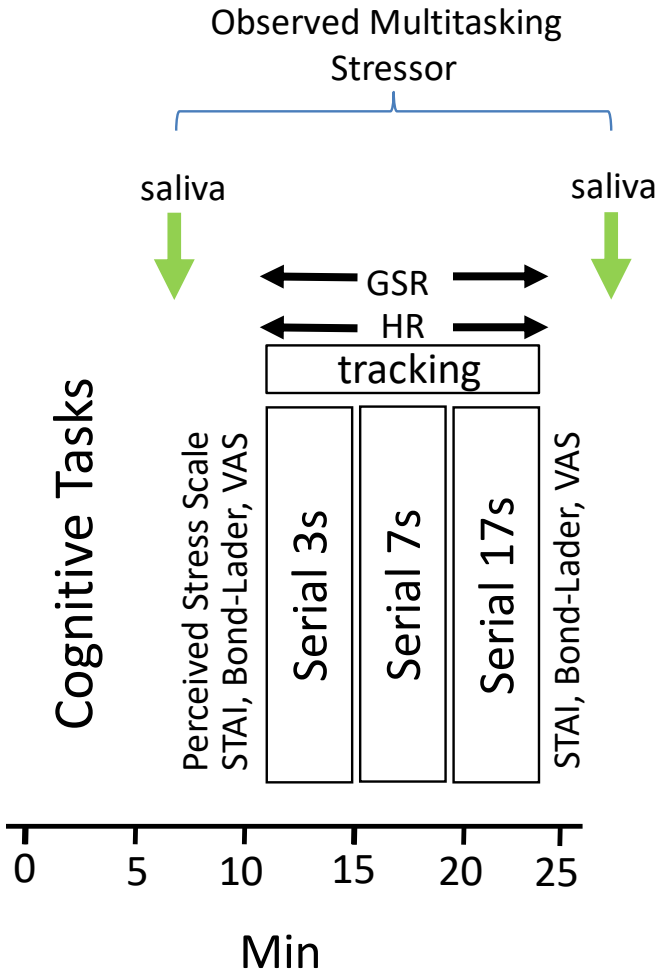

Figure 1. Cognitive, mood and Observed Multitasking Stress assessment. Participants complete a short cognitive assessment (executive function, attention and working memory) in the general laboratory and then move to the interview room. They provide a saliva sample (cortisol/α-amylase) and complete the Perceived Stress Scale. They then complete the STAI, Bond-Lader, and VAS before and after the completion of three 4-minute verbal Serial Subtraction tasks (Serial 3s, 7s, 17s, completed in counterbalanced order) and a concomitant computerised tracking task. Heart rate (HR) and galvanic skin response (GSR) are measured throughout performance of the tasks. A further saliva sample is taken at the end of testing.

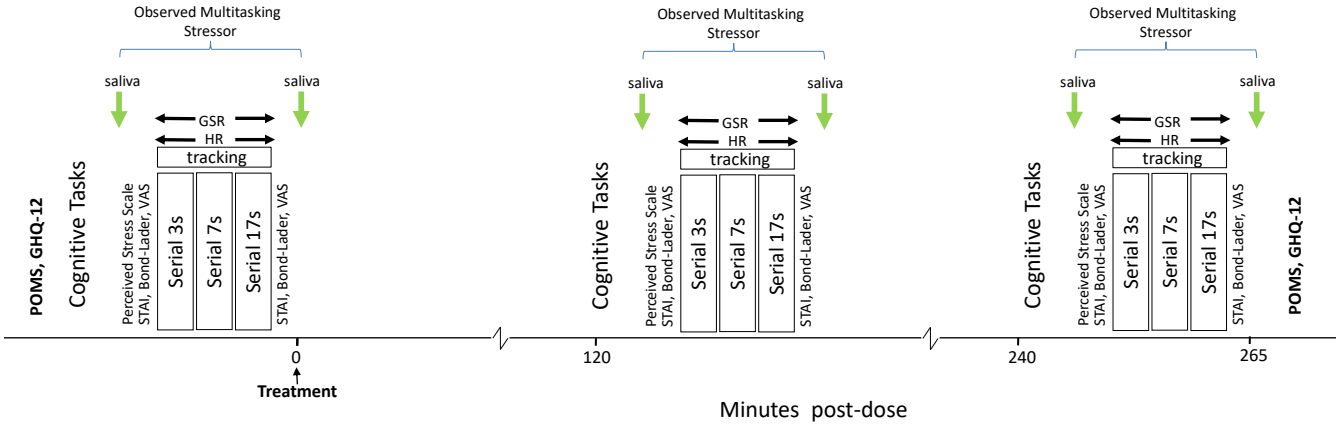

Figure 2. Schedule of each testing session (Day 1, Day 29). Participants complete the POMS and GHQ-12, followed by the cognitive tasks (in the lab). They then move to the interview room and complete the Observed Multitasking Stressor assessment. After this they take their day's treatment, and then complete further identical cognitive/OMS assessments commencing 120- and 240 min post-dose. Between these two post-dose assessments, participants will be provided with a standardised lunch. Before departing they complete the POMS and GHQ-12.

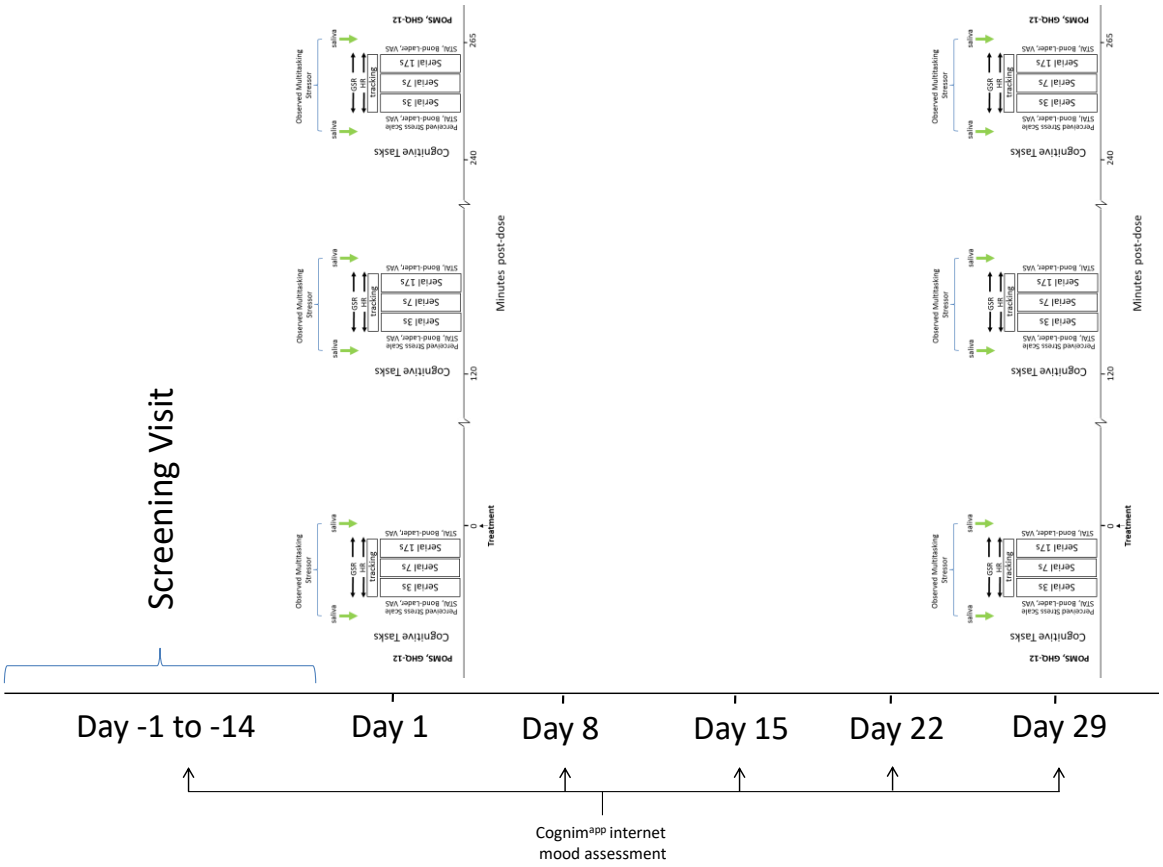

Figure 3. Study timelines. Participants attend an introductory/screening session -1 to -14 days before the first (acute dose) study day (Day 1). They then undergo an identical assessment on Day 29 of the treatment schedule. Brief mood assessments are completed in the participants home using Cognimapp pre-treatment and after 8, 15, 22 days treatment – the final assessment takes place in the laboratory on Day 29.

Study analysis plan

The cognitive, mood, and psychological/physiological stress response data may be subjected to several baseline adjusted ANOVA[1] analyses in order to assess:

[1] Alternative analyses include ANCOVA in the event of any influence of demographic/mood parameters on the outcomes (e.g. age, anxiety) and/or Linear mixed models in the case of missing data.

- The acute effects of treatment (Day 1 data from 120/240 min post-dose baseline adjusted to Day 1 baseline – ANOVA factors: assessment x treatment)
- The effects of chronic supplementation on the acute effects of the treatment (Day 1 and Day 29 data from 120/240 min post-dose baseline adjusted to the respective day's pre-dose data ANOVA factors: day x assessment x treatment)
- The pure chronic effects of treatment (Day 29 pre-dose data baseline adjusted to Day 1 pre-dose AND Cognim^{app} mood data from Day 8, 15, 22 and 29 baseline adjusted to the pre-dose assessment ANOVA factors: day x treatment)
- The chronic with acute superimposed effects of treatment (Day 29 pre-dose, 120, 240 min data baseline adjusted to Day 1 pre-dose - ANOVA factors: assessment x treatment)

The above analyses will be applied, as appropriate, to the following data:

- General mood, including depression and anxiety (POMS, GHQ-12 – measured pre dose and at the end of testing on Day 1 and Day 29.
- General mood/psychological state (GAD-7, Bond-Lader, VAS) measured pre-treatment and on Days 8, 15, 22, 29 using Cognim^{app}.
- Cognitive function – executive [Stroop], attention [RVIP], working memory [Corsi blocks, NWM] –pre-dose [chronic only] and at 120 and 240 minutes post-dose.
- Overall mood and psychological state measured pre-dose and at 120 and 240 mins post-dose (PSS, STAI, Bond-Lader VAS – measured pre-OMS)
- Overall cognitive performance during the OMS pre-dose and at 120 and 240 mins post-dose (accuracy and speed of Serial 3s, 7s, 17s, computerised tracking task)
- Overall stress hormone levels (salivary cortisol, α-amylase) measured before the OMS pre-dose [chronic only] and at 120 and 240 min post-dose.
- Modulation of the psychological response to acute stress (change in: PSS, STAI, Bond-Lader, VAS during the OMS) as a consequence of the OMS pre-dose and at 120 and 240 mins post-dose.
- Modulation of the physiological response to acute stress (change in heart rate, galvanic skin response, salivary cortisol, α-amylase during OMS) as a consequence of the OMS pre-dose and at 120 and 240 mins post-dose.