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Study Protocol

The Effects of Green Rooibos Tea Extract on Anxiety Levels (REAL) Study: Protocol for a Single-center Randomized Controlled Trial

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Abstract

Background and objectives: Anti-anxiety medications may cause adverse effects and can be associated with long-term health risks. Rooibos tea (*Aspalathus linearis*) contains polyphenolic compounds that may confer health benefits. This study aims to investigate the effects of green rooibos extract supplementation on anxiety levels in adults with mild-to-moderate anxiety.

Methods: This double-blind, placebo-controlled, randomized controlled trial will enroll 60 adults aged 18-65 years with mild-to-moderate anxiety. Participants will receive either green rooibos extract (19.25 mg aspalathin per capsule; $n = 30$) or placebo ($n = 30$). They will take one capsule each morning during the first week and two capsules each morning during the subsequent 7 weeks. The anxiety subscale of the 21-item Depression, Anxiety and Stress Scale will be the primary outcome and will be assessed from baseline to post-intervention. Secondary outcomes will include salivary biomarkers, heart rate variability, sleep quality, and dietary intake. Measurements will be collected at baseline, mid-intervention, and post-intervention.

Discussion: The findings may help determine whether green rooibos extract supplementation is associated with reduced anxiety and improvements in related health markers in adults with mild-to-moderate anxiety.

Introduction

Anxiety disorders are among the leading causes of health burden worldwide, affecting an estimated 4% of the global population.[1,2] These disorders are the most prevalent mental health conditions worldwide and impose substantial disability and economic costs, accounting for approximately 370 disability-adjusted life years per 100,000 population.[3] Anxiety disorders are characterized by persistent and excessive anxiety symptoms, including shortness of breath, an elevated heart rate, and insomnia.[1] Anxiety can affect people of all ages, sexes, socioeconomic groups, and regions, and the number of affected individuals increased by 55% between 1990 and 2019.[3,4] Most of these estimates predate the COVID-19 pandemic and may therefore underrepresent the current global burden of anxiety-related disorders. During the pandemic, the global prevalence of anxiety disorders was estimated to have increased by 25.6%, potentially because of social isolation, job loss, domestic violence, and heightened uncertainty, grief, and fear.[5,6]

Although several treatments are available for anxiety disorders, including psychological interventions such as cognitive behavioral therapy and prescription medications, their use is limited by access barriers and stigma.[2] Consequently, only approximately one-quarter of people with anxiety receive treatment.[7] Interest is increasing in nutraceuticals and supplements derived from food products as approaches to prevent or delay disease onset or

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support the management of chronic conditions, including mental disorders.[8,9] Nutraceuticals are often considered convenient and well tolerated, with few reported adverse effects.[10] Evidence also suggests that nutraceuticals may have complementary effects when used alongside standard care for chronic diseases.[11]

Tea is one of the most widely consumed beverages worldwide.[12,13] Historically, tea has been used in traditional Chinese medicine for disease prevention and symptom management.[14] Research suggests that regular tea intake, alone or alongside standard care, may be associated with beneficial cardiometabolic effects, delayed cancer development, weight management, and a lower risk of neurological disease.[14,15] These effects may be attributable to phytochemicals, including polyphenols, flavonoids, dihydrochalcones, and enzymes, found in teas, particularly those derived from *Camellia sinensis*.[12,14,16]

Rooibos (*Aspalathus linearis*), a plant endemic to South Africa and commonly consumed as an herbal tea, is the only known food source of aspalathin.[16] Teas and extracts with higher aspalathin concentrations may exert physiological and psychological effects, including improvements in energy metabolism and anti-inflammatory, antioxidative, and antihyperglycemic activity, that could reduce cardiometabolic risk and alleviate anxiety and stress.[12,17-20] *In vitro* and animal studies, together with limited human observational data, suggest that rooibos extracts may exert broad cytoprotective, antioxidative, anti-inflammatory, antihyperglycemic, and antithrombotic effects, with potential implications for metabolic syndrome, cardiovascular disease (CVD), and neuroprotection.[21] *In vitro* assays also suggest that rooibos extract can inhibit several human cytochrome P450 (CYP450) enzymes, some of which are involved in cortisol biosynthesis.[22] Such inhibition could reduce cortisol production and may therefore be relevant to conditions associated with hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, including anxiety-related conditions.[23] The HPA axis is also closely linked to immune function, energy metabolism, oxidative stress, and glucose regulation.

Although the potential health benefits of rooibos have been widely investigated *in vitro* and in animal models, its anxiolytic potential remains underexplored in humans.[21,24] Aspalathin may influence enzymes involved in HPA-axis activity and cortisol production, providing a plausible mechanism through which rooibos could modulate anxiety symptoms. Clinical studies have also examined nutraceuticals such as probiotics, micronutrients, myo-inositol, and omega-3 fatty acids in a range of populations.[25-29] Despite promising preclinical findings and numerous randomized controlled trials of other nutraceuticals, no human randomized controlled trial has investigated the effects of green rooibos extract on anxiety. Therefore, the primary aim of this trial is to investigate the effects of green rooibos extract supplementation on anxiety levels in adults with mild-to-moderate anxiety. Secondary outcomes will assess physiological, biological, and lifestyle markers associated with anxiety and general health.

Materials and methods

This protocol was prepared in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement and the SPIRIT Outcomes 2022 extension.[30,31] The trial was approved by the University of Canberra Human Research Ethics Committee (approval no. UC HREC 13602) and registered with the Australian New Zealand Clinical Trials Registry on April 2, 2024 (ACTRN12624000373572; Universal Trial Number U1111-1301-6151; https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=387037&isReview=true). At the time of manuscript submission, participant recruitment and data collection had been completed. This article reports the prespecified study protocol, with a focus on the primary outcome and key secondary outcomes, and does not present or analyze trial outcomes. The study was conducted in accordance with the Declaration of Helsinki as revised in 2024. Written informed consent was obtained before participants are screened for eligibility. Eligible participants will be familiarized with the study measures and procedures. Participant information and data will be deidentified in accordance with data-sharing requirements. Participant identities will be protected through a coding system. The code key linking participant names to study identifiers will be stored in a separate protected file accessible only to investigators directly involved in the study. Study records and files will be retained at the University of Canberra for 15 years after study completion. Individual participant data will not be shared or uploaded to a public repository because participants did not provide consent for data sharing. These data-protection procedures apply to all study data. The study will be conducted in a dedicated, appropriately equipped laboratory at the University of Canberra, Australia. Participants may withdraw from the trial at any time without penalty or the need to provide a reason. If a participant becomes distressed during the experiment, testing will cease immediately, and associated data will be discarded. If suspension or early termination is considered, the investigators will follow National Institutes of Health guidance.[32]

Study design

The study, titled "The Effects of Green Rooibos Tea Extract on Anxiety Levels (REAL)," is a single-center, pilot, double-blind, randomized, placebo-controlled, parallel-group trial. Participants will receive either active or placebo capsules for 8 weeks. Each active capsule will contain 250 mg of powdered green rooibos extract, manufactured and supplied by Archer Daniels Midland (ADM), and 19.25 mg of aspalathin. The prescribed dose (two capsules/day; approximately 40 mg aspalathin) is equivalent to approximately six cups of green rooibos tea per day, which has been described as an optimal dose for sustaining health benefits.[33] The placebo will contain maltodextrin, a standardized inert compound commonly used in such studies. Placebo capsules will be matched to active capsules in color and size. All capsules will be stored in the temper-tell, light-protected containers labeled with the study name, investigators' contact details, dosage instructions, and the statement "This container contains green rooibos extract or placebo." At the start of the intervention, participants will take one capsule orally each morning during the first week and two capsules each morning during the subsequent 7 weeks to reduce the risk of gastrointestinal discomfort. Adherence will be assessed by counting returned capsules at the end of the trial.

Participants

Eligible participants will be 18-65 years old and have mild-to-moderate anxiety according to the Beck Anxiety Inventory (BAI), defined as a self-reported score of 11-25.[34] Participants will be excluded if they have a body mass index ≥ 35 kg/m²; blood

pressure ≥ 160/95 mmHg; are taking anxiolytics, antidepressants, sleep medications, gastrointestinal or cardiovascular medications, or any medication or supplement that may affect anxiety symptoms, gut microbiota, autonomic measures, or sleep quality; or have a known chronic disease, including CVD, diabetes, cancer, kidney disease, gastrointestinal disease, a clinically diagnosed mental illness, or immunocompromise. Participants must not have taken antibiotics within 8 weeks before enrollment or probiotics, prebiotics, or postbiotics within 2 weeks before enrollment.[35] Individuals who are pregnant or lactating or who have an injury that precludes exercise testing will also be excluded.

Participants will be recruited through advertisements in local and national media outlets (radio, television, and newspapers), social media campaigns, the University of Canberra campus and website, and the research team's website (https://www.ffnrlaboratory.org/home/therealstudy). The target sample size is 60 participants, with 30 assigned to each group, and includes an anticipated dropout rate of 13%. The sample size was calculated using G*Power version 3.1.9.7 (Universität Düsseldorf) based on the primary outcome of anxiety level.[36] The calculation was based on detecting a between-group difference in the change in DASS-21 anxiety scores from baseline to post-intervention using a two-tailed independent-samples t test, 80% power, a type I error rate of $\alpha = 0.05$, and Cohen's $d = 0.80$. Although the primary analysis will use linear mixed-effects models to account for repeated measurements over time, the sample size calculation was based on the between-group difference in change scores as an approximation of the treatment group x time interaction.

Intervention

Randomization will be stratified by age and gender through www.randomizer.org, using permuted blocks of 10, with participants allocated in a 1:1 ratio to the active and placebo groups. The target sample size is 60 participants, with 30 participants allocated to each group. Allocation concealment will be managed by a researcher who is not involved in data collection. Investigators directly involved in the trial, participants, data analysts, funders, and other trial personnel will be blinded to allocation. Participants will attend four clinic visits (Fig. 1). Questionnaires will be completed in person and through take-home packets (Table 1). Participants will also receive a wrist-worn actigraphy device (MotionWatch 8; CamNtech, Fenstanton, UK) and cryovials with instructions for at-home saliva collection. Anthropometric, cardiorespiratory fitness, and physiological measurements will be obtained during clinic visits (Table 1).

At Clinic 1, participants will complete the BAI and a health questionnaire to determine eligibility. Blood pressure will be measured after 10 min of seated rest, and body composition will be assessed using a portable body composition analyzer (Tanita, MI, USA). Eligible participants will then be familiarized with all relevant study materials and instructions.

At Clinic 2, participants will return saliva samples, actigraphy devices, and completed questionnaires. They will be fitted with a Polar H10 chest strap and asked to rest in a supine position for 10 min.[37] Heart rate variability (HRV) will be recorded during the final 5 min of this period.[38] After HRV recording, supine blood pressure will be measured using an electronic device (ProBP 2000 Digital Blood Pressure Device; Welch Allyn, Guangdong, China).[39] Cardiorespiratory fitness will be assessed with a maximal exercise test ($\dot{V}O_2max$) using a Vyntus CPX metabolic cart (Vyaire Medical, Mettawa, IL, USA) and a stationary bicycle ergometer (SRM, Jülich, Germany). Immediately after the $\dot{V}O_2max$ test, participants will remain seated on the bicycle without pedalling for assessment of HRV reactivity.[40] After testing, participants will receive their assigned active or placebo capsules, saliva collection kits, and a second set of take-home questionnaires, together with detailed instructions on capsule consumption, activity recording, and sample collection and storage.

At the start of Clinic 3, participants will submit frozen saliva samples and completed questionnaires. Resting supine HRV and blood pressure will be measured. Participants will also schedule a brief appointment to collect the wrist-worn actigraphy device 1 week before Clinic 4.

At Clinic 4, participants will submit frozen saliva samples, the wrist-worn actigraphy device, unused capsules, and completed questionnaires. Physiological, anthropometric, and cardiorespiratory fitness measurements will be obtained using the same procedures as at previous visits.

For each participant, Clinics 2-4 will be scheduled at approximately the same time of day to standardize HRV and physiological measurements. Before Clinics 2-4, participants will be instructed to abstain from caffeine, alcohol, and smoking for 12-24 h because stimulants and depressants may modulate physiological systems.[41-44] Participants will also be asked to avoid rooibos tea throughout the trial and to limit exercise to light intensity, if any, during the 24-36 h before each visit because moderate-to-vigorous intensity exercise may acutely affect autonomic nervous system activity.[45]

Procedures

The effects of green rooibos extract will be assessed using questionnaires on anxiety, diet, lifestyle, and sleep, together with biological, physiological, anthropometric, and physical measurements. Sleep, lifestyle, and general health are associated with anxiety and may provide important context for interpreting trial outcomes.[46-52]

Primary outcome

The primary outcome will be the change in anxiety level from baseline to post-intervention, assessed using the anxiety subscale of the 21-item Depression, Anxiety and Stress Scale (DASS-21). The DASS-21 is a brief self-report questionnaire that correlates strongly with the BAI and has good validity and reliability.[53,54] It uses a 4-point Likert scale ranging from 0 ("Did not apply to me at all") to 3 ("Applied to me very much, or most of the time") to indicate the extent to which each statement applied during the preceding week.[55]

Secondary outcomes

Assessment of autonomic regulation

Heart rate variability, a non-invasive neurophysiological marker of autonomic regulation, will be assessed using the Polar H10 Heart Rate Sensor, which detects intervals between electrical signals corresponding to cardiac R waves. Signal processing will convert recorded data into interbeat intervals, which will be saved as text files. Raw interbeat-interval data will be manually corrected and analyzed with Kubios HRV software (version 4.1.0; Biosignal

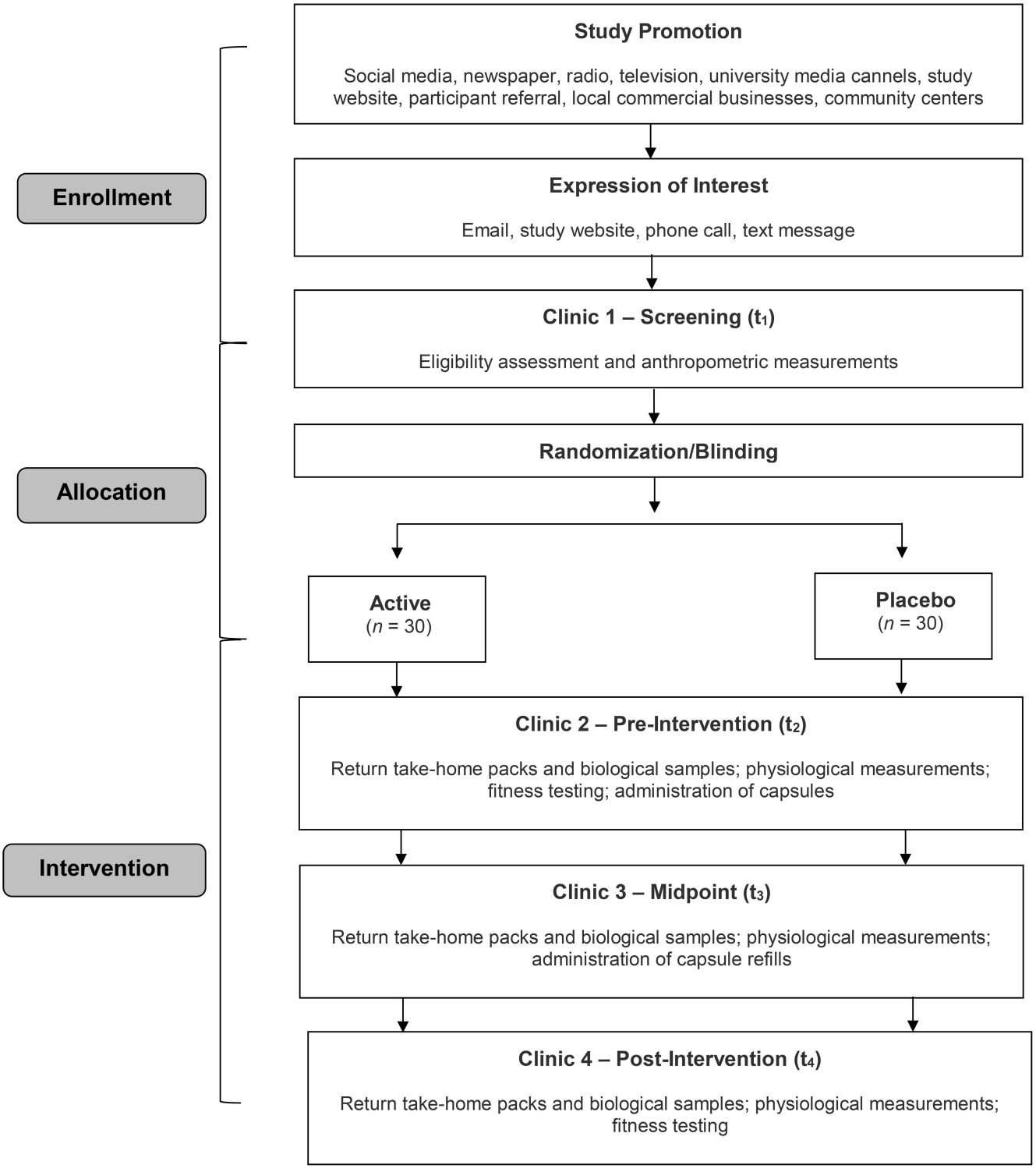

Fig. 1. Timeline of participant involvement from enrollment through completion of the trial. *n*, number of participants; t1-t4, clinic time points.

Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) using previously described methods.[56,57] Short-term (5-min) HRV analysis will include standard time- and frequency-domain measures: the root mean square of successive differences, standard deviation of interbeat intervals, low-frequency power (LF), high-frequency

Table 1. Timeline of study procedures

Clinic	Consent	Screening	Capsule administration	Height (cm)	Weight (kg)	Waist and hip circumference (cm)	BC	BP (mmHg)	HR (bpm)	HRV	$\dot{V}O_2$max (mL/kg/min)	Saliva sample return	MW8 issue/return	Take-home questionnaires
1	X	X		X	X	X	X	X	X				X	X
2			X					X	X	X	X	X	X	X
3			X					X	X	X		X		X
4					X	X	X	X	X	X	X	X	X	X

A brief appointment was made for each participant one week prior to Clinic 4 to reissue the MW8. BC, body composition; BP, blood pressure; HR, heart rate; HRV, heart rate variability; MW8, MotionWatch 8; $\dot{V}O_2$max, maximal oxygen uptake.

power (HF), and the LF/HF ratio. HRV is a valid and reliable measure of parasympathetic regulation of heart rate and may provide insight into the development and progression of psychopathology, including anxiety.[58] Participants will remain supine throughout the 10-min recording, including a 5-min stabilization period. Arterial blood pressure will be measured immediately afterwards using a Welch Allyn ProBP 2000 monitor.

Saliva collection

Saliva samples (1 mL) will be collected using the passive drool method.[59] Nine samples will be collected from each participant: three at baseline, three at the midpoint, and three post-intervention. Participants will be instructed to collect samples at 8:00 p.m. on the evening before each clinic visit, immediately upon awakening on the day of the visit, and 45 min after the first morning collection. Samples will be stored in participants' freezers and transported on ice to the investigators at the next clinic visit. They will then be stored at −80°C until analysis. Salivary cortisol and salivary alpha-amylase (sAA), non-invasive markers of HPA-axis and sympathetic nervous system activity, respectively, will be measured using a Salivary Cortisol Enzyme-Linked Immunosorbent Assay kit and an sAA Kinetic/Enzymatic Assay kit (Salimetrics, USA) according to the manufacturer's instructions.[46,50,60]

Actigraphy evaluation

Real-time sleep data will be collected using a MotionWatch 8 (MW8) actigraphy device (CamNtech, UK) for 1 week before Clinic 2 and 1 week before Clinic 4. The wrist-worn MW8 device is waterproof, lightweight, and validated for quantifying sleep and physical activity in adults.[61,62] Sleep variables, including sleep-onset latency, wake after sleep onset, total time in bed, total sleep time, and sleep efficiency, will be quantified.[62]

Dietary intake

Dietary intake will be monitored using a 3-day food record covering two weekdays and one weekend day. This type of record has been used in studies with similar designs to monitor dietary intake.[63,64] Dietary intake will be analyzed using FoodWorks software (version 10; Xyris Software, Queensland, Australia) and the AUSFOODS 2017 database.

Exploratory and contextual covariates

The depression and stress subscales of the DASS-21 will be included as exploratory outcomes. Standing height, weight, waist and hip circumferences, and body composition will be measured with participants lightly clothed and without shoes, following standard anthropometric procedures. Body composition will be assessed using a calibrated digital body composition analyzer (Tanita, MI, USA), and height will be measured using a stadiometer (ADE, Sydney, Australia). Body mass index will be calculated as weight divided by height squared (kg/m²), and the waist-to-hip ratio will be calculated from waist and hip circumference measurements.

Cardiorespiratory fitness will be measured using an incremental maximal exercise test to volitional exhaustion ($\dot{V}O_2$max). Participants will exercise on an SRM High Performance Cycling Ergometer with an SRM Science PowerMeter, which has a reported power-measurement accuracy of ± 0.5%. Expired gases will be measured breath by breath through indirect calorimetry using a Vyntus CPX metabolic cart (Vyaire Medical, Mettawa, IL, USA). Participants will wear a mask designed for this test, and the gas analyzer will be calibrated automatically before each assessment. The continuously increasing ramp protocol will begin with a 2-3-min resting phase to allow adaptation to the mask.[65] This phase will be followed by a 3-min warm-up at 50 W and an approximately 10-min incremental exercise phase with increases of 10-30 W/min, rounded to the nearest 5 W/min and individually calculated according to age, height, and weight.[66] Carbon dioxide production ($\dot{V}CO_2$, L/min), oxygen

consumption (VO_2, L/min), minute ventilation (L/min), and the respiratory exchange ratio (RER; VCO_2/VO_2) will be calculated from gas analysis data averaged over 20 s. Oxygen consumption values near the end of the test will be used to calculate VO_2max, whereas RER will indicate the relative contributions of fat and carbohydrate to energy production.[67] This protocol has previously been used in adults with mental illness.[56]

Additional questionnaires will include the Alcohol Use Disorders Identification Test-Concise (AUDIT-C) to assess current alcohol consumption and the American Psychiatric Association recommended Patient-Reported Outcomes Measurement Information System (PROMIS) instrument to assess self-reported sleep quality.[68,69]

Investigators will monitor possible side effects, including gastrointestinal discomfort, headache, and fatigue, as well as adverse events and serious adverse events potentially related to green rooibos extract. Participants will record these events in diaries provided at Clinics 2-4 and will have the contact details of the principal investigators for use between visits. They will be advised to contact their general practitioner or emergency services when necessary. Investigators will follow up with participants 2 weeks after trial completion to assess their well-being and any adverse events. Adverse events will be graded and reported according to version 6.0 of the National Cancer Institute Common Terminology Criteria for Adverse Events.[70]

Data analysis

Statistical analyses will be performed using SPSS version 29.0.2.0 (IBM Corp., Armonk, NY, USA). Before analysis, all variables will be examined for suitability for parametric or non-parametric methods using histograms and the Kolmogorov-Smirnov test. Mixed-effects models will assess changes over time within and between groups, with random intercepts for participants and fixed effects for time, treatment group, and their interaction.

The primary analysis will follow the intention-to-treat principle, with participants analyzed according to their original treatment allocation. Linear mixed-effects models will use all available outcome data and include participants with incomplete follow-up under the assumption that data are missing at random. This approach accommodates missing observations without requiring imputation or excluding participants with partially observed data. Complete-case and per-protocol analyses may be conducted as sensitivity analyses to assess the robustness of the primary findings.[71] Within- and between-subject comparisons will be performed, with statistical significance set at $P < 0.05$.

For secondary outcomes, the false discovery rate will be controlled using the Benjamini-Hochberg procedure because the analyses include several correlated physiological variables. For repeated measures, Bonferroni-adjusted pairwise comparisons will be performed after significant omnibus tests. Results will be expressed as the mean ± standard deviation, and statistical significance will be set at $P < 0.05$. Percentage change from baseline will be calculated for DASS-21 scores at all follow-up assessments. Cumulative frequencies of treatment responders will be generated; responders will be defined as participants who achieve and maintain a ≥ 15% reduction in anxiety scores.[72] A per-protocol analysis will compare treatment groups among participants who complete the trial.

Data management and quality control

Data management will be supervised directly by the chief investigators. A master list will link participant names to the identification numbers used for study data. Collected data will be labeled only with participant identification numbers. All data will be stored on a dedicated password-protected drive on the University of Canberra server and will be accessible only to the chief investigators. All investigators will share responsibility for monitoring data accuracy and completeness. Data management activities will be communicated to the research group through periodic reports and regular meetings. Data quality will be supported by standardized training for all investigators who collect data.

Discussion

Clinical utility

Aspalathin, a major polyphenol in green rooibos tea, has shown diverse health-promoting potential.[73] This protocol describes the scientific rationale and multifaceted design of a study investigating green rooibos extract supplementation in adults aged 18-65 years with mild-to-moderate anxiety. Persistent anxiety symptoms are associated with disrupted physiological homeostasis and changes in biological stress systems, including neuroendocrine, metabolic, autonomic, and immune pathways.[23,74] Long-term dysregulation of these processes may contribute to the onset or exacerbation of comorbid conditions, including CVD, hypertension, diabetes, obesity, cancer, major depressive disorder, systemic inflammation, gastrointestinal dysfunction, impaired sleep, and autoimmune disease.[74-78]

Physiological mechanisms

Anxiety will be assessed primarily using the self-reported DASS-21, which is recommended for research in clinical and non-clinical populations and has been used in studies of the anxiolytic effects of nutraceutical interventions.[55,79] Preclinical studies suggest that rooibos polyphenols, including aspalathin, may have immunomodulatory effects and influence enzymes involved in the stress response.[80] Rooibos may also exert neuromodulatory and cardioprotective effects, potentially by increasing hippocampal taurine levels and thereby supporting anxiolytic activity.[18,81] Diurnal salivary cortisol and sAA will therefore be measured as biomarkers of stress-related neuroendocrine and autonomic activity.[46,50,60,82] Cortisol levels are typically highest in the morning, peak approximately 30 – 45 min after awakening, and then decline throughout the day.[83] In contrast, sAA levels decrease sharply upon awakening and increase gradually during the day.[82] Anxiety has been associated with deviations from these typical diurnal patterns, including altered secretion at different time points.[82,84] Cardiorespiratory fitness will be considered when interpreting HPA-axis responses because higher fitness in healthy individuals is associated with lower diurnal cortisol secretion and may buffer HPA-axis dysregulation.[85] HRV will provide an additional measure of autonomic regulation related to anxiety.[86] Participants receiving green rooibos extract may show improved resting HRV indices compared with the placebo group, although this hypothesis requires confirmation.[23,86]

The effects of green rooibos extract on anxiety will also be

considered in relation to sleep quality, physical activity, and diet.[87] Lower-quality diets are associated with poorer metabolic function and a greater risk of mood and anxiety disorders, whereas higher-quality diets that include prebiotic fibers have been associated with lower anxiety levels.[88] *In vitro* evidence suggests that rooibos tea may have prebiotic activity because of its polyphenolic content and composition.[73] Prebiotics may influence the intestinal microbiota and thereby affect immune regulation, sleep quality, performance, stress, and anxiety.[89]

Nutraceutical development

Green rooibos tea is commercially available and culturally accepted. Findings from this trial may inform the development of rooibos extract as a functional food or complementary therapeutic supplement and could support future regulatory evaluation. Establishing an effective dose and intervention duration may also inform the formulation of capsules, teas, or functional beverages intended to support anxiety management. Because encapsulated rooibos extract may be cost-effective, stable, and readily scalable, it could offer an accessible adjunctive strategy for people who are underserved by conventional care or reluctant to use pharmaceuticals. These potential applications will require confirmation of efficacy and safety.

Future research directions and limitations

This trial focuses on people with subclinical-to-moderately elevated anxiety symptoms; therefore, its findings may not generalize to people with diagnosed anxiety disorders, such as generalized anxiety disorder or panic disorder. Although these symptoms are not diagnostic, they can impair quality of life and are often undertreated.[90] Demonstrating benefit in this population could support early-intervention strategies, but larger trials would be needed to determine whether green rooibos extract can prevent progression to more severe mental health conditions. If efficacy is supported, healthcare providers and nutrition professionals may consider green rooibos extract as an adjunct to broader lifestyle and dietary strategies. Public health communication would need to present its safety, natural origin, and traditional use without overstating the evidence. Subsequent trials could enroll people with clinically diagnosed anxiety disorders or compare responses among populations with different gut microbiota profiles or stress biomarkers. Omics approaches, including metabolomics and proteomics, could further clarify the biological pathways through which rooibos may exert anxiolytic effects.

Conclusions

This trial will provide preliminary evidence on the potential anxiolytic effects of green rooibos extract in adults with mild-to-moderate anxiety. The findings may also provide insight into physiological and psychological pathways relevant to nutraceutical interventions for anxiety. If clinically relevant effects are observed, green rooibos extract could represent an accessible and well-tolerated adjunct to current anxiety management strategies. Further research will be required to validate the findings in clinical populations and assess long-term outcomes, safety, and integration into routine care or consumer health products.

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Conflict of interest

Nenad Naumovski serves as an Associate Editor of *Exploratory Research and Hypothesis in Medicine*. Kathryn E. Speer is a postdoctoral researcher at the University of Canberra working on a project funded by Archer Daniels Midland Pty Ltd. Nenad Naumovski and Andrew J. McKune have received funding from Archer Daniels Midland Pty Ltd. All funds are administered through the University of Canberra Research Office (UC-R01583). Funders did not have any role in the study design, data collection, analysis, interpretation, manuscript preparation or in the decision to publish this manuscript.

Author contributions

Conceptualization (KES, AJM, NN); data curation (KES); formal analysis (KES, AJM, NN); funding acquisition (NN, AJM); investigation (KES); methodology (KES, AJM, NN); project administration (KES); resources (NN, AJM); supervision (NN, AJM); validation (KES, AJM, NN); visualization (KES); writing—original draft (KES); writing—review and editing (KES, AJM, NN). All authors have approved the final version and publication of the manuscript.

Ethical statement

The study was approved by the University of Canberra Human Research Ethics Committee (approval no. UC HREC 13602) and was conducted in accordance with the Declaration of Helsinki as revised in 2024. Written informed consent was obtained from all participants before screening. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12624000373572).

Data sharing statement

Individual participant data will not be made available. Participants did not provide consent for their data to be shared beyond the purposes of this study.

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