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Effects of kombucha consumption on well-being, induced stress, and inflammation

Submission date	**Recruitment status**	[X] Prospectively registered
01/03/2024	No longer recruiting	[X] Protocol
Registration date	**Overall study status**	[_] Statistical analysis plan
06/03/2024	Completed	[_] Results
Last Edited	**Condition category**	[_] Individual participant data
05/03/2024	Other	[_] Record updated in last year

Plain English summary of protocol

Background and study aims
Studies have linked tea drinking to a decreased incidence of cognitive decline and lower levels of emotional distress. Probiotic, fermented products such as kombucha (fermented with a symbiotic culture [scoby] of bacteria and yeasts) have attracted significant interest due to their potential beneficial health-giving and wellness properties. Kombucha has multiple functional properties, including promoting favourable intestinal microbiome (bacteria) colonisation to improve gastrointestinal (digestive system) function, and anti-inflammatory, antioxidant, and metabolic activity including reduced cholesterol levels and blood pressure. The aim of this study is to assess the effects on kombucha on well-being, working memory, inflammation, and stress.

Who can participate?
Healthy volunteers from Ceredigion and surrounding areas, between 18 and 60 years of age, mixed gender, mixed ethnicity.

What does the study involve?
There will be two groups of participants randomly allocated to consume a specified volume (330 ml) of either probiotic (kombucha) or placebo (flavoured carbonated water) control drink, daily for 8 weeks.
Participants will be asked to collect first morning void urine samples before testing day 1 (baseline) and the day before day 56 (post-intervention). The researchers will also collect blood samples before test days 1 and 56.
During testing days 1 (baseline) and 56 (post-intervention), participants will be invited to have their height, weight, heart rate, and blood pressure taken. They will then be asked to complete a series of self-report questionnaires. Completion of the questionnaires will take about 20 minutes.
Following the questionnaires, participants shall take their first saliva sample for cortisol measurements (pre-stress). Saliva samples will be taken before (0 minutes) a stress test where ice water is used to create a stress experience (observed as elevated blood pressure, heart rate, inflammatory and electrodermal activity). Electrophysiological (BIOPAC) recording will measure heart rate and electrodermal activity. After the test, participants' heart rate and blood pressure will be measured and they will again complete questionnaires to report how stressful, painful, and unpleasant the test was. They shall also begin taking their saliva (cortisol) samples at timed

intervals.
They shall follow the same procedure for testing day 56 (post-intervention). The total approximate duration of each testing day is 1 hour.

What are the possible benefits and risks of participating?
Participants who complete all sessions will be awarded £20 for every session they spend on campus.
If they are an Aberystwyth University Psychology student, then they will also be awarded 5 SONA credits for each completed visit.
In addition to the financial incentive, by participating in this research, they will allow researchers to gain important insight into the effects of kombucha on well-being, working memory, and inflammation, which will strengthen our understanding of the physiological benefits of post-biotics on the gut and gut-brain axis.
The stress test is designed to create low levels of acute, manageable pain via temperatures of 2oC using ice water. To ensure that the pain levels are suitable for this type of experiment and to ensure the safety of participants, the same procedure developed by Smeets et al., 2012 will be used. In addition, participants will remain completely in control of their hand submersion, so if the pain becomes too much, they can easily withdraw their hand from the water. If they decide they would like to continue with the test, they can choose to submerge their hand again in the water during the next submersion interval, and continue doing so (submerging and retrieving) until the end of the experiment. The researcher will make a note of any 'failed' submersion trials. The participant's heart rate will be continuously monitored throughout the test, so if the participant's heart rate exceeds the upper threshold, then the experiment will be stopped. Participants will be explained the procedure before taking part and be allowed to ask any questions before taking part to provide informed consent. They will also be reminded of their right to withdraw and to stop the experiment at any point. Additionally, participants shall be given thorough instructions before they are requested to provide urine, blood, and saliva samples. If participants wish to no longer participate in the stress test but still wish to continue with the rest of the trial (providing blood, urine and questionnaire data and consuming the kombucha/placebo for the 8-week intervention period), then they will be permitted to do so. Due to the nature of the topics assessed in this study participants will be given contact details for Mind, the Samaritans, MentalHealth.org and the student well-being services in the participant information sheet (PIS) and debrief sheet. There will also be first-aid-trained individuals available throughout the study to account for any pain-related issues that may arise due to exceptional circumstances beyond the known level of pain from ice water.
Part of the Mast protocol is to state to participants that they are being video recorded, but they will not be. It is done to increase levels of stress, based on findings that the perception of being videotaped whilst performing a mental arithmetic task reliably elicits strong stress responses, including a 2- to 3-fold increase in salivary cortisol concentrations, rendering it an essential (and therefore necessary) social-stress component of the stress test. This choice of, and reason for, deception will be explained to participants in person, during their debrief, at the end of session 2. Participants will have plenty of time to ask questions to ensure they are happy with how all components of the study were conducted.

Where is the study run from?
Aberystwyth University (UK)

When is the study starting and how long is it expected to run for?
September 2023 to February 2025

Who is funding the study?
Innovate UK

Who is the main contact?
Amanda J Lloyd, abl@aber.ac.uk

Contact information

Type(s)
Public, Scientific, Principal investigator

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Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
10074259

Study information

Scientific Title
Physiological and self-reported effects of Kombucha on well-being, induced stress, and inflammation

Acronym
INBK

Study objectives

The researchers will conduct a randomised, double-blinded, parallel clinical trial with healthy 18-60-year-olds, mixed gender and ethnicity. There will be two groups consuming a specified volume (330 ml) of either probiotic (kombucha) or placebo (flavoured carbonated water) control drink, daily, for 8 weeks.

The hypothesis is that between the start and the end of the intervention, and between placebo and active intervention, there will be changes in the following:
1. Self-report questionnaires: the Carol Ryffs (1989) Psychological Well-Being (PWB) Scale (18-item), the Depression Anxiety Stress Scale (DASS; 21-item), the Positive and Negative Affect Scale (PANAS)-GEN, the Visual Analogue Scale (VAS), and the EQ5D-5L, and the PANAS-NOW
2. Cortisol measurements before and after the Maastricht Acute Stress Test (MAST)
3. Chemical composition of urine samples using metabolomics and targeted analysis
4. Inflammation (cytokines) measured in blood using ELISA

Ethics approval required
Ethics approval required

Ethics approval(s)
approved 07/02/2024, Aberystwyth University Research Ethics Panel (Aberystwyth University, Reception, Penglais, Aberystwyth, SY23 3FL, United Kingdom; +44 (0)1970 621694; lif1@aber.ac.uk), ref: 25618

Study design
Randomized double-blinded parallel clinical trial

Primary study design
Interventional

Study type(s)
Prevention, Quality of life

Health condition(s) or problem(s) studied
Inflammation and well-being

Interventions
Epidemiological studies have linked tea consumption to decreased incidence of cognitive decline and lower levels of emotional distress. Probiotic, fermented products such as kombucha, (fermented with a symbiotic culture (scoby) of bacteria and yeasts), have attracted significant interest due to their potential beneficial health-giving and wellness properties. Kombucha has multiple functional properties, including promoting favourable intestinal microbiome colonisation to improve gastrointestinal function, and anti-inflammatory, antioxidant, and metabolic activity including reduced cholesterol levels and blood pressure.

There will be two groups consuming a specified volume (330 ml) of either probiotic (kombucha) or placebo (flavoured carbonated water) control drink, daily, for 8 weeks. A 4-week washout period eliminating probiotic-containing products in the daily diet will be implemented before the intervention. Participants will be asked to collect 2 x 4 ml urine samples using at-home-urine sampling kits both before testing day 1 (baseline) and the day before day 56 (post-intervention). The chemical composition of urine samples will be assessed using metabolomics. The researchers will also collect venous blood samples (3 x 4.5 ml) 48 hours before test days 1 and 56 to investigate the chemical composition using metabolomics and inflammation status (ELISA).

During testing days 1 (baseline) and 56 (post-intervention), participants will be invited to have their height, weight, heart rate, and blood pressure taken. They will then be asked to complete a series of self-report questionnaires: the Carol Ryffs (1989) Psychological Well-Being (PWB) Scale (18-item), the Depression Anxiety Stress Scale (DASS; 21-item), the Positive and Negative Affect Scale (PANAS)-GEN, the Visual Analogue Scale (VAS), and the EQ5D-5L, and the PANAS-NOW. Completion of the questionnaires will take approximately 20 minutes:
1. PWB-18 (3-5 min)
2. DASS-21 (3-5 min)
3. PANAS-GEN (2-3 min)
4. VAS (<1 min)
5. EuroQol -5D-5L (5 min)
6. PANAS-NOW (2-3 min)

Following the self-report questionnaires, participants shall be guided through taking their first saliva sample for baseline cortisol assessments (pre-stress). Saliva samples will be taken before (0 minutes) the Maastricht Acute Stress Test (MAST). Sarstedt cortisol salivates will be administered into, and removed from, the mouth by participants themselves after a prior demonstration of the method before they start the testing.

Participants will engage in a validated and reliable stress-inducing pain test, the MAST, which, via the use of a cold pressor, is designed to create an event-related stress experience (observed as elevated blood pressure, heart rate, inflammatory and electrodermal activity – EDA). Electrophysiological (BIOPAC) recording will measure HR and EDA during the MAST. After completion of the MAST, participant HR and BP will be measured, and they will again complete the PANAS-NOW and visual analogue scale (VAS) to report how stressful, painful, and unpleasant the MAST was (scale = 0 = ‘‘not at all’’ to 100 = ‘‘extremely’’). They shall also begin taking their saliva (cortisol) samples at timed intervals (T0m, T10m, T20m, T30m, T40m). Finally, they shall be provided instructions for consuming 330 ml of Kombucha or placebo control drink (carbonated placebo matching in taste, texture, and quality) daily, across the 8-week intervention period. They shall follow the same procedure for testing day 56 (post-intervention). The total approximate duration of each testing day is 1 hour.

Intervention Type
Supplement

Primary outcome(s)
Quality of life is measured by the EuroQol -5D-5L at baseline and after 8 weeks

Key secondary outcome(s))
1. Psychological well-being measured using the Carol Ryffs (1989) Psychological Well-Being (PWB) Scale (18-item) at baseline and after 8 weeks
2. Emotional states of depression, anxiety and stress measured using the Depression Anxiety Stress Scale (DASS; 21-item) at baseline and after 8 weeks
3. Mood or emotion measured using the Positive and Negative Affect Scale (PANAS)-GEN at baseline and after 8 weeks
4. Pain measured using the Visual Analogue Scale (VAS) at baseline and after 8 weeks
5. Near global metabolomics of first morning void urine measured by flow injection using electro-spray mass spectrometry instrumentation at baseline and after 8 weeks
6. Stress measured by cortisol concentrations in saliva before and after the Maastricht Acute Stress Test (MAST) at baseline and after 8 weeks
7. Near global metabolomics of fasting plasma measured by flow injection using electro-spray

mass spectrometry instrumentation at baseline and after 8 weeks
8. Inflammation measured by cytokine analysis (TFN-alpha) of fasting serum using ELISA at baseline and after 8 weeks

Completion date
28/02/2025

Eligibility

Key inclusion criteria
1. Subjects over 18 years of age and under 60 years of age
2. Subjects who are able to commit to multiple visits to WARU
3. Subjects who can provide venous blood samples, urine samples, and saliva samples
4. Subjects able to provide written informed consent prior to performing any study procedures

Participant type(s)
Healthy volunteer

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 years

Upper age limit
60 years

Sex
All

Key exclusion criteria
1. Subjects with a diagnosis of Alzheimer’s disease or other forms of dementia
2. Subjects taking medication for the treatment of dementia (such as acetylcholinesterase inhibitors (Aricept, Excelon), memantine (Namenda) or other medications with similar mechanisms of action) or medical foods (such as Cerefolin, Souvenaid, Axona) for the treatment of dementia.
3. Subjects who are pregnant or lactating
4. Subjects with medical condition or disease that is life-threatening
5. Subjects diagnosed with diabetes.
6. Subjects already consuming pro-biotics who will not comply with the 4-week washout
7. Subjects with any cardiovascular diseases
8. Subjects with severe physical illnesses (e.g., fibromyalgia)
9. Subjects experiencing hypertension (high blood pressure)
10. Subjects with endocrine disorders
11. Subjects suffering from substance abuse
12. Subjects who heavily smoke (>10 cigarettes/day)
13. Any medication known to affect the HPA axis.

Date of first enrolment
20/03/2024

Date of final enrolment
31/07/2024

Locations

Countries of recruitment
United Kingdom

Wales

Study participating centre
Well-being and Health Assessment Research Unit (WARU)
Carwyn James Building
Penglais Campus
Aberystwyth University
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Sponsor information

Organisation
Aberystwyth University

ROR
https://ror.org/015m2p889

Funder(s)

Funder type
Government

Funder Name
Innovate UK

Alternative Name(s)
Technology Strategy Board

Funding Body Type

Government organisation

Funding Body Subtype
National government

Location
United Kingdom

Results and Publications

Individual participant data (IPD) sharing plan
The data-sharing plans for the current study are unknown and will be made available at a later date

IPD sharing plan summary
Data sharing statement to be made available at a later date

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Participant information sheet			04/03/2024	No	Yes
Protocol file			05/03/2024	No	No