Cover page

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NCT Number: NCT ID not yet assigned

Document Date: 23rd May, 2025

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Study protocol of randomized controlled trial investigating the impact of Honey-sweetened black tea, green tea, and coffee on blood pressure, heart rate and blood glucose level in healthy young female adults

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Abstract

Background: Coffee and tea are known for their diverse phytochemical composition and potential physiological effects. While honey is a natural sweetener that has long been celebrated for its potential health benefits. This manuscript describes a randomized controlled trial designed to investigate the acute effect of honey-sweetened coffee, black tea, and green tea on blood pressure, heart rate, and blood glucose level in healthy young female adults.

Methods: The study will be conducted over a 3-day period, involving healthy young female adults. The participants will be randomly assigned to seven groups: Control (250 mL of warm water); Coffee Group (2.25g of coffee per 250ml of hot water); Honey-sweetened coffee group (2.25g of coffee per 250 mL of hot water with 20 mL of honey); Black tea group (2g black tea diffused in 250 mL hot water); Honey-sweetened black tea (2g black tea diffused in 250 mL hot water with 20 mL honey; Green Tea group (2g green tea diffused in 250 mL hot water); and Honey-sweetened green tea group (2g green tea diffused in 250 mL hot water with 20 mL honey. The basal measurements of each participant will be taken, after which the participants will be given their

designated beverages. The blood pressure and heart rate of each participant will be measured at 15, 30, 45, and 60 minutes, and blood glucose at 30 and 60 minutes after consumption.

Discussion: This research aims to provide significant insights regarding the possible health benefits or risks linked to the consumption of coffee, black tea, green tea and honey-sweetened coffee, black tea, and green tea . It will specifically focus on their effects on critical physiological factors such as blood pressure, heart rate, and blood glucose level. Understanding these factors is essential for maintaining individual health, as any adverse changes in them can lead to detrimental health consequences. By examining the relationship between these beverages and these vital health indicators, the study seeks to contribute to the broader understanding of dietary influences on cardiovascular and metabolic health.

Keywords: Coffee, Black tea, green tea, Honey-sweetened coffee, Honey-sweetened tea, Blood pressure, Heart rate, Blood glucose

Introduction

Coffee and tea stand out as globally favoured and versatile beverages of choice. Their nutritional significance primarily derives from their bioactive constituents. Coffee and tea are enriched with different polyphenols, known for their wide array of biological functions, encompassing antioxidant capabilities, potential cancer prevention, inflammation suppression, and protective properties against conditions like cardiovascular diseases, diabetes, hyperlipidemia, and obesity [1]. The polyphenols in coffee are chlorogenic acid, diterpenes, and trigonellin, while those in tea are catechins [2]. The catechins in green tea are epigallocatechin-3-gallate, gallocatechingallate,

epicatechin, epigallocatechin, gallocatechins, and epicatechin-3-gallate [3]. Those in black tea are theaflavins, amino acids L-theanine, epigallocatechingallate, and thearubigins [4]. Besides the polyphenol constituents, coffee and tea are rich in caffeine. Caffeine is a natural stimulant well-known to stimulate the central nervous system. There are varying quantities of caffeine in coffee and tea. Coffee contains 95-200 mg, black tea 14-70 mg, and green tea 24-45 mg of caffeine [5].

Green tea is known for its potential impact on blood pressure and blood glucose. Several studies have explored the relationship between green tea consumption and blood pressure [6,7]. In addition, green tea has also been associated with glucose metabolism. It has been documented to improve tissue insulin sensitivity [8,9]. Some studies suggest that the consumption of green tea decreases postprandial blood glucose spikes [10,11]. Black tea might have the potential to prevent cardiovascular diseases. Studies have also linked black tea consumption to a reduction in blood pressure and a lower risk of developing hypertension [12,13]. Furthermore, black tea has also been documented to have an impact on blood glucose levels. The consumption of black tea has the potential to lower the risk of developing type 2 diabetes. Black tea and its components have been shown to have the potential to lower blood glucose and reduce the risk of diabetes mellitus [14]. Coffee, as a rich source of caffeine, has been studied for its impact on blood pressure and blood glucose. The consensus among studies is that caffeine can lead to a transient increase in blood pressure due to its stimulatory effects on the central nervous system [15,16]. Additionally, coffee consumption can influence blood glucose levels. Studies have reported that coffee consumption increases blood glucose levels, decreases glucose disposal, and reduces insulin sensitivity [17,18]. While other studies have reported that coffee consumption improves insulin sensitivity, contributes to better blood glucose regulation, and reduces the risk of diabetes mellitus [19,20].

Honey has a rich history of utilisation dating back to ancient times, thanks to its nutritional and therapeutic properties [21]. Honey is produced globally and is primarily composed of carbohydrates, particularly in the form of monosaccharides like fructose and glucose. Honey is enriched with different biological compounds, like flavonoids and other types of antioxidants, which have been suggested to have cardioprotective benefits [22]. It has a lower glycemic index compared to regular sugar, which may result in a slower release of glucose into the bloodstream and potentially have a favourable impact on blood sugar levels [23].

Coffee and tea are either taken plain or sweetened with sugars, such as white sugar or artificial sweeteners. However, the use of natural sweeteners like honey is becoming more and more popular. Research on the possible health impacts of coffee, tea, and honey has been done separately; nevertheless, a significant gap persists in the research regarding the synergistic effects of tea and coffee when sweetened with honey. This study will investigate whether a three-day consumption of coffee and tea sweetened with honey will significantly influence the blood pressure and blood glucose level in healthy young female adults.

Study goals and objectives

Goals

The primary aim of this study is to determine the effect of acute consumption of honey-sweetened coffee, black tea, and green tea on systolic blood pressure, diastolic blood pressure, heart rate, and blood glucose level in healthy female participants.

Objectives

- To determine whether a three-day consumption of honey-sweetened black tea, green tea, and coffee will have an effect on blood pressure in healthy female individuals.
- To determine whether a three-day consumption of honey-sweetened black tea, green tea, and coffee will have an effect on heart rate in healthy female individuals.
- To determine whether a three-day consumption of honey-sweetened black tea, green tea, and coffee will have an effect on blood glucose level in healthy female individuals.

Study design

The study is an experimental study; specifically, it is a randomized controlled trial that will involve healthy young female adults between the ages of 18 and 26 years. The participants will consume honey-sweetened black tea, green tea, and coffee, or plain black tea, green tea, and coffee, or warm water, one cup a day for 3 days. The study will be carried out in the Physiology Laboratory, University of Uyo, and will be done within the period of July to October 2023. Each participant will fill out a consent form prior to the study, after which the participants will be given the beverages and observed for 60 minutes, during which their blood pressure, heart rate, and blood glucose level will be measured. This procedure will be repeated for the 3 days of the study. Prior to the consumption of the beverages, baseline measurements of blood pressure, heart rate, and blood glucose will be taken. For this study, a minimum of 200 volunteers will be recruited, and out of this, the eligible participants will be selected.

Methodology

The randomized controlled trial will be carried out among female students studying at the University of Uyo. The participants will be randomly grouped as follows:

Group 1 (Control) will be given 250 mL of water.

Group 2 (Coffee group) will be given 2.25 g of coffee per 250 mL of hot water.

Group 3 (Honey-sweetened coffee group) will be given 2.25 g of coffee per 250 mL of hot water with 20 mL of honey.

Group 4 (Black tea group) will be given one tea bag (2 g) per cup (250 mL) of hot water.

Group 5 (Honey-sweetened black tea) will be given one tea bag (2 g) per cup (250 mL) of hot water with 20 mL of honey.

Group 6 (Green tea group) will be given one bag (2 g) per cup (250 mL) of hot water.

Group 7 (Honey-sweetened green tea) will be given one bag (2 g) per cup (250 mL) of hot water with 20 mL of honey.

Study procedure

● Participants will be screened for eligibility criteria and will be provided with informed consent to participate in the study.

● The participants will be divided into seven groups, as specified above, through random allocation.

● After arrival, the subjects will be allowed to rest for 30 minutes. Then, baseline measurements of their blood pressures, heart rates, and blood glucose levels will be taken. This will serve as the 0-minute measurement. The subjects will then be given the standardized drinks according to their groups.

● Blood pressures and heart rates of the subjects will be measured and recorded 15 minutes, 30 minutes, 45 minutes, and 60 minutes after consumption of the drinks, while blood glucose levels of the subjects will be measured and recorded 30 minutes and 60 minutes after consumption.

● Blood pressure and heart rate will be measured using a digital sphygmomanometer. Blood pressure is measured in millimeters of mercury (mmHg) and recorded with the systolic number first, followed by the diastolic number. Heart rate is measured in beats per minute (bpm).

● Blood glucose level will be measured with a glucometer. Blood glucose level will be measured in mg/dL.

Intervention

Coffee, black tea, green tea, and honey

The coffee that will be used for this study is Nescafé Gold Blend, manufactured by Nestlé Coffee Brand, Nestlé Global, Vevey, Switzerland. The black tea is Lipton Yellow Label Black Tea, manufactured by Lipton Teas and Infusions, Rotterdam, Netherlands, and the green tea is Qualitea Natural Green Tea, packed by Qualitea Ceylon (PVT) LTD, Wattala, Colombo, Sri Lanka. These will be obtained from Market Square, Ibom Tropicana Mall, Uyo, Akwa-Ibom State, Nigeria. Raw dark amber honey produced by Apis mellifera adansonii will be used for this study, and it will be obtained from Vika Farms located in Mbak Etoi, Akwa-Ibom State, Nigeria.

The participants will be given a cup per day for 3 days. A tea cup will be prepared by infusing a tea bag in 250 mL of hot water (95°C for black tea and 80°C for green tea). The infusion will be done for 7-10 minutes with constant stirring. A cup of coffee will be prepared by dissolving 2.25 g of instant coffee in 250 mL of 95°C water with proper stirring.

The water will be allowed to boil and cooled down to a respectful temperature before the addition of either a tea bag or coffee. The beverages will then be allowed to cool to approximately 35 degrees before honey is added and given for consumption. The control group will be given 250 mL of water at 35°C.

Measurement of blood pressure, heart rate, blood glucose

Blood pressure and heart rate will be recorded on the left arm using an automated blood pressure monitor (Shanghai Little Doctor Electronics CO. LTD, Nantong 226007, China). First, the cuff

will be firmly placed around the arm (biceps). Then the power button will be pressed, and the automatic machine will read the blood pressure through the cuff. The reading will be taken three times, and the average value will be determined.

Blood glucose level will be measured by the oxidase method using a fine test glucometer (Osang HealthCare (Infopia), 132 Anyangcheondong-ro, Dongan-gu, Anyang-si, Gyeonggi-do, Korea). Firstly, the fingertip will be cleaned with an alcohol wipe and allowed to dry completely. The glucometer will be turned on, and a test strip will be inserted into the designated slot. A lancing device will be used to gently prick the fingertips of the subjects. The finger will be gently squeezed to help produce a small blood drop. Then the edge of the test strip will be used to touch the blood droplet until the meter beeps or indicates that it has received enough blood. The glucometer will process the blood sample and display the blood glucose level on the screen.

Sample size

The sample size was determined to assess the number of participants required to measure the mean difference between groups. The sample size was calculated using the formula explained by Charan et al. [24].

Sample size = $[2SD^2(1.96 + 0.84)^2]\div d^2$

Where:

SD = standard deviation from previous studies or pilot study

d = effect size- difference between mean values

However, a sample size of 70 will be used in this study based on available funds, equipment, manpower, and data from the literature.

Randomized controlled trial procedures

Recruitment and screening

The recruitment of participants will occur at the University of Uyo, Uyo, Akwa-Ibom State. Specifically, healthy female students studying at the University will be recruited for this study.

The recruitment process will involve all the departments within the Town Campus and Annex Campus of the University. The contact details of the class representatives of each level in each department within the aforementioned campuses will be obtained. The recruitment invitation for the study will be posted on the WhatsApp group page of each class by their class representative. In addition, flyers will also be distributed throughout the classrooms and hostels. A referral incentive of N100 per three referrals will be added to the recruitment invitation. The potential participants will be attended to by two experienced and trained research staff who are accustomed to all the study procedures. Participants who meet the preliminary eligibility criteria (living in the university hostel or living in close proximity to the university) and state clear interest in study participation, will then be invited to voluntarily complete the screening questionnaire and informed consent process.

Eligibility, informed consent, and enrollment processes

The participants who meet all the pre-screen requirements will be taken through the enrollment process, which includes eligibility for the study by filling out a questionnaire; filling out and signing the informed consent form; and measurement of their vitals (temperature, pulse rate, respiration, blood glucose level, and blood pressure). All participants that meet all the requirements for this stage will be considered fully enrolled for the study.

Eligibility criteria

Inclusion criteria are as follows:

(1) Age between 18 and 26; (2) Non-habitual coffee and tea drinkers who consume coffee, green tea, or black tea 2-5 times in the last two months; (3) Non-allergic to coffee, green tea, black tea, or honey; (4) Absence of medication use; (5) Will refrain from ingesting energy drinks, carbonated beverages, caffeinated beverages, or food for 24 hours before the study and throughout the study periods; (6) Will not consume anything except water after 11 p.m. during the experimental days; (7) Will be available and cooperate throughout the study duration.

Exclusion criteria are as follows:

(1) Presence of cardiovascular disorders, hypertension, or diabetes; (2) Blood pressure above 120/80 mmHg and random blood glucose above 125 mg/dL; (3) Alcohol intake; (4) Smoking; and (5) Habitual coffee or tea drinkers.

Randomization

Participants who meet the eligibility criteria, possess medically acceptable vital signs, and submit a signed informed consent form will undergo randomization conducted by research staff. A simple randomization method will be employed. Each group will be assigned a distinct number, which will be inscribed on slips of paper corresponding to the total number of participants. Each slip, bearing one of the assigned group numbers, will be securely folded. These will then be put in a basket, which will be thoroughly jostled. All these procedures will be executed by a trained research personnel. Each participant will have the opportunity to randomly select one slip of paper. The selection process will be overseen by another research staff member who is not involved in the initial phase. The group number selected by each participant will determine their assigned group.

Compensation

At the end of the study, the participants will be compensated for cooperating and following through with all the research procedures. A token of one thousand naira will be given to each participant.

Safety considerations

This study involves healthy participants, and all the procedures involved in this study are generally safe, with minimal risks. To ensure the safety of the participants, all the parameters will be measured by well-trained and experienced research personnel. However, if any unfavorable or unintended sign or symptom arises, it will be noted as an adverse event. All research personnel will be trained to recognize, document, and report adverse events.

Follow-up

Although the study involves healthy participants and the study procedures are generally safe, the participants will be followed up via a phone call. All the participants will be requested to give their contact details and those of their family and friends. The follow-up call will be made twice (3 days and 10 days after the completion of the study). If an adverse event is recorded, a follow-up visit will be arranged with the participant to ensure their well-being.

Data management and quality control

To ensure protection of confidentiality, the data will be entered into the study database with no personal identity, and the source documents will refer to participants solely by their study identification number. Access to the data will be restricted to the research investigators. The study database will be securely stored in the central library of the University of Uyo and Faculty of Basic

Medical Sciences library. Additionally, all data will be compiled into a thesis that will not disclose any participant identities or information. Three copies of the thesis will be generated, which will be distributed as follows:

One copy will be submitted to the Faculty of Basic Medical Sciences library for preservation.

One copy will be retained in the Department of Physiology.

One copy will be kept in the University of Uyo library.

Ethics

All data collected during this study will be treated as strictly confidential. The personal information will be anonymized, and any published results will not include identifiable information. Participation in this study is entirely voluntary. The participants have the right to refuse to participate or withdraw from the study. The participants will consent to participate by filling out and signing an informed consent form. The protocol of this study will be submitted to the Akwa-Ibom State Health Research Ethics Committee, Ministry of Health, Akwa-Ibom State, Nigeria, for approval.

Statistical analysis

The results will be presented as mean ± standard error of the mean (SEM). All data collected from the research will be analyzed using the Microsoft Excel (MS Excel) application and GraphPad Prism Software (Version 8.1). One-way and two-way analysis of variance (ANOVA), as well as Tukey's post-hoc comparison, will be used to determine the significant differences between the mean values. The statistical significance will be accepted at the level of $P < 0.05$.

Discussion

The role of diet in maintaining and promoting cardiovascular and metabolic health has long been a subject of scientific investigation and public interest. Among the diverse dietary components, coffee and tea have received attention for their potential health benefits. Their health advantages are primarily derived from their bioactive constituents, particularly polyphenols and caffeine. Honey, a natural sweetener, has been used for ages and has received attention for its ability to decrease blood pressure. It contains bioactive compounds such as flavonoids, which have vasodilating properties.

High blood pressure and elevated blood glucose level are significant risk factors for chronic diseases, including heart disease and diabetes. These conditions have become global health concerns, contributing to substantial morbidity and mortality. While pharmaceutical interventions are commonly used to manage these conditions, there is a growing interest in natural dietary approaches to support health. Coffee, tea, and honey both contain bioactive compounds that may influence blood pressure, blood glucose, and heart rate. Research on the possible health impacts of coffee, green tea, black tea, and honey has been done separately; however, there remains a notable lack of research examining the combined effects of these beverages with honey. This study protocol describes a randomized controlled trial designed to investigate whether a three-day consumption of either coffee, green tea, or black tea sweetened with honey can significantly change some physiological parameters in healthy young female adults.

This research aims to provide significant insights regarding the possible health benefits or risks linked to the consumption of coffee, black tea, green tea and honey-sweetened coffee, black tea,

and green tea . It will specifically focus on their effects on critical physiological factors such as blood pressure, heart rate, and blood glucose level. Understanding these factors is essential for maintaining individual health, as any adverse changes in them can lead to detrimental health consequences. By examining the relationship between these beverages and these vital health indicators, the study seeks to contribute to the broader understanding of dietary influences on cardiovascular and metabolic health.

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PARTICIPANT ELIGIBILITY QUESTIONNAIRE

Personal information

1. What is your name? _____

2. Gender? M / F
3. How old are you?

[ ] Less than 18 years [ ] 18-26 years [ ] 26 years and above

4. Weight: _____
5. Height: _____
6. Phone number: _____

Medical history

7. Do you have a history of hypertension (high blood pressure)? (Yes/No)
8. Do you have a history of diabetes or impaired glucose tolerance? (Yes/No)
9. Do you have any cardiovascular problem? (Yes/No)

If yes, please specify: _____

10. Are you on any medication? (Yes/No)

If yes, please specify the medication(s):

Lifestyle

11. Do you smoke? (Yes/No)
12. Do you consume alcohol? (Yes/No)
13. Are you currently following any specific diet plan? (Yes/No)

If yes, please provide details:

Dietary Habits

14. How often do you consume coffee/black tea/green tea?

[ ] Daily [ ] Several times per week [ ] Occasionally [ ] Rarely/Never

15. Do you regularly consume honey as a sweetener? (Yes/No)
16. Do you have any known allergies or intolerances to any of the following? (Yes/No)

[ ] Honey
[ ] Coffee
[ ] Black tea
[ ] Green tea

General Health

17. Have you been diagnosed with any chronic medical conditions? (Yes/No)

If yes, please provide details:

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18. Are you currently pregnant or breastfeeding? (Yes/No)
19. Have you undergone any recent surgeries? (Yes/No)

If yes, please specify:

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20. Are you willing to comply with the study protocol, including the consumption of the assigned tea for the specified duration? (Yes/No)

INFORMED CONSENT FORM

1. Title of research work: Effect of honey-sweetened coffee, black tea and green tea on blood pressure, heart rate and blood glucose level in healthy young female adults.
2. Location: Physiology laboratory, University of Uyo.
3. Time of research: In the morning, 8 am to 11 am
4. What is required of the participants:

No breakfast

Avoid soda drinks and caffeinated beverages throughout the period of the experiment

5. What the experiment is all about:

You will be given either water, coffee, green tea, black tea, honey-sweetened coffee or honey-sweetened tea. Your blood pressure will be measured before the commencement of the experiment and after 15 minutes, 30 minutes, 45 minutes, and 60 minutes of consumption of any of the beverages listed above. Your blood glucose will also be measured before the commencement of the experiment and after 30 and 60 minutes of consumption of any of the beverages.

6. Study Procedures:

i. Measurement of blood pressure: Your blood pressure will be measured using a digital blood pressure monitor. The measurement involves placing an inflatable cuff around your upper arm and inflating it to briefly restrict blood flow. This process may cause temporary discomfort or a feeling of pressure on your arm.

ii. Measurement of blood glucose levels: Your blood glucose levels will be measured using a glucometer. A small drop of blood will be obtained by pricking your finger with a sterile lancet. The blood sample will be placed on a test strip, which will be inserted into the glucometer for analysis. You may experience a brief pinprick sensation during this process.

7. Risks and Discomforts:

The procedures involved in this study are generally safe, and the risks are minimal. However, it is important to note the following potential risks and discomforts:

i. Temporary discomfort or pressure on your arm during blood pressure measurement.

ii. A brief pinprick sensation and potential minor bleeding at the finger prick site during blood glucose level measurement.

8. Confidentiality and Voluntary Participation

All data collected during this study will be treated as strictly confidential. Your personal information will be kept anonymous and any published results will not include any personal information. Participation in this study is entirely voluntary. You have the right to refuse to participate or withdraw from the study at any time

9. Consent

I _____ have read the procedures of the experiment explained above, and I hereby voluntarily consent to be a participant in the project. I will make myself available throughout the duration of the project, and I will do all that is required of me.

_____ _____

Name Signature and date