NATIONAL POLITECHNIC INSTITUTE
BIOTIC PRODUCTS DEVELOPMENT CENTER

AUTONOMOUS UNIVERSITY OF BARCELONA

RESEARCH PROTOCOL

NCT ID Not yet assigned

"Trial of acceptability, tolerability and biochemical indicators of *Hibiscus sabdariffa* L. drink in pregnant women"

APRIL 2024

1. INTRODUCTION

When developing foods with potential health benefits, it is necessary to evaluate their acceptability and tolerability to determine whether the product will be consumed by the intended population. (Yang & Lee, 2018), In addition to ensuring that its consumption does not compromise the consumer's health, these tests allow the developer to make the necessary modifications to increase the likelihood of consumption.

The acceptability of a product can be studied through surveys or interviews and the way to apply these resources is through sensory tests oriented to the consumer, these tests allow to obtain information about the aspects that are liked and disliked about the product in question (Ramírez-Nava, 2012). To achieve this, sensory analysis is used, which is defined as the scientific discipline used to measure, identify and interpret food characteristics perceived by the senses of sight, smell, taste, touch and hearing (Lawless & Heymann, 2010). Among consumer-oriented tests, there are two main ones: preference tests and acceptance tests. In preference tests, consumers are asked to indicate which sample they prefer. While in acceptance tests, consumers are presented with the products and asked to indicate their level of liking them on a scale that can be 3, 5, 7, and 9 points (Sharif *et al.,* 2017). Acceptance tests are often used to compare two or more products or used on a single product to measure the level of liking.

Pregnancy is a stage that is marked by physiological changes related to hormones that modify sensory perception (Muluh *et al.,* 2024), mainly affecting taste (Choo & Dando, 2017) and smell (Haddad-Tóvolli *et al.,* 2023); However, this has been very little studied and most of the knowledge on the subject is empirical (Kölble *et al*., 2001), but it has now begun to be studied as the work conducted by Matsuda *et al.* (2024) who have suggested that oxytocin has an effect on the olfactory sensitivity of pregnant women, meanwhile Muluh *et al.* (2024) found moderate evidence of sensory changes during pregnancy, such as a reduction in the ability to identify odors and changes in the perception of the intensity of odors and hedonic responses to flavors.

The proposed beverage to be evaluated is made entirely from natural compounds, which in theory should not cause gastrointestinal intolerance effects, but to reaffirm this in the

acceptability test, a gastrointestinal tolerability questionnaire was included evaluating the presence or absence of symptoms such as nausea, regurgitation, vomiting, diarrhea, constipation and flatulence. (De Luis *et al.,* 2015).

In this work, a phase I pilot test was carried out, which consists of testing on a smaller scale the execution logistics planned for a higher-level project and thereby reducing the probability of errors in future studies (Díaz-Muñoz *et al.,* 2020). This work was approved by the Research Ethics Committee of the Biotic Products Development Center of the National Polytechnic Institute.

The study evaluated the gastrointestinal acceptability and tolerability of the proposed beverage developed to help control hypertensive disorders associated with pregnancy. Indicators such as blood pressure, blood markers of glucose, cholesterol, triglycerides and creatinine, and general urine test were also assessed. This trial was conducted on pregnant women, because this is the group to which the drink is directed and at this stage physiological changes occur that modify the sensory perception of food (Matsuda *et al.,* 2024), This is why it is important to conduct it in this group. This trial aims to lay the groundwork for defining the logistics used in a double-blind, randomized, controlled clinical trial in which the beverage will be given for a longer period of time. This is why it is important to know the acceptability of the beverage, in case it is necessary to make changes to the beverage that allow for increased consumption (Lawless & Heymann, 2010).

2. OBJECTIVES

2.1. General objective

Evaluate the acceptability, tolerability, blood pressure and biochemical indicators of a beverage with *Hibiscus sabdariffa* L. in pregnant women.

2.2. Specific objectives

- To evaluate the acceptability of the beverage by sensory analysis in a 4-week trial with a 7-point hedonic scale.
- Determine the tolerability of the beverage by the presence of gastrointestinal symptoms.
- Evaluate biochemical indicators, blood pressure and general urine test before and after the beverage consumption trial.

3. MATERIAL AND METHODS

A 4-week phase I pilot trial was conducted in 10 women in their second trimester of pregnancy, who consumed a daily dose of *H. sabdariffa* beverage. Acceptability and tolerability were assessed. Blood pressure, urinalysis, and blood biomarkers of glucose, cholesterol, triglycerides, and creatinine were also measured.

Figure 1 presents the experimental diagram of the beverage acceptability and tolerability test.

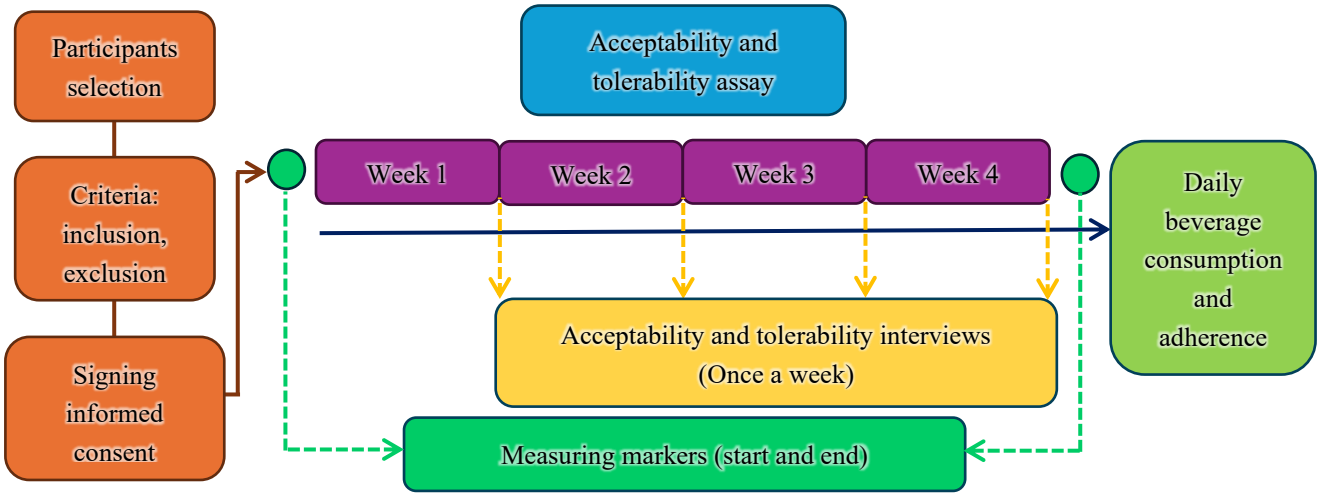

Figure 1. Experimental diagram of the acceptability and tolerability test of a beverage with *Hibiscus sabdariffa* L.

3.1. Ethical considerations

This research project complies with the International Ethical Guidelines for Research Related to Human Health, developed by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the WHO.

All participants in this trial received dignified treatment and were not discriminated against under any circumstances based on their ethnic or national origin, social or economic status, religion, opinions, or marital status, in accordance with the Federal Law to Prevent and Eliminate Discrimination. Participants were never recruited against their will, nor did they suffer any form of disapproval or retaliation if they decided not to participate or withdrew from the study. It was emphasized that participation was completely voluntary and that they could refuse to participate or withdraw from the research at any time if they so desired.

The results obtained were used solely by the research team. The privacy of the participants, as well as the confidentiality of their personal information, was protected. All collected samples were identified by the participant's initials and a consecutive number, and only the researchers had access to their personal information. This information was kept safe with their file and will not be disclosed or published at any time. The results may be published for scientific purposes, but personal and identifying data of the participants will not be disclosed at any time. The results obtained were analyzed using Past software through pairwise analysis to determine statistical differences between the indicators measured before and after treatment with a significance level of $P < 0.05$. Descriptive statistics with means, standard deviations, and percentages were also applied to characterize the group.

The researchers of this protocol declare that there is no conflict of interest and that the objective is to generate knowledge that allows us to evaluate the effect of a beverage with *H. sabdariffa*, which can be used in the prevention and/or treatment of hypertensive disorders associated with pregnancy, as well as to determine its degree of acceptability and tolerability.

3.2. Materials

The proposed beverage was made with *H. sabdariffa* calyces from the Temixco cultivar in Morelos, and was given to participants as a powder for beverage preparation. The powder contained 5.7 g of powdered *H. sabdariffa* calyces and 5 g of standard sugar.

3.3. Methods

3.3.1. Microbiological analysis of *Hibiscus sabdariffa* L. calyces

Prior to the acceptability and tolerability test of the beverage, a microbiological analysis of the powdered calyces of *H. sabdariffa* was performed, measuring mesophilic counts, total coliforms, molds, yeasts, *Escherichia coli* and *Salmonella* spp. to ensure the safe consumption of the hibiscus. All indicators were within permissible values according to Mexican standards.

3.3.2. Participants selection

Because this was a phase 1 pilot study, where participants were self-controlled, with marker measurements taken before starting to consume the beverage and at the end of the 4-week treatment period, the sample size was based on convenience. All women who met the inclusion criteria and did not meet the exclusion criteria were invited to participate. At least ten pregnant women were recruited through the consultation of PhD. Edmundo González Vargas, a specialist in obstetrics and gynecology and maternal-fetal medicine at the "MEDOMAI" Comprehensive Health Services Clinic located in Cuernavaca, Morelos, México.

Participants were selected according to the following criteria:

Inclusion criteria

- Pregnant women aged 18 to 35
- Those in their second trimester of pregnancy (14 – 28 gestation weeks)
- Blood pressure <140/90 mmHg

Exclusion criteria

- Multiple or high-risk pregnancy
- Having suffered from a hypertensive disorder in previous pregnancies

- Having chronic non-communicable diseases such as diabetes mellitus, cardiovascular disease, cancer, and chronic kidney disease
- Body Mass Index ≥ 32 kg/m²

Elimination criteria

- Develop any pregnancy complications that prevent you from consuming the beverage and/or attending the measurements
- Deciding of your own volition to withdraw from the study

The patient records were reviewed to select patients who met the criteria for pregnancy weeks and age. They were contacted by telephone, where their medical history was taken to confirm that they met the remaining criteria. The procedures to be performed and how to prepare the beverage were explained in detail. In this conversation, the candidates were asked about their interest in participating and the date they should arrive to sign the informed consent form, receive the powdered doses to prepare the beverage along with its preparation instructions, and perform blood pressure measurements, biochemical indicators, and provide a urine sample.

3.3.3. Acceptability and beverage consumption adherence

Beverage acceptance was assessed through a sensory analysis once a week for 4 weeks using a 7-point hedonic scale, considering the characteristics of color, odor, flavor, mouthfeel, aftertaste, and overall appearance. The scoring system was as follows: 1 = I dislike it a lot, 2 = I dislike it moderately, 3 = I dislike it a little, 4 = I neither like it nor dislike it, 5 = I like it a little, 6 = I like it moderately, and 7 = I like it a lot. To conduct this assessment, participants were sent a Google Form via WhatsApp. This form also included a section to indicate any other sensations they perceived when consuming the beverage, as well as their percentage of daily consumption of the beverage, and thus assess attachment.

Beverage acceptance is assessed using the hedonic scale, and the score is evaluated whether the score is maintained over the duration of the trial. The feedback provided by participants allows adjustments to improve beverage acceptance, if necessary.

3.3.4. Gastrointestinal tolerability due to consumption of the beverage.

Once a week for the 4 weeks of the trial, participants completed a gastrointestinal tolerability assessment form as proposed by De Luis *et al.* (2015), where participants indicated the presence or absence of gastrointestinal symptoms such as nausea, regurgitation, vomiting, constipation, diarrhea, flatulence, and abdominal pain. These symptoms were assessed using 5 points where: 0=absent symptom, 1=symptom present but not bothersome, 2=annoying symptom but does not interfere with daily activities or sleep, 3=a symptom present and bothersome but does interfere with daily activities and sleep, and 4=a symptom present that requires medical attention.

3.3.5. Blood pressure, urinalysis and biochemical markers

Blood pressure, urinalysis, and biochemical indicators of serum glucose, cholesterol, triglycerides, and creatinine were measured before the start of the trial and after the four weeks of consumption of the beverage were completed, to compare these parameters before and after treatment. The measurements and blood samples were taken by trained and experienced personnel. Blood pressure was measured using a noninvasive technique using the Korotkoff auscultation method.

Blood samples were obtained by puncture in the antecubital region of the participants' non-dominant arm, using sterile, disposable materials. Blood was placed in anticoagulant-free tubes for clinical chemistry, extracting 6 ml of blood. These tubes were kept refrigerated until the determinations were performed in the Biomarker Measurement Laboratory of the Biotic Product Development Center by personnel from the Department of Nutrition and Functional Foods, who are trained and experienced in sample collection and processing. Blood chemistry was measured using the Spinlab series 5-5312 semi-automated turbidimetry equipment for clinical chemistry.

The biological materials were used only for the aforementioned tests and were kept refrigerated until their disposal as Biologically Infectious Hazardous Waste (BIHW) in accordance with Mexican Official Standard NOM-087-SEMARNAT-SSA1-2002. The

results of the biochemical indicators were reported in writing to the participants, who received free medical and nutritional guidance, if necessary.

The general urine test was performed by the Jacobo Curiel external clinical analysis laboratory in Cuernavaca, Morelos. Participants submitted their samples to the "MEDOMAI" clinic on the indicated date, and the samples were then transferred to the external laboratory.

The researchers of this protocol declare that there is no conflict of interest and that the objective is to generate knowledge that allows us to evaluate the effect of a beverage with *H. sabdariffa*, which can be used in the prevention and/or treatment of hypertensive disorders associated with pregnancy, as well as to determine its degree of gastrointestinal acceptability and tolerability.

4. SCHEDULE OF ACTIVITIES

Activity	2024				
	March	April	May	June	July
Information meeting with potential participants	X	X			
Interviews to verify inclusion criteria		X			
Participants selection		X			
Measurement of blood pressure and biochemical indicators before the test.		X			
Beverage acceptability and tolerability test			X		
Measurement of blood pressure and biochemical indicators after the test			X		
Analysis of results			X	X	
Preparation and submission of the report to the Research Ethics Committee					X

5. BIBLIOGRAPHY

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6. Ethics Committee Approval

Affair: Dictum 2024-1 CEI-CEPROBI.

PhD. PERLA OSORIO DÍAZ
PRINCIPAL INVESTIGATOR
BIOTIC PRODUCTS DEVELOPMENT CENTER

In relation to your request for re-evaluationand preparation of the opinion, on the following research protocol:

"ACCEPTABILITY, TOLERABILITY AND BIOCHEMICAL INDICATORS OF HIBISCUS SABDARIFFA L. DRINK IN PREGNANT WOMEN"

Discussed at the first extraordinary meeting of the CEI-CEPROBI on April 8th, 2024.

Le informamos que con base en los **artículos 41 Bis, el artículo 98 de la Ley General de Salud, la Norma, Oficial Mexicana NOM-012-SSA3-2012 y la Guía nacional para la integración y el funcionamiento de los Comités de Ética en Investigación**, que establecen los criterios para la ejecución de proyectos de investigación para la salud en seres humanos; su proyecto de investigación ha sido evaluado por este comité, resultando:

Approved

Therefore, you may begin the corresponding work with the commitment to address the minor modifications indicated in the appendix. We inform you that this opinion is valid for one year from its receipt. In accordance with the internal procedures of the Research Ethics Committee, and based on the regulations of the **General Health Law on Research (Art. 116),** we request that you send this committee reports on the progress of your project, such as: the start of collection work, modifications, amendments, interruptions during development and/or definitive suspension, as well as a report on the completion of the research (Annex 5).

C. Javier Villanueva Sánchez
President of the CEPROBI
Research Ethics Committee

ANNEX

1. From the protocol:
   a) Align the title with the study objective. It should match the statements in the three reviewed documents (protocol, consent form, and study summary). Ensure that it addresses the study variables, both dependent and independent. It was suggested to simplify it with the following: "Evaluation of blood pressure, tolerability, and acceptability of Hibiscus sabdariffa L. in pregnant women."
   b) Clarify the research design and its scope, based on the maturity of the project, recognizing, if applicable, that before implementing the study with the appropriate design and sample, an evaluation of the instruments, resources to be employed, and the feasibility of the designed logistics is intended.
   c) With the current design, and in order to expand the sample size, it is suggested, if appropriate for the research group, that a control group with regular consumption of Jamaican be included. This would accurately demonstrate the comparability or efficacy of the product.
   d) Add available quality information of the product (technical sheet): average anthocyanin content (ds), variety to be used, origin of the material and the minimum physical-chemical specifications for its adequate characterization..
   e) It is recommended to include the training of human resources in postgraduate studies in the contributions section of the study.
   f) In any case, any changes or modifications to the original protocol must be noted and notified to the committee.

2. Informed consent.
   a) Standardize information in the protocol and consent forms regarding benefits for participants (nutritional, medical, or both) and duration (whether it will be only during the study or until the end of the pregnancy).

3. Summary of previous studies.
   b) A systematic review and clinical trial strategy should be undertaken to justify and clarify the sample size and selection with greater evidence. The review should consider the type of subjects, recruitment strategy and inclusion, exclusion, and elimination criteria, the number and size of groups, the method and quantity of the product being evaluated, and the effect size and precision. It is suggested that searches be conducted in databases such as the Cochrane Library and PubMed.