PILOT CLINICAL STUDY PROTOCOL

Broccoli Extract Supplementation and Gastrointestinal Health in Older Adults with Active Alcohol Use and Low Diet Quality

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Sponsor

National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health

Protocol Version

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SYNOPSIS

Study Purpose

To identify the effect of broccoli sprout extract (BSE) supplementations to mitigate alcohol-induced gut leak, inflammation, and oxidative stress.

Primary Objective

To test the ability of BSE to preserve gut barrier integrity. By assessing serum levels of biomarkers such as intestinal fatty acid-binding protein (iFABP), zonulin and lipopolysaccharide binding protein (LBP).

Secondary Objectives

To test the effect of BSE to decrease peripheral blood pro-inflammatory mediators. Isolated plasma will be assessed for pro-inflammatory cytokines (IFN-gamma, IL-2, IL-10, and TNF, IL-4, IL-6 and IL-17). To test the effect of BSE to decrease oxidative stress in peripheral blood. We will measure serum levels of lipid peroxidation, as well as enzymatic and non-enzymatic antioxidants.

Study Design

This will be a prospective, randomized, double-blind placebo-controlled clinical pilot study.

Study Date Range and Duration

January 2024, through January 2025.

Number of Study Sites

01 - LSU Health School of Medicine, 433 Bolivar Street, New Orleans, LA 70112.

Primary Outcome Variable

iFABP concentration.

Secondary Outcome Variables

IL-6 and total antioxidant capacity.

Study Population

Subjects ≥ 50 years of age at enrollment, with active alcohol drinking and low diet quality.

Number of Participants

40.

Study Schedule

1. Remote Screening and Invitation to Participate (estimated time = 10 minutes)
2. Informed Consent and First Visit (estimated time = 4 hours)
3. Second Visit (estimated time = 4 hours)

Contents

1. Statement of Compliance

This document is a protocol for a human research study. The purpose of this protocol is to

ensure that this study is to be conducted according to the Common Rule at 45CFR46 (human

subjects) and other applicable government regulations and Institutional research policies and

procedures.

2. Background & Significance

2.1 Background

Aging is associated with an increased risk of comorbidities. The close connection between nutrition, intake of bioactive nutrients/supplements, and inflammation validates the key role of dietary strategies as regulators of the immune response and inflammatory status[1, 2]. Age-related changes in physiology and lifestyle can lead to decreased intestinal enzymatic activity and changes in the gut epithelium and microbial communities, contribute to nutrient malabsorption and gut barrier integrity disruption[3, 4]. According to the National Survey on Drug Use and Health (NSDUH, 2020), the prevalence of alcohol use disorder (AUD) has increased in the last 20 years in older adults in the U.S. AUD has been linked to a broad spectrum of medical conditions with tissue and organ injury, including gastrointestinal-related diseases[5]. Chronic alcohol consumption leads to perturbations in gut microbiome balance (dysbiosis) and disruption of gut barrier integrity[6]. As a result*, bacteria, toxins, and metabolites can enter the blood stream and reach distant organs, triggering inflammation and oxidative stress[5, 7].* Through this mechanism gut leak is closely related to the onset of metabolic diseases, such as nonalcoholic fatty liver disease (NAFLD) and diabetes.

Intestinal barrier integrity can be evaluated in clinical, using serum biomarkers such as zonulin, iFABP/FABP2 (intestine fatty acid binding protein) and LBP (Lipopolysaccharide Binding Protein). Zonulin is a modulator of intracellular tight junctions (TJ), and increased zonulin serum concentrations correlates with intestine permeability. Whereas iFAB is present inside of the intestinal epithelial cells, transporting and regulating fatty acid absorption, and it is widely accepted as a biomarker of epithelial injury[8]. LBP has a key role in inflammation, by biding to bacterial lipopolysaccharide and triggering immune responses[9]. Despite the prominent role of diet and alcohol in the pathogenesis of metabolic diseases, there is a lack of treatments to mitigate their effects in triggering systemic inflammation and oxidative stress. *Novel treatments using generally recognized as safe (GRAS) compounds focused on restoring the intestinal barrier to mitigate metabolite endotoxemia are sorely needed.* This project will test the potential of broccoli sprouts extract (BSE) as a GRAS treatment to minimize the combined effect of poor nutrition and alcohol on the gut. Broccoli sprouts are rich in sulforaphane, a bioactive compound derived from the glucosinolate glucoraphanin with anti-inflammatory and antioxidant proprieties[10]. BSE supplementation has been used in preclinical and clinical studies as a health-promoting food, showing significant positive changes in the gut microbiota composition, protection against colitis, cardiometabolic improvement, and lower inflammation[11-13]. We believe that BSE is a viable alternative therapeutic approach for patients who are resistant to lifestyle changes such as healthy eating and reducing alcohol use.

2.2 Purpose of Study/Potential impact

To test BSE supplementation in human subjects with poor nutrition compounded by alcohol use, specifically in older adults who we believe will receive greater benefit from this approach.

At the completion of the proposed study, we expect to have determined that treatments using generally recognized as safe (GRAS) compounds can be useful to restore the gut barrier integrity, and as consequence of reduced gut leak we expect to observe lower inflammation and oxidative stress.

2.3 Potential Risks

There are small, but real risks associated with some of the tests to be performed:

Interview & Completing Questionnaires: risks include emotional stress and fatigue.

Anthropometrics and Vital Sign: risk of fatigue, falls and device malfunction.

Phlebotomy: risk of discomfort and bruising. Some people may feel lightheaded or faint. There is also slight risk of infection.

Pills intake: risk of allergy, diarrhea or other gastrointestinal intolerance.

Unforeseen complications: there may be other complications that occur as a result of participation in this research study.

In order to minimize the potential risks, all the procedures will be performed by a trained professional, the materials and electronic devices to be used will be checked before the visits, sterile and single use materials for venipuncture, the participant's will be asked if they already consume vegetables from the Cruciferous vegetable's family (broccoli, arugula, bok choi, brussels sprouts, collard greens, cauliflower and cabbage).

2.4 Potential Benefits

There will be no direct benefit to the participants of this study. However, this study will help the researchers and society to find strategies to mitigate alcohol effects in the gut.

3. Study Objectives

3.1 Hypothesis

Broccoli sprout extract (BSE) supplementation reduces gut leak and systemic biomarkers of inflammation and oxidative stress caused by alcohol consumption in older adults with unhealthy diets.

3.2 Primary Objective

To test the ability of BSE to preserve gut barrier integrity. By assessing serum levels of biomarkers such as intestinal fatty acid-binding protein (iFABP), zonulin and lipopolysaccharide binding protein (LBP).

3.3 Secondary Objectives

To test the effect of BSE to decrease peripheral blood pro-inflammatory mediators. Isolated plasma will be assessed for pro-inflammatory cytokines (IFN-gamma, IL-2, IL-10, and TNF, IL-4, IL-6 and IL-17). To test the effect of BSE to decrease oxidative stress in peripheral blood. We will measure serum levels of lipid peroxidation, as well as enzymatic and non-enzymatic antioxidants.

4. Study Design

4.1 General Design Description

This will be a prospective, randomized, double-blind placebo-controlled clinical pilot study. The participants will be recruited by convenience by printed advertising and word of mouth.

4.1.1 Study Date Range and Duration

January, 2024, through January 1st, 2025.

4.1.2 Number of Study Sites

01 - LSU Health School of Medicine, 433 Bolivar Street, New Orleans, LA 70112.

4.1.3 Primary Outcome Variable

iFABP concentration.

4.1.4 Secondary Outcome Variables

IL-6 and total antioxidant capacity.

4.1.5 Study Population

Subjects ≥ 50 years of age at enrollment, with active alcohol drinking and low diet quality.

4.1.6 Number of Participants

40.

4.1.7 Eligibility Criteria

- Subjects ≥ 50 years of age at enrollment.
- Healthy Eating Index (HEI) score below 51 (poor diet).
- Consume at least 8 alcohol drinks/week. AUDIT-C score >8.

4.1.8 Exclusion Criteria

- Bowel-related diseases
- Diagnosed Diabetes

- Allergy or intolerance to broccoli.
- Any acute illness within the last 6 weeks.
- Chronic anti-inflammatory use or antibiotic treatment in the last 7 days.
- Acute illness withing the preceding six weeks (defined as fever, new antibiotic use or unscheduled healthcare visit – for illness).
- Acute alcohol intoxication upon arrival on the day of study visit.

Additional exclusion criteria:

- Any health issue that, the study investigator's judgement, confers excess risk for participation.

5. Study Methods

5.1 Study procedures

Study procedures are listed in the Schedule of Activities (Appendix 2).

5.2 Data Collection

We will use the REDCap (Research Electronic Data Capture) software toolset and workflow methodology designed for building and managing online surveys and databases to allow electronic data collection from multi-site clinical research studies. Data will be collected by interview and observer-assessments, and multiple levels of quality control will be used to ensure the highest level of data quality and security. The REDCap system allows for restricted access, read-and-write privileges for each database and data entry form. Routine data quality reports are produced evaluating range checks, valid fields, and missing fields. All changes to the database are logged and time stamped. In our prior work, the error rate was <1%. The participants will be randomized into placebo or BSE supplementation group, blood samples and data will be collected in two time-points, baseline day zero of treatment (Visit 1) and after 28 days of treatment (Visit 2). For the supplementation, the participants will be advised to take 2 capsules per day of the AVMACOL® (Nutramax Laboratories) supplement for a 28-days period. The placebo group will receive capsules with no BSE and will be instructed to take 2 capsules per day for the 28-days period.

5.3 Adverse Events Definition and Reporting

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life- threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. The following guidelines will be used to describe severity of adverse events (AEs).

- **Mild** — Events require minimal or no treatment and do not interfere with the participant's daily activities.

- **Moderate** — Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- **Severe** — Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

All adverse events (AEs) will have their relationship to study activities assessed by a study investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** — There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study activities and cannot be explained by concurrent disease or other drugs or chemicals. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- **Probably Related** — There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after study activities and is unlikely to be attributed to concurrent disease or other drugs or chemicals.

- **Potentially Related** — There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after study activities). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- **Unlikely to be related** — A clinical event, including an abnormal laboratory test result, whose temporal relationship to study activities makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after study activities) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** — The AE is completely independent of study activities, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

The Study Investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described in the informed consent form for the study intervention.

The investigators will record nonserious adverse events and report them to the sponsor regulatory agencies following the site's IRB guidance but no later than 7 days after the investigator becomes aware of the AE.
The study principal investigator will immediately report any serious adverse event, whether or not considered study activities related, including those listed in the protocol or informed consent and must include an assessment of whether there is a reasonable possibility that a study activity caused the event.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

5.4 Study Schedule/Study Visits

1. Remote Screening and Invitation to Participate (estimated time = 20 minutes)
2. Informed Consent and First Visit (estimated time = 3 hours)
3. Second Visit (estimated time = 3 hours)

Reminder text and/or telephone messages will be sent the day before and a phone call will be made the morning of the scheduled first and second visits.

During the 28-days study duration, the participants will receive daily phone calls or text, as reminders for the daily pill's intake. They also will be asked to fill out a weekly pill's intake report (Appendix 1) and send a picture of the filled report every 7 days to via e-mail or text message to the researcher contact.

Refer to Schedule of Activities (Appendix 1) and Study Schema (Appendix 1) for the detailed information.

Biospecimens:

40mL of blood will be collected by phlebotomy at the first and second visit. Plasma, peripheral blood mononuclear cells and serum will be separated either from EDTA, heparin or preservative free tubes. Roughly 300 microliters of whole blood will be taken out from the EDTA tubes, and used to measure Phosphatidylethanol (PEth). Peth is direct alcohol biomarker which is found in human blood following alcohol consumption.

Standard Operating Procedures (SOPs): Detained guidance on study procedures can be found in specific SOPS. See the following documents in the appendix:

- SOP_Blood Processing_2023

Survey / Questionnaire Forms are in the appendix.

5.4.1 Screening and Recruitment

Participants will be recruited by word of mouth, printed advertising (flyers), social media. In the advertising, office phone number and project's email will be available for contact.

Once the individuals reach out (phone/email), they will answer the questionnaires (see in schedule of activities, appendix 1). Once the potential participant met the eligibility criteria, they will be scheduled for a in person visit at the research site, LSU Campus Multispecialty Clinic/Seton Building (478 S Johnson St – New Orleans).

5.4.2 Informed Consent and Enrollment

After an invitation to participate in the research study is issued, potential research participants that are scheduled for a visit at the testing facility for formal informed consent followed by first visit schedule. All participants will be provided with information sheets in lay language and a complete verbal explanation regarding the reasons for conducting the study, the potential benefits of the information collected, and the risks involved. Health literacy sensitive consent and information documents will be used. Individuals not meeting basic literacy metrics will have the entire consent document read to them. Participants will be provided a copy of the informed consent and the consent process documented in study record.

5.4.3 Compensation

The participants will be paid as a compensation for their time and effort for their participation in this study.

The total amount of $400 will be paid for each enrolled participant, the payment will be arranged as follows:

Day 0 ($50): First visit, anthropometrics, vital signs and questionnaires.

Day 7 ($75). Day 1 to day 7 of the diet intervention.

Day 14 ($75): Day 8-14 of the diet intervention.

Day 21 ($75): Day 15-21 of the diet intervention.

Day 28 ($75): Day 22 to day 28 of the diet intervention.

Day 28 ($50): Second visit, blood, anthropometrics, vital signs and questionnaires.

Each subject will receive a ClinCard in their first visit, and the credits will be released accordingly to the participation in the schedule above. The participants will be responsible for any taxes assessed on the compensation.

5.4.4 Monitoring plan for pills intake adherence

The participants will receive daily text messages as a reminder to take the pills and will be asked to confirm when the pills were already taken. Also, they will be given a weekly pill's intake report document (please, see on Appendix 1), and asked to send a picture of the filled document every 7 days. Finally, the participants will be asked to bring the tablets container back by the end of the study (Full test visit 2) and the pills left will be counted by the researcher[14]. The compliance to the intervention's schedule needs to reach 80% of the total 28 days, meaning that if the enrolled subject did not take the pills for more than 5 days they are going to be considered as drop out.

5.5 Statistical Method

The data obtained will be analyzed using R and SAS software. After performing normality tests, we will compare group changes in each biomarker according to the most appropriate statistical test: either t-test (normal distribution) or Wilcoxon-Mann-Whitney (non-normal distribution). Multivariate methods will be applied to verify the relationship between BSE supplementation and gut leak, BSE supplementation and inflammation and BSE supplementation and oxidative stress. The data will be adjusted for following confounders: sex, age, BMI, race/ethnicity and substance use.

Scientific Rigor: The principal investigator (PI) will be blinded for the randomization; the pill bottles will be labeled as "Tablets A" or "Tablets B" by the manufacturer. The PI will be responsible for the data collection's quality control, as well as the experiments execution, assuring proper negative and positive controls and appropriate replicates within experiments. All the findings of the present research will be analyzed and reported with full transparency. The product's manufacturer will not have any authority over the study design, results or scientific publication.

5.5.1 Sample Size Consideration

The sample size was calculated using the preliminary data for gut leak, the estimated number of participants was 13 (power= 0.8; alpha= 0.05; beta= 0.02). However, to increase the power in transitioning to humans, we plan to recruit 20 subjects for each group. In total, 40 participants will be recruited by convenience by printed advertising, post in social media and word of mouth.

5.5.2 Planned Analysis

The present study will allow us to collect important data and shed light on BSE diet supplementation as an adjuvant to reduce gut leak, decrease inflammation and improve the antioxidant status. Furthermore, the published results from this clinical study will contribute to the scientific literature by supporting the feasibility of cost-effective and simple interventions for a complex issue in alcohol using patients.

6. Trial Administration

6.1 Ethical Considerations: Informed Consent

Consent forms will be Institutional Review Board (IRB)-approved and the participant/legally authorized representative (LAR) will be asked to read and review the document. The PI will explain the research study to the participant and answer any questions that may arise. This conversation will take place in a private room. Participants/LAR will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants/LAR should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants/LAR must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants/LAR for their records.

6.2 Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol will require an approved IRB amendment before implementation. The IRB will determine whether informed consent and HIPAA authorization are required.

A study closure report will be submitted to the IRB after all research activities have been completed.

6.3 Subject Confidentiality

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s)/funding agency. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict

confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible. The study sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to records (office, clinic, or hospital) for the participants in this study. The study site will permit access to such records. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or, if applicable, sponsor/funding agency requirements. Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in a locked container. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number (study ID). The study data entry and study management systems used will be secured and password protected.

6.4 Deviations/Unanticipated Problems

A protocol deviation is any noncompliance with the study protocol. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.
It is the responsibility of the site investigator to identify and report deviations within 7 working days of identification of the protocol deviation. All deviations must be addressed in study source documents, reported to the study sponsor, and the reviewing Institutional Review Board (IRB) per their policies.
Unanticipated problems involving risks to participants or others include, in general, any incident, experience, or outcome that meets all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied.

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the study sponsor. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number.
- A detailed description of the event, incident, experience, or outcome.
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP.
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs will be reported to the IRB and study sponsor within 7days of the investigator becoming aware of the event.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) in accordance with policy of the IRB's receipt of the report of the problem from the investigator.

6.5 Data Quality Assurance

Data will be quality checked by screening for out-of-range variable results and by random audits prior to releasing data for analysis.

6.6 Study Records

The study records will be kept in the RedCap database. Printed informed consents, after participant’s signature will be maintained in a designated in a locket cabinet, in the laboratory MEB room 3205.

6.7 Access to Source

Source data will be maintained per Medical Records policy in a password protected, secure, Health Insurance Portability and Accountability Act (HIPAA) compliant, web-based electronic database with a built-in audit trail.

Only Institutional Review Board (IRB) approved research team members who have current HIPAA and human subjects' protection training will be authorized to access records.

6.8 Data or Specimen Storage/Security

The data will be maintained in the RedCap database. Physical copy of signed informed consents will be kept in a locker cabinet. The biological samples will be identified by the respective study ID and will be stored on MEB room 3205 -80 ºC or nitrogen tank.

6.9 Retention of Records

Federal regulations require research records to be retained for at least 3 years after the completion of the research (45 CFR 46).

6.10 Study Monitoring

Ensuring adherence to IRB approved procedures in the study is the responsibility of the Principal Investigator. Once a week the PI will review the accuracy of the data collected and the protocol compliance.

6.11 Study Modification

All modifications to the study protocol and procedures require IRB approval prior to implementation.

6.12 Funding Source

National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number U24DK132740.

6.13 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the appropriate conflict of interest review committee has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. Any investigator who has a

conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the LSU Health administration with a committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All investigators will follow the applicable conflict of interest policies.

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