Cover letter

Protocol Title:

Effect of Multi-Strain Probiotics as an Anti-Obesity among Overweight and Obese Saudi Adults

Issued on

6/8/2021

PROTOCOL TEMPLATE

Instructions to User:

1. **Sections and text that are in regular font and that have not been highlighted in grey** represent standard language. In general, these sections should be present in your final protocol and the language should not be changed. However, every protocol is unique and changes to standard sections and language may be necessary to meet the needs of your protocol. Please review the language carefully to make sure that it is accurate for your study.
2. **Sections that are highlighted in grey, but that have regular font**, represent sections or information that needs to be customized as applicable to your study, but the language that is present is generally considered to be standard if that section (or procedure) applies to your protocol.
3. **Sections that are highlighted in grey, and where the text is italicized**, represent instructions with some example text. All require complete customization for your study.
4. As you customize each section of the protocol, **remove the highlighting and restore the font to regular (from italics)** to denote that section as having been completed.
5. When your protocol is complete, **review** it to ensure that all highlighting and italics have been removed.

SAMIRA MOHAMMED ALMALKI

Clinical Research Protocol

Academic study (Doctor of Philosophy degree)

Protocol Number:	E-20-5503
Version Date:	5/8/2021
Investigational Product:	Hexbio Probiotic supplementation
Development Phase:	3 phace
Sponsor:	Samira Almalki College of Food Science and Agriculture, King Saud University Riyadh
Funding Organization:	Non-fund
Principal Investigator:	Name: Prof. Hanan Abdullah Al Fawaz Telephone: 0118055890 E-mail: halfawaz@ ksu.edu.sa
Medical Monitor:	Name: Dr. Mariam Eid Al- Juhani Telephone: 058 144 4264 E-mail: maljohani@ksu.edu.sa
Coordinating Center:	If applicable

Approval:

Health Science College on Human Subjects (H-01-R-002)

PI. Prof. Hanan Abdullah Al Fawaz *6/8/2021*

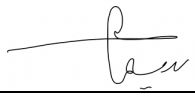
This confidential information about an investigational product is provided for the exclusive use of investigators of this product and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing Samira Almalki with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: E-20-5503

Protocol Title: Effect of Multi-Strain Probiotics as an Anti-Obesity among Overweight and Obese Saudi Adults



Investigator Signature — *Date*: 5/8/2021

PhD student: Samira Mohammed Almalki

Print Name and Title

Site #: 1

Site Name: Occupational clinic, King Khaled Hospital University

Address: *King Saud University*

Phone Number: *0542839259*

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Add all other abbreviations referenced in the protocol and delete any not referenced in the protocol.

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CRF	case report form
CRP	C-reactive protein
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	forced expiratory flow
FEV_1	forced expiratory volume over one second
FVC	forced vital capacity
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IL-8	Interleukin-8
IRB	Institutional Review Board
IV	intravenous
LDH	lactate dehydrogenase
mEq	milliequivalent
PI	Principal Investigator
PK	pharmacokinetic
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase

PROTOCOL SYNOPSIS

TITLE	Effect of Multi-Strain Probiotics as an Anti-Obesity among Overweight and Obese Saudi Adults
SPONSOR	Self-Funded (Investigator Investment)
FUNDING ORGANIZATION	Non-funded
NUMBER OF SITES	One
RATIONALE	Regulating microbiota is considered a potential therapeutic avenue for obesity through many different mechanisms. Exacerbate obesity and associated risk factors are noticed in our society among many social segments for different ages. On the treatment side, most obesity surgery treatments are expensive, prone to failure, and have many side effects, which is a health and economic burden on our government. Since limited studies have been conducted internationally on human subjects, more investigation is needed in this field. Locally, much fewer works on humans are done. Therefore, this study sheds light on the investigation of the anti-obesity effect of probiotic supplementation.
STUDY DESIGN	This is a randomized, double-blind, placebo-controlled phase 3 study.
PRIMARY OBJECTIVE	Study the anti-obesity effect of consuming multi-Strain probiotic supplementation on weight loss for overweight and obese adults.
SECONDARY OBJECTIVES	1. Investigate the effect of probiotics on reducing Lipopolysaccharides LPS. 2. Determine the effect of probiotics on liver functions. 3. Study the association between probiotics and food intake. 4. Evaluate the role of gender difference in response to probiotic.
NUMBER OF SUBJECTS	90
SUBJECT SELECTION CRITERIA	Inclusion Criteria: 1. Adult's male and female ages between 19 to 40 years. 2. BMI (in kg/m^2) from 25 to 35, (WHO, n. d.) WC > 88 cm (women) or > 102 cm (men) (lean et al, 1995). 3. Stable body weight in the previous month of the trial. Exclusion Criteria:

	• Participants who suffer from diseases and on treatment, such as immune system diseases or thyroid disorders. • Pregnant women or who plans to be pregnant. • Participants who had gastrointestinal surgery. • Hormone replacement therapy. • Participants who taking antibiotic. • Participants who consume probiotic or prebiotic supplementation regularly.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	**Product: (HEXBIO®) containing MCP® BCMC® strains** Each sachet containing 3g / 90 billion CFU Oral consumption. Two sachets per day, by dissolving the sachet content in a glass of room-temperature water, one before breakfast and one before going to bed, for three months length.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	The placebo sachets containing only the excipients, i.e., maize starch and maltodextrins. The placebo was indistinguishable in color, smell, and taste from the probiotic formulation. All participants will be asked to consume two sachets per day, one before breakfast and one before going to bed, after dissolving the content in a glass of room-temperature water.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to 3 months Screening: In the beginning of study Treatment: 3 months Follow-up: 3 months The total duration of the study is expected to be 6 months for subject recruitment and 6 for final subject follow-up.
PRIMARY ENDPOINT	• The primary outcome is the relative change in WC measurement from baseline to end-of-treatment.
SECONDARY ENDPOINTS	• Other obesity-related outcomes included body weight, WC, and hip circumference (HC).
STATISTICS **Primary Analysis Plan**	The primary outcome of this study is the difference in waist circumference between groups after 12 weeks of supplementation. The analysis of this study will include all the patients using intention to treat (ITT) methodology. Waist circumference of two groups after 12 weeks of supplementation will be examined for statistical significant using repeated measures ANOVA.

Rationale for Number of Subjects	The study population depends on the study result of Gomes and his colleagues who used the G-Power software (version 3.0.10) to calculate the sample size. The primary outcome in that study is the difference in waist circumference between groups. The power calculation requires 17 participants in each group (95% power; 5% type I error) to detect a difference in waist circumference (Gomes et al, 2018). In this study, to avoid any later drop-off, both groups will be set to have 45 participants.

1 BACKGROUND

There are many studies indicated that some strains are involved successfully in weight reduction especially *Bifidobacterium* and *Lactobacillus* species. HEXBIO® is a formulation containing six microorganism strains (Lactobacillus acidophilus BCMC®12130, Lactobacillus casei subsp BCMC®12313, Lactobacillus lactis BCMC®12451, Bifidobacterium bifidum BCMC®02290, Bifidobacterium infantis BCMC®02129, and Bifidobacterium longum BCMC®02120). All the six microorganism strains in HEXBIO® have been proven to be gastric acid resistant and bile salt resistant, thus able to survive via intestinal tract transit. The product has been tested in 18 clinical trials and thus far there has been no adverse effects for human consumption reported.

1.1 Overview of Non-Clinical Studies

The acute toxicity of HEXBIO® have been tested via acute oral toxicity study and test reports concluded that the product can be considered as Category 5 (unclassified); whereby the LD_{50} cut- off value is equal or more than 5000mg/kg ($\geq$ 5000mg/kg) body weight. The 6 microorganism strains in HEXBIO® have been assessed via *in-vitro* studies for their tolerance to bile salt, tolerance to gastric acid and their ability to exhibit the Bile Salt Hydrolase (BSH) enzyme. All 6 microorganism strains has been proven to be both bile salt tolerant, gastric acid tolerant, and able to exhibit the BSH enzyme.

1.2 Overview of Clinical Studies

There are many published Clinical Trials with HEXBIO®. For example, Mahadzir, et. al., (2017) conducted a double-blind randomized controlled trial to study the effect of HEXBIO® on body weight. The result was insignificant; however, the length of study was only 4 weeks. The Investigator's Brochure is attached in the CD.

2 STUDY RATIONALE

There are growing interests in the effectiveness of probiotics for treating chronic diseases. Regulating microbiota is considered a potential therapeutic avenue for obesity through many different mechanisms.

Exacerbate obesity and associated risk factors are noticed in our society among many social segments for different ages. On the treatment side, most obesity surgery treatments are expensive, prone to failure, and have many side effects, which is a health and economic burden on our government.

Since limited studies have been conducted internationally on human subjects, more investigation is needed in this field. Locally, much fewer works on humans are done. Therefore, this study sheds light on the investigation of the anti-obesity effect of probiotic supplementation.

2.1 Risk / Benefit Assessment

Understanding the composition of gut microbiota helps to find appropriate solutions to control the increasing weight. In animal studies, physical and biochemical parameters, metabolic and inflammatory markers, and alterations in GM diversity revealed beneficial results against obesity whereas the results in humans are still rare. Alteration of the GM via applying natural or supplementation probiotic is considered as a new and promising therapeutic intervention treating human obesity.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to Study the anti-obesity effect of consuming multi-Strain probiotic supplementation on overweight and obese adults.

3.2 Secondary Objectives

1. Evaluate the anti-obesity effect of multi-Strain probiotic supplementation
2. Investigate the effect of probiotics on reducing Lipopolysaccharides LPS.
3. Determine the effect of probiotics on liver functions.
4. Study the association between probiotics and food intake.
5. Evaluate the role of gender difference in response to probiotic.

4 STUDY DESIGN

4.1 Study Overview

This is a single center, double-blind, placebo-controlled, randomized, trial. Subjects number will be 90 participants. Each subject will be administered a one dose of probiotic supplementation, two times per day, for three months, consisting each time of stable doses of active or placebo.

Subjects will be assigned to the treatment in random order. Evaluations will be taken at baseline and at the end of study.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used:

• Experimental treatment is Hexbio sachet containing 3g / 90 billion CFU

• Placebo sachets containing only the excipients, i.e., maize starch and maltodextrins. The placebo was indistinguishable in color, smell, and taste from the probiotic formulation.

Total duration of subject participation will be three months. Total duration of the study is expected to be six months.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

The primary outcome is the relative change in WC measurement from baseline to end-of-treatment.

5.2 Secondary Efficacy Endpoints

Other obesity-related outcomes included body weight, WC, and hip circumference (HC).

5.3 Safety Evaluations

- Increasing in Antioxidants enzymes including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx).
- Decreasing in both Aspartate Transaminase (AST) and Alanine transaminase (ALT).
- Decreasing in lipopolysaccharide LPS, which is known also as endotoxin.
- Decreasing in blood analysis including glycated hemoglobin (HA1c) and lipid profile (TC, HDL-C, and TG)

5.4 Other Evaluations (include only if applicable)

Primary Efficacy Endpoint: The primary outcome is the relative change in WC measurement from baseline to end-of-treatment.

Secondary Efficacy Endpoints: Other obesity-related outcomes included body weight, WC, and hip circumference (HC).

6 SUBJECT SELECTION

6.1 Study Population

Subjects with BMI (in kg/m2) from 25 to 35 who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Adult's male and female ages between 19 to 40 years.
2. BMI (in kg/m^2) from 25 to 35, or WC > 88 cm (women) or > 102 cm (men).
3. Stable body weight in the previous month of the trial. That can be detected by asking the participants about their weight; slight change doesn't matter. Also, case report study includes questions related to the weight, for example: Have you

enrolled in any diet during the past three weeks? That can also help to detect the stability of weight.

4. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.

6.3 Exclusion Criteria

- Participants who suffer from diseases and on treatment, such as immune system diseases or thyroid disorders.
- Pregnant women or who plans to be pregnant. That can be achefed by asking participant wether they are pregnant or planning to become pregnant, If the answer is yes, the participant will be excluded. Other females, who are not planning to be pregnant, should use any of contraceptive methods. The studies didn't mention to any interfering between probiotic and contraceptive, so woman who use contraceptive will not be excluded.
- Participants who had gastrointestinal surgery.
- Hormone replacement therapy.
- Participants who taking antibiotic.
- Participants who consume probiotic or prebiotic supplementation regularly.

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

All subjects should be free from any chronic diseases which require medications.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

In allocation process, a randomization center will be involved in randomizing the participants in two groups, also there will be allocation concealment, according to the following steps:

- Hexbio company will provide sachets which only have alphabet Q or R.

- In the beginning of the study, participants will be given a unique number by the research phlebotomist.
- The randomization scheme will be computer generated in which all participants will be allocated (1:1) in two different groups, group Q and group R.
- After completing the intervention, a request letter will be sent to the company in order to unblind the study.
- Then the company lab will send an unblinding letter to inform us which alphabet is probiotic or placebo.

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will not be known to investigators, research staff, or patients. The following study procedures will be in place to ensure double-blind administration of study treatments:

- Access to the randomization code will be strictly controlled.
- Packaging and labeling of test and control treatments will be identical to maintain the blind.
- Due to necessity of evaluate the activity of treatment product, both packages(with different letters) will be sent to microbiology lab to determine the activity of strains, the result will be blind without mention to any letter.
- Full blinding is done after the last participant of the intervention is done. The investigator will send an email to the company asking for unblinding.

The study blind will be broken on completion of the clinical study and after the study database has been locked. Investigators will be made aware of their subjects treatment assignments after all participant finish the treatment duration, three months.

During the study, the blind may be broken **only** in emergencies when knowledge of the patient's treatment group is necessary for further patient management. When possible, the Investigator should discuss the emergency with the Medical Monitor prior to unblinding.

For blinding in emergency case, the investigator has 2 options:
1. Have direct contact with the company (mobile) personnel in charge of blinding (preferrable)
2. Assign a 3rd individual not included in the team to whom the company will share the blinding information.

8.3 Formulation of Test and Control Products

Company's name: B-CROBES LABORATORY SDN. BHD.

Product : HEXBIO® MCP® Granule (Original Flavour)
Generic Name : MCP® BCMC® strains
Trade Name : HEXBIO®

8.3.1 Formulation of Test Product

HEXBIO® is a cream-coloured, original flavoured granular powder containing 6 microorganism strains. The microorganisms / active ingredients in HEXBIO® are; *Lactobacillus acidophilus* BCMC®12130, *Lactobacillus casei* subsp BCMC®12313, *Lactobacillus lactis* BCMC®12451, *Bifidobacterium bifidum* BCMC®02290, *Bifidobacterium infantis* BCMC®02129, and *Bifidobacterium longum* BCMC®02120.

Table X: Formulation and Measured pH of XXX and Placebo

	Hexbio	**Placebo**
Active Ingredient, mg/mL	*Lactobacillus acidophilus* BCMC®12130, 107 mg *Lactobacillus casei* subsp BCMC®12313, 107 mg *Lactobacillus lactis* BCMC®12451, 107 mg *Bifidobacterium bifidum* BCMC®02290, 107 mg *Bifidobacterium infantis* BCMC®02129, 107 mg *Bifidobacterium longum* BCMC®02120. 107 mg	excipients, i.e., maize starch and maltodextrins 3 g

8.3.2 Formulation of Control Product

A placebo containing similar granule appearance as the treatment without 6 probiotic strains will be provided by the Sponsor in a plain box containing 6 zipper bags (10 sachets in each zipper bag). Each sachet is for single use and ready for administration.

8.3.3 Packaging and Labeling

Packaging: Study drug is supplied in cartons containing 1,200 single use sachets. The sachets will be packaged in sets of 10 enclosed within a transparent zipper bag. Six zipper bags will be contained in one plain box labeled with instruction for use and storage. Twenty plain boxes will be contained in each carton.

Labeling: Each **plain box of study drug (not carton)** will be labeled with the required FDA warning statement, the protocol number, a treatment number, the name of the sponsors, and directions for patient use and storage. Each sachet will be labeled with OA-R-01 or OA-Q-01 and Expiry date.

8.4 Supply of Study Drug at the Site

The Sponsor will ship the supplementation after all required regulatory documentation has been received by the Sponsor and a contract has been executed.

8.4.1 Dosage/Dosage Regimen

B-CROBES LABORATORY SDN. BHD. (588778-K) 18 & 20, Lintasan Perajurit 17G, Taman Teknologi Industri & Perusahaan Ipoh, 31400 Ipoh, Perak, Malaysia. Contact Number: +603-5636 1708	OA-R-01
Study no.	
Treatment no.	
Dosage form	3g of granules per sachet
Instructions for Use	1 sachet to be taken twice daily. Can be taken before or after meal. Drink a glass of water.
Route of Administration	Oral
Storage Conditions	Storage below 25°C. Protect from sunlight and moisture.
Pack size	60 sachets per box
Expiry Date	
NOT FOR SALE. KEEP OUT OF REACH OF CHILDREN	

8.4.2 Dispensing

Investigator will dispense the product at study site.

8.4.3 Administration Instructions

One sachet to be taken orally twice daily. Before or after meal, pour one sachet directly into the mouth. Drink a glass of water.

8.5 Supply of Study Drug at the Site

Once the sponsor received the written request from the investigator, the sponsor will arrange the production of study drug which takes 1-2 months. The investigator will need to send a written notice and request for additional study drug if it is necessary in advance so the sponsor will arrange for production accordingly.

8.5.1 Storage

Study drug should be stored by the study site at room temperature below 25ºC with air conditioning. If the temperature of study drug storage in the clinic/pharmacy exceeds 25ºC, this should be reported to the Sponsor or designee and captured as a deviation. Subjects will be instructed to store the medication in original packaging (plain box and protected from sunlight) at room temperature below 25ºC according to the instructions outlined on the Drug Administration Instructions.

8.6 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study supplement for each subject if there is any side effect. All those details will be recorded by a member of the study site staff. The study monitor will verify these documents specially in the first week of the intervention.

8.7 Measures of Treatment Compliance

Subjects will be asked to keep a participant's diary noting the day and date they take their study probiotic supplement and any adverse events. They will be asked to bring their diary to next study visit along with all used and unused probiotic or placebo sachets.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability must be signed and dated by the subject.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

Inapplicable, participants should be healthy.

9.1.2 Demographics

Demographic information (date of birth, gender, income, education, professional status, and social status) will be recorded at screening.

9.1.3 Medical History

Relevant medical history will be recorded at Screening.

9.1.4 Physical Examination

A complete physical examination will be performed by co-investigator who is a physician at Visit 1. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam at next visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at Visits 1 &2.

9.1.6 Oximetry

Inapplicable

9.1.7 Spirometry

Inapplicable

9.1.8 Other Clinical Procedures

Inapplicable

9.1.9 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to the supplementation will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements (include sections as appropriate)

9.2.1 Hematology

Inapplicable

9.2.2 Blood Chemistry Profile

The blood analysis including glycated hemoglobin (HA1c) and lipid profile (TC, HDL-C, and TG). Other blood analysis include: LPS, Aspartate Transaminase (AST), Alanine transaminase (ALT), superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx).

9.2.3 Pregnancy Test

Inapplicable

9.2.4 Urinalysis

Inapplicable

9.3 Pharmacokinetic Measurements

All analyses will be done in the CBCD, KSU using colorimetric method. Regular blood analysis will be taken at baseline and at the end of the study, then are kept into plan red covered tubes. All blood samples will be centrifuged (3500 × 5 mins) to separate serum samples. All tubes should be placed vertically for 45 minutes until the sample is completely coagulated, then transported to CBCD, KSU for further analysis.

9.4 Research Laboratory Measurements (include sections as appropriate)

Inapplicable

9.4.1 Cell Count and Differential

Inapplicable

9.4.2 Sputum Cytokine Measurements

Inapplicable

10 EVALUATIONS BY VISIT

10.1 Visit 1 in the beginning of study:

1. Review the study with the subject and obtain written informed consent.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record medical history, including:
    - Stability of weight in the last three months prior the study.
    - Having any of the following diseases: Thyroid disorders, Diabetes type-1, Diabetes type-2, (Any type of disease) Cancer, Neurological disorders, psychiatric disorders, or other.
    - Having any of weight loss surgeries.
    - Enrolling in any diet during the past three weeks.
    - Taking antibiotics in the past 3 weeks.
    - Taking any probiotic supplements regularly for the past 3 months.
    - For ladies:
        -Are you planning to become pregnant in the next three months?
        - Are you taking any hormonal therapy?
5. Perform a complete physical examination.
6. Explain the possible side effect.
7. Perform and record results of blood pressure testing.
8. Collect blood for clinical laboratory.
9. Allocation process which includes randomizing the participants in two groups and allocation concealment.
10. Taking food frequency record for probiotics & prebiotics.
11. Taking 24-hour record.

12. Taking anthropometric measurements.
13. Taking physical activities.

10.2 Visit 2 at the end of the study (3 months length)

1. Perform an abbreviated physical examination
2. Perform and record results of blood pressure testing.
3. Collect blood for clinical laboratory.
4. Taking 24-hour record
5. Taking anthropometric measurements.
6. Taking physical activities.

10.3 Early Withdrawal Visit

1. Record any Adverse Experiences and/or review subject diary for adverse experiences and exclusionary medication use.
2. Record changes to concomitant medications.
3. Perform complete physical examination.
4. Perform and record vital signs.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

In general, using probiotic products may cause mild bowel discomfort and diarrhea in the first few days or maximum one week for some people. All these symptoms will disappear later.

AE Severity

Probiotic products are considered over the counter drugs (OTC) which don’t require doctor's prescription. Many clinical trials proved that Hexbio product doesn't cause any AE severity, that clearly explained in investigator's brochure. However, participant can stop participating at any time, recording reasons as possible.

AE Relationship to Study Drug

AE include possible bowel discomfort and mild diarrhea.

11.2 Serious Adverse Experiences (SAE)

Using probiotic products doesn't cause any SAE.

11.2.1 Serious Adverse Experience Reporting

Probiotic products are safe, however, if there are any SAEs, they will be documented (whether related to study drug or not). The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

11.3 Protocol Defined Important Medical Findings Requiring Real Time Reporting

Inapplicable

11.4 Medical Monitoring

Dr. Mariam Eid Al- Juhani should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: 0581444264

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

1. Subject withdrawal of consent (or assent)
2. Subject is not compliant with study procedures
3. Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
4. Protocol violation requiring discontinuation of study treatment
5. Lost to follow-up
6. Sponsor request for early termination of study
7. Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

12.3 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to Visit 2) can have an early discontinuation visit.

12.4 Replacement of Subjects

Subjects who withdraw from the study treatment will not be replaced.
Subjects who withdraw from the study will not be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

1. Failure to meet inclusion/exclusion criteria
2. Use of a prohibited concomitant medication
3. Not follow the role, (for example do not follow the health guideline).
4. Not use the probiotic according to the description.

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 DATA SAFETY MONITORING (OPTIONAL SECTION – INCLUDE WHEN APPROPRIATE)

Inapplicable

15 STATISTICAL METHODS AND CONSIDERATIONS

The primary outcome of this study is the difference in waist circumference between groups after 12 weeks of supplementation. The analysis of this study will include all the patients using intention to treat (ITT) methodology. Waist circumference of two groups after 12 weeks of supplementation will be examined for statistical significant using repeated measures ANOVA.

15.1 Data Sets Analyzed

All eligible subjects will be included in each analysis.

15.2 Demographic and Baseline Characteristics

Demographic information (date of birth, gender, income, education, professional status, and social status) will be recorded at screening.

15.3 Analysis of Primary Endpoint

ANOVA

15.4 Analysis of Secondary Endpoints

ANOVA

15.5 Interim Analysis

All analysis will be done at the end of the intervention.

15.6 Sample Size and Randomization

The study population depends on the study result of Gomes and his colleagues who used the G-Power software (version 3.0.10) to calculate the sample size. The primary outcome in that study is the difference in waist circumference between groups. The power calculation requires 17 participants in each group (95% power; 5% type I error) to detect a difference in waist circumference (Gomes et al, 2018). In this study, to avoid any later drop-off, both groups will be set to have 45 participants.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic, Case Report Form (eCRF) and paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study documents to be collected by the Sponsor (or designee) but will be identified by a subject number.

For eCRFs: If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. For paper CRFs: If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator

Data Management Procedures

The data will be kept in paper documents and e-documents, the study doesn't have database.

16.2 Data Quality Control and Reporting

Inapplicable

16.3 Archival of Data

All data will be kept in both:
Electronic documents, in which can be reached by principal investigator and other investigators only.
Paper documents which can be kept in a locked file cabinet until 2 years at least after the study, then they will be disposed.

16.4 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), as IRB and S FDA, inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following study. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.5 Monitoring

Appropriate regulatory authorities, such as the SFAD and Clinic Manager, may conduct monitoring visits to conduct on-site monitoring and/or audits of all appropriate study documents.

16.6 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the SFDA. The Investigator must also comply with all applicable privacy regulations.

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form are reviewed and approved by the IRB prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information)

will be submitted to the IRB. The IRB written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

The Investigator will prepare the informed consent form and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB -approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject will also be obtained. A copy of the signed consent form will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines.
4. Report to the Sponsor or designee any AEs that occur in the course of the study.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements.

APPENDIX 1. EXAMPLE OF SCHEDULE OF STUDY VISITS

	VISIT 1 (Day/Week/Month #)[a]	VISIT 2 (Day/Week/Month #)[a]
Informed Consent	X	
Medical History	X	
Complete Physical Exam	X	
Abbreviated Physical Exam		X
Height	X	X
Weight	X	X
Vital Signs	X	X
Oximetry		
Spirometry		
Pharmacokinetics		X
Chemistry	X	X
Pregnancy Test (Urine or Serum)		
Hematology		
ESR		
C-Reactive Protein		
Urinalysis		
Randomization	X	
Dispensing or Administration of Study Drug	X	X
Counting of Returned Study Drug		X
Initiate Subject Diary	X	

Subject Diary Review		X
Concomitant Medication Review	X	X
Adverse Experiences		

[a] ±2 days

Required Components of Informed Consent

Informed consent is not a single event or just a form to be signed; it is an educational process that takes place between the investigator and the prospective subject. The basic elements of the consent process include:
full disclosure of the nature of the research and the participant's involvement,
adequate comprehension on the part of the potential participant, and
the participant's voluntary choice to participate.

It is the investigator's responsibility to document that the informed consent process has taken place, and an informed consent form is the standard for documenting the process for research projects involving human participants.

Consent forms must contain all the required components of informed consent as defined in SOP 9: Informed Consent Options, Processes, and Documentation and summarized below. The consent form must be written in language that is easy for a potential participant to understand and assures that individual's comprehension. Therefore, avoiding technical terms and complex sentences, even for the educated layperson, is very important.

When the participant population is not homogeneous, different consent documents may be required for different groups of people. If the research population will include participants under 18 years of age, then the IRB will expect investigators to use an assent form and a parental permission form instead (see SOP 11: Informed Consent, Enrollment, and Other Considerations for Research Involving Children). Similarly, research with cognitively or decisionally impaired individuals will require documented consent from another party—namely that person's legally authorized representative (see SOP 9, section 8 and/or SOP 10: xxx).

The IRB also recognizes that there are instances when documenting written informed consent is not appropriate to a research project. Alternatives to using a signed form for documenting informed consent are detailed in SOP 9.

Regardless of the method of documenting informed consent, however, the process of obtaining informed consent should always contain the same required components.

Helpful guidelines for constructing an effective consent form:
Use common, ordinary language instead of technical, academic terms. Ideally we would like consent forms to be written an 8th grade reading level. A helpful gage is to consider if one's 13 year-old cousin would be able to understand the research after reading the consent form.
Try to keep the sentences as short and simple as possible. Write in the second person using you/your pronouns. For example: "You are being asked to participate in a research project…," "If you have questions later, you may contact…," or "You will be given a copy of this form to keep for your records."

Do not use assumptive statements such as "You understand that ..." or "You have been told that...."
Use adequate white space so that the form is easy to read, and avoid using small fonts to squeeze all the text onto one page.
Headings for paragraphs are helpful and make the form easier to read and understand.

Required Elements of Informed Consent Forms:
1. A clear, concise explanation of the purposes of the research, including the name of the study and prominent use of the term "research." (Note: the IRB can waive this element if the study requires deception. In such cases, a debriefing statement should also be used to inform participants at an appropriate time after their involvement in the study.)
2. An explanation of what will be happening to the participant during the study, and an indication of the participant's time commitment for each component.
3. Description of the risks, side effects or discomforts of the study procedures. For instance, even though it is not considered a risky procedure, a needle stick to draw blood may cause brief pain or discomfort. For social science and behavioral research, though risks usually do not extend beyond the possible loss of confidentiality and/or mild emotional distress, these should also be made clear to prospective participants.

If it appears that there are no real risks to participation, state, "We do not anticipate any risks to you participating other than those encountered in daily life." Please see our Sample Consent Form for an example of appropriate wording of a risk statement.
4. Description of any potential benefits from participating.

For individual participants, these should be limited to direct benefits: information about better coping skills, awareness of available support or resources, or any other personal gain other than financial rewards. (Learning about how experiments are conducted, receiving a gift, or earning extra credit for being a research participant are NOT recognized as benefits. Gifts, extra credit for courses, and reimbursement for expenses are considered compensation.) If there are no direct benefits, simply indicate that there are none.

For indirect benefits to society or scientific knowledge, statements such as "…information from this study may benefit other people now or in the future…" or "…we hope to learn more about _____ …" are appropriate.
5. A statement that the participant's involvement is voluntary, the participant may refuse to participate before the study begins, discontinue at any time, or skip any questions that may make him/her feel uncomfortable, with no penalty to him/her, and no effect on the compensation earned before withdrawing, or their academic standing, record, or relationship with the university.
6. A statement that the participant is allowed to ask questions concerning the study, both before agreeing to be involved and during the course of the study. See required contact information in #11 below.
7. A description of how the participant's confidentiality will be protected.
8. A description of what will be done with the data once the study is completed.

9. An indication that recording devices, audio or visual, are being used (when applicable).

Be sure to describe what will be done with the any video or audio tapes upon the completion of the study (destroyed, erased, archived, etc.), and when (after transcription, 3 years, 5 years, etc.).

Also, provide a separate signature line on the consent form for the participant to agree to be video/audio taped or photographed, if the recording is optional for participation. For example:

Please sign below if you are willing to have this interview recorded on tape (specify audio or video). You may still participate in this study if you are not willing to have the interview recorded.

I am willing to have this interview recorded on tape:

Signed: _____

Date: _____

10. An indication that the participant shall receive a copy of the signed and dated consent form.
11. The name(s) of the investigator(s) and contact information.
12. An indication that the participant may contact the Institutional Review Board for Human Participants (IRB) with any concerns or complaints. Additionally, a statement indicating that participants can report their concerns or complaints anonymously through.
13. A "statement of consent" and the name and signature of the participant.
14. The name signature of the person obtaining consent.
15. At the bottom of the form the following statement: "This consent form will be kept by the researcher for at least three years beyond the end of the study and was approved by the IRB on [date]."