EFFECT OF AQUEOUS EXTRACTS OF CISSUS QUADRANGULARIS AND DICHROSTACHYS GLOMERATA ON GLP-1 CONCENTRATION AND DPP-4 ACTIVITY IN OVERWEIGHT AND OBESE ADULTS

NCT NUMBER: NCT06827002

DOCUMENT DATE: DECEMBER 16, 2023

Protocol Title: A Randomized, Double Blind, Placebo-Controlled, Parallel Clinical Trial To Investigate The Efficacy Of Dichrostachys Glomerata And Cissus Quadrangularis Extracts In Glp-1/Dpp-4 Modulation And Weight Loss In Overweight And Obese Individuals

Protocol Number: 24JACCGD01
Protocol Date: December 16, 2023
Version: 5
Study Design: Randomized, Double-Blind, Placebo-Controlled Trial

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SCHEDULE OF ASSESSMENTS

PROCEDURES / ASSESSMENTS	**V1 - DAY 0 V2 V3 Screening-Baseline**	**V2 Day 28 ± 4 days**	**V3 Day 58 ± 4 days**	**V4 Day 85 ± 5 days**	**V5 Day 115 ±5 days**
Informed consent	X				
Review inclusion/exclusion criteria	X				
Review medical history	X				

Review concomitant therapies	X	X	X	X	X
Height*, weight, heart rate, blood pressure (* Height will only be measured at Visit 1)	X	X	X	X	X
Urine pregnancy test	X				X
Randomization	X				
VAS scales for satiety assessment	X	X	X	X	X
Fasting Blood Glucose, HbA1c, Lipid Profile, GLP-1 and DPP4 activities	X	X	X	X	X
Study diary Dispensed	X	X	X	X	
Study Diary Returned		X	X	X	X
Investigational Product Dispensed	X	X	X	X	
Investigational Product Returned		X	X	X	X
Food Record	X	X	X	X	

Diary Dispensed					
Food Record Returned		X	X	X	X
Compliance Calculated		X	X	X	X
Adverse Events		X	X	X	X

LIST OF ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
BP	Blood Pressure
CRO	Contract Research Organization
CVD	Cardiovascular Disease
DPP-4	Dipeptidyl peptidase 4
Etc.	"and so forth"
E.g.	"for example"
GCP	Good Clinical Practice
GLP-1	Glucagon-Like-Peptide-1
HDL-C	High Density Lipoprotein Cholesterol
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Conference of Harmonization
i.e.	"in other words" IEC Independent Ethics Committee
JIRB	Joint Institutional Review Board for Animal & Human Bioethics
ITT	Intention to Treat
kg	Kilogram
LDL-C	Low Density Lipoprotein Cholesterol
mL	Milliliter
OTC	Over the Counter
PP	Per Protocol
QI	Qualified Investigator
SAE	Serious Adverse Event
TC	Total Cholesterol
TG	Triglycerides
VAS	Visual Analogue Scale

TABLE OF CONTENTS

1. INTRODUCTION

Obesity is a health burden affecting over 2.3 billion people of all ages globally [1]. The development and progression of obesity involve a complex pathogenesis, and several drugs have been developed to target these pathways. In recent years, dipeptidyl peptidase-4 (DPP-4) inhibitors or gliptins, such as sitagliptin, saxagliptin, and vildagliptin, have emerged as viable obesity management options. Gliptins inhibit DPP-4, an enzyme known to deactivate the glucagon-like peptide-1 (GLP-1) hormone, contributing to the development and progression of obesity and other metabolic diseases. GLP-1 is an important incretin hormone secreted in the L-cells of the gut for the maintenance of blood sugar homeostasis. It has other pleiotropic effects through its receptors in the liver, brain, and stomach to delay gastric emptying, reduce appetite, and induce significant weight loss [2]. In healthy individuals, GLP-1 has a half-life of <2 minutes due to the activities of DPP-4 [3]. Some studies have reported increased DPP-4 levels in people with obesity [4], further reducing the incretin effects of GLP-1. Gliptins are primarily used to manage type 2 diabetes, but studies have suggested their weight loss effects through enzymatic activities.

Synthetic drugs used for obesity management are often associated with side effects and contraindications. For conditions such as obesity, patients will often require individualised management options owing to sensitivity and the high likelihood of comorbidities. For example, patients with obesity are more vulnerable to pancreatitis and pancreatic cancer [5], whereas gliptins are associated with a high incidence of acute pancreatitis.[6]. In terms of cost, gliptins are considerably expensive [7]. The off-label use of gliptins for obesity management may lead to increased demand, higher prices, and potential shortages. Therefore, there is a need to explore safe, cost-effective, and potent alternatives for obesity management.

Owing to their potency and low toxicity, natural products continue to emerge as potential drug leads for several metabolic disease conditions. *Dichrostachys glomerata* (Forssk.) Chiov. (Fabaceae) is a popular Cameroonian spice, and *Cissus quadrangularis L*. (Vitaceae) is an ornamental and medicinal plant that grows in Africa and Asia and has shown tremendous effects on weight loss. Youovop et al. [8] reported that *D. glomerata* extract (DGE) induced 22.85% weight loss in 60 subjects at 12 weeks. In a double-blind, placebo-controlled study involving 35 subjects, C. quadrangularis extract (CQE) reduced body fat by 12.8% in 8 weeks [9]. The mechanisms by which these two extracts exert their weight loss effects are not fully understood. It has been suggested that DGE and CQE may

exert weight loss effects through anorectic mechanisms. Studies by Kim et al. [10] and Lee et al. [11] proposed that DGE and CQE may reduce food intake through increased adiponectin secretion and the AMPK pathway. To date, no study has investigated the effects of DGE or CQE on GLP-1 levels or DPP-4 activity.

This randomized, double-blind, placebo-controlled clinical trial will examine the effect of DGE and CQE on GLP-1/DPP-4 modulation from Day 0 to Day 115 in comparison with semaglutide as a primary outcome. Secondary outcomes will assess anthropometric and metabolic outcomes, including changes in body weight, BMI, body fat, fasting blood lipid levels (cholesterol, triglycerides, and HDL-c), fasting blood glucose levels, energy intake and satiety.

Enrolled participants will include overweight and obese adults with a BMI between 25.0 - 34.9 kg/m^2 who have had a stable body weight and have not participated in a weight loss or diet program for at least three months. The inclusion and exclusion criteria is as shown in the table below:

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	**Exclusion Criteria**
Age 25 - 59 years	BMI > 34.9 kg/m^2
BMI 25 - 30 kg/m^2;	Pregnancy
Men and women	Breastfeeding
	overweight or obese as a result of metabolic and/or endocrinologic disorders
	Diabetes mellitus requires daily insulin management.
	Active infection
	Systemic diseases such as HIV/AIDS, active hepatitis
	Signs of active malignancy within the past 5 years
	Use of any other medication or natural health product that might affect the parameters of interest in this study.
	individuals with a surgical history of gastric bypass or history of any other surgery for weight loss purposes

Eligible participants taking any other supplements containing the investigational product ingredients will be required to undergo a washout period unless they have been on a stable dose of the supplements for a period of 3 months or more. These eligibility criteria is to address possible confounders on the outcomes of this study and to ensure participants' safety.

2. STUDY OBJECTIVES

The objective of this study is to investigate the effect of *D. glomerata* and *C. quadrangularis* extracts on GLP-1/DPP4 modulation as well as metabolic and anthropometric parameters.

2.1. Primary outcome:

1. The changes and differences in DPP-4 activities and post-prandial GLP-1 levels from baseline, at Day 28, 58, 85 and 115 between DGE, placebo and Semaglutide
2. The changes and differences in DPP-4 activities and post-prandial GLP-1 levels from baseline, at Day 28, 58, 85 and 115 between CQE, placebo and Semaglutide

2.2. Secondary outcomes:

1. The difference in change in body weight, BMI and body fat from baseline, at Day 28, 58, 85 and 115 between DGE, placebo and Semaglutide
2. The difference in change in body weight, BMI and body fat from baseline, at Day 28, 58, 85 and 115 between CQE, placebo and Semaglutide
3. The difference in change in lipid profile (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, triglycerides (TG), TG/HDL-C, TC/HDL-C and LDL-C/HDL-C) from baseline, at Day 28, 58, 85 and 115 between DGE, placebo and Semaglutide
4. The difference in change in lipid profile (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, triglycerides (TG), TG/HDL-C, TC/HDL-C and LDL-C/HDL-C) from baseline, at Day 28, 58, 85 and 115 between CQE, placebo and Semaglutide
5. The difference in change in energy intake and satiety from baseline, at Day 28, 58, 85 and 115 between DGE, placebo and Semaglutide
6. The difference in change in energy intake and satiety from baseline, at Day 28, 58, 85 and 115 between CQE, placebo and Semaglutide

2.3. Safety outcomes:

1. Incidence of post-emergent adverse events (AE)
2. Clinically relevant changes in vital signs (blood pressure (BP) and heart rate (HR)) after supplementation

3. STUDY DESIGN

The planned sample size for this study is 248 participants with 62 participants per arm. The schedule of assessment in section 1 will be followed to evaluate the primary, secondary and safety outcomes.

Study Arm	**Number of Participants**
Placebo	62
DGE	62
CQE	62
Semaglutide	62
Total	248

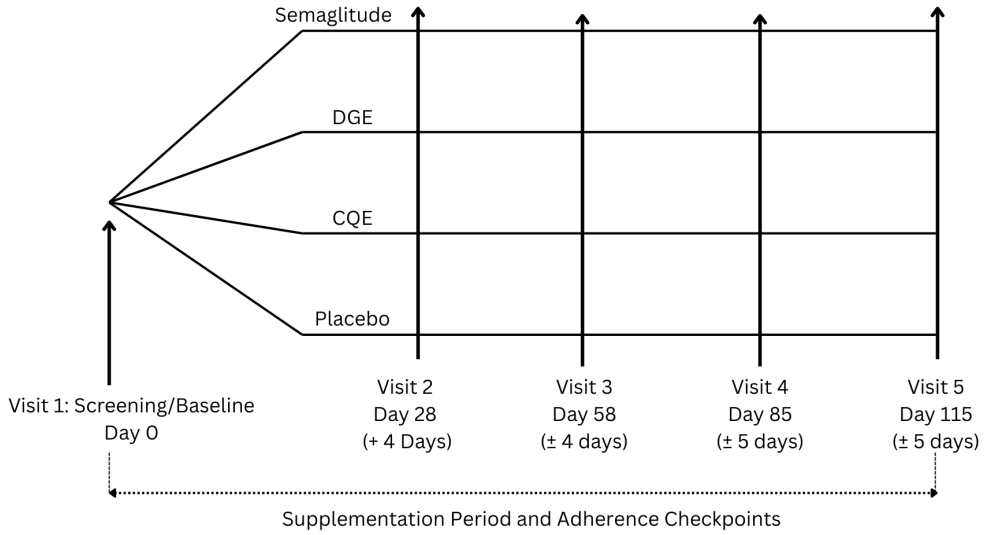

Figure 1: Study Design

4. SELECTION OF STUDY POPULATION

This study will enrol 248 participants who must fulfil the eligibility criteria as described in sections 4.1 and 4.2 below.

4.1 Inclusion Criteria

1. Males and females must be 25 years or older
2. BMI must be between 25.0 - 34.9 kg/m^2
3. Females must be non-pregnant and must agree to use a medically-approved child control if of childbearing potential for the entire duration of the study.
4. Participants must have a stable body weight, defined as <5% change in body weight in the last 3 months before the commencement of the study
5. Be willing to complete records and diaries associated with the study, and complete all study visits
6. Agrees to maintain their current lifestyle habits for the duration of the study
7. Provides their consent to participate in the study through the written informed consent form
8. Must be healthy as determined by medical history, which is assessed by a qualified investigator (QI) of this study

4.2. Exclusion Criteria

1. Individuals who are pregnant, planning to be pregnant or breastfeeding during the study
2. Individuals who have allergies or sensitivities that prevent the consumption of the investigational products or placebo ingredients
3. Has had a gastric bypass surgery or any other surgery to induce weight loss
4. Participated in a weight loss program within the last 3 months or is currently taking weight loss medications
5. Currently or a history of eating disorders
6. Current or history of metabolic diseases, diseases of the gastrointestinal tract or any chronic disease
7. Currently hypertensive

8. Actively enrolled participants residing in the same household
9. Diagnosis of Diabetes
10. Significant cardiovascular event within the last 6 months.
11. Current diagnosis or history of kidney and/or liver diseases
12. Self-reported confirmation or diagnosis of thyroid conditions
13. Had a major surgery in the last 3 months or with a planned major surgery during the course of the study. Participants with minor surgery will be considered on a case-by-case basis by the QI
14. Individuals with cancer. Participants with cancer in full remission for more than 5 years after diagnosis will be considered
15. Individuals who are immunocompromised or have autoimmune diseases
16. Self-reported confirmation or diagnosis of HIV, Hepatitis-B and/or C-positive
17. Self-reported confirmation of blood/bleeding disorders
18. Chronic use of cannabinoid, tobacco or nicotine products. Occasional users will be required to wash out and abstain for the duration of the study period
19. Alcohol or drug abuse within the last 12 months
20. Current use of supplements or OTC medications that may impact the efficacy or safety of the investigational products
21. Use of Antibiotics in the last month from baseline
22. Current use of weight loss medications such as GLP-1 agonists or SGLT-2 inhibitors
23. Blood donation in the last month, planned donation during the entire duration of the study
24. Individuals who are unable to give informed consent
25. Any other condition or lifestyle factor that may prevent the participant from completing the study or pose a significant risk to the participant.

4.3. Concomitant Medications

Participants who are taking any prescribed medication that are not considered to affect the outcome of the study must agree to maintain their dosing regimen unless otherwise recommended by their general practitioner or nurse practitioner.

4.4. Washout Periods

Participants who are currently taking the following OTC medications, supplements, food or drink must undergo the following washout period

OTC, Supplement, Food or Drink	**Washout Period**
OTC weight loss drugs e.g orlistat (alli)	7 days
Weight loss supplements	3 days
Supplements or foods containing the investigational products' ingredients as assessed by the QI	7 days
Prebiotics, probiotics or postbiotics	30 days

4.5. Participants' withdrawal

As stated in the informed consent form, participants may withdraw from the study for any reason and at any time.

The Qualified investigator also has the discretion to discontinue the participation of a participant based on the following reasons:

a. Clinical reasons: If, in the opinion of the Qualified investigator, the participants' health is at risk as a result of a serious adverse event may be discontinued from the study. A participant who requires any of the prohibited medications will be withdrawn. Participants who become pregnant will be withdrawn and followed up until birth.
b. Violation of the protocol: Participants who are inappropriately enrolled (who do not meet the eligibility criteria) will be withdrawn. Participants who have entered the study in violation of the protocol will be discontinued at the discretion of the QI. Non-compliant participants who fail to show up for study visits, take the investigational product as instructed, refuse to follow the study visit procedures or are found taking any of the prohibited drugs without the knowledge of the QI will be discontinued. Any other major protocol deviations that may increase the risk to the participant or compromise the integrity of the study will lead to the affected participant's discontinuation.

c. Participant replacement: At the QI's discretion and consultation of the study sponsor, a participant may be replaced if attrition rates reach 30%. This is to mitigate the effect of attrition on the study, as excessive withdrawal can render results uninterpretable.

In any case of withdrawal, details on the events leading to the withdrawal must be documented and included in the final report.

5. INVESTIGATIONAL PRODUCTS

5.1. Procurement and storage

DGE and CQE will be procured from Gateway Health Alliances, Fairfield, California, USA. Oral semaglutide (Rybelsus®) will be purchased and then repackaged into capsules. Identical-looking placebo capsules containing dextrin will also be procured. The size and shape must be identical so that neither the researcher nor the participant can distinguish the capsules.

The products will be stored in the study site in a lockable, limited-access area only accessible to the study team in compliance with the study regulations. The products will be stored at room temperature and will not be exposed to direct sunlight or heat.

Investigational Product	**Quantity per capsule**
Cissus quadrangularis Extract	300mg
Dichrostachys glomerate Extract	400mg

5.2. Placebo and control

Placebo: Dextrin (400mg)

Control: Semglutide (3mg, 7mg, 14mg)

5.3. Labelling and Coding

Labelling will be according to the requirements of ICH-GCP guidelines and applicable local regulatory guidelines. The products will be randomized and labelled by a study team member who is not involved in data collection or analysis.

5.4. Procedure

Participants will be instructed to take one capsule every morning with a meal daily for 16 weeks. To ensure compliance, participants are instructed to save all unused and open packages and return them during study visits. If a dose is missed, participants are instructed to take the missed dose as soon as they remember. Participants are advised not to exceed one dose per day.

Participants are encouraged to continue their regular lifestyles and diet as normal.

5.6. Randomization

On the first study visit, participants will be randomly assigned in a 1:1:1:1 ratio to CQE, DGE, Semaglutide, or placebo groups using block randomization. This will use a fixed block size of 8 to ensure balanced group sizes throughout enrollment. Randomization sequences will be pre-generated by a statistician not involved in this study.

5.7. Blinding and Allocation

Allocations will be sequentially numbered and concealed in opaque, sealed envelopes opened only after participants are deemed eligible and consented. Investigators and participants do not know the allocation sequence until the patient receives treatment.

Information regarding randomization will be stored in a secure document, which will be readily available for the QI to use in any event when it becomes necessary to know what product a participant is taking for the sake of the participant's health.

Unblinding should only occur in cases of emergency. If a serious adverse effect occurs in a participant, the study arm assigned will be unblinded to identify the investigational product taken. Details of the unblinded participant must be included in the final report.

6. STUDY ASSESSMENTS

6.1. Study Visits

6.1.1. Screening/Baseline (Day -45 to Day 0; Visit 1)

At screening, an informed consent form is given to the potential participant. They will be required to read the information and will be allowed to seek more information if needed or given the option to take the form home to review before making their decision. Upon agreement to be enrolled, the participant will sign the form and receive a duplicate copy of

the signed form. Screening will begin once the consent is obtained, and a screening number will be entered in the screening and enrollment log.

Screening assessment will include:

1. Medical history, concomitant medications or therapies, and current health status
2. Weight and height measurement (For BMI calculation)
3. Urine pregnancy test for individuals of childbearing potential
4. Assessment of inclusion and exclusion criteria
5. Randomization of eligible participants
6. Collect fasting (12h) blood samples for the analysis of
    a. GLP-1 levels
    b. DPP4 activities
    c. Fasting blood glucose
    d. Lipid profile
7. Dispensation of investigational products, study diaries and 7-day food diaries and instructions on use

Eligible participants will be scheduled for the next appointment on Day (28 ± 4 days).

6.1.2 Visit 2 (Day 28 ± 4 days)

Visit 2 assessments include:

1. Return of unused investigational products
2. Return and review of completed study diaries and completed 7-day food record
3. Dispensation of new study diary and investigational products
4. Review of concomitant therapies and Adverse effects
5. Vital signs measurement
6. Collection of fasting (12h) blood samples for the analysis of
    a. GLP-1 levels
    b. DPP4 activities
    c. Fasting blood glucose
    d. Lipid profile
7. Measurement of body weight
8. Satiety test

The next visit will be scheduled for Day 58 (± 4 days).

6.1.3. Visit 3 (Day 58 ± 4 days)

Visit 3 assessments include:

9. Return of unused investigational products
10. Return and review of completed study diaries and completed 7-day food record
11. Dispensation of new study diary and investigational products
12. Review of concomitant therapies and Adverse effects
13. Vital signs measurement
14. Collection of fasting (12h) blood samples for the analysis of
    a. GLP-1 levels
    b. DPP4 activities
    c. Fasting blood glucose
    d. Lipid profile
15. Measurement of body weight
16. Satiety test

The next visit will be scheduled for Day 85 (± 5 days).

6.1.3. Visit 4 (Day 85 ± 5 days)

Visit 3 assessments include:

17. Return of unused investigational products
18. Return and review of completed study diaries and completed 7-day food record
19. Dispensation of new study diary and investigational products
20. Review of concomitant therapies and Adverse effects
21. Vital signs measurement
22. Collection of fasting (12h) blood samples for the analysis of
    a. GLP-1 levels
    b. DPP4 activities
    c. Fasting blood glucose
    d. Lipid profile
23. Measurement of body weight
24. Satiety test

The next visit will be scheduled for Day 115 (± 5 days).

6.1.4. Visit 5 - End of study (115 ± 5 days)

Visit 4 assessments include:

1. Return of unused investigational products
2. Return and review of completed study diaries and completed 7-day food record
3. Review of concomitant therapies and Adverse effects
4. Vital signs measurement
5. Urine pregnancy tests for participants of childbearing potential
6. Satiety test
7. Collection of fasting (12h) blood samples for the analysis of
    a. GLP-1 levels
    b. DPP4 activities
    c. Fasting blood glucose
    d. Lipid profile
8. Measurement of body weight

6.2. Clinical Assessment Procedures

6.2.1. Body weight and BMI

Height measurement will be taken with the participants' shoes removed, knees straightened and head held upright.

Weight will be taken with shoes off and bladder emptied with a calibrated scale. At least two measurements must be taken per visit. With at least a 0.5kg difference in the two weight measurements, a third one must be taken. The closest two measurements will be used for computation. BMI will be calculated as:

$$\text{Body weight (Kg)} / \text{Height (m}^2\text{)}$$

6.2.2. Body Fat Percentage

Body fat percentage (%) will be measured using an impedance meter.

6.2.3. Blood Pressure and Heart Rate

Participants should be made to sit comfortably with the back supported and the upper arm bare without restricted clothing. The feet should be placed flat on the floor and the legs not crossed. The participant is made to rest for about 5 minutes before the first reading.

Participants with elevated BP should be queried about their usual BP. High BPs should be rechecked after the participant is given a glass of water and rested for at least 15 minutes.
At baseline, the BP is taken on both arms and the arm with the higher BP reading will be used for BP measurement for the rest of the study.
Heart rate (HR) will be measured in beats/minute simultaneously during the BP recording.

6.2.4. Blood sample Collection

The venipuncture procedure will be performed by a trained and qualified personnel according to standard phlebotomy techniques.

6.2.5. Study Diary

The study diary contains questions about compliance, lifestyle habits, concomitant medications and adverse events. Participants are instructed to complete the study diary daily.

6.2.6. Compliance

Compliance is calculated based on the number of unused investigational products at each visit.

(Number of dosage units taken/ Number of dosage units expected to be taken) X 100%

Participants with less than 80% compliance or over 120% compliance are counselled for non-compliance.

6.2.7. Food records

Food records are to be completed in the last 7 days leading up to each study visit. The food records will confirm compliance with maintaining dietary habits over the study period. All food records are void of all personal information to maintain confidentiality. The food record will be reviewed by a trained staff at each study visit, and participants will be counselled with dietary suggestions if required. If the food record is inaccessible to the participant, they are instructed to take note of the food they consume with a pen and paper, which must be brought on the next study visit.

7. ANALYSIS

7.1. GLP-1 levels and DPP-4 activities

Blood will be drawn into EDTA tubes containing a DPP-4 inhibitor for GLP-1 stabilization. Samples will be placed on ice immediately, centrifuged at 4°C within 30 minutes of collection, and plasma stored at −80°C until analysis. GLP-1 concentrations will be measured using the Ray Bio® GLP-1 ELISA kit, and DPP-4 activity will be measured using the human DPP-4/CD26 immunoassay kit (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions. All assays will be performed in triplicate, and standard quality control procedures will be followed, including the use of calibration curves and the assessment of intra-assay and inter-assay precision.

7.2. Fasting blood glucose and lipids

Fasting blood glucose levels will be measured using a glucose meter. Fasting blood lipid levels (cholesterol, triglycerides, and HDL-c) will be measured using commercial kits, while LDL-c levels will be obtained using the Friedewald formula [12]

7.3. Energy Intake

Using the food diaries, the quantification of food intake will be calculated in terms of household measures previously calibrated to gram equivalents. The FAO food composition table for Cameroon will then be used to quantify carbohydrate (Carb), lipid (Lip), and protein (Prot) intakes. Energy intake (EI) is calculated using the following formula:

$$EI\ (Kcal/day) = Ecarb + Elip + E\ prot$$

With:

$$Ecarb/prot\ (Kcal/day) = (Amount\ of\ carb/prot\ ingested\ (g) \times 4Kcal) / 7$$

$$Elip\ (Kcal/day) = (Amount\ of\ lip\ ingested\ (g) \times 9\ kcal) / 7$$

Assumption: 1 g carbohydrate or protein = 4 Kcal and 1 g lipid = 9 kcal.

Satiety will be calculated using the visual analogue scale (VAS) questionnaire as described in Cazzo *et al. [13]*.

8. TERMINATION OF STUDY

Investigators/participants and the Joint Institutional Review Board for Animal & Human Bioethics (JIRB) must be promptly informed in the case of an early termination.

9. PROTOCOL AMENDMENTS

Any amendments made to this protocol after its approval must be captured in writing, including the reasons for the change, the signature of the sponsor and the date. The change may be subject to the Joint Institutional Review Board for Animal & Human Bioethics (JIRB) review/approval before implementation, except for the aversion or elimination of an immediate hazard to the participant. In this case, the QI must inform the board within 5 days of implementation. All amendments should be documented and included in the final report.

10. SAFETY INSTRUCTIONS AND GUIDANCE

10.1. Adverse Events

Participants are asked to record any adverse event (AE) in their diary. At each study visit, the Investigator must confirm any health changes since the last study visit and document their responses. Any reported AE will be classified based on description, intensity, frequency and outcome. The cause of the reported AEs is determined by the QI.

The intensity of an AE is graded on a 3-point scale (mild, moderate and severe) and must be reported in detail in the study record.

- **Mild**- Awareness of the event but easily tolerated
- **Moderate** - Event results in discomfort and some interference with usual activity
- **Severe**- Impairment; inability to carry out usual activities.

The causality relationship between the investigational product and the AE will be assessed by the QI as follows

Most probable	The event responds to dechallenge (Withdrawal of IP) and rechallenge (readministration of IP after withdrawal)
Probable	The event only responds to the dechallenge

Possible	Dechallenge information is unclear, but there is a reasonable relationship between the Investigational product and the AE.
Unlikely	No causal relationship between the investigational product and the AE is observed. Although there is a temporal occurrence of AE when the investigational product is first administered
Not related	No temporal relationship is observed between AE and investigational product, and there is no reasonable causal relationship of the AE by the product.

10.2. Serious Adverse Event

This is an AE that results in any of the following:

1. Death
2. Inpatient hospitalization for up to 24 hours or prolongation of existing hospitalization
3. Persistent or significant disability
4. A birth defect of the offspring of a participant who received the study investigational product.

Other important medical events that may not result in any of the serious adverse events listed above may require intervention to prevent safety risks for the participant. Some examples of such events include severe allergic reactions or the development of drug dependency or abuse.

10.3. Laboratory Test Anomalies

The clinical significance of any abnormal laboratory result must be assessed by the QI using a compendium of normal values for reference.

For a laboratory result to be effectively classified as an anomaly, it should present with the following conditions

- Presence of clinical symptoms in the participant
- Leads to the discontinuation or interruption of the investigational product
- Requires the participant to change concomitant therapy

This applies to any laboratory test that is carried out after the first dose of the investigational product. Such anomalies must be reported as an AE in the study record.

10.4. Treatment and Follow-up of Adverse Events

Participants with AEs, especially those in which the relationship with the investigational product is suspected, should be followed up until baseline status is achieved. If baseline status cannot be achieved, an explanation must be documented in the study record. All AEs and follow-up should be recorded in the study record.

10.5. Treatment and Follow-up of Laboratory Anomalies

In case of laboratory test abnormalities, the participant is withdrawn from the investigational product or placebo. The data obtained from the participant will, however, be used in statistical analysis as part of the intent-to-treat and safety populations.

10.6. Reporting Adverse Events

Any AE must be classified by the QI within 24 hours of notification. Causality must be determined prior to reporting to the ethics and regulatory bodies, including information on the AE or unblinded participant and the study arm. The QI must also inform the sponsor in writing.

11. STATISTICAL EVALUATION

11.1. Determination of sample size

Sample size (n) was calculated for the primary endpoint (change in post-prandial GLP-1) using a two-sided two-sample test for difference in means. To control errors across four planned pairwise comparisons (CQE vs placebo, CQE vs semaglutide, DGE vs placebo, DGE vs semaglutide) Bonferroni correction ($\alpha' = 0.05/4 = 0.0125$) was used. The following formula was used.

$$n = 2(Z_{1-\alpha/2} + Z_{1-\beta})^2 \sigma^2 / \Delta^2$$

Where:

- n = required sample size per group,
- α = nominal Type-I error (two-sided) with $Z_{1-\alpha/2}$ the corresponding normal quantile,
- 1−β = desired power (80%), and $Z_{1-\alpha/2}$ was that quantile,
- σ^2= variance,
- Δ = the minimum clinically meaningful difference between groups.

Allowing 15 % dropout increased the number (n) of participants to be enrolled per group ie 62, and the total number (N) was planned to be 248 (N = 62 x 4).

11.2. Analysis Plan

Safety Population: Includes all participants who were given any of the investigational product, and for whom any post-randomization safety information is available.

Intention-to-Treat Population: Consists of all participants who were randomized to any of the study arms.

Per-Protocol Population: Consists of participants who have at least 80% compliance, completed all study visits and with no record of protocol deviations.

11.3. Statistical analysis plan

For each primary and secondary endpoint, descriptive statistics, including means ± standard error of the mean, will be presented for each study visit and for changes from baseline (Week 0 to Week 16). Changes in endpoints will be calculated as:

Change in endpoint = Value at Week 16 – Value at Week 0

Between-group differences will be analyzed using one-way ANOVA, followed by post hoc Tukey tests where appropriate. Associations between GLP-1, DPP-4, body fat, and energy intake will be assessed using Pearson correlation. Probabilities of ≤ 0.05 are considered statistically significant. All statistical analyses will be performed using SPSS v.24 (IBM Corp., Chicago, IL, USA).

11.4. Description of Premature Discontinuation

The following information should be listed for each participant withdrawn from the study:

1. Participant number

2. Start and end dates of participant enrolment
3. Reason for discontinuation

12. DATA COLLECTION AND STORAGE

Data will be collected and stored in compliance with the ICH-GCP Guidelines and applicable local regulatory guidelines.

13. ETHICAL ASPECTS

This clinical study will follow the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH GCP) and the Declaration of Helsinki. It will be conducted after approval of this protocol and the informed consent form by the University of Yaounde I Joint Institutional Review Board for Animal & Human Bioethics (JIRB). Written consent form will carry elements of informed consent as described in the Declaration of Helsinki, the ICH Guidelines for GCP, in accordance with applicable local laws and regulations. Consent will be obtained from participants after clearly explaining the study's nature, scope and expected results. The consent form will also explain potential risks.

The investigational products, Cissus quadrangularis extract and Dichrostachys glomerata extract, have been shown in safety studies to be well-tolerated without the report of adverse events.

14. DATA PRIVACY

By agreeing to enrol in this study, participants must give their consent to collect and use their health information as described in the Informed Consent Form. The following regulations will be followed to maintain participants' privacy and confidentiality:

1. All health information will be kept confidential to the extent permitted by the law
2. Forms used in collecting participants' information will not contain their names, and all research data will be kept in a secure location.
3. Electronically stored data will be password-protected and be accessible to authorised personnel. Unless required by law, only the following people can have access to confidential study data:
    a. The study staff
    b. The sponsor
    c. Members of the Ethics Boards
    d. Government Regulatory authorities, including the Ministry of Public Health, Cameroon and other foreign regulatory bodies

4. Participants will not be identified in any publications resulting from this study
5. Personal identifying information will not be included in the study information shared with the Sponsor
6. Participants have the right to their own information and may request changes if the information is incorrect.

15. MONITORING AND AUDITING

During or after the completion of this study, all study materials are subject to quality control. The sponsor or any regulatory body may conduct a monitoring and auditing process to assess compliance with this protocol and review study documents for information verification.

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