Phenolic acid fractions of *Anisopus mannii* (PhAM) as a novel add-on therapy for type 2 diabetes: a single-center, double-blind, randomized, placebo-controlled, feasibility trial

1. Supplementary details and results

2. Clinical Study Protocol and Statistical Analysis Plan

**Supplementary details and results**

**Preparation of Anisopus mannii extract and Phenolic Acid Fractions (PhAM)**

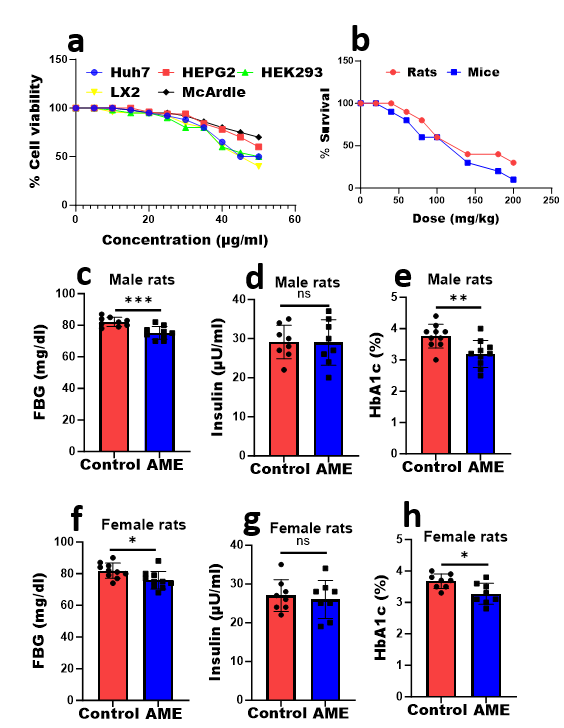
After 6 months of planting, the fresh leaves of A. mannii was harvested and specimens deposited at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri, and registered under the herbarium number IMSU/HBN/202160x. The leaves were pulverized and submerged in distilled water containing 10% DMSO. The extract was concentrated using a rotary evaporator. Phenolic acids were fractionated from Anisopus mannii using a simultaneous rotary extraction method and Microwave-assisted extraction (MAE). The process was optimized to enhance the extraction of phenolic acids and exclude the extraction of other phytochemical classes. The Anisopus mannii leaves were milled and freeze dried for 6h to optimize the retention of phenolic acids. 10g of the dried powder was transferred to 1 litre flask containing 500ml of extraction medium made up of 80% ethanol, acetic acid, and sulfated H 2 0 in a ratio of 6:3:1 v/v. The flask was transferred to a rotary shaker for 2h in an incubator set at 37 0 c. Afterwards, the flask was transferred to a Milestone Ethos EX Microwave Extraction System, set at 250 W microwave power, 5 mins irradiation time, and 50ml/g liquid to solid ratio. After two simultaneous runs, the extract was filtered through 0.45 μm nylon filter and evaporated to dryness, under reduced pressure at 37 °C. Internal standards containing known concentrations of Gallic acid (phenolic acid), linalool (terpenoids), Quercetin (flavonoids), and caffeic acid (hydroxycinnamic acid) was used for the process optimization.

**Chromatograms and output file of bioactive components of PhAM**

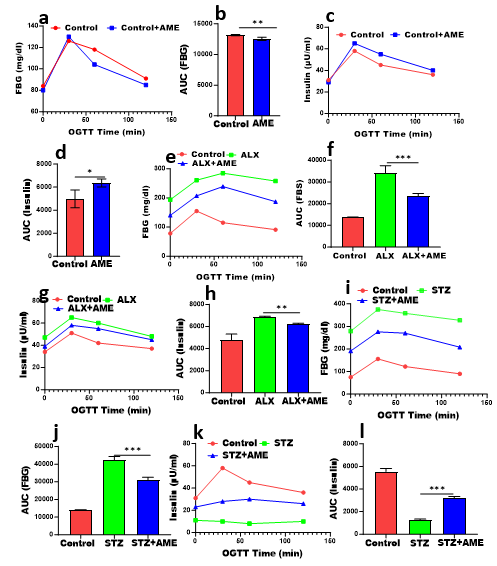
A screenshot of a computer

Description automatically generated

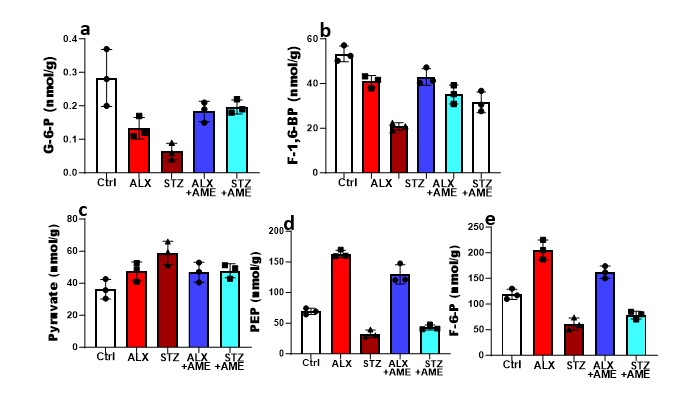
Supplementary file A: Chromatograms showing bioactive components of PhAM



Supplementary Figure 2. a): cell viability assay using different cell lines, (b): LD50 of AME on rats and mice (c-h): fasting blood glucose (FBG), insulin, and HbA1c levels of normoglycemic male and female rats with or without AME treatment.



Supplementary Figure 3 a-c): Fasting glucose, insulin, and AUC after oral glucose tolerance test (OGTT) of normoglycemic rats treated with or without AME (e-h): glucose, insulin, and AUC of rats with or without AME administration for 28 days before alloxan injection. (i-l): glucose, insulin, and AUC of rats with or without AME administration for 28 days before STZ injection.



**Supplementary Figure 4:** Effect of AME administration on metabolites of glycolysis/gluconeogenesis, **a)** Glucose-6-phosphate (G-6-p), **b)** Fructose-1,6-Bisphosphate (F-1,6-BP), c) pyruvate, **d)** Phosphoenolpyruvate (PEP), **e)** Fructose-6-phosphate (F-6-P).

A comparison of a graph

Description automatically generated with medium confidence

**Supplementary Figure 5:** Effect of PhAM administration on **a)** Fructose-2,6-bisphosphate (F-2,6-BP), **b)** Phosphofructokinase-2 (PFK-2)

**Key resource table**

|  |  |  |
| --- | --- | --- |
| **Reagent or resource** | **Source** | **Identifier** |
| **Antibodies** | | |
| **Kits** | | |
| Rat Microsomal Triglyceride Transfer Protein (MTTP) Kit | Abbexa | abx258102 |
| Free Fatty Acid Assay Kit | Abcam | ab65341 |
| Fructose-1,6-Bisphosphatase Activity Assay Kit | Abcam | ab273329 |
| Fructose-1,6-Bisphosphate Assay Kit | Abcam | ab284537 |
| Fructose-6-Phosphate Assay Kit | Sigma-Aldrich | MAK020 |
| Glucose-6-Phosphate Assay Kit | Sigma-Aldrich | MAK014 |
| Glucose-6-Phosphate Assay Kit | Sigma-Aldrich | MAK014 |
| Hexokinase Colorimetric Assay Kit | Sigma-Aldrich | MAK091 |
| Ketone Body Assay Kit | Sigma-Aldrich | MAK134 |
| Phosphoenolpyruvate Assay Kit | Abnova | KA3745 |
| Phosphoenolpyruvate Carboxykinase Assay Kit | Sigma-Aldrich | MAK408 |
| Phosphofructokinase (PFK) Activity Colorimetric Assay Kit | Sigma-Aldrich | MAK093 |
| Pyruvate Assay Kit | Abcam | ab65342 |
| Pyruvate Kinase Activity Assay Kit | Sigma-Aldrich | MAK072 |
| Rat C-peptide Elisa Kit | Sigma-Aldrich | #EZRMCP2-21K |
| Rat GAD2/GAD65 ELISA Kit | Assay Genie | RTFI00792 |
| Rat GLUT2 (Glucose Transporter 2) ELISA Kit | Elabscience | E-EL-R0354 |
| Rat Insulin Elisa Kit | Sigma-Aldrich | RAB0904 |
| **Chemicals, reagents, Recombinant DNA, and diets** |  |  |
| 2-Deoxy-D-glucose | Sigma-Aldrich | D8375 |
| 3-Mercaptopropionic acid | Sigma-Aldrich | M5801 |
| Acetic acid | Sigma-Aldrich | 64-19-7 |
| Alloxan monohydrate | Sigma-Aldrich | A7413 |
| Alloxan monohydrate 98% | Sigma-Aldrich | A7413 |
| Ammonium molybdate | Sigma-Aldrich | 277908 |
| bpV(pic) | Sigma-Aldrich | SML0885 |
| Cacodylic acid | Sigma-Aldrich | C0125 |
| Cetilistat | Selleckchem | S4930 |
| Dextrose Monohydrate | YSHC Doris | YSHC210420 |
| Dulbecco′s Modified Eagle′s Medium - high glucose | Sigma-Aldrich | D5796 |
| Ethylenediaminetetraacetic acid tetrasodium salt dehydrate | Sigma-Aldrich | E6511 |
| Fetal Bovine Serum | Sigma-Aldrich | TMS-013 |
| Formalin Solution | Thermofisher | 50-00-0 |
| Gibco™ Insulin-Transferrin-Selenium (ITS -G) (100X) | Thermofisher | 41400045 |
| Gibco™ Opti-MEM™ | Thermofisher | 51985034 |
| Glucose 6-phosphate disodium salt hydrate | Sigma-Aldrich | G7250 |
| High-Capacity cDNA Reverse Transcription | Thermofisher | 4368814 |
| Hydrochloric Acid, ACS, | Thermofisher | 7647-01-0 |
| L-Ascorbic Acid (Crystalline/Certified ACS) | Thermofisher | A61-100 |
| Shikonin | Sigma-Aldrich | S7576 |
| Sodium (meta) arsenite | Sigma-Aldrich | S7400 |
| Sodium Citrate | Specialty Che. | CN-021Kg |
| Sodium Citrate Dihydrate | Thermofisher | 6132-04-3 |
| Sodium Hydroxide Solution | Thermofisher | 1310-73-2 |
| Sodium orthovanadate | Sigma-Aldrich | S6508 |
| Sodium taurocholate hydrate | Sigma-Aldrich | 86339 |
| Streptozocin | Sigma-Aldrich | S0130 |
| Streptozocin | Sigma-Aldrich | S0130 |
| Sucrose (Crystalline/Certified ACS) | Sigma-Aldrich | 57-50-1 |
| Teklad Global Rat Food Pellets | Inotiv | 2018c, 88137, 02028 |
| Trichloroacetic acid, 99% | Thermofisher | 76-03-9 |

**Study design and statistical analysis**

In this supplement, details of the study protocol, as well as the statistical analysis will be presented. All details regarding the composition of the interventional therapy are redacted, and available only on reasonable request.

**Study protocol**

Phenolic acid fractions of *Anisopus mannii* (PhAM) as a novel add-on therapy for type 2 diabetes: a single-center, double-blind, randomized, placebo-controlled, feasibility trial.

Version number: 1

Date: 2024 – 09 – 01

PACTR Number: PACTR202206531545729

Principal investigator: Peter Amadi

**Confidentiality Statement**

This study protocol and revelations are only for reference purposes only. The confidential information in this protocol belongs to the principal investigator and the Imo State University, and is only provided to you as a principal investigator, co- investigator, and applicable Institutional Review Board/ regulatory authorities for review. It is strictly prohibited to disclose any information herein to any irrelevant third party without prior written authorization from the sponsor, except that explanation for potential subjects to sign informed consent form is necessary.

Table 1: Study administration responsibilities

|  |  |
| --- | --- |
| Roles | Person Responsible |
| Principal Investigator  Co-Investigator  Trial hub/enrolment coordinators  Sponsor/Contract Organization | Peter Uchenna Amadi, PhD  Department of Biochemistry,  Imo State University, Owerri  Tel: +2348061159916  Email: [amadi@imsu.edu.ng](mailto:amadi@imsu.edu.ng); [amadi@imsuonline.edu.ng](mailto:amadi@imsuonline.edu.ng)  pamadi@ualberta.ca  Celestine E. Ekweogu, MD PhD, Professor  Department of Medical Biochemistry,  Imo State University, Owerri  Tel: +2348065213873  Email: [ekweogucelestinen@imsu.edu.ng](mailto:ekweogucelestinen@imsu.edu.ng)  Justice O. Osuoha, PhD  Chidi N. Ekweogu, PhD, MD (Chairman Steering Committee)  Prince C. Odika, PhD  Emmanuel N. Agomuo, PhD  Oluchi Aloy-Amadi, PhD  Imo State University, Owerri  Okigwe Road, IMSU Jnc, Owerri, Imo State, Nigeria  Fobs@imsuonline.edu.ng |
| Study location | Central clinical trial/outpatient center, Department of Medical Biochemistry, Imo State University, Owerri. |

**Abbreviations**

PhAM – Phenolic acid fractions of Anisopus mannii

AME – Anisopus manii

T2D – Type 2 diabetes

IMSU – Imo State University

FBG – Fasting Blood Glucose

HbA1c – Glycated hemoglobin

AUC – Area Under Curve

STZ – Streptozotocin

OGTT – Oral Glucose Tolerance Test

ALX – Alloxan

HFD – High fat diet

G-6-P – Glucose 6 Phosphate

F -1,6-BP – Fructose-1,6-Bisphosphate

F-6-P – Fructose-6-Phosphate

PEP – Phosphoenolpyruvate

PFK-1 – Phospohofructokinase 1

HOMA-IR - Homeostatic model assessment of insulin resistance

BMI - Body mass index

HIV – Human immunodeficiency virus

ALT – Alanine amino transferase

AST – Aspartate amino transferase

ALP – Alkaline Phosphatase

GGT – Gamma glutamyl transferase

TB – Total Bilirubin

CB – Conjugated Bilirubin

TP – Total protein

ALB – Albumin

IgM anti-HBc - IgM antibody to hepatitis B core antigen

HBsAg - Hepatitis B surface antigen

TC - Total cholesterol

LDL-C - Low density lipoprotein cholesterol

TG – Triglyceride

VLDL-C - Very low density lipoprotein cholesterol

HDL-C- High density lipoprotein cholesterol

SOD - Superoxide dismutase

HCG - Human chorionic gonadotrophin test

FDA – U.S. Food and Drug Administration

PPAR-a – Peroxisome proliferator-activated receptor alpha

CPT1-a - Carnitine palmitoyltransferase I alpha

**Study authentication**

On behalf of every co-investigator, I append my signature in affirmation that this protocol clearly describes all the activities undertaken in this study, and that I take responsibility of describing this study to every other personnel contributing to this study. By signing this declaration, I agree that this study in all its forms complies with the study protocol, informed consent, institutional and registry approval, and all relevant national and international laws and regulation.

A reasonable concession to make is that PhAM stimulates PFK-1 as a potential mechanism for potent glycemic control as observed in the preclinical results. Because PhAM contains the several phenolic acids, it is possible that these individual phytocompounds contribute in several ways to produce potent glycemic control.

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Peter Amadi Date

Principal Investigator

**Protocol Synopsis**

|  |  |
| --- | --- |
|  |  |
| Study title | Phenolic acid fractions of *Anisopus mannii* (PhAM) as a novel add-on therapy for type 2 diabetes: a single-center, double-blind, randomized, placebo-controlled, feasibility trial |
| Trial Number | PACTR202206531545729 |
| Principal Investigator | Peter Amadi |
| Background of study | As of 2020, 6 million Nigerians are living with diabetes mellitus. In 2015 alone, 40,000 Nigerians died as a result of diabetes and its complications, a figure likened to a tip of an iceberg given that that two-thirds of diabetes cases in Nigeria are yet undiagnosed. As a result, the use of herbal medicine alone or alongside prescription drugs for its management is quite common in Nigeria and most African countries. *Anisopus mannii* is a native Nigerian herb called the "diabetes killer" as it is popularly used for the management of Type 2 diabetes. Using various cell lines, our group discovered that the phenolic acid fractions of this herb (PhAM) serves as an agonist for the glycolysis enzyme called phosphofructokinase 1. In mice, the PhAM protected beta cells from streptozotocin induced destruction, justifying the current aim of this trial to investigate the PhAM in patients with Type 2 diabetes. |
| Primary objective | The primary objective of this study is to assess the potentials of combining PhAM with regular antidiabetic drugs, on the glycemic control of patients with type 2 diabetes (T2D). The primary endpoint will be the changes in HbA1c levels, and fasting blood glucose levels, in response to six months administration of PhAM, relative to the placebo group. |
| Secondary objectives | The secondary objectives of this study will be to measure the effects of the six months administration of PhAM on insulin levels, The homeostatic model assessment of insulin resistance (HOMA-IR), and the body mass index (BMI) levels of the patients with T2D, relative to the placebo. |
| Study design | This study is designed as a proof of concept, single-center, double-blind, randomized, placebo-controlled trial. We will recruit 108 study participants with clinical diagnosis of type 2 diabetes, with HbA1c >6.5% and history of good compliance to first line diabetes medications. One third of these participants will be randomized into the placebo group that will instead of PhAM, receive placebo with similar physical features of the intervention. The remaining patients will be randomized into two groups that will either receive 12 mg PhAM or 20 mg PhAM every 48h for 6 months |
| Study population | Patients with T2D, confirmed through the standard clinical diagnosis for T2D. |
| Number of subjects | Study participants were recruited to generate data that can sufficiently power a Phase 3 trial. The study is set out to recruit 108 participants including a 20% attrition. |
| Inclusion criteria | Evidence of clinical diagnosis of T2D;  - On the spot HbA1c ≥6.5%, and fasting blood sugar > 130 mg/dl  - Clinical history of compliance with first line T2D treatment  - between 40 – 75 years of age  - willingness to take PhAM  - written informed consent |
| Exclusion criteria | - History of diabetic ketoacidosis, type 1 diabetes, or secondary diabetes.  - Involved in planning or dissemination of this study or the participation in other clinical trials.  - Treatment with insulin therapy or incretins  - Smoking, pregnancy, breastfeeding, use of steroids or any immunosuppressive or immunomodulatory therapies.  - History of acute cardiovascular events, heart failure, chronic pancreatitis, or liver cirrhosis.  - Use of any herbal drugs including *Anisopus mannii*, 2 months before trial commences  - Blood pressure above 170/110 mm Hg  - Lactose intolerance or malabsorption  - History of any other metabolic disease that complicates the pathological features of type 2 diabetes  - Active hepatitis b or c, HIV, tuberculosis, and systemic infections.  - Any condition or treatment that in the judgment of the investigator makes it difficult or unsafe to participate in the study. |
| Study medication | Oral administration of a dose titration of 5.0 mg weekly up to 20 mg of PhAM for a month, and maintained on either 12 mg or 20 mg of PhAM, every 48h for the 6 months of administration. Saline in the form of sodium chloride 0.9% w:v solution was orally administered as placebo. |
| Study duration | December 2022 – January 2024 |

Table 2: Study Schedule

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Screening | | Run-in | Randomization | | Intervention | | | | | | | | | Follow-Up | |
| Visit number | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visit 7 | Visit 8 | Visit 9 | Visit 10 | Visit 11 | Visit 12 | Visit 13 | Visit 14 | Visit 15 | Visit 16 |
| Visit time (weeks) | -5 | -3 | - 3 | -1 | 1 | 2 | 4 | 6 | 10 | 14 | 18 | 22 | 24 | 28 | 36 | 44 |
| **Study Procedures** | | | | | | | | | | | | | | | | |
| Demographics | X | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Medical history | X | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Inclusion/exclusion criteria | X | X |  | X | X |  |  |  |  |  |  |  |  |  |  |  |
| Informed consent | X | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Randomization |  |  |  | X | X |  |  |  |  |  |  |  |  |  |  |  |
| Treatment emergent AE’s |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Physical assessments | X | X |  |  |  | X | X | X | X | X | X | X | X | X | X | X |
| **Laboratory assessments** | | | | | | | | | | | | | | | | |
| Fasting blood glucose | X | X |  |  |  | X | X | X | X | X | X | X | X | X | X | X |
| HbA1c | X | X |  |  |  | X |  |  |  | X |  |  |  | X | X | X |
| Urinalysis | X | X |  |  |  | X | X | X | X | X | X | X | X | X | X | X |
| Liver function test | X | X |  |  |  | X |  |  |  | X |  |  |  | X | X | X |
| Renal function test | X | X |  |  |  | X |  |  |  | X |  |  |  | X | X | X |
| Liver echogeneicity | X | X |  |  |  |  |  |  |  |  |  |  |  | X |  |  |
| Virology test | X | X |  |  |  |  |  |  |  |  |  |  |  | X |  | X |
| Lipid Profile | X | X |  |  |  | X |  |  |  | X |  |  |  | X | X | X |
| Antioxidant enzymes |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Pregnancy test | X | X |  |  |  |  |  |  |  |  |  |  |  | X |  |  |
| **Quality assurance** | | | | | | | | | | | | | | | | |
| Sensitization | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |
| Subject diary | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |
| Distribution of drugs |  |  | X | X | X | X | X | X | X | X | X | X | X | X |  |  |
| Drug compliance check |  |  | X | X | X | X | X | X | X | X | X | X | X | X |  |  |

* The visitation should only involve a selected recruitment team (appointed by the trial steering committee chairman), made up of clinicians not recognized as contributors with 1 trial hub managers. This is a blinding requirement.
* The visitation team should be blinded from distinguishing between placebo and the interventional drug.
* All assessments are to occur during normal working hours. In case of any unforeseen or holiday causes of disruption of the visit or working schedule, the next working day will become an acceptable visitation. A note about the disruption must be documented and sent to the trial monitoring unit of the institution.
* Any intentional disruption of the normal working schedule will be recorded as protocol deviation.
* All assessment pre or post intervention must be in fasting state. i.e. overnight fast, with the assessment taking place from 8 am to 9 am.
* An option of receiving a self monitoring glucose device should be offered to all the study participants.
* No incentives are to be offered. Willingness to participate is a mandatory inclusion criterion.
* At each study visit, an overview of the trial should be availed to the participants.
* Only two visitations are allowed to complete the official study recruitment. All the inclusion criteria tests must be concluded within these two visitations. Costs for additional recruitment visitation are not covered, except only by concession by the study principal investigator.
* Medical history is collected through the participant’s hospital records PLUS the questionnaire provided on the spot. Informed consent must be provided before any assessment or recruitment can occur.
* Before run-in or randomization, all inclusion/exclusion criteria must be satisfactorily addressed. In case of a required rescreening for any of the less weighted inclusion criteria, this must be rescheduled for the second visit.
* Randomization is the duty of the IT team of the University, in keeping with the University’s oversight on reducing bias in clinical trials. Randomization is typically done using an interactive response technology.
* Only the placebo is to be administered during the run-in period.
* Fasting blood sugar must be performed on the spot using a glucometer. Plasma samples are to be safely stored for laboratory analysis.
* HbA1c – glycated hemoglobin must be conducted on the spot during visit 1 and 2, and after every 3 months’ post intervention until the completion of follow-up visitations.
* Urinalysis includes: protein, ketones, pH, glucose, and erythrocytes.
* Liver function tests include: Liver enzymes; ALT – Alanine amino transferase, AST – Aspartate amino transferase, ALP – Alkaline Phosphatase, GGT – Gamma glutamyl transferase, TB – Total Bilirubin, CB – Conjugated Bilirubin, TP – Total protein, ALB – Albumin, while liver echogenicity measurement includes liver ultrasound.
* Renal function test included: Urea and creatinine, plasma electrolytes, erythrocyte sedimentation rate
* Virology test include: human immunodeficiency virus antibody, hepatitis A virus IgM antibody, IgM antibody to hepatitis B core antigen (IgM anti-HBc), and hepatitis B surface antigen (HBsAg).
* Lipid profile include: Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglyceride, very low density lipoprotein cholesterol (VLDL-C), and high density lipoprotein cholesterol (HDL-C).
* Antioxidant enzymes test include: Catalase, superoxide dismutase (SOD), and malondialdehyde (MDA).
* Pregnancy test: Human chorionic gonadotrophin test (HCG).
* Sensitization and compliance: The clinicians that manage the administration of the intervention are tasked to assess the lifestyle habits that could compromise the efficacy of diabetes medications in general. Where there are concerns, the clinicians should establish regular engagements with the affected participants to moderate the lifestyle habit and or consideration of exercise.
* In the same manner the trial hub managers are tasked with ensuring adequate compliance to the administration of this intervention. Being an oral intervention, the trial hub managers are to coordinate the compliance field staff to ensure any of the dedicated compliance mechanisms like telephone and personal visits are achieved at least, once every week. The visit team are to assess compliance records at every visit, and submit a brief summary of the compliance report to the Chairman of the trial steering committee.
* For any participant that misses the target dose for a week or less, the drug can be resumed for the next week. If dose is missed for two consecutive weeks, a special dosing calendar must be initiated to include more frequent in-person administration, until a match is made with the actual dosing calendar. The trial hub clinician must initiate adequate monitoring and make a report on this available to the visitation team, at the next visit. A missed dose case of three weeks or more meets the criteria for study exclusion, decided by the visitation team, the chairman of the trial steering committee, and the principal investigator.

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**BACKGROUND AND RATIONALE**

Currently, many popular anti-hyperglycemic monotherapies are becoming less successful in achieving the glycemic goals of patients with type 2 diabetes (T2D), and has even become dire as the glycemic targets are frequently lowered.1,2 Due to the complexities and complications associated with T2D, more patients even when the glycemic goals are achieved, require additional treatment plans to maintain a long-lasting stable glycemic control.3 As recommended by standard guidelines, metformin is the most preferred first-line medication for T2D.4 Metformin targets hepatic glucose output as a predominant mechanism for its anti-hyperglycemic effects.5 However, the progressive deterioration of β-cell function often causes the loss of efficacy of metformin monotherapy, which necessitates the combinatorial administration of additional anti-hyperglycemic medications.6,7 Several combinatorial treatment plans involving other drugs with various mechanisms of actions are approved by the FDA. Yet, the achievement of glycemic goals to the recommended levels remains an unmet goal in the treatment of T2D as approximately 537 million adults worldwide are still living with diabetes.8

The use of naturopathic medicine, especially herbal medicine, has emerged as a promising option for the management of diseases with complex pathological features, such as T2D.9-12 Many current orthodox drugs have their origin from herbal medicines. The history of metformin may be traced to the use of *Galega officinalis* (goat's rue or French lilac) in medieval Europe to treat symptoms of diabetes.13 Despite this, most popular herbs are yet to gain acceptance into orthodox care because research on these herbs are often carried out by researchers with infrastructural limitations, and as such, do not apply convincing scientific rigor required to gain global acceptance. Herbs contain well characterized bioactive components, and unlike orthodox monotherapies, the potentials of the synergistic effect of several bioactive constituents of these herbs, can be harnessed to provide better efficacy for diseases with multiple aetiologies, such as T2D.

There is increasing evidence to show that the anti-hyperglycemic activities of various herbs are closely related to their insulin-secretagogue activities.14-16 However, most of these studies fail to progress to clinical trial due to the inability to identify which bioactive compounds elicit these anti-hyperglycemic effects, and in addition identify their mechanisms of actions. *Anipsopus mannii*, popularly known as the “diabetes killer” in Nigeria, deserves recognition for its therapeutic benefits. The flowering species grows in the tropical environments of central Africa, and is a renowned traditional Nigerian medicine applied for its potent anti-hyperglycemic effect.17-19 Interestingly, our preliminary data have shown that the herb contains compounds that potently activates phosphofructokinase 1 (PFK-1), among other possible mechanisms, which leads to a significant glycemic control. We further uncovered that the phenolic fractions of this herb, contains therapeutic doses of ferulic acid, gallic acid, protecathechuic acid, and syringic acid that activates PFK-1 and synergistically produces potent anti-hyperglycemic effects. PFK-1, an allosteric enzyme controlled by many activators and inhibitors, is the rate limiting enzyme of the glycolytic pathway.20 This means that the activities of PFK-1 directly controls glucose uptake and breakdown, and indirectly controls other pathways involved in glucose metabolism.

Several drugs developed to target enzymes of the glycolytic pathways have shown promising efficacies in improving glucose sensitivities in patients.21,22 Small molecule activators of glucokinase are popular drugs in this category, and are well studied for their add-on effects on insulin, glucagon, and incretins thereby improving the maintenance of glycemic homeostasis in patients with T2D.23-25 Notwithstanding the promising anti-hyperglycemic outcomes of targeting glycolytic enzymes, the druggability of PFK-1 is underexplored leading to scarce literature on the efficacy of modulators of PFK-1 in type 2 diabetes.

Given our robust preclinical trial, we test the hypothesis that PhAM combined with metformin achieves a glycemic control better than metformin monotherapy.

**Summary of results from pilot study**

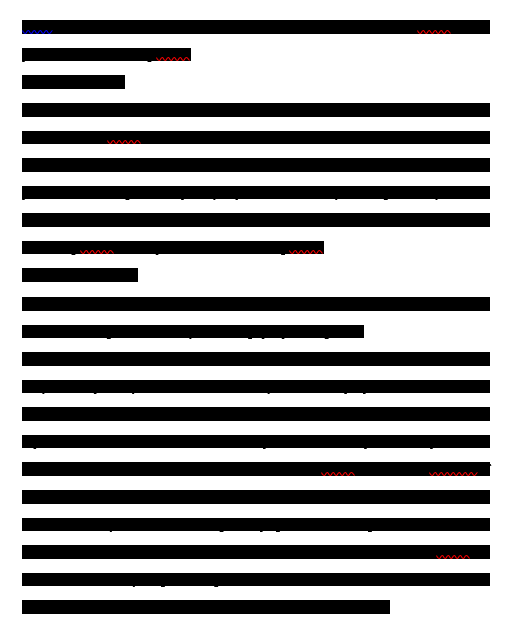
This is the first study to discover that phenolic acid fractions from *Anisopus mannii* (PhAM) can modulate the activities of phosphofructokinase 1 (PFK-1), the rate limiting step of glycolysis. Till this study, the druggability of PFK-1 is not yet reported, hence no study has been undertaken to assess the potentials of agonist of this enzyme to improve glycemic control. Hexokinase, another regulatory enzyme of the glycolytic pathway, is a target of metformin, a well studied drug used as a first line treatment for type 2 diabetes. In addition to this, PhAM also showed potent protection to beta cells in both alloxan and streptozotocin models of diabetes. Another separate study is currently exploring the potentials of PhAM to decrease the dependence on insulin gargine in patients with type 1 diabetes.

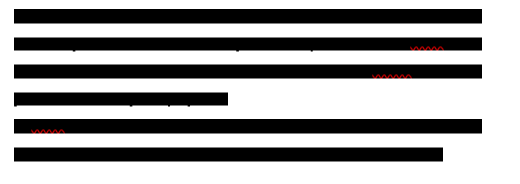
* Among other compounds, PhAM contains 2.14 mg/100g of protocatechuic acid, 0.05 mg/100g vanillic acid, 0.02 mg/100g p-hydroxybenzoic acid, 7.24 mg/100g gallic acid, 19.3 mg/100g ferulic acid, 5.3 mg/100g syringic acid, 0.007 mg/100g piperic acid, and 0.02 rosmarinic acid.
* Diabetic rats treated with the whole extract of Anisopus mannii (AME), showed significant improvements in hexokinase (HK), phosphofructokinase (PFK-1), and pyruvate kinase (PK). However, in vitro, at all concentrations, AME showed no significant effect on HK and PK in the presence and absence of 2-Deoxy-D-glucose (a potent HK inhibitor) or shikonin (a potent PK inhibitor). However, AME counteracted the inhibitory effect of sodium orthovanadate on PFK-1 activity. This implied that the bioactive components of AME may modulate the activities of PFK-1 without a direct effect on hexokinase or pyruvate kinase. Different fractions of AME was then examined for any potential agonist effect on PFK-1, and the phenolic acid fractions (PhAM) significantly modulated the activities of PFK-1.
* Using a Western type diet model of diabetes, a clinically relevant model of diabetes, PhAM administration significantly lowered the fasting blood sugar levels and HbA1c, with a marked reduction in the OGTT glucose levels after 90 min.

**Description of the interventional drug**

**Composition**







**Potential benefits**

Patients from poor resource settings do not have the sufficient resources or access to rigorous management for T2D. More so, the first line therapy for T2D still leaves more to be desired with regards to achieving target glycemic control in majority of the populace. As a result, the discovery of potent new drugs to supplement the first line treatment is overdue. Currently, no drug has shown any potentials to exploit the druggability of phosphofructokinase 1, despite being the rate limiting step of the glycolytic pathway. An important benefit of this study was the development of a new therapy that exploits a new druggable target to achieve a significant glycemic control. Based on the preclinical results, PhAM has the potentials of being integrated into orthodox diabetes management due to its profound preclinical efficacy on ameliorating type 2 diabetes.

**AIMS OF THE STUDY**

**Primary objective**

The primary objective of this study is to assess the potentials of combining PhAM with regular antidiabetic drugs, on the glycemic control of patients with type 2 diabetes (T2D). The primary endpoint will be the changes in HbA1c levels, and fasting blood sugar levels, in response to six months’ administration of PhAM, relative to the placebo group. These changes in the primary objective will be analyzed using One-Way ANOVA independently comparing across the HbA1c levels or fasting blood sugar levels of the placebo, the patients treated with 12 mg PhAM, and the patients treated with 20 mg PhAM.

**Secondary objective**

The secondary objectives of this study will be to measure the effects of the six months’ administration of PhAM on insulin levels, the homeostatic model assessment of insulin resistance (HOMA-IR), and the body mass index (BMI) levels of the patients with T2D, relative to the placebo. These changes in the primary objective will be analyzed using One-Way ANOVA comparing across the insulin levels, HOMA-IR, or BMI levels of the placebo, the patients treated with 12 mg PhAM, and the patients treated with 20 mg PhAM.

**Exploratory objectives**

The bi-weekly continuous glucose monitoring 1 month before and after the study will be the measurement for glucose variability, and average postprandial glucose.

Because we see a significant improvement in the plasma and hepatic triglycerides in the preclinical study, other exploratory measurements of this study will include lipid profile, liver ultrasound to measure the liver echogenicity, liver function enzymes, and blood ketone bodies. We intend to explore the blood ketone bodies because in the preclinical trial, we performed hepatic VLDL secretion assay and oral lipid tolerance assay to verify that PhAM did not affect lipidation of chylomicrons or secretion into the lymph or hepatic TG secretion, but rather upregulates hepatic mitochondrial fatty acid oxidation through the upregulation of PPAR gamma and CPT1a. This upregulation then increased the levels of plasma ketone bodies. The rats administered PhAM also showed decreased body weight with significant fecal fat accumulation, and as a result we will collect stool samples from the patients to measure fecal fat and triglycerides.

Our collaborative effort with Fiona Gribble of Cambridge revealed that STC-1 cells treated with this herb improved GIP secretion, and in our preclinical study, rats administered PhAM showed marked improvements in GIP and GLP-1, and for this we will assess the incretin levels of these patients as additional exploratory objectives.

As PhAM is a potential drug not currently used in orthodox clinical practice, these exploratory measurements are aimed to reveal any additional findings for potential follow-up studies.

**Potential risks**

The preclinical study clearly shows that the steatorrhic stool was a major adverse effect, leading to weight loss. PhAM inhibited intestinal lipase in rats leading to the impairment of intestinal uptake, with steatorrhic stool as a major consequence. This consequence is common among weight loss drug classes, a popular one being orlistat. The treatment-emergent adverse events (TEAEs) related to PhAM treatment were listed in Table 1 below. 12 mg of PhAM (PhAM 1) caused severe adverse effects on 13 % of participants with 9 % hospitalized, whereas 20 mg of PhAM (PhAM 2) caused severe adverse effects in 16 % of participants, 11 % of whom required hospitalization. All hospitalizations were reported to be due to severe diarrhea. No deaths or adverse effects requiring study withdrawal occurred from PhAM administration. The most reported TEAES was diarrhea and nausea, reported by 50 % of participants in PhAM 1 and 72 % of participants in PhAM 2, the majority of which occurred < 4 months without any dose adjustment. Approximately 5 % of participants in the placebo group experienced appetite loss. Therewas also an increased appetite loss (14 % and 39 % in PhAM 1 and in PhAM 2 respectively). All these effects were transient and mildly severe. Feverish condition and sleepiness reported by participants did not appear to be related to the study, as the majority occurred in the placebo group. 27 % of participants in PhAM 1 and 33% of participants in PhAM 2 reported occasional tiredness, while one participant in the PhAM 2 group showed tiredness with occasional vomiting. Two patients in PhAM 1 or 2 experienced constipation and abdominal distension, while 4 patients reported flatulence and eructation. None of these participants had dyspepsia, palpitations, or hypoglycemia.

**Table 1: Baseline demographic characteristics of study participants**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Placebo (n=21)** | **12mg PhAM (n=22)** | **20mg PAEAM (n=18)** |
| Sex (%) |
| Male | 13 (62) | 7 (32) | 8 (45) |
| Female | 8 (38) | 15 (68) | 10 (55) |
| Average Age (yr) | 52.9 | 50.8 | 52.0 |
| Average duration of diabetes (yr) | 7.5 | 8.6 | 7.9 |
| Drugs |  |  |  |
| Metformin | 17 | 14 | 13 |
| Metformin +  Sulphonylureas | 3 | 8 | 5 |
| Incretins | 0 | 0 | 0 |
| Insulin therapy | 0 | 0 | 0 |
| Herbal drugs | 0 | 0 | 0 |
| Body weight (g) | 94.3 | 96.7 | 94.7 |
| Height (cm) | 166.2 | 170 | 167.6 |
| BMI (kg/m2) | 33.8 | 33.3 | 33.5 |
| HbA1c (%) | 7.6 | 7.7 | 7.7 |
| Glucose (mg/dl) | 183 | 184 | 182 |
| Insulin (μU/mL) | 18.0 | 17.8 | 17.2 |
| HOMA-IR | 8.1 | 8.0 | 7.7 |
| Blood pressure (mm/Hg) |  |  |  |
| Systolic | 127.9 | 126.8 | 127.0 |
| Diastolic | 74.3 | 75.6 | 74.4 |
| Pulse rate (bpm) | 77.7 | 77.6 | 76.2 |

Data are n (%) or mean. HOMA-IR = (glucose x insulin)/405. BMI, body mass index. HbA1c – glycated hemoglobin

**Primary efficacy outcome**

The primary efficacy outcome of this study is the change in HbA1c levels and fasting blood sugar levels between the placebo and treatment groups.

**Secondary and exploratory efficacy variables**

The secondary efficacy variables of this study measures the changes that occurs in insulin levels, insulin sensitivity index, and BMI of patients treated with 12 mg or 20 mg of PhAM compared to the placebo group, over the course of 6 months’ intervention. Exploratory variables will include changes in average postprandial blood glucose and time in range as measured by continuous glucose monitoring, GLP-1, and GIP, lipid profile; total cholesterol, low density lipoprotein cholesterol, triglycerides, and high density lipoprotein cholesterol, liver function enzymes; alanine transaminase, aspartate transaminase, gamma glutamyl transaminase, alkaline phosphatase, total bilirubin, conjugated bilirubin, total protein, and albumin, kidney function indices including urea and creatinine and plasma electrolytes, and hematological indices including red blood cell counts (RBC), hemoglobin concentration (HB), white blood cell count (WBC) and differentials, and blood platelets count (PLT). These changes will be assessed through the comparison among the placebo and the two treatment groups of this study.

**Safety variables**

As this is the first study to explore the therapeutic effect of PhAM, a robust and thorough safety profile will be required to enable any potential integration of this drug into the clinical management of diabetes. Among the placebo group, and the patients administered 12 mg or 20 mg of PhAM, we will monitor the occurrence of serious adverse effects with or without hospitalization, any deaths, adverse effects that leads to study withdrawal, the occurrence of persistent diarrhea and diarrhea greater than 6 months, loss or decrease in apetite, nausea, cough, fever, muscle and back pain, sleepiness, tiredness, weakness, vomiting or constipation, abdominal distension, flatulence and eructation, dizziness, rash, dyspepsia, palpitations, and hypoglycemia. Other safety variables will include measurement of blood pressure, and pulse rate as indicators of cardiovascular health.

Patients who are excluded from this study because of any of these criteria, will be offered a follow-up by the physicians affiliated to Imo State University or their own physician with additional screening to determine if the adverse effects were indeed related to the administration of PhAM, and thus to be administered relief medications in line with standard clinical practice. Any patient excluded from these study due to adverse reactions will not be included in the study, however the data collected will be recorded for any future reference.

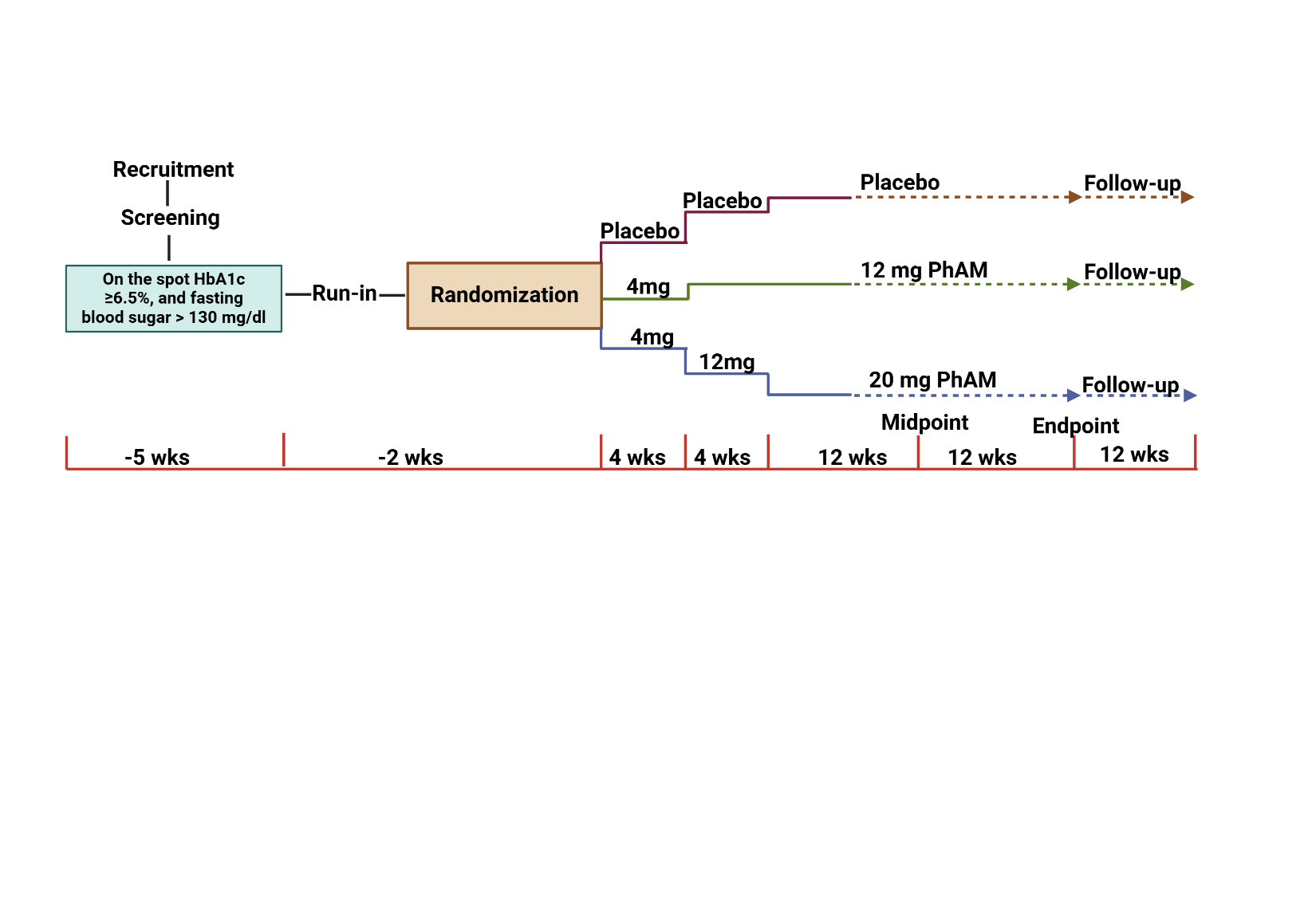
**Other variables that are not study outcomes**

Together with the primary, secondary, and exploratory measurements, we will also collect demographic information on sex, age, diabetes duration, type of first-line diabetes treatment currently used, any incretin or herbal drug use, and height to ensure that none of these variables can confound the results of the primary, secondary, and exploratory measurements.

In addition to the outcome and safety variables, we will collect data on age, gender, time since diabetes diagnosis, diabetic complications, metformin dose, Hb (to ensure that changes in Hb do not confound).

**Study design**

This study is a randomized placebo-controlled trial that will be open labelled with fixed stratification variables in the dosing of the interventional drug. The study compares the glycemic control of patients either exclusively on first line anti-diabetic medications or in combination with PhAM intervention, randomly stratified into 20 mg or 40 mg dosing arms. Approximately 72 subjects that meets the inclusion criteria will be recruited and will receive a 2 week placebo run-in before randomization into the placebo, 12 mg, or 20 mg study arms in a ratio of 1:1:1. Participants in the intervention arm will receive the 4 mg of PhAM for 4 weeks, and if no safety concerns were recorded, the patients will receive an increased dose of 12 mg. The dose will be increased for the patients in the 20 mg PhAM arm, to 20 mg PhAM, if no safety issues was recorded with 12 mg PhAM. In brief, either 12 mg or 20 mg intervention will be titrated from 4 mg PhAM every 4 weeks based on the absence of any major adverse outcomes. The final dose, either 12 mg PhAM or 20 mg PhAM will be maintained for 24 weeks. The participants will attend three screening visit until the administration of the last intervention followed by follow-up visits at 0, 2, and 4 months. At the first and last study visit they will undergo an OGTT. HbA1c will be measured at all study visits. Also, some biological samples like stool, blood, and urine will be and stored for analyses of metabolites and proteins as exploratory parts of the study.



**Study flow chart**

**Dose Modification Criteria:**

1. A study participant in the 20 mg group who after reaching the required dose titration cannot tolerate the 20 mg of PhAM. An anonymized dose reduction case should be initiated by the clinician in charge of the patient and submitted to the lead of the visitation team who will then consult with the trial steering committee. The case will be considered for reduction to 12 mg PhAM and the patient will be considered in the 12 mg PhAM arm of the study.
2. A study participant in the 12 mg group who cannot tolerate the 12 mg of PhAM, even after reaching the titration dose will have two options. An exclusion from the study will be upheld if recommended by the managing clinician. A second option will entail that the patient will remain on 4 mg PhAM if tolerated and only provided 12 mg PhAM for 12 weeks. The information obtained from the patient must only be used for midpoint data. A recommendation for exclusion is most encouraged, especially if adverse effects cannot be managed.

**Screening visit**

Study participants will be recruited through the established information dissemination mechanisms of the institution, as coordinated by the clinical trials office. Participant who show up for the study will receive oral and written information and by signing the informed consent the patient will undergo screening process. As a first step the capillary HbA1c will be measured and analysed at the study site. Only those who have HbA1c ≥6.5% and >130 mg/dl fasting blood glucose will proceed to further control of study criteria, and if they are fulfilled, the venous blood will be sampled. This procedure is to make screening as effective as possible. Re- screening will be allowed once in the next study visit. The managing clinician of the hub will obtain written informed consent and check study criteria while capillary and venous sampling will be done by research nurses or phlebotomists. After completion of screening and signing of informed consent, the participant will get a screening number, all the specified inclusion and exclusion criteria are checked by the clinician|, the medical history is collected including time since diabetes diagnosis, diabetic complications (retinopathy, neuropathy, nephropathy and other complications and current medication (name and dose. The vitals should then be measured especially the blood pressure and pulse rate are measured. Then, the venous blood samples should be drawn for analysis of liver and kidney function, lipid profile, and antioxidant assays (later in the laboratory). Not more than 4 ml of blood volume should be drawn at any point. A pregnancy test is done for premenopausal women. The participant should then be scheduled for run-in which will normally be within 4 weeks of meeting the inclusion criteria. The participant should be offered a hand held glucometer and strips for glucose monitoring.

If any necessary information from the medical history is unavailable to the screening personnel, efforts should be made to have the patient consent to obtaining this information from the hospital where the screening was done. After the screening exercise, if any of the primary, secondary or exploratory measurements that can confound the study enrolment, do not meet the study criteria the patient will be contacted for rescreening. Any cancellation decision will be made by the clinical steering committee except when there are abundance of suitable study participants. If cancellation is initiated, a letter is sent to the patient’s physician managing his/her diabetes with information on the study and that the patient has been discontinued from study participation, and all patient data made available to the physician for any intended follow-up. Data from the screening visit are anonymized and recorded on the study sheets, which will be entered into the trial database. The participant receives a screening number (with D-(hub number)-(patient number). For instance, if the hub number is 3 and patient is 11, the code is D-3-11.The code list for screening number and personal ID is kept at the study site. Only subjects who have signed the informed consent form should be included on the logs and receive a screening number.

When screening failure is encountered, those who attend the screening visit, sign the informed consent but are not eligible to participate will continue to be managed by their regular physician. If necessary, the subject will be referred to relevant healthcare clinic to follow up abnormalities discovered at the screening visit. All reasons for screening failure should be documented, including those that occur during run-in.

**Randomization Visit**

The participants are asked not to attend any randomization visit if contraindications to the inclusion criteria has been met. In addition, intense physical activity or above moderate alcohol consumption 24 h before the visit is discouraged. The participants must be fasting from 10pm the previous day. Nicotine users should not have used nicotine the same day. Data collected will form the baseline data.

The following will be done at randomization visit:

• Personal ID is checked

• Information on any changes of medication or disease status since last visit is collected

• The participant receives a study ID (different from the screening ID)

• The samples with morning urine is collected and frozen for subsequent analyses of urine biomarkers.

•Recording of AEs

• Physical characteristics measurement including: length, weight, waist circumference, blood pressure and pulse rate are measured

•Venous fasting blood samples are drawn for analysis of the primary, secondary, and exploratory outcomes. Total blood volume should not exceed 4 ml.

•An oral glucose tolerance test (OGTT) is conducted at which patients drink 75 g glucose dissolved in water. Venous blood samples are drawn at 0, 30, 60, 90 and 120 minutes for glucose, insulin, c-peptide, and glycated hemoglobin.

• Participants complete a questionnaire on lifestyle habits

• Any fresh stool sample is collected.

•The patient is enrolled to receive either placebo, 12 mg PhAM, or 20 mg PhAM. Enrolment is the responsibility of the visitation team led by trial hub coordinators who are expected to be blinded from the participants.

•The patient receives study medication for the coming 3-month period and information about the medication. However, as a consequence of blinding, the intervention bears similar appearance with the placebo, and only the identification drug code A, B, or C will be available to the administering physician. The patient also receives a diary to mark each time they have taken a dose.

• A time is scheduled for weekly telephone contact or in person visit by the compliance field workers.

• The patient also receives a card about the study to show in contacts with healthcare professionals, including the regular physician and nurse managing their diabetes. Data from the visit are recorded on the report form, except for blood data analyzed at the central laboratory, which will be entered into a secure database. Subsequent visits should ideally be scheduled on the same weekday as the first visit. If that is not possible, e.g. because of public holidays or unavailability of the participant to come on an assigned day, the subsequent visit will be scheduled as soon as possible three months after the previous visit. Reasonable measures should be taken to schedule the visits at three month intervals but a delay of up to 2 weeks is acceptable to accommodate for travels, sudden illness etc. The participant will be reminded by email or mobile text message before next visit.

In between visits, the participant continues taking all their regular medications and those offered by the compliance field officers during the study. They are instructed not to change their metformin dose or general lifestyle habits (e.g. overall dietary pattern) during the study and contact us before any such changes are done. The study team will contact the participants by phone on account of a report of adverse effects from the field compliance officers. Adverse events are noted, and appropriate follow-up is initiated. Reasonable efforts should be undertaken to get in contact with the participants by calling back if no reply and/or sending emails. Participants could contact us at their own initiative in case they have any suspected side effects of the treatment or experience problems with the intervention, including the placebo. Patients may also be contacted by phone at additional time points to follow-up on adverse events, compliance or injection problems. Patients who at any visitation during the intervention period meet any of the above listed safety concerns will be followed up with additional physical or metabolic screens. If any value is still above these limits appropriate medication will be initiated, and both their original physician and the trial physician will workout the best practice for the participant.

**Questionnaire**

The participants are instructed not to make any major lifestyle changes during the study (e.g. major changes of dietary pattern, initiation of weight reduction programmes etc.). We will assess lifestyle habits by a questionnaire, which will assess the following:

• Physical activity using the short IPAQ (international physical activity questionnaire), which is a commonly used scale to assess physical activity.

• Dietary habits using items that have been validated in Stockholm health questionnaire 2010.

• Tobacco and alcohol, by validated items previously used by the Nigerian Agency for Food Drug Administration and Control (NAFDAC).

• Control of Eating Questionnaire will comprise 21 items designed to assess the intensity and type of food cravings, as well as subjective sensations of appetite and mood. We will use 19 of its items to assess the subscales of craving control, positive mood, craving for savoury and craving for sweet. This is of relevance because both PhAM may affect appetite. The questionnaires take an estimated 15-20 minutes to complete.

**Blood sampling**

The clinicians should make an assessment of the available results with regard to clinically significant abnormalities. The laboratory reports should be signed and retained as source data for laboratory variables. Venous HbA1c, Hb, creatinine, cystatin C, Na, K, albumin, ASAT, ALAT, ALP, bilirubin, lipids (HDL, LDL, total cholesterol, triglycerides), insulin as well as urine albumin/creatinine index are analysed at the Medical Biochemistry Lab of the Imo State University or at the University of Port Harcourt Teaching Hospital, depending on closeness to trial hub. Venous blood glucose is analysed directly by BC300 semi automatic biochemistry blood analyzer. Capillary HbA1c at screening visit is analysed by a Bayer DCA 2000+ Blood Analyzer according to the manufacturer’s instructions. All these samples are analysed within the same day as the visit and destroyed immediately. The extra blood tubes that are obtained for later analyses will be handled in accordance with the approved institutional handling policies for biological samples. The samples will be pseudonymized using a study ID. The code list for study ID/personal ID will be stored securely and separately at the study site and after the study by the sponsor to prevent unauthorized persons to access them. If a patient withdraws consent to the use of donated biological samples, the samples will be destroyed, and the action documented. If samples are already analysed, the sponsor is not obligated to destroy the results of this research.

**Statistical analyses**

As a proof-of-concept trial a reasonable sample size will be intended to be recruited, with the sole aim of sufficiently powering a Phase 3 trial. Only a full data set comprising of post randomization baseline data, and midpoint and/or endpoint data will be used.

- Demographic and baseline characteristics will be summarized, using frequency distributions and summary statistics based on the full data set, for each arm; placebo, 12 mg PhAM and 20 mg PhAM..

- Safety analyses will also be summarized, using frequency distributions and summary statistics based on the full data set, for each arm; placebo, 12 mg PhAM and 20 mg PhAM..

- Efficacy analyses for the primary endpoint will entail a comparison between the average value of the HbA1c and fasting blood glucose of participants in the 12 mg or 20 mg PhAM group compared to the placebo. Both baseline, midpoint and endpoint measurements will be compared and comparison will be analyzed using a One-Way ANOVA model. Specifically, for the values of baseline HbA1c and fasting blood glucose of participants in placebo vs 12 mg PhAM vs 20 mg PhAM will be compared by One-Way ANOVA model, so also the midpoint and endpoint data. A disaggregation table will be presented which will compare the frequency distribution of defined changes from baseline to midpoint and endpoint. For HbA1c, the frequency data will show changes that worsened the condition, resulted in no change, decrease between 0.1 and 0.4, decrease between 0.5 and 1.0, decreased between 1.1 and 2.0. For fasting blood glucose, the frequency data will show changes that worsened the condition, decrease < 10 mg/dl, decrease between 11 and 20 mg/dl, decrease between 21 and 40 mg/dl, decrease between 41 and 70 mg/dl, and decrease > 70 mg/dl.

- Efficacy analyses for the secondary endpoint will entail a comparison between the average value of the fasting insulin, HOMA-IR, and BMI of participants in the 12 mg or 20 mg PhAM group compared to the placebo. All of the baseline, and endpoint measurements will be compared and comparison will be analyzed using a One-Way ANOVA model. Specifically, for the values of baseline fasting insulin, HOMA-IR, and BMI of participants in placebo vs 12 mg PhAM vs 20 mg PhAM will be compared by One-Way ANOVA model, as well as the endpoint data. Presentation of the mid-point secondary or exploratory out come data is not required. A disaggregation table will be presented which will compare the frequency distribution of defined changes from baseline to endpoint. For all of the secondary outcome measurement, the frequency data will show changes that worsened the condition, decrease between 0.5 and 1.0, decrease between 1.1 and 2.0, decrease between 2.1 and 3.0, decrease between 3.1 and 4.0, decrease between 4.1and 5.0, and decrease > 5.0.

**Data Management Plan**

|  |  |
| --- | --- |
| **0. Proposal name** | |
| Phenolic acid fractions of Anisopus mannii (PhAM) as a novel add-on therapy for type 2 diabetes: a single-center, double-blind, randomized, placebo-controlled, feasibility trial | |
| **1. Description of the data** | |
| **1.1 Type of study**  The three phases of this study will recruitment, intervention, and follow – up of study participants administered phenolic acid fractions of Anisopus mannii (PhAM). The study involves the recruitment of 108 study participants who will be randomized into placebo, low dose (12 mg), and moderate dose (20 mg).  **1.2 Types of data**  Quantitative data: Chromatogram peak properties, data from self-completed and assisted questionnaires, data from clinical records, biochemical data from clinical measurements, analysis of biological samples (fluids and tissues), and image-derived data in clinical settings (liver echogeneicities of the study participants).  **1.3 Format and scale of the data**  File Formats: Pdf, csv, sav, jpeg, xml software used; SPSS, Graphpad Prism, Ms Office Packages  Scale: Electronic entries containing chromatograms and peak properties of a sample will be generated and saved. 108 participants clinically diagnosed with type 2 diabetes will enrol in the study and data will be collected from each of them, throughout the study for approximately 60 weeks. 108 questionnaires (self-completion and assisted) will be circulated for both qualitative and quantitative data, scanned and saved. Electronically captured data (scanned or digitally obtained) from participants will entail 3260 entries for each parameter cases assuming all no repeated measurements due to a re-screen criterion. Physical data will be stored in secure lockable cabinets and soft copies will be stored at IMSU’s central research data storage which is secure, centrally managed, reliable, and backed up. | |
| **2. Data collection / generation** | |
| **2.1 Methodologies for data collection / generation**  Interviews, questionnaires and surveys, image files such as ultrastound scans, observations, documents and records, focus groups discussions, experimental observations, clinical laboratory measurements, and controlled observations.  **2.2 Data quality and standards**  Data entry will occur in two stages; real time and repository transfer. Validation and control for bias will be ensured by repeat clinical measurements of a subset of the participants. Double entry of data is a standard operation procedure to ensure consistency and detect incorrect entries of values as enshrined in the IMSU data entry, management, and curation policy. Auto-range checks are automatic built-in tools at IMSU data management centre that flags outliers and ambiguousness. Reference papers will be used as tools to validate assessment scales in questionnaires. Interview records will be obtained by trained personnel and subjected to standard expert checks before repository storage. Clinical procedures will undergo regular auditing. All non-personal data mandatorily undergoes peer review (by research project staff) as a data quality check protocol before storage in central repository. | |
| **3. Data management, documentation and curation** | |
| **3.1 Managing, storing and curating data.**  Institutional policy entails that data cleaning and repository storage for funded projects be carried out by the ICT data management teams and statisticians using established guidelines. Original copies are also stored unaltered to maintain data integrity. Data is conventionally stored in PI’s account according set out conventions and accessible to the PI and specific institution’s data managers. Both hard copies and soft copies of stored files are stored and accessible to the PI, soft copies are routinely backed up.  **3.2 Metadata standards and data documentation**  Generation of metadata is mandatory according to institutional policy for data management and sharing. A unified metadata scheme is used that creates an aggregate of catalogues of data objects. Complete and detailed study protocol and real time instrument metadata must also be made available and searchable with a common metadata schema for clinical research data objects (primary outcomes), and stored with links to research data. All codes and variables must also be fully described and must accompany the study protocols.  **3.3 Data preservation strategy and standards**  Back up storage of research data is done on the basis for indefinite retention, and several copies are also made to mitigate the consequence of disc failure or file corruption. Routinely, data format migrations are made to achieve longevity and future availability using safe protocols. Obsolete file tagging is only done when necessary, and in line with the Institutional procedures. Hard copies are mandatorily scanned and the soft copies stored indefinitely while the hard copies are stored for a foreseeable period according to an easily identifiable storage procedure. | |
| **4. Data security and confidentiality of potentially disclosure information** | |
| **4.1 Formal information/data security standards**  Imo State University is compliant with ISO/IEC 27001:2013  **4.2 Main risks to data security**  IMSU guards against risks such as accidental disclosure, unchecked access and computer hacks and thievery. A strict policy on confidentiality is enforced regarding personal data. Personal data are logged only on receipt of written and endorsed express permission of the subject and concerned PI. Personal data is logged in separately (with unique and confidential id only available to the project PI and director of data management) and not linked to metadata or research data. Personal identifiers are not accessible to researchers, and personal data is not input in either shareable or network linked databases. Access logs are automatically generated to ensure traceability of access for disks containing personal data. | |
| **5. Data sharing and access** | |
| **5.1 Suitability for sharing**  Yes, on reasonable request  **5.2 Discovery by potential users of the research data**  Data management team ensures designated staff work with PI’s to develop easily discoverable metadata with reasonable data-sharing agreements notice to potential users, is upeld, and to be deposited at widely accessible open access data repositories like Figshare and Mendeley depending on the funder’s data management policies.  **5.3 Governance of access**  Less sensitive information are made freely available at depositories, whereas decisions regarding access to data with considerable access restriction are subject to consideration by the data access committee (must include the PI except for when dead or clearly inaccessible) on receipt of an official request from a requester. Nonetheless, requests are denied without review if it compromises the confidentiality of study participants. The university research office management team exercises the right of oversight of data access and sharing. Open access journals with authors securing full copyright access are relied upon to disseminate and provide access to peer reviewed research data and supplementary files.  **5.4 The study team’s exclusive use of the data**  It is the policy of IMSU that the consideration of request for data access to public is made timely, as soon as data has been deposited in the database. However, a clause to grant the principal investigator exclusive access for up to 12 months is upheld within which the decision on access depending on the data value and needs relies on the discretion of the PI. Nevertheless, the university senate retains an overriding authority to the clause.  **5.5 Restrictions or delays to sharing, with planned actions to limit such restrictions**  Before study commencement, it is required to receive participants consent agreements with clear and detailed descriptions of plans to widely but procedurally circulate non sensitive details and outcomes. With these agreements in place, the delays to data sharing is mitigated with assent from the PI.  **5.6 Regulation of responsibilities of users**  Normally, signing a confidentiality form applies to all external users of data with restricted access. External users in addition to data sharing agreements are prohibited to ‘lift’ files from open access data depositories and deposit in their own databases. Requests for access to biological samples (where available) are subjected to extra checks and considered based on a convincing intent of use. External users are expected to secure access for IMSU to data emanating from the use of data approved to them. | |
| **6. Responsibilities** | |
| The director of data management unit of the ICT is also responsible for the securing of study data management and data security while designated data managers/personnel is responsible for metadata creation and quality assurance of data. | |
| **7. Relevant institutional, departmental or study policies on data sharing and data security** | |
| **Policy** | **URL or Reference** |
| Data Management Policy & Procedures and sharing | PI: [amadi@imsu.edu.ng](mailto:amadi@imsu.edu.ng), [amadi@imsuonline.edu.ng](mailto:amadi@imsuonline.edu.ng) |

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