CHAPTER 3

Rationale and Objectives

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3 Objective, rationale, and plan of work

3.1 Objective

Diabetes mellitus (DM), a metabolic and endocrine condition, poses a serious threat to human health and longevity around the world. DM, which is marked by insulin resistance, the progressive dysfunction of pancreatic β-cells, and endocrine abnormalities diagnosed by elevated glucose levels coupled with disturbances of carbohydrate, protein, and fat metabolism, is observed as an inflammatory disorder. Hyperglycemia causes β-cells in the pancreas to die off (a process called apoptosis), leading to abnormalities in insulin secretion as well as glucose uptake. In addition, hyperglycemia encourages the increased formation of reactive oxygen compounds via NADPH oxidase activity, which in turn amplifies the effects of pro-inflammatory biomarkers. In chronic hyperglycemia, glucose oxidation is a key generator of free radicals.

3.2 Rationale

Many anti-diabetic drugs, such as insulin, insulin stimulants, insulin secretagogues, and glucose absorption blockers, have been used to treat hyperglycemia and its complications, despite the fact that they can cause a number of undesirable side effects. It is increasingly evident that drugs aiming at a single target may be inadequate for treating complex diseases such as DM, which is multifactorial. The currently approved medications for DM therapy give symptomatic relief or decrease the worsening of symptoms, but they do not slow or stop disease development. There is practically no drug for DM treatment that can address the basic pathophysiological factors responsible for the disease. Therefore, the need for new potential medicine options derived from natural sources with minimal side effects and therapeutic benefits that will have disease-modifying effects is of supreme importance.

It's really interesting to learn that over a thousand medicinal herbs have reportedly been used traditionally to manage DM. Ancient Indian medicines, which are defined through systematic and fundamental regulation by multiple medicinal plants, such as polyherbal formulations containing multiple active components, acting on multiple pathways, and targeting multiple targets, are thus having an increasing impact on the development of anti-diabetic drugs. The Indian traditional medical system has developed several anti-diabetic medicines for the treatment of diabetes in accordance with advances in medicinal technology, for example, Madhumegha churna, Naval churna, Seenthil churna, Avarai kudineer, Vilva kudineer, Abarga parpam, Abraka chendooram, Diabecon, Manomanichooranam, GSPF kwath, PF-4, Dashmoolarishta, Triphala, etc. With this concept in mind, we initiated our drug discovery approach to formulate a novel poly herbal extract (PHE) formulation that has multifunctional agents (polyphenolic compounds) possessing α-amylase and α-glucosidase enzyme inhibitory activity along with antioxidant property, anti-inflammatory, and anti-diabetic activities (down regulation of TNF-α). One of the major goals of this study is to validate the anti-diabetic activity study of PHE with reshaping of gut microbiota dysbiosis and enrichment of beneficial microbiomes to produce effective short-chain fatty acid metabolites in the treatment of DM. With the findings of this study, we concluded that PHE was found to be a beneficial remedy in the treatment of DM, both by bioinformatic approaches and in vitro as well as in vivo studies on animal models.

3.3Plan of work

It is divided into the following headings-

1. Literature survey
2. Selection of medicinal plants
3. Collection,identification/authentification, and processingof plants source
4. Method of extract preparation/calculation of yield
5. Phytochemical investigation
   A) Preliminary phytochemical study of each extract

B) Phytochemical study of each extract
   i) Quantification of polyphenols
   ii) Quantification of flavonoids

6. Antioxidant capacity of each extract
7. Preparation of poly herbal extracts (PHE)
   A) PHE1
   B) PHE2
   C) PHE3
   D) PHE4
8. Standardization of PHE
   A) Polyphenol content
   B) Flavonoid content
   C) Antioxidant capacity
9. Selection and validation of PHE
   A) Heavy metalsdetermination of PHE through ICP-MS
   B) In vitro anti-inflammatory activity of PHE
      i) Membrane stabilization assay
      ii) Albumin denaturation inhibition assay
   C) In vitro anti-diabetic activity of PHE
      i) α- amylase inhibition assay
      ii) α- glucosidase inhibition assay
   D) Anti-hyperglycemic activity through oral glucose tolerance test (OGTT)
10. Standardisation of PHE through highly sophisticated instruments for analysis of bioactive compounds
   A) GC-MS analysis
   B) LC-MS analysis
   C) HR-MS analysis
   D) HPTLC analysis
11. Bioinformatics approach of bioactive compounds present in PHE
   A) In silico study of bioactive compounds
   B) Molecular docking test
   C) Network pharmacology study
12. Toxicity study of PHE
   A) Acute toxicity study (OECD-423)

B) Sub acute toxicity study (OECD-407)

13. In vivo anti-diabetic activity of PHE in Wistar rat model
    A) Determination of animal body weight
    B) Determination of fasting blood glucose (FBG)
    C) Determination of insulin and HbA1C
    D) Biochemistry of blood samples
    E) CBC, LFT, KFT, and lipid profiling of blood samples
    F) Molecular gene expression study (RT-PCR) of tissues samples
    G) Immunohistology study of tissue samples
14. Gut microbiota dysbiosis study of PHE in diabetic rat model
    A) Analysis of fecal samples of rat groups
    B) Determination of short chain fatty acids (SCFAs) in the fecal samples
    C) Identification of gut microbiota producing SCFAs
    D) Correlation of gut microbiota with diabetic indices/markers
15. Statistical analysis of data and its presentation

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1 **Objective 1: Phytochemical Investigation**

Materials and Method

Collection and processing of plant sources

1. Leaves, *Nyctanthes arbor-tristis* L.
2. Leaves, *Premna integrifolia* L.
3. Stem, *Tinospora cordifolia*
4. Root, *Citrullus colocynthis* L.
5. Fruit, *Emblica officinalis* Gaertn.
6. Roots, *Cyperus rotundus* L.
7. Rhizome, *Picrorhiza kurroa* Royle ex Benth.
8. Fruit, *Terminalia chebula* Retz.
9. Fruit, *Terminalia bellerica* Roxb.
10. Stem, *Berberis aristata* DC.
11. Whole herb, *Andrographis paniculata* Nees.

Crude material & Taxonomical authentication

Extraction (Cold maceration) and condensation

Determination of phytochemical (TPC, TFC, and antioxidant capacity of each extract)

PHE 1
PHE2
PHE3
PHE4

PHE contains 6 extract in equal ratio

Preparation of PHE and selection of PHE on the basis of TPC, TFC, antioxidant capacity , In vitro anti-inflammatory and In vitro anti-diabetic activities of each PHE

PHE analysis
LC-MS
HR-MS
HPTLC
GC-MS

Biomarker-based standardization

Evaluations of PHE
Heavy metals (ICP-MS)
In-vivo anti-hyperglycemic (OGTT) activity

Pharmacological evaluations of PHE

2 Bioinformatics based prediction

I. Bioinformatics approach of bioactive compounds identified in GC-MS, LC-MS, HR-MS, and HPTLC

1) ADMET, drug-likeness score and potential target genes of indentified bioactive compounds

II. Bioinformatics approach of metabolites screened using gut microbiota (gutMGene database)

1) Acquisition of gut metabolites through top selected microbiome and their potential targets

2) Intersection of potential target genes and selection of common target genes related to DM

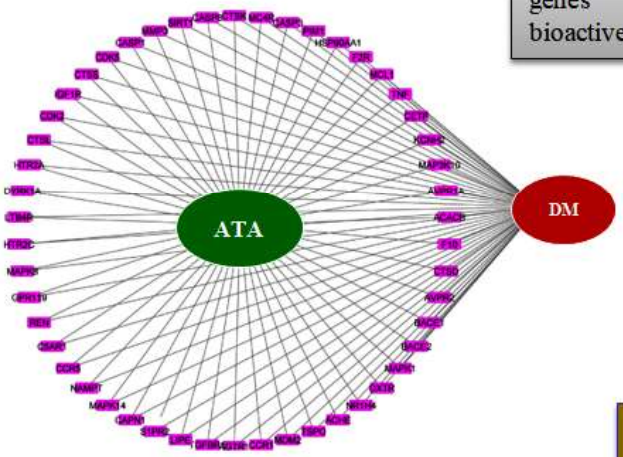
3) Selection of hub genes derived from top score from target genes; network construction by STRING web server and Cytoscape updated software

4) Functional annotation (Gene Ontology and KEGG pathway analysis) of hub genes

5) Binding affinity score between hub gene as protein and bioactive compounds as ligand by molecular docking test (MDT)

6) Molecular docking simulation, molecular mechanics/generalized born surface area (MM/GBSA) analysis

A



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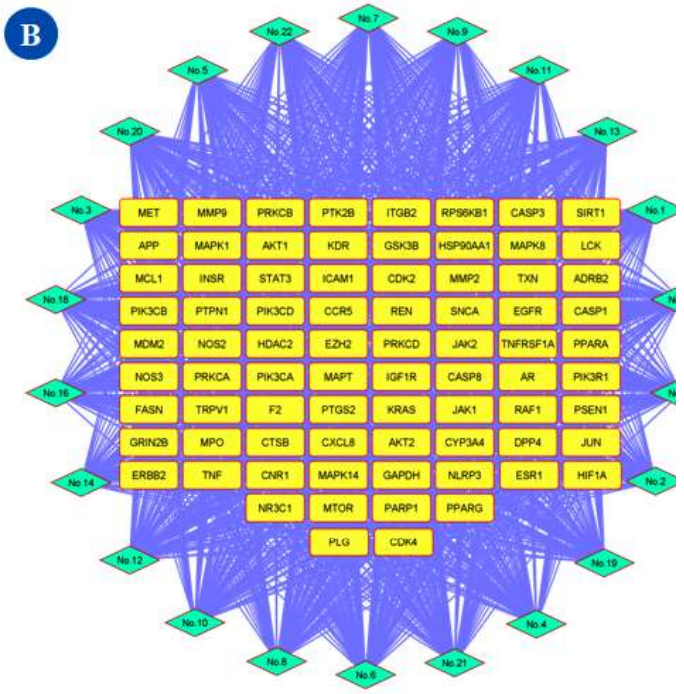

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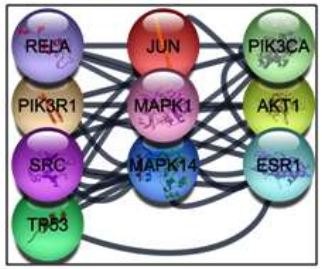

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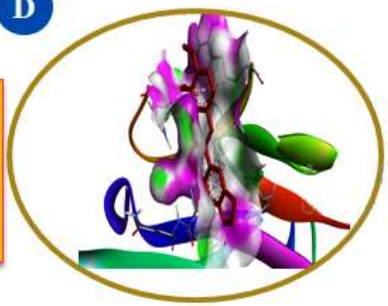
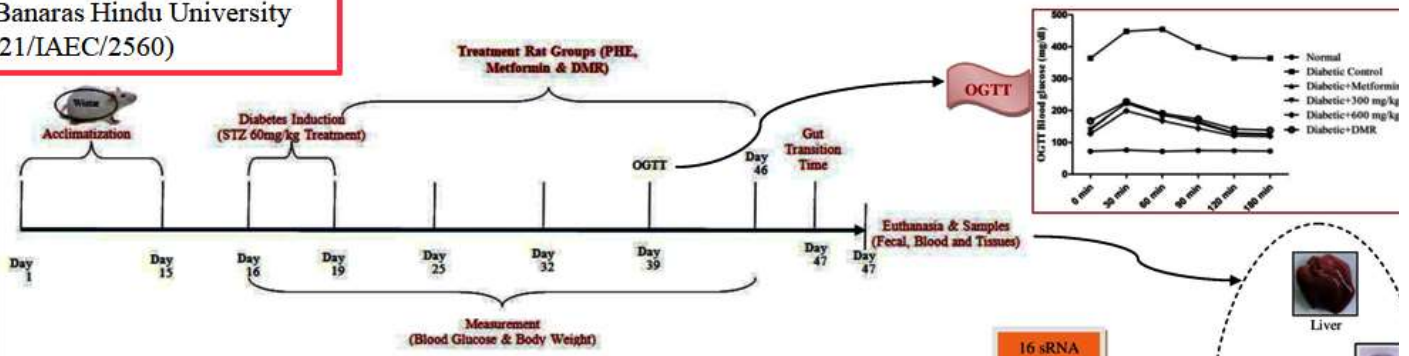


3 Objective 3: Toxicity study and anti-diabetic activity

All experimental protocols for animal studies
Central Animal Ethical Committee of Banaras Hindu University
(Approval number: Dean/2021/IAEC/2560)

Animals
average weight 150 ± 5 g Wistar rats
Acclimatization
15 days
room temperature (23-25 °C)
55 ± 10% of relative humidity
a light-dark cycle for 12-12 hours
a recommended condensed diet
allowed unlimited access to sterile tap water



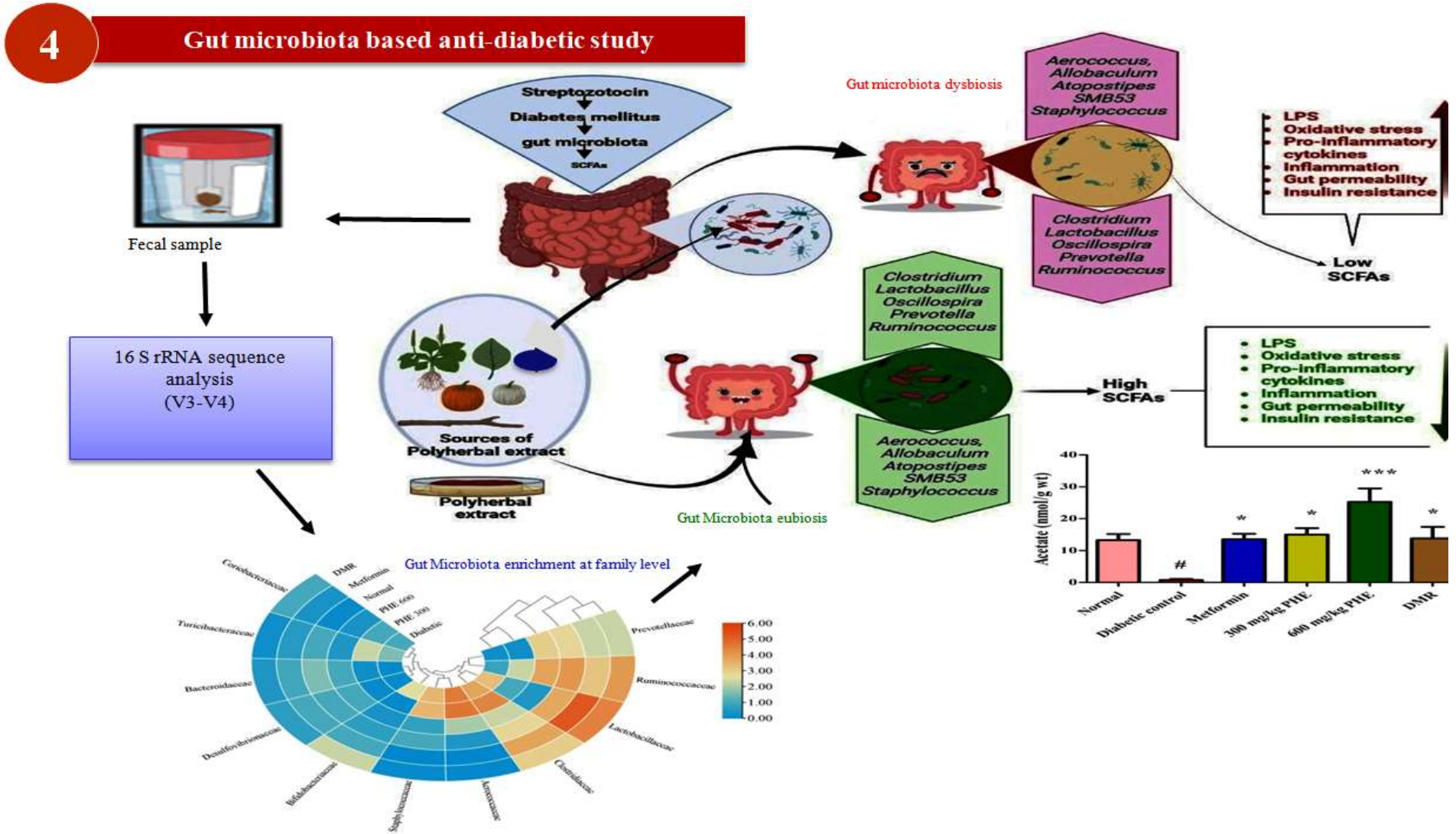
1.Acute, sub acute toxicity evaluations

Acute toxicity assessment of PHE (2000mg/kg) on Wistar rats
OECD guideline 423

Sub acute toxicity assessment of PHE (100, 200, 400 mg/kg) on Wistar rats
OECD guideline 407
The doses were selected as per OECD 1/5th, 1/10th, and 1/20th accordingly from the acute toxicity dose.

2. Comprehensive anti-diabetic activity evaluation

GP-1: Normal rats + drinking water
GP-2: Diabetic control untreated rats
GP-3: Diabetic rats + 250 mg/kg body weight of metformin
GP-4: Diabetic rats + 300 mg/kg body weight of PHE
GP-5: Diabetic rats + 600 mg/kg body weight of PHE
GP-6: Diabetic rats + 1700 mg/kg body weight of DMR


4
Gut microbiota based anti-diabetic study
Streptozotocin
Diabetes mellitus
gut microbiota
SCFAs
Fecal sample
16 S rRNA sequence analysis (V3-V4)
Sources of Polyherbal extract
Polyherbal extract
Gut microbiota dysbiosis
Aerococcus, Allobaculum Atopostipes SMB53 Staphylococcus
Clostridium Lactobacillus Oscillospira Prevotella Ruminococcus
LPS
Oxidative stress
Pro-inflammatory cytokines
Inflammation
Gut permeability
Insulin resistance
Low SCFAs
Gut Microbiota eubiosis
High SCFAs
Gut Microbiota enrichment at family level
DMR
Metformin
Normal
PHE 600
PHE 300
Diabetic
Coriobacteriaceae
Turicibacteraceae
Bacteroidaceae
Desulfovibrionaceae
Bifidobacteriaceae
Staphylococcaceae
Actinomycetaceae
Clostridiaceae
Lactobacillaceae
Ruminococcaceae
Prevotellaceae
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Acetate (nmol/g wt)
40
30
20
10
0
#
*
*
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Normal
Diabetic control
Metformin
300 mg/kg PHE
600 mg/kg PHE
DMR