Efficacy and Safety of Chinese Herbal Medicine (T50) added to Metformin Monotherapy in Patients with Type 2 Diabetes Mellitus:

A 12-week randomised, double-blind, placebo-controlled pilot clinical trial

A thesis submitted for the Degree of Master of Science

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Yi Zhao declare that this thesis, is submitted in fulfilment of the requirements for the award of master's degree, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a complex set of metabolic diseases that has reached pandemic proportions globally. The multifactorial nature of its pathogenesis makes patient management particularly challenging. Currently, there is a growing body of evidence supporting the efficacy of medicinal plant supplements in the prevention and management of T2DM. Previous clinical studies have demonstrated that T50 (initially coded as TM81), a traditional Chinese medicine formula, is both effective and safe for patients with newly diagnosed T2DM. However, there are no studies examining the use of T50 in patients with T2DM who have inadequate glycaemic control with metformin monotherapy. Building on prior research, the primary aim of this pilot clinical trial is to evaluate the effectiveness of T50 as an adjunct therapy to metformin in managing T2DM.

The study was primarily designed to evaluate changes in HbA1c levels over a 12-week period, recognizing HbA1c as a crucial indicator of long-term glycaemic control and diabetes management. The secondary aims were to assess the impact of T50 on various metabolic factors related to T2DM control. Additionally, the safety and tolerability of T50 were monitored through the measurement of hemodynamic parameters and assessments of liver and kidney function.

Fifty-two patients were screened and forty-two patients on metformin monotherapy with unsatisfactory results (HbA1c 7-9%) were randomized to receive either T50 pills ((7.5 g, twice daily) or a placebo (3:1 ratio of T50 to placebo) in addition to their metformin regimen. Clinical assessments, including blood pressure, heart rate, BMI, and waist circumference, as well as blood sampling for laboratory measurements including fasting glucose and insulin levels, lipid profiles, and liver and kidney function, and laboratory testing for GIP levels were conducted at baseline and at four-week intervals until the end of the 12-week treatment period.

This study found a reduction in HbA1c levels by 0.36% compared to baseline, while the placebo group exhibited an increase of 0.13% in HbA1c levels ($p<0.05$ for T50 compared to placebo after 12 weeks of treatment). Conversely, no significant change in fasting glucose levels was detected, which can be attributed to their susceptibility to recent food intake and diurnal variation. Thus, fasting glucose levels may not accurately reflect long-term diabetes control. Furthermore, an improvement in insulin resistance was evidenced by a reduction in HOMA-IR from baseline to the 12-week point (4.02 ± 0.51 vs. 3.40 ± 0.41, $p<0.05$) without significant changes in HOMA-Beta values, indicating that T50 may improve glycaemic control through its action on peripheral insulin target tissues rather than pancreatic β cell function. The T50 treatment did not alter other metabolic parameters, such as BMI, total cholesterol, and triglycerides, nor did it affect liver and kidney functions. Additionally, no significant adverse effects were observed throughout the 12-week study.

To elucidate the mechanism by which T50 affects glycaemic control, serum levels of glucose-dependent insulinotropic polypeptide (GIP), an incretin hormone that stimulates insulin secretion postprandially were measured. While the placebo group exhibited a slight decrease in serum GIP levels and the T50 group a slight increase, neither change reached statistical significance. This finding suggests that the impact of T50 on glucose metabolism and insulin secretion might be mediated through mechanisms other than GIP modulation.

In conclusion, this study addresses a gap in the use of naturally occurring medicines as adjuncts to pharmaceutical antidiabetic treatments for diabetes control. The results demonstrate that T50 provides beneficial effects as an add-on to metformin for glycaemic control in patients with T2DM. However, the trial had a small sample size and a relatively short treatment duration. To further confirm the efficacy and safety of T50, studies with a larger number of patients and longer durations are needed.

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ABBREVIATIONS

ADA	American Diabetes Association
AE	Adverse Event
AIHW	Australian Institude of Health and Welfare
ALT	Alanine Aminotransferase
AMPK	AMP-activated protein kinase (AMPK)
ANOVA	Analysis of Variance
AQOL	Assessment of Quality of Life
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
BW	Body Weight
CRP	C-Reactive Protein
CTN	Clinical Trial Notification
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DPP-4i	Dipeptidyl Peptidase-4 Inhibitors
ECG	Echocardiogram
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
FBG	Fasting Blood Glucose
FFA	Free Fatty Acids
GDM	Gestational Diabetes Mellitus
GIP	Glucose-Dependent Insulinotropic Polypeptide
GIPR	GIP Receptor
GLP-1	Glucagon-like Peptide-1
GLP-IRA	Glucagon-like Peptide-1 Receptor Agonists
GP	General Practitioner
HbA1c	Glycated Haemoglobin

HDL	High-Density Lipoprotein
HDL-C	High-Density Lipoprotein Cholesterol
HF	Heart Failure
HFD	High Fat Diet
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOMA-β	Homeostatic Model Assessment of Beta-Cell Function
HPLC	High-performance Liquid Chromatography
HR	Heart Rate
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
InsR	Insulin Receptor
IP	Investigational Products
IRS-1	Insulin Receptor Substrate-1
ISI	Insulin Sensitivity Index
ITT	Intention to Treat
LDL	Low-Density Lipoprotein
LDL-C	Low-Density Lipoprotein Cholesterol
MACE	Major Adverse Cardiovascular Events
MS	Mass Spectrometry
OGTT	Oral Glucose Tolerance Test
PI-3K	Phosphatidylinositol 3-kinase
QOL	Quality of Life
RACGP	Royal Australian College of General Practitioners
RCT	Randomized Controlled Trial
ROS	Reactive Oxygen Species
SAA	Serum Amyloid A
SAE	Serious Adverse Events

SE	Standard Error
SGLT2i	Sodium-glucose
STZ	Streptozotocin
SU	Sulfonylureas
T2DM	Type 2 Diabetes Mellitus
T50	Tang-Min-Ling-Wan (Tangminling Pills)
TC	Total Cholesterol
TCM	Traditional Chinese Medicine
TG	Triglycerides
TGA	Therapeutic Goods Administration
TNF-α	Tumour Necrosis Factor Alpha
WHO	World Health Organisation

CONTENTS

CHAPTER 1

General Introduction

Chapter 1: General Introduction

1.1 Epidemic of Diabetes Mellitus and Global Burden

Over the past few decades, the prevalence of diabetes mellitus (DM) has increased significantly, primarily due to a continuous rise in the incidence of typ2 diabetes mellitus (T2DM). According to World Health Organization (WHO) statistics, more than 422 million adults globally were afflicted with DM in 2014, with a continuous increase in prevalence anticipated (WHO 2020). The International Diabetes Federation (IDF) Diabetes Atlas (2024) reports that 537 million adults aged 20-79 are currently living with diabetes, equating to 1 in 10 individuals. This number is projected to escalate to 643 million by 2030 and 783 million by 2045, representing 1 in 8 individuals (IDF 2024). The rising burden of DM constitutes a major concern for global healthcare systems.

In Australia, the estimated diabetes population stands at 1.7 million, marking a 220% increase since 2000 (Diabetes Australia 2024). The economic burden associated with diabetes is substantial. By 2050, the number of Australians living with diabetes is projected to exceed 3.1 million. The annual cost associated with diabetes is expected to rise significantly, reaching approximately $45 billion per year during this period. This growth underscores the substantial impact that diabetes will have on both the healthcare system and the economy in Australia over the coming decades (Diabetes Australia 2024). There are mainly four clinical classes for diabetes which are type 1 diabetes, results from b cell dysfunction and insulin deficiency; type 2 diabetes, results from a progressive insulin secretory defect due to insulin resistance; gestational diabetes mellitus (GDM) results from glucose intolerance during pregnancy and other specific types of diabetes such as monogenic diabetes and diabetes secondary to other causes (RACGP 2020).

1.2 Type 2 Diabetes Mellitus and Its Pathophysiology

Type 2 diabetes mellitus (T2DM) accounts for 95% or more of all diabetes cases (WHO 2024). T2DM is associated with a range of microvascular and macrovascular complications resulting from poorly controlled blood glucose levels, ultimately shortening life expectancy. Therefore, interventions to control diabetes and prevent its complications are a priority for public health.

Three laboratory tests can be used to diagnose of T2DM: fasting blood glucose (FBG), glycated haemoglobin (HbA1c), and the oral glucose tolerance test (OGTT). If the OGTT test results show 6.1-6.9 mmol/L for fasting glucose and less than 7.8 mmol/L for two-hour glucose, it suggests impaired fasting glucose (IFG). If the OGTT test results show less than 7 mmol/L for fasting glucose and greater than 7.8 mmol/L for two-hour glucose, it suggests impaired glucose tolerance (IGT) (RACGP 2020). The prevalence of T2DM is likely underestimated because patients may be unaware of conditions such as IFG and IGT. These asymptomatic individuals may develop T2DM if blood glucose levels remain uncontrolled (Goyal et al. 2023).

Clinical symptoms suggesting diabetes include lethargy, polyuria, polydipsia, frequent fungal or bacterial infections, blurred vision, loss of sensation, poor wound healing, and weight loss. A diagnosis can be made in symptomatic patients when one of the following is confirmatory: a single elevated FBG greater than 7.0 mmol/L, a single HbA1c level greater than 6.5%, a random blood glucose level greater than 11.1 mmol/L, or the presence of a hyperglycaemic crisis (RACGP 2020).

Despite extensive research on T2DM that has enhanced our understanding of its numerous risk factors, the precise causes of T2DM remain largely unclear (Roden & Shulman 2019). The pathophysiology of T2DM involves a complex interplay of genetic, epigenetic, and lifestyle factors, all of which interact with a broader physical and sociocultural environment (Hu et al.

2015). Contributing factors to the development of T2DM include genetic predisposition, overweight and obesity, decreased physical activity, smoking, and heavy alcohol consumption (Burhans et al. 2018). During the compensatory stage of T2DM, also known as pre-diabetes, hyperinsulinemia occurs. Experimental and clinical studies suggest that insulin resistance, associated with central obesity, accelerates glucose and lipid abnormalities. This progression impairs insulin secretion from pancreatic islet β cells, leading to hyperglycaemia and the manifestation of overt diabetes (Figure 1).

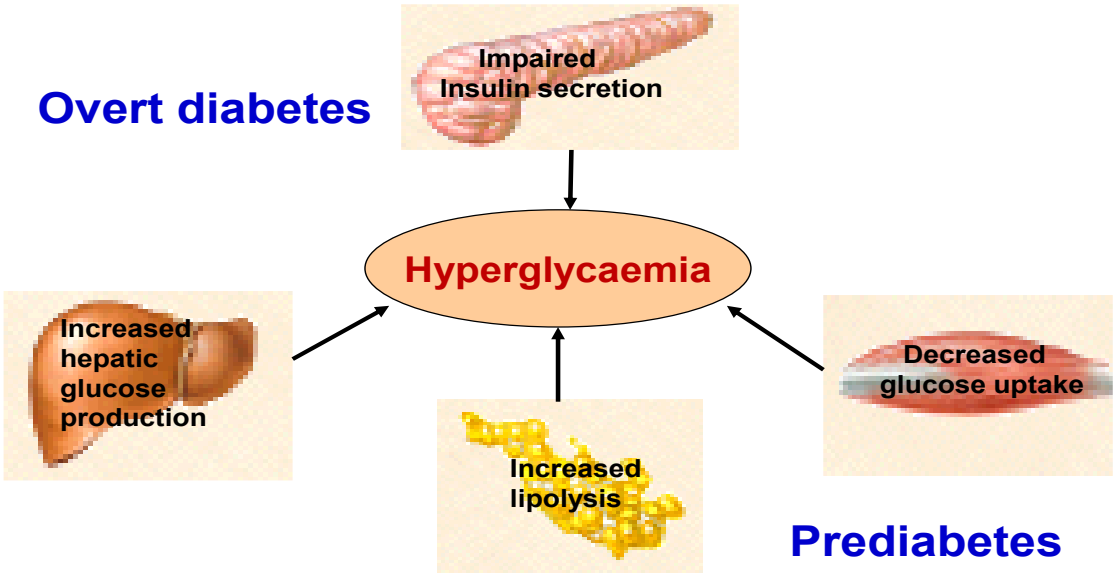

Figure 1. Pathophysiology of hyperglycaemia in T2DM.

Normally β-cells procedure insulin which lead to a decreased glucose production by liver and increased glucose uptake by skeletal muscle and adipose tissue. However, when β-cell dysfunction occurs in the liver, and/or insulin resistance occurs in the skeletal muscle and adipose tissue, it contributes to hyperglycaemia and an excessive amount of glucose circulating in the blood stream.

Insulin resistance is a condition where cells exhibit a decreased response to insulin compared to normal controls. This condition manifests in various tissues, such as the liver, skeletal muscle, and white adipose tissue (Petersen & Shulman 2018). Normally, insulin secretion from pancreatic islet β-cells reduces glucose output by the liver and increases glucose uptake by

skeletal muscle and adipose tissue. Insulin resistance impairs the liver's suppression of endogenous glucose production, reduces glucose uptake by skeletal muscle, and increases the influx of free fatty acids (FFA) to the liver for glucose production, leading to elevated glucose levels in the bloodstream (Wilcox 2005; Stumvoll et al. 2016). Elevated blood glucose levels stimulate further insulin secretion, and compensatory hyperinsulinemia initially maintains glycaemic levels. However, as β-cell function gradually declines, glucose responsiveness decreases, resulting in a progressive functional defect in β-cells and hyperglycaemia (Wilcox 2015).

When the feedback mechanism between insulin action and insulin secretion malfunctions, the effects of insulin in insulin-sensitive tissues such as the liver, muscle, and adipose tissue are disrupted, leading to abnormal glucose levels (Laakso 2016). Additionally, β-cell dysfunction contributes to reduced insulin release, which is inadequate to maintain normal glucose levels (Laakso 2016).

In most developed countries, the prevalence of T2DM is rising in parallel with the increasing incidence of obesity. Evidence suggests that adipose tissue, liver, muscle, and pancreas are sites of inflammation in the presence of obesity. Under insulin resistance, adipose tissue secretes adipokines, cytokines, and chemokines (Burhans et al. 2019).

Recently, the role of glucose-dependent insulinotropic polypeptide (GIP) in the development of T2DM and its potential in diabetes treatment has garnered significant attention. GIP is a hormone secreted by enteroendocrine cells in the small intestine at the onset of meal consumption (Hayes et al. 2021). GIP plays a crucial role in regulating postprandial insulin secretion. In healthy individuals, GIP enhances insulin secretion from pancreatic β-cells, contributing to the regulation of blood glucose levels. However, in diabetic patients, the effect of GIP is significantly reduced due to defects in GIP receptor signalling in pancreatic β-cells

(Holst & Rosenkilde 2020). Moreover, patients with T2DM often have elevated GIP levels that are ineffective due to receptor desensitization. This impairment highlights the complex role of GIP in the pathogenesis of T2DM. GIP affects not only insulin secretion, but also other metabolic processes mediated by the GIP receptor (GIPR). Research indicates that GIPR exists in various tissues, including adipose tissue, where GIP can promote lipid uptake and fat storage (Holst & Rosenkilde 2020).

Studies have yet to show that GIP monotherapy can effectively reduce body weight (Holst & Rosenkilde 2020; Campbell 2021). Despite these challenges, there is ongoing interest in modulating GIP activity as a therapeutic strategy for T2DM. One approach target both GIP and GLP-1 receptors. Dual GIP/GLP-1 receptor agonists have shown positive results in clinical studies, demonstrating improved glycaemic control, enhanced insulin secretion, and reduced body weight (Zhou et al. 2023).

1.3 Current Drug Therapies for T2DM

T2DM necessitates a multifaceted approach that combines behavioural and pharmacological interventions to control hyperglycaemia and mitigate complications. In more advanced stage of T2DM, lifestyle modification still pays an important role in managing glycemia and CVD risks (Wass et al 2011). If a patient fails to achieve the glycaemic target using lifestyle modification alone within three months, glucose-lowing medication should be dispended for hyperglycaemia (RACGP 2020). When blood glucose levels consistently elevating, it can damage blood vessels and nerves. It can lead to medical problems affecting the retina, kidneys, heart and feet. T2DM is correlated with an increasing risk of cardiovascular disease, and there have been research showing that some antidiabetic medications may increase cardiovascular risks, and some may reduce cardiovascular risks.

The clinical management goals for T2DM include achieving an HbA1c level of less than 7.0%, total cholesterol less than 4.0 mmol/L, HDL-C greater than 1.0 mmol/L, LDL-C less than 2.0 mmol/L (and less than 1.8 mmol/L if cardiovascular disease is present), non-HDL-C less than 2.5 mmol/L, triglycerides less than 2.0 mmol/L, and blood pressure lower than 140/90 mmHg (RACGP 2020).

There is no single universal treatment plan for all T2DM patients, as many factors need to be considered prior to treatment. These factors may include the patient's treatment goals, age, general health, presence of other medical conditions, concurrent medications, drug efficacy and tolerance, and socioeconomic status (IQWiG 2023). The aim of treatment is to prevent the development of diabetes-related complications. It is recommended by diabetes organizations that to set individualized treatment goals and choices of medications will be safe and effective and benefit to prevention of diabetic complications (Quattrocchi et al. 2020) using the pharmaceutical drugs listed in the table 1, which provides an overview of oral and injectable non-insulin medications used to control blood glucose levels. It includes the classifications of the medication groups and specific drugs within each group, as well as information on their effects, side effects, and safety profile.

Table 1. Pharmaceutical medications used in T2DM management

Drug Group	Specific Drug	Effects	Adverse Effects	Safety
Biguanides	Metformin	HbA1c↓ Body weight↓→ Cancer↓? Cardiovascular↓?	Gastrointestinal disorders↑ Reversible vitamin B12 deficiency↑ Lactic Acidosis↑	None
Glinides	Repaglinide Nateglinide	HbA1c↓ Body weight↑	Hypoglycaemia↑ Headache↑ Upper respiratory tract infection↑	None
Alpha-Glucosidase inhibitor	Acarbose	HbA1c↓ Body weight↓→	Gastrointestinal disorders Serum transaminases (AST, ALT) ↑	None
SGLT2-i	Empagliflozin Dapagliflozin Canagliflozin	HbA1c↓ Body weight↓ BP↓ MACE↓ Hospitalization for HF↓ Progression of renal disease ↓	Diabetes ketoacidosis↑ Genital infection↑ Urinary tract infection↑ Acute kidney injury↑ (related to hypovolemia) Canagliflozin: Amputation↑ Bone fracture↑	None
Thiazolidinediones	Pioglitazone	HbA1c↓ BP↓ NAFLD↓ MACE↓	Body weight↑ Peripheral oedema↑ Anaemia↑ Hospitalization for HF↑ Bone fracture in women↑	Cancer?
DPP-4 inhibitors	Sitagliptin, Saxagliptin, Alogliptin	HbA1c↓	Saxagliptin: Hospitalization for HF?	None
Sulfonylureas	Glimepiride Gliclazide Glibenclamide Glipizide	HbA1c↓	Body weight↑ Hypoglycaemia↑ Lack of durable effect	Glibenclamide Gliclazide: Cardiovascular events?
GLP-1 RA	Liraglutide Dulaglutide Semaglutide Orforglipron	HbA1c↓↓ Body weight↓ Systolic BP↓ MACE↓ HF?	Gastrointestinal disorders↑ Semaglutide: Macular oedema	Pancreatitis Bike stones Thyroid carcinoma
Combination therapy	Tripeptide Retatrutide	Quality of life for patients with HF (KCCQ-CSS)↓ NAFLD↓→		

The table is adapted from Sibony et al. (2023). ' ↑ 'Aggravation/increase, ' ↓ ' improvement/decrease, '?' Not confirmed. HF = Heart Failure, MACE = Major Adverse Cardiovascular Events, BP = Blood Pressure, NAFLD = Non-alcoholic fatty liver disease

According to the data published by Australian Institute of Health and Welfare (AIHW) 2024, prescriptions for thiazolidinediones and alpha glucosidase inhibitors have been decreasing over time from 2017 to 2021. In contrast, all other diabetes medications have shown an increase

during the same period, which may reflect the increasing needs for diabetic patients. Notably, there has been a 36% increase in the use of a combination therapy including metformin from 2017 to 2021. This shift from older medications to newer or combination therapy may reflect positive patient management outcomes.

1.4 The Role of Metformin in T2DM Management

In the 1940s, metformin's glucose-lowering properties were discovered by chance during its use for treating influenza (Garcia 1950). By 1957, French physician Jean Sterne published findings showing metformin's superior ability to reduce blood glucose level safely, leading to its approval for treating T2DM in Europe, where it was called "glucophage" due to its glucose-lowering effects (Baker et al. 2021). During that period, phenformin and buformin were commonly used to treat T2DM. However, these drugs were removed from the market due to a high risk of lactic acidosis. Many clinical studies on metformin efficacy and safety were done between 1990-1998 and results showed metformin has lower risk of side effects compared to other biguanide, while still offering substantial therapeutic benefits (Baker et al. 2021). Metformin has been used as the first line treatment for patients with T2DM since the American Diabetes Association recommended it as the primary treatment in 1998, owing to its proven effectiveness, favourable safety profile, and affordability (American Diabetes Association, 2019).

Recent research by Lin et al. (2023) elucidates the anti-inflammatory mechanisms of metformin, primarily via AMP-activated protein kinase (AMPK)-dependent and -independent pathways. AMPK activation reduces pro-inflammatory cytokine production and inhibits NLRP3 inflammasome activation, as shown in models of myocardial ischemia-reperfusion injury. Additionally, metformin activates liver kinase B1 (LKB1), which modulates inflammatory responses. Deletion of LKB1 significantly elevated pro-inflammatory cytokine expression in

LPS-stimulated bone marrow-derived macrophages, underscoring its regulatory role (Fei et al. 2020).

These findings suggest metformin may mitigate chronic inflammation, a key factor in T2DM pathophysiology.

Furthermore, Ramesh et al. (2024) conducted a systematic review of 28 eligible studies, identifying multiple antiproliferative mechanisms through which metformin exhibits anticancer activity. These include AMPK activation, mTOR pathway inhibition, and modulation of the insulin/IGF-1 axis. Clinical evidence links metformin use with reduced cancer risk in both diabetic and non-diabetic individuals, suggesting its potential as an adjunct in cancer therapy. However, future trials are needed to clarify optimal dosing and patient selection.

Galal et al. (2024) reported similar findings but noted inconsistencies in clinical efficacy, particularly for breast and lung cancers. Variability in response appears linked to cancer subtypes, genetic profiles, and glycaemic status. Preclinical studies show promise, but translational challenges remain, particularly due to differences between experimental and clinically achievable concentrations and emerging resistance mechanisms.

Metformin has also demonstrated moderate but sustained weight loss effects, primarily through reduced caloric intake (Malin & Kashyap, 2014). It activates AMPK to enhance energy metabolism and reduce visceral fat. Additionally, it improves leptin sensitivity, increases GLP-1 secretion, and modulates circadian genes related to energy homeostasis (Shurrab & Arafa, 2020). These effects extend to obese individuals without insulin resistance.

Among various antidiabetic medications, metformin remains the first-line treatment for T2DM. It is associated with lower risks of hypoglycaemia and offers potential benefits for patients with stable cardiovascular conditions (ADA 2019 & RACGP 2020). Metformin helps reduce glucose release from the liver and increases glucose uptake by tissues (Baker et al. 2021). Despite its widespread use, a significant portion of patients do not achieve optimal glycaemic control with metformin monotherapy (Baker et al. 2021).

Research data have reported risks associated with the long-term use of metformin. Various studies have documented associated side effects, with gastrointestinal upset commonly experienced by many patients (McCreight et al. 2016; Bonnet et al. 2017). A systematic review by Chapman et al. (2016) concluded that metformin usage leads to a significant deficiency in vitamin B12, with a decrease of 57 pmol/L, necessitating close monitoring of patients at risk for B12 deficiency.

If patients are unable to achieve glucose targets, second line and subsequent medical options, in addition to existing metformin, might be necessary. Other agents such as sodium-glucose cotransporter-2 inhibitors (SGLT2i), dipeptidyl peptidase-4 inhibitors (DPP-4i), sulfonylureas (SU), glucagon-like peptide-1 receptor agonists (GLP-1RA), and insulin work through different mechanisms and should be considered according to patients' clinical profiles. Factors to consider include renal function, cardiovascular disease risk, the magnitude of glycaemic lowering required, cost, tolerability, weight control, and safety (RACGP 2020). A systematic review by Maruthur et al. (2016) indicated that the results for add-on therapies were similar to those for monotherapies.

Despite advances in diabetes management with conventional therapy, many patients with diabetes are not achieving the target glycaemic control of HbA1c <7% (53 mmol/mol), which is necessary to prevent microvascular and possibly macrovascular complications. Thus, there

is growing interest in complementary and alternative medicine, particularly Traditional Chinese Medicine (TCM), for its potential role in managing diabetic condition and add-on to pharmaceutical drug as an integrative approach for T2DM (Maruthur et al. 2016).

1.5 The Role of GIP and GLP-1

Incretins are hormones secreted by the gut that help regulate insulin secretion from the pancreas after eating, thus controlling blood sugar levels. The main known incretins are glucose-dependent insulinotropic polypeptide (GIP) and Glucagon-Like Peptide-1 (GLP-1).

The concept of incretins has been around for over 100 years. In 1902, Bayliss and Starling discovered a hormone called "secretin," which inspired Moore et al. to hypothesize that the gut might contain hormones that regulate the pancreas. They found that extracts from the gut could lower urinary sugar levels in diabetic patients. In 1929, La Barre isolated a substance from gut extracts that could lower blood sugar, calling it "incretin." Although this concept was not given much attention in the following decades, new insulin measurement techniques developed in the 1960s brought renewed interest to the field (Seino et al. 2010). In 1971, research conducted by McIntyre and colleagues showed that oral glucose triggered a stronger insulin response than intravenous glucose, understood to be due to incretins secreted by the gut after eating, helping the pancreas release more insulin. Gastric inhibitory polypeptide (GIP) was initially identified by Brown and Dryburgh in 1971 and named due to its ability to inhibit acid secretion in canine gastric pouches; however, it's insulinotropic effects was identified by Dupre and Brown and renamed "glucose-dependent insulinotropic polypeptide (GIP)" because it's inhibitory effects on gastric secretion is not physiologically relevant (Holst et al. 2021).

GIP is a 42-amino-acid hormone secreted by K cells in the upper small intestine (primarily in the duodenum and upper jejunum) (Seino et al. 2010). GIP acts through its receptor, GIPR,

which is part of the seven-transmembrane G protein-coupled receptor superfamily, distributed in various tissues such as the pancreas, stomach, small intestine, adipose tissue, adrenal cortex, lungs, pituitary gland, heart, testicles, vascular endothelium, bones, and brain. Glucagon-Like Peptide-1 (GLP-1) is a 31-amino-acid hormone secreted by L cells in the lower small intestine and colon, capable of directly stimulating insulin secretion, thus identified as the second incretin. Both GIP and GLP-1 play an important role in controlling blood sugar (Seino et al. 2010).

The plasma concentrations of GIP and GLP-1 are very low in the fasting state and increase within 10-30 minutes after eating, peak at 45 to 90 minutes, and then gradually decline. The incretin effect is short-lived as they are inactivated by the enzyme DPP-4 within 1-2 minutes of secretion. However, in patients with T2DM, the incretin effect is almost completed lost, making the restoration of this function important (Holst and Rosenkilde 2020). Given the multiple roles of gut hormones in glucose homeostasis, scientists have developed several diabetes treatments that mimic the action of gut hormones, including GLP-1 receptor agonists and DPP-4 enzyme inhibitors, which enhance the effectiveness of GLP-1 and prevent the degradation of GIP and GLP-1. DPP-4 is a protein in the body that serves multiple functions. One particularly important role of DPP-4 is that it breaks down GLP-1. For DPP-4 inhibition to be an effective treatment for T2DM, DPP-4 must be the primary enzyme degrading GLP-1. If other metabolic pathways are involved in GLP-1 degradation, blocking DPP-4 might not significantly increase intact GLP-1 levels (Deacon 2019). Early research showed that although several DPP-4 inhibitors existed, none were suitable for humans. Preclinical studies in anesthetized pigs using the prototype DPP-4 inhibitor valine pyrrolidide confirmed that inhibiting DPP-4 effectively protected GLP-1 from degradation, enhancing insulin secretion and confirming the critical role of DPP-4 in limiting GLP-1's insulinotropic action. This brief action initially limited enthusiasm for the potential use of incretins in T2DM treatment,

promoting the development of DPP-4 inhibitors, which can prolong the half-life of endogenous incretins (Deacon 2019). These medications are now widely used in the treatment of type 2 diabetes. Real-world evidence suggests that GLP-1 receptor agonists have a lower incidence of adverse events compared to DPP-4 inhibitors in patients with baseline cardiovascular risk; the GLP-1 drug semaglutide (Ozempic®) reduces the risk of major adverse cardiovascular events in a broad T2DM population.

In healthy individuals, both GIP and GLP-1 stimulate insulin secretion based on glucose levels. However, even at high concentrations, GIP can only stimulate minimal insulin secretion at blood glucose concentrations of 2.4 mmol/L (43 mg/dL). The glucose threshold for GLP-1 to stimulate insulin secretion is about 3.7 mmol/L (66 mg/dL). When blood glucose levels rise, the insulinotropic effects of GIP and GLP-1 increase. In the past, some studies showed that infusion studies indicate that only GLP-1 can stimulate insulin secretion in T2DM patients, while GIP has little to no effect (EI & Campell 2020 & Mentis et al. 2011). Another key difference is that GLP-1 can suppress appetite and food intake, leading to weight loss with long-term use, whereas GIP is generally thought to have no impact on food intake. Recent studies using high-affinity antagonists (GIP and exendin) confirmed that GIP contributes more significantly to the insulinotropic effect after oral glucose (Nauck & Muller 2023). Overall, these hormones enhance glucose-induced insulin secretion, making blood glucose levels the determinant of their insulinotropic effects.

Both GIP and GLP-1 are involved in regulating food intake by influencing neurons in the brain's satiety centre (Liu 2024). They also promote insulin secretion from pancreatic β-cells; however, they have different effects on glucagon production by pancreatic α-cells. GIP increases glucagon levels during hypoglycaemia, while GLP-1 suppresses glucagon secretion during hyperglycaemia. Additionally, GIP directly promotes lipogenesis, whereas GLP-1

indirectly facilitates lipolysis (Liu 2024). This combination helps maintain healthy adipose tissue, reduce ectopic fat deposition, and increase adiponectin production and secretion from adipocytes. These actions collectively contribute to metabolic balance, helping to prevent both high and low blood sugar levels, mitigate dyslipidaemia, and reduce cardiovascular disease risk in people with type 2 diabetes and obesity.

Tirzepatide is the first dual GIP and GLP-1 receptor agonist, composed of 39 amino acid sequence, with a half-life of about five days, suitable for once-weekly subcutaneous injection (Chavda et al. 2022). In an open-label, 40-week phase 3 study conducted by Frias et al. (2021), 1879 patients were randomly assigned to receive either tirzepatide (at doses of 5 mg, 10 mg, or 15 mg) or semaglutide (1 mg), in a 1:1:1:1 ratio. The participants had an average HbA1c level of 8.28%, were on average 56.6 years old, and weighed 93.7 kg at the beginning of the trial. The primary endpoint was the change in HbA1c levels from baseline to 40 weeks. The results showed HbA1c reduced 2.01%, 2.24%, and 2.30% for the 5 mg, 10 mg, and 15 mg doses of tirzepatide, respectively, compared to a 1.86% reduction with semaglutide. The differences in HbA1c reduction between tirzepatide and semaglutide were -0.15 % for the 5 mg dose, -0.39% for the 10 mg dose, and -0.45% for the 15 mg dose. The conclusion was that Tirzepatide was found to be both noninferior and superior to semaglutide at all doses tested. Additionally, weight loss was greater with tirzepatide compared to semaglutide, with estimated differences in weight reduction of -1.9 kg, -3.6 kg, and -5.5 kg for the 5 mg, 10 mg, and 15 mg doses.

Cost considerations are crucial when choosing and transitioning medications. A cost-effectiveness analysis conducted in Saudi Arabia revealed that semaglutide (Ozempic®) is the most cost-effective option among GLP-1 receptor agonists, achieving the target HbA1C level for blood glucose control at the lowest cost compared to liraglutide, dulaglutide, exenatide, and

lixisenatide (Alkhatib et al. 2022). Another study conducted in Taiwan, studied from a payer's perspective found that the cost per patient for GLP-1 receptor agonist therapy was higher than for insulin. However, the overall medical costs for the GLP-1 receptor agonist group were lower than for the insulin group, primarily due to reduced emergency visits and hospitalizations (Yang et al. 2021).

Interestingly, another study conducted by Veniant et al. (2024) showed that a GIPR antagonist conjugated to GLP-1 analogues promotes weight loss with improved metabolic parameters in preclinical and phase 1 settings. The combination of GIPR antagonism and GLP-1R agonism was first validated in animal models, which supports its progress to clinical trials. The outcomes in human studies generally mirrored those observed in cynomolgus monkeys. (Veniant et al. 2024).

1.6 Traditional Chinese Medicine and T2DM

TCM is a holistic system of medicine that has been practiced for thousands of years. TCM aims to restore balance and harmony in the body by addressing the root causes of illness rather than just treating symptoms. TCM doctors had been treating Xiao Ke (Diabetes ancient term) by using herbal medicine for more than two thousand years (Xia et al. 2016). Herbal medicine is a cornerstone of TCM. Several studies have explored the efficacy of various herbs and herbal formulations in managing T2D. Some commonly used herbs include:

- Berberine: Found in plants like Coptis chinensis, berberine has been shown to improve glucose and lipid metabolism by activating AMP-activated protein kinase (AMPK) (Yin et al. 2008)
- Ginseng: Both American and Asian ginseng have demonstrated potential in improving insulin sensitivity and reducing blood glucose levels. (Vuksan et al. 2000)

- Bitter Melon (Momordica charantia): This herb is traditionally used to lower blood sugar and has shown promise in both animal and human studies (Basch et al. 2003).

Recent clinical and experimental studies have demonstrated that herbal medicine manage T2DM through its anti-inflammatory, anti-oxidation, blood lipid regulation, and anti-glucose effects (Pang et al. 2019). Yu et al. (2018) conducted a systematic review of fifty-eight RCTs which involved 6637 participants with T2DM and examined 132 different Chinese herbal medicines. The result showed that 42 out of 56 trials showed statistically significant improvement of blood glucose control by Chinese herbal medicine as an add-on therpay to western medicine compared with western medicine monotherapy (Yu et al. 2018).

1.7 Tangminling – a Chinese Herbal Formulation for T2DM

A distinctive feature of Chinese herbal medicine is its use of complex formulations that act on multiple physiological pathways simultaneously. In recent years, the development of evidence-based herbal products for type 2 diabetes mellitus (T2DM) has become an increasingly attractive area of research. Tangminling pill, coded as T81/T50, is a formulation derived from a classic formula described in the ancient treatise on exogenous febrile diseases, written over one thousand years ago. This pill, manufactured by Tasly Pharmaceutical Group Co., Ltd (Tianjin, China), comprises ten active ingredients using modern manufacturing techniques.

1.7.1 Medicinal Plants and Active Ingredients in Tangminling - T50 Pill

The pharmaceutical composition of the manufactured T50 pills includes herbal extracts from the following plants: Dahuang (Rhei Radix et Rhizoma), Huanglian (Coptidis Rhizoma), Baishao (Paeoniae Radix Alba), Chaihu (Bupleuri Radix), Huangcen (Scutellariae Radix), Zhishi (Aurantii Fructus Immaturus), Qingbanxia (Pinelliae Rhizoma Praeparatum Cum Alumine), Shanzha (Crataegi Fructus), Wumei (Mume Fructus), and Tianhuafen

(Trichosanthis Radix). The proportions of each component in the T50 formulation are based on the dosage ranges specified in the Chinese Pharmacopeia (see Table 2).

Table 2. Description of Chinese medicinal herbs in T50 formulation

Herbal Name	Major TCM Actions	Active Compounds	Pharmacological Effects	Clinical Applications
Huang Lian Rhizoma Coptidis	Clearing damp-heat, Cooling heat and purging fire, Counteracting toxin	Berberine Palmatine	Inhibitory effect against G-bacteria, Anti-inflammation and antipyretic effect, Lowing blood lipids and glucose, Anti-platelet aggregation and vasodilation	Gastritis and diarrhea, Infections disease and fever, Hyperlipidemia and diabetes, Hypertension
Da Huang Radix et Rhizoma Rhei	Purgative effects, Cleaning heat in the blood, Dispelling toxins	Anthraquinone Derivatives, Emodin, Rhein	Promote gastrointestinal peristalsis, Reducing glucose and lipids absorption, Hepatoprotective and choleretic effects	Constipation, Hyperlipidemia, diabetes, Fatty liver disease, obesity
Bai Shao Radix Paeoniae Alba	Nourishing blood, Smoothening Liver Qi	Paeoniflorins	Anti-inflammatory effects, Sedative effects	Blood deficiency syndrome in TCM
Chai Hu Radix Bupleuri	Relieving pathogens invasion, Regulating Liver Qi	Bupleurumol	Hepatoprotective effect against liver damage, antihyperlipidemic effect	Infection related fever, hyperlipidemia, fatty liver
Huang Qin Radix Scutellariae	Clearing away the heat, Dissolving dampness, purging the fire and detoxification	Wogonin Baicalin	Antibacterial and antiviral activities, hepatoprotective and choleretic effects, Antioxidant against b cell damage	Various infectious diseases, Hyperlipidemia and prevent diabetes
Zhi Shi Fructus Aurantii Immaturus	Resolving phlegm and reliving stuffiness in chest and abdomen	Citric Acids Flavonoids	Enhance gastrointestinal peristalsis, Promote metabolism and circulation	GI bloating, constipation, Indigestion, obesity
Ban Xia Rhizome Pinelliae	Removing dampnes, Resolving phlegm	Essential Oils	N/A	Phlegm and dampness, associated with GI bloating
Shan Zha Fructus Crataegi	Stimulating digestion, Invigorating blood circulation for blood stasis syndrome	Crategolic Acids Flavonoids	Increase digestive enzyme activity, Vasodilation & lowering blood pressure, Lowing blood lipids, anti-atherosclerosis	Digestive disorder, Hypertension & hyperlipidemia Prevent diabetic complications
Wu Mei Fructus Mume	Promoting production of body fluid, reducing dry cough	Organic Acids	Stimulating salivary gland secretion	Dry mouth related to symptom of diabetes
Tian Hua Fen Radix Trichosanthis	Promoting production of body fluid, moisture the dryness	Triterpene Saponins	N/A	Thirsty and polydipsia related symptom of diabetes

1.7.2 Experimental Studies: Effects of T50 and Its Herbal Ingredients on Animal Models of Diabetes

The anti-diabetic effects of T50 have been investigated using animal models of diabetes induced by streptozotocin (STZ) and a high-fat diet (HFD). According to Zhen et al. (2011), rats were administered 5 g/kg of body weight per day of T50 via oral gavage for two weeks. This treatment significantly reduced fasting blood glucose levels in diabetic rats compared to those given water. Additionally, the insulin sensitivity index (ISI) increased significantly in T50-treated animals, suggesting that T50 has glucose-lowering activity, in part by enhancing insulin sensitivity. T50 was also tested on oral glucose tolerance in STZ-HFD treated rats. After two weeks of treatment, blood glucose levels were significantly lower both before and two hours after glucose loading in T50-treated rats compared to those treated with water.

1.7.3 Human Study: Effect of T50 in Patients with Early-Stage T2DM

Tong et al. (2013) investigated the antidiabetic effects of T50/T81 through a multi-center clinical trial. This trial recruited 480 patients with newly diagnosed T2DM who had not received any pharmaceutical drug intervention. The participants received 6 g of T50 or a placebo, three times daily for 12 weeks. After 12 weeks of treatment, the reduction in HbA1c level was 1.02% in the treatment group compared to 0.47% in the control group. The treatment group also showed improved β-cell function and increased homeostatic model assessment (HOMA)-β. This large-scale randomized, double-blind, placebo-controlled study demonstrated the efficacy and safety of T50 as an initial monotherapy for newly diagnosed T2DM. Additionally, there were no significant differences in the types and frequency of adverse reactions between the T81 group and the placebo group. The results indicated that the Chinese herbal formula T50 is effective in controlling hyperglycaemia and is safe for use in participants with early-stage T2DM.

Cheng et al. (2019) conducted an analysis comparing the effects of Tangminling on clinical outcomes in patients with T2DM. They reviewed four studies that included 767 T2DM patients, comparing Tangminling against a placebo. The findings revealed that Tangminling treatment exhibited better efficacy than the placebo in reducing HbA1c, fasting plasma glucose, 2-hour postprandial glucose, HOMA-β, waist circumference, and body weight index. These results suggest that Tangminling pill might reduce glucose levels and body weight and improve β-cell function in T2DM patients.

1.8 Aims of This Study

Clinical studies in China have established the glucose-lowering efficacy and generally good tolerability of T81/T50 when used as monotherapy instead of metformin for patients with newly diagnosed T2DM. However, no studies have examined the use of T50 in patients with T2DM who have inadequate glycaemic control with metformin monotherapy. In Western countries, metformin (1000 mg-2000 mg daily) is often prescribed to patients after T2DM diagnosis and is considered the first drug of choice for T2DM treatment. As diabetes progresses, metformin may become ineffective in achieving optimal glycaemic control, necessitating combination therapy in many cases (Kalra & Gupta 2015).

Building on prior experimental and clinical studies, the primary aim of this pilot clinical trial was to evaluate the effect of T50 as an add-on to metformin in patients with T2DM and unsatisfactory glycaemic control. The secondary aim was to assess the impact of T50 on various metabolic factors related to T2DM control. Furthermore, the mechanism of T50 on diabetes control needs to be clarified. The safety and tolerability of T50 were monitored through the measurement of hemodynamic parameters and assessments of liver and kidney function. Tolerability and other adverse reactions were observed through participant interviews.

CHAPTER 2

Research Methodology

Chapter 2: Research Methodology

This study on Chinese Herbal Medicine (T50) as an adjunct to Metformin monotherapy in patients with Type 2 Diabetes Mellitus was a 12-week, randomized, double-blind, placebo-controlled pilot clinical trial conducted at a single-site medical center in Sydney, Australia.

2.1 Investigational Products

Investigational Product (IP) of T50 pill (Tang-Min-Ling-Wan, Tangminling Pills) is a formulation derived from a classic formula described in the Treatise on Exogenous Febrile Diseases over 1000 years ago. The ingredients in manufactured T50 include herbal extracts of Dahuang (Rhei Radix et Rhizoma), Huanglian (Coptidis Rhizoma), Baishao (Paeoniae Radix Alba), Chaihu (Bupleuri Radix), Huangcen (Scutellariae Radix), Zhishi (Aurantii Fructus Immaturus), Qingbanxia (Pinelliae Rhizoma Praeparatum Cum Alumine), Shanzha (Crataegi Fructus), Wumei (Mume Fructus), and Tianhuafen (Trichosanthis Radix). The proportion for each component in the T50 formulation is based on dosage ranges in the Chinese Pharmacopeia (Chinese Pharmacopoeia Commission 2010) Importantly, the T50 formula and its individual herbal components all belong to permitted herbal ingredients for clinical use in Australia (Chinese medicine Board of Australia 2012). T50 pills was manufactured and supplied by Tasly Pharmaceutical Group Co., Ltd (Tianjin, China). The formulation contains 10 herbal ingredients. In brief, raw herbs were mixed and extracted according to GMP procedures and 1 kg of finished pills is made from 5 kg of raw materials (concentrated pill: raw herbs 1:5 w/w).

The contents of the placebo sachets included 95% polyethylene glycol, 0.5% of caramel pigment, 4% of opadry and 4.5% of water. The appearance (colour, size, shape and weight) of the matching placebo pills looks the same as T50 pills. The smell of the placebo is slightly

lighter, and the taste is less bitter than T50. Both T50 pills and placebo pills were sealed in sachets with identical external appearances.

2.2 Interventions

During the treatment phase for 84 +/-3 days (12 weeks), participants took a total of 15 g per day (4 sachets per day). Participants received 4-weeks supply of supplements plus 8 additional sachets in case of visiting on a viable day. Neither the participants nor the study staff knew to which sachet each participant was randomised, therefore each participant took exactly the same number of pills in sachet. Each participant took 2 sachets of pills 30 minutes prior to breakfast and dinner, two times per day.

2.3 Research Objectives

The primary objective of study is to s to evaluate how effective T50 is when used in addition to metformin for individuals with T2DM who have suboptimal glycaemic control on metformin only. This will be measured by comparing the changes in HbA1c levels from the start of the study to 12 weeks, comparing T50 to a placebo.

Secondary Objectives of the study included:

- To determine the effects of T50 in combination with metformin on fasting plasma glucose and insulin levels, and on improvements in insulin sensitivity and insulin secretion by calculating HOMA-IR and HOMA-β relative to baseline and compared to placebo.
- To investigate any changes in fasting lipids (total cholesterol, triglyceride, HDL, LDL) from baseline compared to placebo.
- To assess changes in body weight, waist circumference and BMI.

Safety was assessed by measuring liver and kidney function and adverse events between the two groups.

2.4 Study Design, Ethical Approval and Clinical Trial Registration

The study protocol was designed by the principal investigatory with endocrinologist and clinical pharmacologist in the field. This clinical trial was approved by Human Research Ethic Committee at University of Technology Sydney (UTS HREC REF No. ETH18-2850) and investigating product T50 pills was approved by Australian Therapeutic goods Administration (TGA) for research purpose. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12619001041145).

A total of 42 participants were recruited and randomised into two study groups (3:1, T50 and placebo). During the screening session, a series of assessments and measurements were conducted to thoroughly evaluate each participant. This included measuring height and weight to establish baseline physical parameters. A detailed medical history was collected to understand the participant's health background, followed by measurements of waist circumference (WC), electrocardiogram (ECG), blood pressure (BP) and heart rate (HR) to evaluate cardiovascular health. Additionally, a blood sample was taken for laboratory analysis to screen for eligibility criteria and establish baseline biological markers.

Participants who met the eligibility criteria based on these assessments are then presented with a consent form detailing the study's purpose, procedures, potential risks, and benefits. Upon reviewing and understanding the information provided, eligible participants had the option to sign the consent form to officially enrol in the clinical trial.

After the screening visit, participants were randomized to either placebo or investigating product (T50 pills) for 12-weeks of intervention, in a 1:3 ratio, using simple block

randomization on the computer-generated randomization program (FileMaker Pro), which ensures that randomization is truly random. Each participant received a unique study identification number upon enrolment. According to the study protocol, physical examination, TCM, and life quality questionnaire were conducted, and blood and urine samples were collected for pathology analysis by Laverty (North Ryde, NSW, Australia). The study flow and schedule of procedures for enrolment, intervention and assessments are illustrated in Figure 2 and Table 3.

2.5 Participants

Patients who are greater than 18 years old and less than 80 years old have had a confirmed diagnosis of T2DM and had been having Metformin as monotherapy (>1000mg/d) for at least 1 month prior to screening. Most importantly, patients' HbA1c level was 7-9% at the screening visit. The study had also established exclusion criteria in the study protocol.

The eligible participants were randomised in a 3:1 ratio to receive either 7.5g T50 twice daily or 7.5g placebo for 12 weeks. During the study period, the dosage of metformin, as well as dietary and lifestyle factors remain unchanged.

All patients' information was strictly confidential. Patients could make informed decision by the information provided and made informed consent to agree to participate this project.

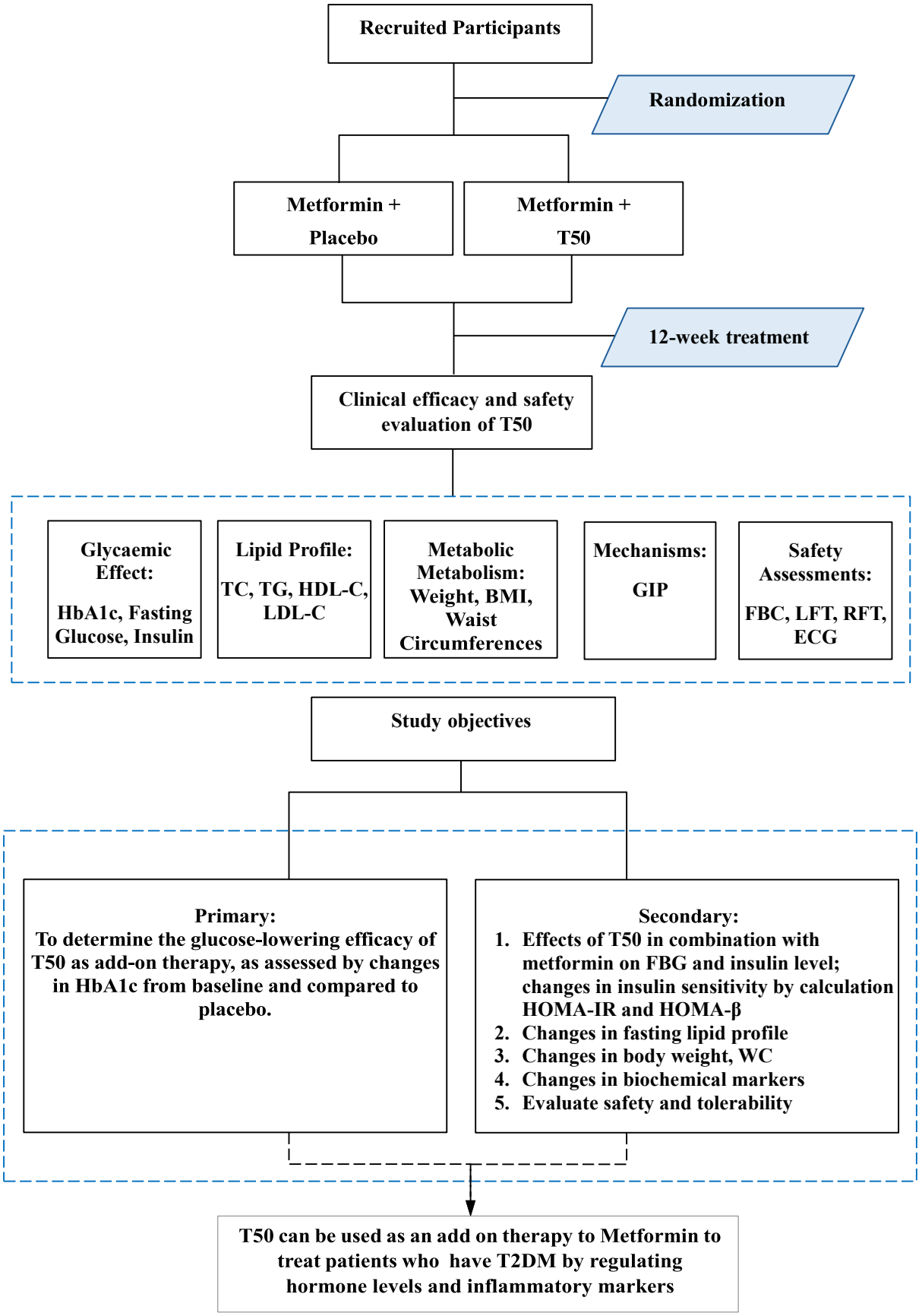

Figure 2. Study design and participant flow during the study.

Table 3. Schedule of procedures for enrolment, intervention and assessments

Visit Number	1	2	3	4	5	6
Week	-1 wk	Day 0±3	4	8	12	16
Days	Screen	Baseline	29±3 days	57±3 days	85±days	113±3days
Sign Consent	✓					
Medical History & Height	✓					
Record Current/New Medications	✓	✓	✓	✓	✓	✓
Record TCM Symptoms and Signs		✓	✓	✓	✓	✓
Completion of Questionnaires		✓			✓	
AE Monitoring		✓	✓	✓	✓	✓
Urine Collection[1]	✓				✓	
Blood Collection[2]	✓		✓	✓	✓	✓
Electrocardiogram[3]	✓				✓	
Physical Examination[4]	✓	✓	✓	✓	✓	✓
Vital Signs[5]	✓	✓	✓	✓	✓	✓
Dispense Study Product		✓	✓	✓		
Prescription of metformin		✓	✓	✓	✓	
ESTIMATED TIME (minutes)	90	40	40	40	60	40

1. Urinalysis (pH, protein levels, glucose, blood, leukocytes, and erythrocytes) and a pregnancy test.
2. Fasting HbA1c, glucose, insulin, C-peptide, lipid profile, liver and kidney function, Hs-CRP, inflammatory markers and adipokines, and gut hormones.
3. Heart rate, P duration, PR interval, QT interval, QRS complex, QTC interval, QTCF.
4. Physical Examination: height, body weight, and waist circumference.
5. Vital Signs: heart rate and blood pressure

2.6 Inclusion Criteria and Exclusion Criteria

Participants were considered for the study if they meet the following inclusion criteria: 1) Male or Female, aged ≥18 and <80 years; 2) T2DM taking Metformin (≥1000 mg/day) as monotherapy for at least 1 month; 3) HbA1c: 7.0% - 9.0% (53 – 75 mmol/L) at screening visit; 4) Females of childbearing potential must have a negative pregnancy test and using a reliable method of contraception; 5) Participants who can understand study related procedures & who give written consent; 6) Participant with ability to read and write English.

Patients were excluded from the recruitment procedure when they: 1) are type 1 diabetes; 2) are taking any glucose-lowering drugs other than metformin, and those on insulin. 3) have uncontrolled hypertension (BP ≥200 mmHg systolic and/or ≥110 mmHg diastolic); 4) have a history of unstable angina or acute coronary syndrome within 3 months of screening. 5) have moderate-to-severe heart failure; 6) have a history of pancreatitis; 7) have significant liver dysfunction (serum ALT or AST >2.5 times the upper limit of the normal range); 8) have severe kidney dysfunction (eGFR ≤ 45 mL/min/1.73 m^2); 9) have untreated thyroid disease; 10) have a history of systemic malignancy; 11) have a history of alcohol or illicit drug abuse; 12) have a history of allergy to herbal medicine products; 13) have other medical conditions that may adversely affect the outcome of the study, including diabetic retinopathy; 14) participated in another clinical trial in the last 2 months; 15) are unable to commit to the appointment schedule required in the study (Fridays).

All women of childbearing age must have a negative pregnancy test before enrolling in this trial and using a reliable method of contraception during the period of a clinical trial.

2.7 Measurements

Efficacy Assessments

The primary efficacy endpoint in the randomized, double-blind phase was the change in HbA1c from screening test (week 0; baseline) to the completion of the treatment period (week 12). Secondary endpoints included fasting serum glucose and insulin concentrations at each assessment point, Other endpoints included: (1) fasting C-peptide, fasting serum lipids total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), body weight, waist circumference, HOMA-R (homeostasis model assessment of insulin resistance), HOMA-β (homeostasis model assessment of β-cell function), and active GIP concentrations measured at baseline and the end of 12 week treatment.

Safety Assessments

Safety was assessed by recording all vital signs, BP, 12-lead ECG, and laboratory tests including haematology, serum chemistry for liver and kidney function and urinalysis parameters. At each visit, participants were asked to report any health-related symptoms or events. All adverse events were fully documented in the case record form (CRF), and the medical investigator were assessed the likelihood of any relationship to study medication, or not related.

2.8 Study Procedures

Schedule of study procedures for enrolment, intervention and assessments was listed in the table 2.

2.8.1 Screening Evaluation (Visit 1) (Day -7)

At screening (1 week prior to baseline visit), the following measurements and personal history were recorded:

- Consent
- Medical history (including any forthcoming procedures)
- Concomitant medications
- Height
- Body weight
- Waist circumference
- Blood pressure and heart rate
- Electrocardiogram (ECG)
- Venous blood sample

The medical history assessment invoiced specific questions on cardiac history, angina, breathlessness, swollen ankles, sleep apnoea, urinary incontinence, impaired fertility, depression, anxiety and functional limitations such as joint pain and decreased mobility.

At screening visit, participants' diabetes status including microvascular and macrovascular complications were confirmed. The blood sample were used to test the following agents:

- Fasting blood glucose
- Fasting insulin
- Glycated Haemoglobin (HbA1c)
- Full lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, total cholesterol: HDL ratio, triglycerides)
- Liver enzymes (ALT, AST)
- Creatinine and eGFR calculation
- Full blood count (white cell, platelet, red cell)
- Glucose-dependent insulinotropic polypeptide (GIP) (extra serum sample were stored at -80℃ for further analysis).

Female participants who were at childbearing age were collected a urine sample to confirm the absence of pregnancy.

Each participant had a folder labelled with unique study number. All information and procedure were documented and saved in patient's folder. All study folders were securely stored in a locked file storage.

2.8.2 Baseline Visit (Visit 2) (Day 0)

At the baseline visit 2 (7+/-3 days after screening), procedures and assessments included:

- Body weight
- Waist circumference
- Dispensing of 4 weeks supply of IP (treatment or placebo)
- Completion of questionnaires for AQOL survey and TCM assessment
- Reviewing and recording any changes to concomitant (non-diabetes) medications and dosage
- Monitoring AE
- Providing a booklet with instruction of administration of study product, date of next visit and diary for recording health condition and AE if any

During this visit, test results from the screening visit were checked and confirmed the eligibility of study. IP were dispensed and instruction on how to take the study medication were given to participants. The participants were reminded to keep the metformin therapy regimen unchanged throughout the study. The participants were encouraged to document on dairy any adverse events they encountered. The appointment time were made for the next visit. All documents were kept in the patient's folder and were kept in the locked file cupboard.

2.8.3 Treatment Visit (Visit 3) (Day 29)

At Visit 3 (28 days after the baseline visit +/-3), the following assessments and interventions were conducted:

- Body weight
- Waist circumference
- Blood pressure and heart rate
- Review and record changes to concomitant medications and dose
- AE monitoring
- Completion of TCM assessment
- Collection of diaries and dispensing of new diary
- Dispensing of 4 weeks supply of T50 supplement (active or placebo)
- Reconcile returned study medication

The blood samples were taken to measure the following:

- Fasting blood glucose, insulin and C-peptide
- Glycated Haemoglobin (HbA1c)
- Full lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, total
- cholesterol: HDL ratio, triglycerides)
- Liver enzymes (ALT, AST)
- Creatinine and eGFR calculation

Participants were asked to return the study medication box, and the leftover pills were counted and documented. Participants were asked about any side effects they experienced during this period. The blood samples were taken and sent to Laverty pathology for testing.

2.8.4 Treatment Visit (Visit 4) (Day 57)

At Visit 4 (56 days after the baseline visit +/-3), the following measurements were conducted:

- Body weight
- Waist circumference
- Blood pressure and heart rate
- Reviewing and recording changes to concomitant medications and dose
- AE monitoring
- Completion of TCM assessment
- Collection of diaries and dispensing of new diary
- Dispensing of 4 weeks supply of supplement (active or placebo)
- Reconcile returned study supplements

The following laboratory samples were taken to test on the following:

- Fasting blood glucose, insulin and C-peptide
- Glycated Haemoglobin (HbA1c)
- Full lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, total
- cholesterol: HDL ratio, triglycerides)
- Liver enzymes (ALT, AST)
- Creatinine and eGFR calculation

Participants were instructed to return their study medication box for pill counting and documentation of remaining pills. Previous test results were explained to the patients, any questions related to the progress of the study were answered. They were also queried about any side effects encountered during the study period. Blood samples again were collected and sent to Laverty Pathology for testing.

2.8.5 Treatment Visit (Visit 5) (Day 85)

At Visit 5 (84 days after the baseline visit +/-3), participants were no longer be required to take the investigational products. The following activities occurred during the visit:

- Body weight
- Waist circumference
- Blood pressure and heart rate
- Electrocardiogram (ECG)
- Collection of diaries
- Completion of questionnaires for AOL survey and TCM assessment
- Review and record changes to concomitant medications and dose
- AE monitoring
- Reconcile returned study supplements

Blood sample collected for below tests:

- Fasting blood glucose, insulin and C-peptide
- Glycated Haemoglobin (HbA1c)
- Full lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, total
- cholesterol: HDL ratio, triglycerides)
- Liver enzymes (AST, ALT)
- Creatinine and eGFR calculation
- Full blood count
- Glucose-dependent insulinotropic polypeptide (GIP) (extra serum sample were stored at -80℃ for further analysis).

Participants were instructed to return their study medication box for pill counting and documentation of remaining pills. Previous test results were explained to the patients, any questions related to the progress of the study were answered. Any side effects were encouraged to be reported. The test results and QOL assessment forms were kept in the patient's file. Blood was taken with additional samples transferred to the UTS laboratory for GIP testing.

2.8.6 Final Visit (Visit 6) (Day 113)

One month after completion of the study, participants had a follow-up visit (113 days after the baseline visit +/-3). The following measurements were conducted:

- Body weight
- Waist circumference
- Blood pressure and heart rate
- Review and record changes to concomitant medications and dose
- AE monitoring

Blood collection for laboratory tests:

- Fasting blood glucose and insulin
- Glycated Haemoglobin (HbA1c)
- Full lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, total cholesterol: HDL ratio, triglycerides)
- Liver enzymes (AST, ALT)
- Creatinine and eGFR calculation

This visit was to measure the long-term effects of T50. Blood was taken to measure biological markers and results were interpreted by the GP. The study was finished, and all documents were finalised and kept in patient records. The patient data were entered to the system (OpenClinica).

2.9 Data Collection

This study was a double blinded randomisation to eliminate selection bias. The study used electronic data capture (EDC) systems to manage data electronically. Biomarkers and laboratory tests were performed by Laverty Pathology (North Ryde, NSW Australia). Clinical data were collected through patient interviews, blood tests and research laboratory testing. This study also included a follow-up period to assess long-term effects. Compliance with the assigned interventions was monitored throughout the 12-week intervention period using self-reported adherence.

This holistic data collection strategy included a wide range of health indicators, such as participants' physical examinations, 12-lead ECG and laboratory testing enabled a thorough evaluation of the T50's impact on T2DM management. The procedures of measurements were detailed in Appendix A. This study aimed to provide comprehensive insights into the effectiveness and safety of T50 as an adjunct treatment for T2DM by comparing HbA1c, glucose and insulin levels, liver and kidney functions at week 12 to baseline results.

Each participant had a folder labelled with unique study number. All information, procedure and pathology report were documented and saved in patient's folder. All study folders were securely stored in a locked file storage at MediCentral (Sydney, Australia).

2.10 Statistical Analysis

In the randomized, double-blind study, the primary efficacy analysis (change in HbA1c from baseline to week 12), summary statistics (means, standard errors) of clinical and biomedical parameters were calculated for each treatment group. HbA1c changes at week 12 from baseline as a dependent variable, HbA1c at baseline as a covariate, and different treatment group as an independent variable. For analysis of differences between groups, analysis of covariance (ANOVA) was used, followed by Tukey's test to determine significant differences between the two treatments using Prism version 10 (GraphPad Software Inc, CA, USA). $p\text{-value} < 0.05$ was considered statistically significant.

CHAPTER 3

Effect and Mechanism of T50 on Glycaemic Control for Diabetic Patient with Unsatisfied HbA1c Levels in Metformin Monotherapy

Chapter 3: Effect and Mechanism of T50 on Glycaemic Control for Diabetic Patient with Unsatisfied HbA1c Levels in Metformin Monotherapy

3.1 Brief introduction

Previous clinical study has shown that the T50 (originally coded as TM81), a traditional Chinese medicine formula, was effective and safe to use in patients with newly diagnosed Type 2 Diabetes Mellitus (T2DM) (Tong et al. 2013) but there is no study utilizing T50 for patients with T2DM and inadequate glycemic control with metformin monotherapy. The primary aim of this pilot clinical trial is to assess the effectiveness of T50 as an adjunct therapy to Metformin in managing T2DM. The study design is to focus primarily on evaluating changes in HbA1c levels over a 12-week period, recognizing HbA1c as a pivotal indicator of long-term glycaemic control and diabetes management (RACGP 2020) The secondary aims were focused on assessing the impact of T50 on various biological factors regarding glycaemic control and metabolic parameters, such as insulin levels, body weight and lipid profile. By examining these impacts of T50, we aim to provide a holistic understanding of T50's effects on glycaemic control and to assess the safety and tolerability of T50 as an add-on therapy to Metformin.

Moreover, this study investigated the molecular mechanisms of antidiabetic action of T50 product. It was hypotheses that T50 may improve insulin sensitivity, as evidenced by changes in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Homeostatic Model Assessment of Beta-cell function (HOMA- β) from previous study (Son et al. 2022). T50 formula contains active ingredients that improve diabetic condition through and anti-inflammation and regulating gastrointestinal function (Zhang et al. 2019). Gut hormone released by enteroendocrine cells in response to T50 treatment was detected in this study. Specifically, the laboratory test was conducted on changes in the secretion of Glucose-

Dependent Insulinotropic Polypeptide (GIP), one of the key hormones involved in the regulation of glucose homeostasis and appetite control (Seino et al. 2010). Through this investigation, we aim to gain insights into the mechanisms of T50 effects on glycaemic control for T2DM.

3.2 Research Plan and Methods

3.2.1 Participants

The research plan employed a randomized, double-blind, placebo-controlled design to investigate the efficacy and safety of the T50 herbal formulation as add-on therapy for T2DM patients with metformin (1000 mg - 2000 mg/daily) and unsatisfied glycaemic control (HbA1C 7 – 9%). Detailed inclusion and exclusion criteria to ensure a representative sample of the T2DM population was demonstrated in Chapter 2 (Refer Chapter 2.6). Participants were recruited from the existing medical data base at medical centres (Sydney CBD, Chatswood, and Hurstville, NSW Australia) and from advertising at the University of Technology Sydney. Eligible participants were randomly assigned to receive either T50 or a placebo, in a 3:1 ratio, in addition to their standard metformin treatment.

There were 53 participants screened initially, of whom 42 met the eligibility criteria and were subsequently randomized into the T50 treatment group or the placebo group, alongside the same diabetes medications and lifestyle interventions. The randomization process followed a 3:1 ratio of treatment to placebo (Figure 3). The intervention period was set for 12 weeks, with 4 visits to monitor glycaemic control, liver and kidney function, overall well-being, and tolerability.

During the twelve weeks of the trial, two participants withdrew from the study. One participant withdrew the study because of the diarrhoea from the placebo group and the other participant withdrew from T50 group due to intolerance to the bitter taste of the IP.

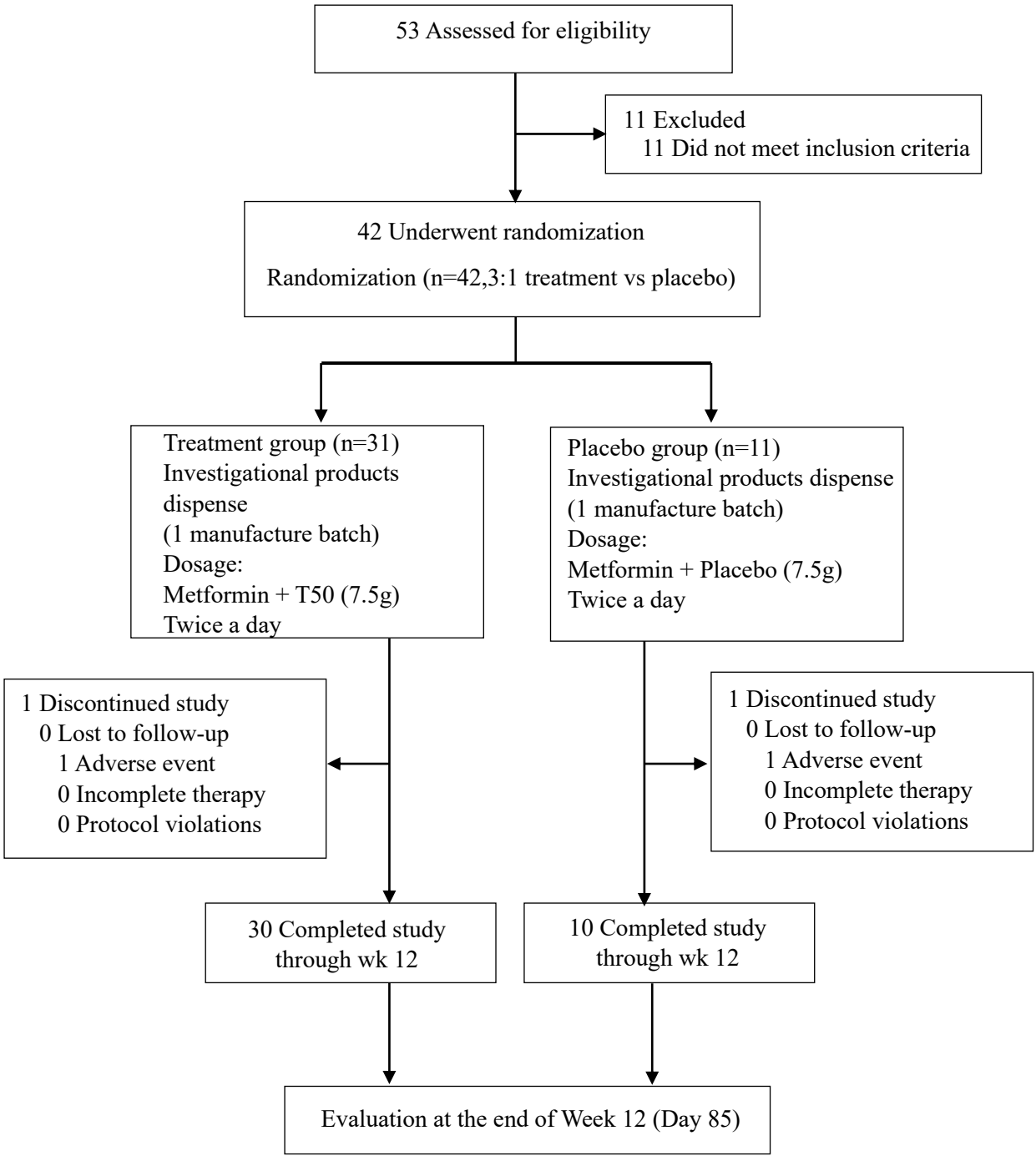

Figure 3. Flow diagram of participant screening, randomization, treatment, and observation. A total of 30 subjects in treatment group and 10 subjects in the placebo group, 2 subjects dropped out from the study due to reasons described in the text.

3.2.2 Investigational Products (IP)

The chemical composition of T50 was measured by using a high-performance liquid chromatography/mass spectrometry (HPLC/MS) method. An example chromatographic fingerprint is Figure 4. Chemical structures of six major compounds (berberine, albiflorin, paeoniflorin, naringin, hesperidin and baicalin) in the finished dosage are also shown. These compounds were also used as the quality control markers in the T50 formulation.

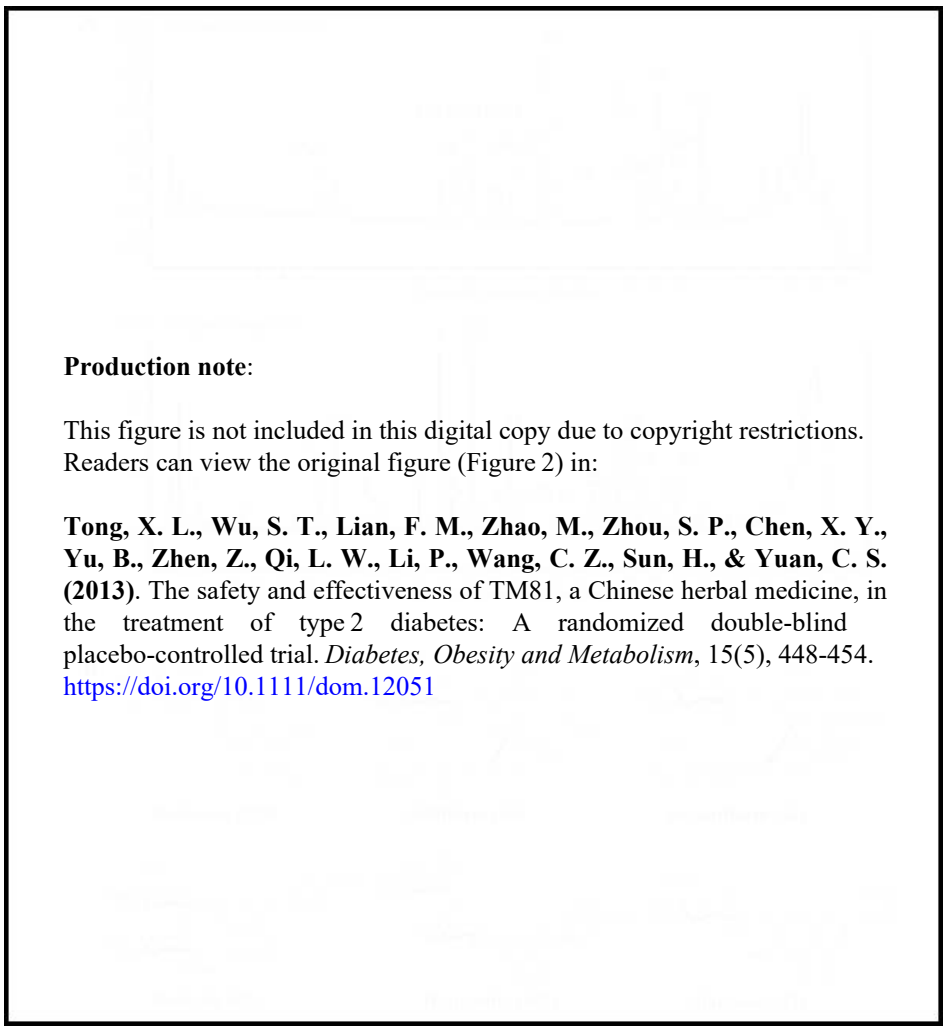

Figure 4. (A) High-performance liquid chromatography/mass spectrometry analysis of TM81 extract. Representative total ion current (TIC) chromatograms of the TM81 extract obtained in positive (upper panel) and negative (lower panel) ion electrospray. Visual inspection of the two TIC plots shows that the two modes of ionization generate different constituent information based on the ionizability differences of the components in TM81. A total of 31 peaks were observed. (B) Six chemical structures of some most abundant compounds identified in TM81: berberine (peak #19) in upper panel of (A), and albiflorin (peak #3), paeoniflorin (peak #4), naringin (peak #7), hesperidin (peak #12) and baicalin (peak #17) in lower panel of (A).

3.2.3 Treatment Administration

Upon successful enrolment and randomization, participants began the treatment phase of the trial. The intervention group received the T50 investigating product in conjunction with their prescribed metformin regimen, while the control group was administered a placebo alongside metformin.

Investigational products (IP) were packed as 120 sachets in one box for 4 weeks. There were three study IP visit packs for each participant. In each visit pack, there were 120 sachets, including 112 sachets for both morning and evening doses of 4 week's supply of IP during the treatment phase, and additional eight sachets for visiting on variable days. Each sachet contained 3.75g of T50 pills or placebo pills and were administered twice daily orally; hence four sachets (15g) were administered daily.

The placebo has the same size and same taste as T50 pills. Dosage for the T50 herbal medicine (7.5g) was standardized across the intervention group, administered twice daily at a dose determined to be clinically effective and safe in previous studies (Tong et al. 2013). The participants in the placebo group will take the same dosage placebo products (7.5g) twice daily in addition to their regular Metformin regimen.

All IP and placebo were shipped from Tasly and the study site confirmed the receipt of the IP and placebo. The study site checked the packs, and no damaged packs found. All IP and placebo were stored at room temperature.

The products were properly labelled with the study name, study participation number, storage instructions, pack number, batch number, date dispensed, expiry date, visit number, sponsor name, for "clinical trial use only" and "keep out of reach of children" signage. All participants were instructed on proper medication intake procedures by the research team to maximize

adherence. The instruction was attached in the medication box contains information such as directions for use, sponsor contract details and ingredients of IP.

Adherence rates were monitored through a combination of self-reported medication diaries, pill counts at scheduled study visits. Instances of non-adherence were recorded, with reasons for missed doses categorized and analysed. Protocol deviations, either in dosing or administration, were systematically tracked. This thorough approach to treatment administration was designed to ensure the integrity of the trial's findings and to facilitate a clear understanding of the T50 herbal medicine's impact when used as an adjunct therapy for T2DM.

3.2.4 Clinical Assessments and Pathological Tests

Regular follow-up visits, scheduled every four weeks (Table 3), included clinical assessments conducted to measure patients' height, weight, BMI, heart rate, and blood pressure. Biochemical measurements such as HbA1c level, fasting glucose, insulin level, full blood count, and lipid profile, creatinine and eGFR were conducted by the Laverty Pathology. Additionally, participants were encouraged to report any adverse events or side effects experienced during the study. With systematic collection and evaluation of this information the tolerability and safety of T50 have been monitored.

3.2.5 Measurement of Glucose-Dependent Insulinotropic Peptide

To elucidate whether T50 influences secretion of incretin hormones (Holst et al. 2009) Plasm levels of GIP were measured at the baseline and after 12-weeks intervention. Briefly, blood samples for GIP were drawn from participants and centrifugated immediately. The plasma was stored for -80 °C until analysis. The high-quality enzyme immunoassay for the quantification of GIP in plasma were measured using chemiluminescent ELISA protocols from Crystal Chem (Catalog number 81515, Crystal Chem, IL, USA). 630 Determining the total GIP concentration

in plasma was determined using Quantitative Flow Cytometry (BD FACSLyric™ Flow Cytometry, BD Bioscience, NJ, USA) at wavelength A450 and 630.

3.2.6 Data Collection and Statistics

The randomization codes were kept sealed and inaccessible to the researcher until the completion of the clinical study. Unblinding occurred when the primary analysis was completed.

Data were collected through patient interviews, blood tests, continuous glucose monitoring and research laboratory testing. This study also included a follow-up period to assess long-term effects. Compliance with the assigned interventions was monitored throughout the 12-week intervention period using left counts and self-reported adherence.

This holistic data collection strategy as detailed in Chapter 2.9, encompassing a wide range of health indicators, enabled a thorough evaluation of the T50's impact on T2DM management. Through the execution of standardized protocols and the utilization of specialized equipment and expertise, this study aimed to provide comprehensive insights into the efficacy and safety of T50 as an adjunct treatment for T2DM. The statistical analysis plan is structured to handle both within-group and between-group comparisons. Analysis of variance (ANOVA) was the primary statistical technique employed to detect any significant differences in continuous outcome measures, such as HbA1c levels, fasting plasma glucose, and other metabolic parameters, followed by followed by Tukey's test to determine significant differences between the two treatments using Prism version 10 (GraphPad Software Inc, CA, USA). $p\text{-value} < 0.05$ was considered statistically significant.

3.3 Results

3.3.1 Baseline Characteristics

In this study, descriptive statistics provide a detailed snapshot of the baseline characteristics of the enrolled participants, as well as the changes observed in key clinical parameters over the 12-week intervention period. Baseline characteristics of the enrolled participants revealed an average age of 63 years, with a relatively balanced distribution between genders. The mean duration of diabetes among participants was 7 years, and the average baseline HbA1c level was 7.45%. Additional baseline parameters, such as body weight, BMI, fasting blood glucose levels, insulin, and lipid profile, were also assessed at the beginning of the study to establish a comprehensive understanding of the participants' health status (Table 3). It was noted that the baseline characteristics of participants were similar across both groups, with no significant differences in weight, BMI, duration of diabetes or baseline HbA1 levels.

Table 4. Baseline demographic characteristics of patients randomized to treatment in the double-blind study.

Characteristics	**Placebo+ Metformin (n=10)**	**T50 pills+ Metformin (n=30)**	**Total (n= 40)**
Age, years (Mean ± SE)	68.29 ± 0.42	58.43 ± 2.07	63.36 ± 1.25
Gender			
Male (n, %)	5 (50.00)	16 (53.33%)	21 (52.50)
Female (n, %)	5 (50.00)	14 (46.67%)	19 (47.50)
Body Weight (kg)	78.57 ± 6.72	79.44 ± 3.70	79.00 ± 5.21
Height, cm	165.71 ± 3.74	169.66 ± 1.67	167.69 ± 2.70
BMI, kg/m^2	27.1 ± 5.46	28.3 ± 4.13	27.7 ± 4.80
Duration of diabetes, years	7.86 ± 6.21	6.12 ± 8.21	6.99 ± 7.21
HbA1c, %	7.36 ± 0.20	7.54 ± 0.11	7.45 ± 0.16
Fasting glucose (mmol/L)	7.71 ± 0.79	8.04 ± 0.30	7.88 ± 1.84
Insulin (mU/mL)	12.43 ± 3.84	11.03 ± 1.38	11.73 ± 2.61
Total cholesterol (mmol/L)	4.30 ± 0.42	4.05 ± 0.17	4.18 ± 0.30
Triglycerides (mmol/L)	1.87 ± 0.37	1.76 ± 0.18	1.82 ± 0.28

Data were presented as Mean ± SE. Abbreviations: BMI, body weight index, HbA1c, Glycated haemoglobin, n=40.

3.3.2 Primary Outcome Measurements

3.3.2.1 Changes in HbA1c Levels

The primary objective of the study was to assess the alterations in HbA1c levels at week 12 for the placebo group, which received Metformin (1000 mg - 2000 mg) as monotherapy, in comparison to the treatment group, which received T50 pills (7.5 g, twice daily) in addition to Metformin.

In a comparative analysis of the HbA1c levels at the end of 12-week treatment between the placebo group and the treatment group, statistical results showed the T50 combined with Metformin significantly reduced HbA1c compared with the placebo ($p < 0.05$, T50 vs. placebo). implying that the T50 pill may have a distinct advantage in glycaemic control (Figure 5).

Furthermore, a comparison of HbA1c levels from baseline to the 12-week point showed a statistically significant reduction ($p < 0.05$, baseline vs. 12 weeks-results) (Figure 5).

Following 12 weeks of treatment, the mean HbA1c level in placebo group has increased 0.13 ± 0.28% versus the T50 treatment group the HbA1c levels decreased by 0.36 ± 0.17% (Figure 5).

These data demonstrate that the T50 plus Metformin has some effect in lowering HbA1c levels over the duration of the study.

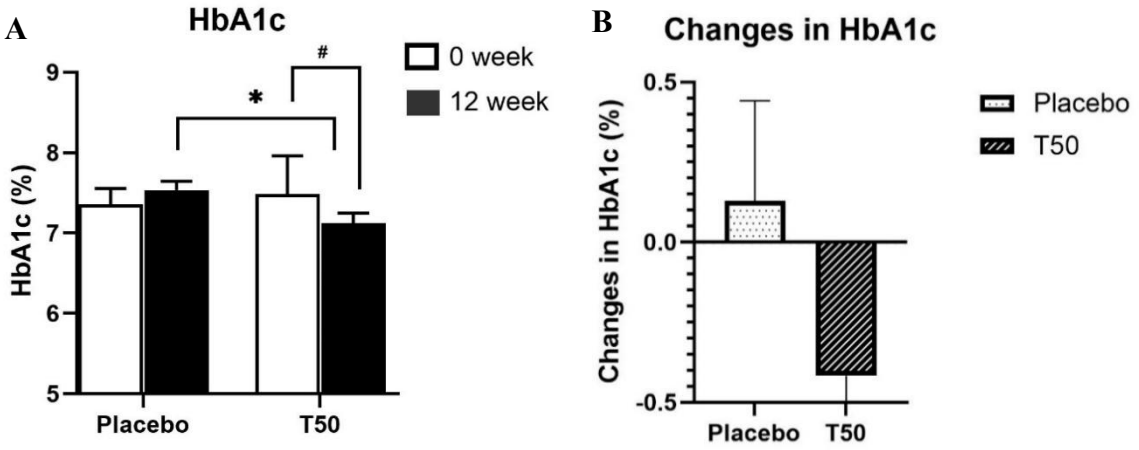

Figure 5. Changes in HbA1c Levels. Left panel A: HbA1c level between a placebo group and a T50 treatment group at week 0 and week 12. Right panel B: Changes in HbA1c in T50 group and placebo group at week 0 and week 12. Data were presented as Mean ± SE. *p < 0.05, #p < 0.05

3.3.2.2 Blood Glucose Level Variations

The fasting glucose level was 7.71 mmol/L in the placebo group versus 7.49 mmol/L in the T50 group. After 12 weeks treatment, the fasting glucose level was 8.06 ± 0.94 mmol/L in the placebo group versus 7.74 ± 0.29 mmol/L in the T50 treatment group. Compared to their baseline values, the fasting glucose level increased by 0.34 ± 0.6 5mmol/L versus decreased by 0.30 ± 0.37mmol/L in the T50 group (Figure 6). The findings suggest that the treatment did not significantly affect fasting glucose levels over time.

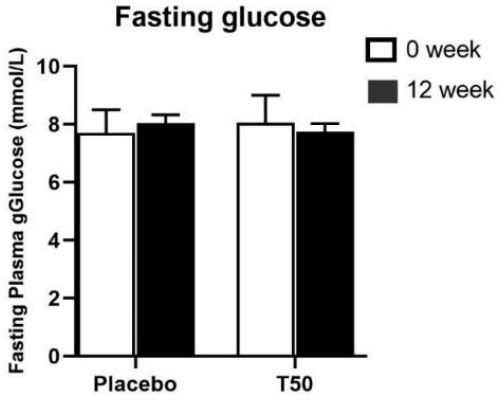

Figure 6. Changes in fasting glucose level. Fasting glucose level (mmol/L) between a placebo group and a T50 treatment group over a period o to 12 weeks. Data were presented as Mean ± SE.

3.3.2.3 Insulin Level Variations

Figure 7 shows fasting insulin levels at baseline and at the end of 12 weeks treatment in placebo group and T50 group. At baseline, the insulin level was 12.43 mU/mL in placebo group and 11.03 mU/mL in treatment group. There was no statistically significant difference. After 12 weeks' intervention with placebo or T50 pills, insulin level was 12.57 ± 3.16 mU/mL in the placebo group, 10.04 ± 1.26 mU/mL in the T50 treatment group.

Compared to their baseline values, the fasting insulin level increased by 0.34 ± 0.65 mU/mL in placebo group versus decreased by 0.30 ± 0.37 mU/mL in the T50 group (Figure 6). The results suggest T50 can lower the insulin secretion; however, these changes did not reach statistical significance.

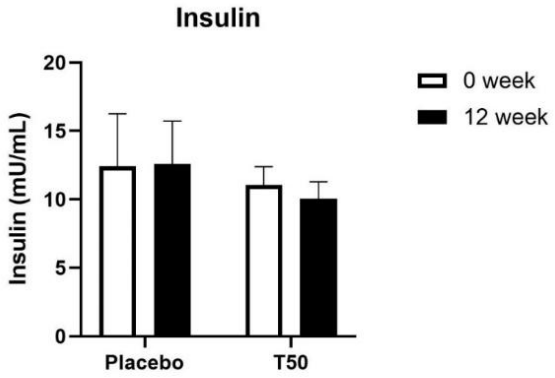

Figure 7. Comparative Analysis of Insulins Levels in Placebo vs T50 at week 0 and week 12. insulin level (mU/mL) between a placebo group and a T50 treatment group at week 0 and week 12. Data were presented as Mean ± SE.

3.3.2.4 Changes in HOMA-IR and HOMA-β

HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) is used to estimate insulin resistance, which is a condition where the body's cells become less responsive to insulin. HOMA-IR is calculated using fasting glucose and fasting insulin levels. Higher HOMA-IR values indicate greater insulin resistance.

The baseline HOMA-IR score is 4.42 ± 1.46 in placebo group and 4.02 ± 0.51 in treatment group. After 12 weeks treatment, the HOMA-IR was 4.33 ± 0.93 in the placebo group and 3.4 ± 0.41 in the treatment group. In the placebo group, there was a minimal reduction but was not statistically significant. In the treatment group, a comparison of HOMAR-IR from baseline to the 12-week point showed a statistically significant reduction ($p < 0.05$, baseline vs. 12 weeks-results) (Figure 8). As indicated by data, there was a significant decrease in HOMA-IR scores (Table 5). This suggests that T50 has the potential to improve insulin sensitivity.

HOMA-Beta (Homeostatic Model Assessment of Beta-cell function) is to estimate β-cell function, which refers to the ability of the pancreas to produce insulin in response to glucose levels in the blood. HOMA - β is also calculated using fasting glucose and fasting insulin levels. Higher HOMA-β indicates better β cell's function. Both groups showed an increase in HOMA-β from baseline to week 12, suggesting a stable β-cell function.

The findings indicate that T50 has effects of reducing insulin resistance, as demonstrated by the reduction in HOMA - IR scores. The lack of effect on HOMA - β suggests that its therapeutic benefits are likely through mechanisms other than altering β-cell function.

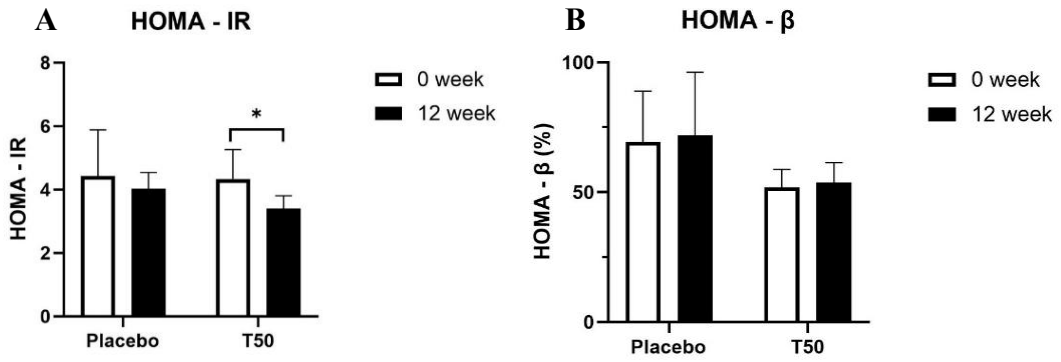

Figure 8. HOMA-IR and HOMA-β Measurements. Left pancel A: HOMA-IR between a placebo group and a T50 Treatment group at week 0 and week 12; Right pancel B: HOMA-β between a placebo group and a T50 treatment group at week 0 and week 12. Data were presented as Mean ± SE. Abbreviations: HOMA-IR, homeostatic model assessment to quantify insulin resistance, HOMA-β, homeostatic model assessment to quantify β-cell function. *p=0.00012 (p<0.001)

Table 5. Changes in HOMA - IR and HOMA - β values after 12 weeks treatment with T50 and group.

Items	Change	Metformin + T50	Metformin + Placebo
HOMA-IR	Decrease	-0.66 ± 0.41	-0.09 ± 1.20
HOMA-β	Increase	2.26 ± 5.1	4.41 ± 9.66

Data presented as Mean ± SE. Abbreviations: HOMA - IR, homeostatic model assessment to quantify insulin resistance, HOMA - β, homeostatic model assessment to quantify β-cell function.

3.3.3 Secondary Outcome Measurements

3.3.3.1 Lipid Profiles Measurements

Lipids profiles typically include parameters such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG).

Both the placebo and treatment groups showed a slight decrease in TC and TG levels, but there was a slight increase in HDL-C levels and a slight increase LDL-C levels after the 12-week intervention period in treatment group.

Total cholesterol (TC)

The TC was 4.30 ± 0.42 mmol/L in the placebo group versus 4.05 ± 0.17mmol/L in the treatment group at week 0. After 12-week intervention, the TC was 4.20 ± 0.35 mmol/L in the placebo group and 4.02 ± 0.19 mmol/L in the treatment group.

HDL cholesterol (HDL-C)

The HDL-C was 1.31 ± 0.11 mmol/L in the placebo group and 1.15 ± 0.06 mmol/L in the treatment group at baseline. After 12-week intervention, the HDL-C was 1.39 ± 0.1 mmol/L in the placebo group and 1.19 ± 0.06 mmol/L in the treatment group.

Triglycerides (TG)

At baseline, the TG was 1.87 ± 0.37 mmol/L in the placebo group and after 12-week intervention, the TG was 1.63 ± 0.29 mmol/L. The TG was 1.77 in the treatment group at week 0 and become 1.52 ± 0.1mmol/L after 12-week intervention.

LDL cholesterol (LDL-C)

At baseline, the LDL-C was 2.11 ± 0.33 mmol/L in the placebo group and after 12-week intervention, the LDL-C was 2.07 ± 0.25 mmol/L. The LDL-C was 2.18 ± 0.15mmol/L in the treatment group at week 0 and became 2.20 ± 0.15 mmol/L after 12-week intervention.

However, these changes were not statistically significant, indicating that T50 supplementation did not lead to significant alterations in total cholesterol levels compared to the placebo group (Figure 9).

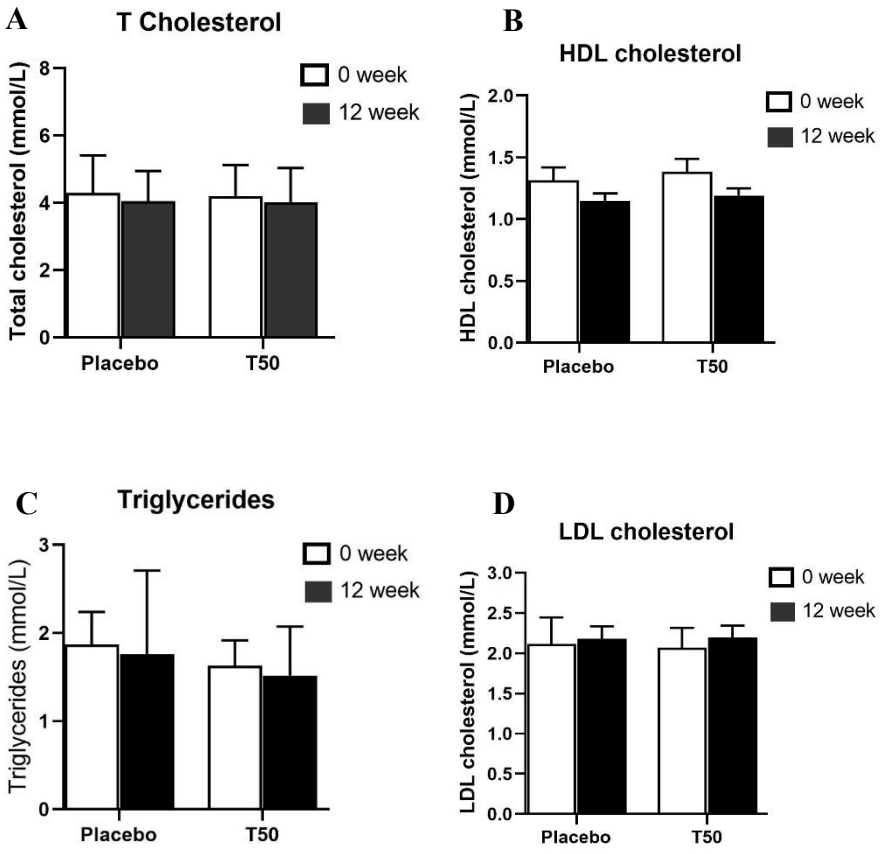

Figure 9. Lipids profile measurements. Upper left pancel A, comparative analysis of total cholestrol levels in placebo group vs T50 group at week 0 and week 12; Upper right pancel B, comparative analysis of HDL-C levels in placebo vs T50 at week 0 and week 12; Lower left panel C, comparative analysis of Triglycerides levels in placebo vs T50 at week 0 and week 12; Lower right panel D, comparative analysis of LDL-C Levels in placebo vs T50 at week 0 and week 12. Data were presented as Mean ± SE.

3.3.3.2 Heart Rate and Blood Pressure Measurements

The changes in BP, systolic and diastolic blood pressure between before and after the intervention for placebo group and T50 group were not statistically significant as illustrated in Figure 10.

The heart rate (HR) in the placebo group was 73 ± 4 bpm at baseline and was 72 ± 3 bpm at week 12. In placebo group, the systolic blood pressure was 138 ± 9 mmHg at week 0 and 133 ± 8 mmHg at week 12. The diastolic blood pressure was 83 ± 5 mmHg at week 0 and 74 ± 4 mmHg at week 12.

Compared with the treatment group, the heart rate (HR) was 76 ± 2 bpm at baseline and was 74 ± 2 bpm at week 12. The systolic blood pressure was 131 ± 3 mmHg at week 0 and 126 ± 6 mmHg at week 12. The diastolic blood pressure was 81 ± 3 mmHg at week 0 and 80 ± 2 mmHg at week 12.

This suggests that T50 did not lead to significant alterations in heart rate, systolic and diastolic blood pressure at baseline and at the end of study (Figure 10).

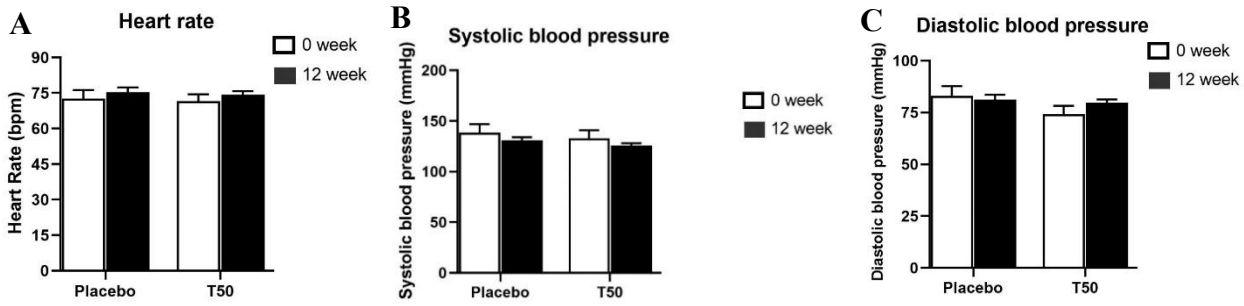

Figure 10. Heart Rate and Blood Pressure measurements. Left panel A: Heart Rate (bmp) between placebo group and T50 treatment group at week 0 and week 12; middle panel B: Systolic Blood Pressure (mmHg) between placebo group and T50 treatment group at week 0 and week 12; Right panel C: Diastolic Blood Pressure (mmHg) between between placebo group and T50 treatment group at week 0 and week 12. Data were presented as Mean ± SE.

3.3.3.3 Body Weight, BMI, and Waist Circumference Measurements

The changes in body weight (BW), body mass index (BMI), and waist circumference (WC) as secondary outcome measures to access the broader impact of T50 on metabolic health beyond glycaemic control were also closely observed.

The BW in placebo group was 78.57 ± 6.72 kg at baseline and 77.4 ± 6.57 kg at week 12. The BW in treatment group was 79.44 ± 3.7 kg at baseline and 78.25 ± 3.66 kg at week 12.

The BMI in placebo group was 28.34 ± 1.56 kg/cm^2 at baseline and 27.94 ± 1.55 kg/cm^2 at week 12. The BMI in treatment group was 27.33 ± 1.01 kg/cm^2 at baseline and 26.91 ± 0.98 kg/cm^2 at week 12.

The WC in placebo group was 100.64 ± 5.04 cm at baseline and 99.21 ± 5.42 cm at week 12. The WC in treatment group was 96.22±2.71 cm at baseline and 95.59 ± 2.55 cm at week 12.

Throughout the 12-week period, participants' body weight had a slight decrease, with no statistically significant differences observed between the T50 treatment group and the control group. Slightly reduced BMI values and waist circumference in both placebo group and T50 treatment group have been observed, however, these changes did not reach statistical significance (Figure 11).

Overall, the findings suggest that T50 has slight decrease but did not lead to significant alterations in body weight and BMI in individuals with T2DM.

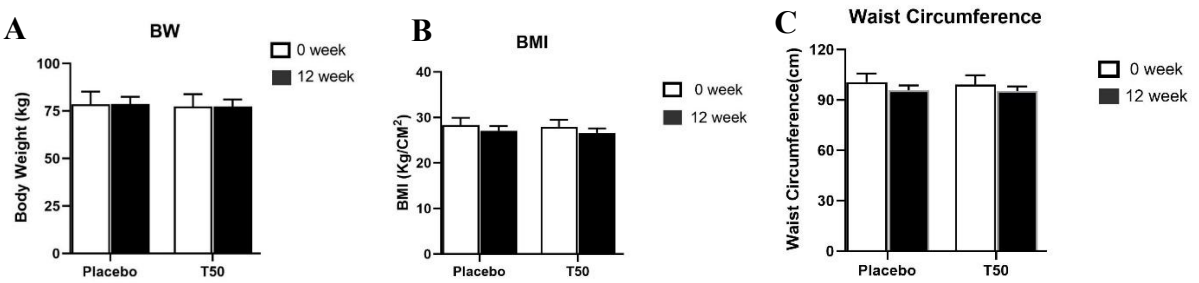

Figure 11. Body weight (BW), BMI, and Waist Circumference Measurements. Left panel A: body weight (kg) in placebo group and T50 treatment group at week 0 and week 12; Middle panel B: BMI in placebo group and T50 treatment group at week 0 and week 12; Right panel C: waist circumference (cm) in placebo group and T50 treatment group at week 0 and week 12. Abbreviations: BW, body weight (kg); BMI, body weight index (kg/cm2). Data were presented as Mean ± SE.

3.3.4 Impact of T50 on Glucose-Dependent Insulinotropic Polypeptide

Glucose-dependent insulinotropic polypeptide (GIP) is one of the incretin hormones, released by the gut in response to the ingestion of nutrients, particularly glucose and fats. The primary

role of GIP is to potentiate insulin secretion from the β cells of the pancreas. The release of GIP could cause an increase in the amount of insulin release.

To illustrate the mechanism by which T50 affects glycaemic control serum GIP levels were measured at baseline and at the end of 12-week treatment in both groups. Comparison of baseline to after 12-week treatment there were no significant changes in GIP levels in placebo intervention and T50 treatment, although there was a slightly decreased serum GIP level in placebo group and a slightly increased GIP level in T50 group. None of these changes reached statistical significance. This finding suggests that the impact of T50 on glucose metabolism and insulin secretion might be mediated through different mechanisms beyond GIP modulation.

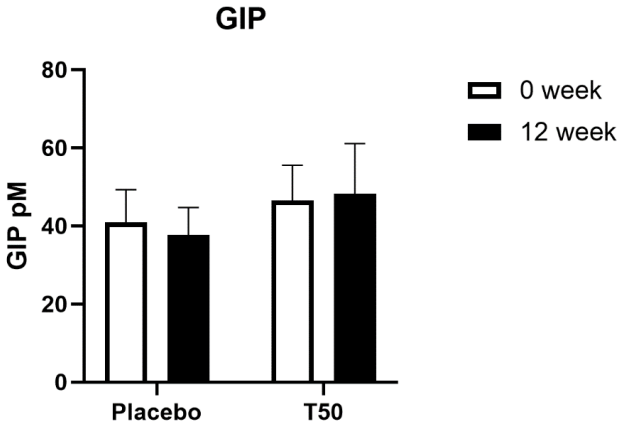

Figure 12. GIP level measurements. Serum glucose-dependent insulinotropic polypeptide (GIP) levels in placebo group and T50 treatment group at baseline (week 0) and at the end of treatment (week 12). Data were presented as Mean ± SE.

3.5 Discussion

In this 12-week, randomized, placebo-controlled, double-blind clinical trial, patients with T2DM inadequately controlled with metformin received either Chinese herbal medicine T50 pills or a placebo. Measurements were collected at regular intervals following randomization and intervention. T50 as an add on therapy to metformin treatment had improved glycaemic

control and reduced HOMA-IR compared to the placebo group who used metformin monotherapy. The reductions in HbA1c observed in patients treated with T50 combined with metformin in this trial suggest that integrating Chinese medicine T50 with metformin may provide a better approach to glycaemic control for T2DM.

Metformin has been the first-line treatment option for T2DM management since the American Diabetes Association (ADA) recommended it as the primary treatment in 1998, owing to its proven effectiveness, favourable safety profile, and affordability (ADA 2019). However, the efficacy of metformin monotherapy tends to diminish over time; 42% of patients may experience treatment failure within approximately 24 months of initiation (Brown et al. 2010) and a similar result showing 32.9% of patients with T2DM experienced a loss of glycaemic control at 24 months was also concluded by Weiss et al. (2021). This trend is reflected in our study's participant data, where the average duration since diagnosis is 6.99 ± 7.21 years, suggesting that many participants are at a stage where they may require additional therapeutic support beyond Metformin.

Alternative monotherapies for treating T2DM, such as sulfonylureas, thiazolidinediones (glitazones), alpha-glucosidase inhibitors, and insulin injections, are also in use. These treatments tend to be costlier and carry the risk of weight gain, a particularly concerning side effect given that approximately 95% of individuals with T2DM are overweight (ADA 2022). Weight gain is one of the most unwanted side effects of antidiabetic medications. In this study, the average body weight of participants was 79.44 ± 3.7 kg at baseline and 78.25 ± 3.66 kg at week 12. Though weight loss was not significant, notably, T50 treatment does not elevate weight gain.

Recent research underscores the trend of utilizing combination therapy early in the treatment process to improve glycaemic control and address the multifaceted pathophysiology of T2DM

(Greiver et al. 2021). Janumet® contains sitagliptin (a dipeptidyl peptidase-4 inhibitor) and metformin (a biguanide). The combination was approved for medical use in the United States in 2007 (FDA 2007) and has since become a popular medication for diabetes management. Janumet® may cause side effects such as kidney dysfunction, low vitamin B12, hypoglycaemia, joint pain, and skin reactions (Merck & Co. Inc. 2023).

Traditional medicine, despite being less researched in the context of T2DM, represents a potentially valuable complement to conventional treatment modalities (Cheng et al. 2019) This study sought to fill this gap by evaluating the efficacy and safety of the T50 herbal formulation as an add-on therapy to metformin monotherapy.

HbA1c is recognized as the definitive marker of glycaemic control over a period of 2-3 months and is less influenced by short-term fluctuations compared to fasting glucose measurements, affirming its use as a primary endpoint in this trial (Wang & Hng 2021). In line with this, our study observed a reduction in HbA1c levels, indicating an improvement in glycaemic control over time with the use of T50. Conversely, no significant effect on fasting glucose levels was detected, which may be attributed to their susceptibility to recent food intake and diurnal variation. Thus, fasting glucose levels may not reliably represent long-term diabetes management.

Insulin, generated by the β cells within the pancreatic islets, is secreted in response to elevated blood glucose levels following a meal. Its primary function is to promote the absorption of glucose into cells, thereby reducing blood sugar levels (Rahman 2021). The liver and muscles utilize absorbed glucose for immediate energy or store it as glycogen for future use (Rahman 2021).

Insulin resistance plays a crucial role in the pathogenesis of T2DM (Defronzo et al. 2015). Insulin resistance is characterized by an impaired biologic response to insulin's stimulation of tissues, primarily affecting the liver, muscles, and adipose tissues (Freeman et al. 2023). Insulin resistance and β cell function were evaluated using HOMA-IR and HOMA-β, respectively, methods introduced by Matthews et al. in 1985, which have become standard in clinical and epidemiological studies (Son et al. 2022). The study revealed an improvement in insulin resistance among participants treated with T50, without a corresponding enhancement in β cell function. Insulin resistance can manifest in various organs, including adipose tissues, muscles, and the pancreas (Freeman et al. 2023). This distinction is critical; the mechanism of action may primarily target pathways involved in enhancing insulin sensitivity outside the pancreas. The observed reduction in insulin resistance suggests that T50 effectively improves the body's sensitivity to insulin, potentially facilitating better glucose uptake and utilization in peripheral tissues such as muscles and adipose tissues. The alignment of the study's results with previous research conducted by Zhang et al. (2016) underscores the potential of T50 from preclinical models to human applications. Such findings are significant as they contribute to our understanding of T50's role in T2DM management. While improving insulin sensitivity is a valuable therapeutic goal, the inability of T50 to enhance β cell function suggests that it may be most effective when used in conjunction with other treatments aimed at preserving or augmenting β cell capacity. This dual approach could offer a more comprehensive strategy for managing T2DM, addressing both major pathophysiological aspects of the disease.

Dyslipidaemia is commonly observed in T2DM patients and contributes significantly to the risk of cardiovascular disease. The lipid profile as a secondary outcome measure was deemed essential for a holistic evaluation of T2DM management (Thambiah & Lai 2021). For all participants, the mean value for total cholesterol was 4.18 ± 0.30 mmol/L. It remained at a similar level at week 12, which was 4.11 ± 0.27 mmol/L. In the study population, participants

with lipid abnormalities were already receiving lipid-lowering agents before enrolment. The primary goal of lipid management is to lower LDL cholesterol to mitigate cardiovascular risks (Grundy et al. 2019). The study findings indicate a slight increase from 2.18 ± 0.15 to 2.2 ± 0.15 mmol/L among participants treated with T50. While these changes remained within clinically accepted normal ranges, the implications of such an increase warrant careful consideration, particularly in the context of long-term cardiovascular health. The slight uptick in LDL levels observed in our study does not immediately signal a cause for alarm, yet it underscores the complexity of managing lipid profiles in T2DM patients. Given that individuals with T2DM are at an elevated risk for cardiovascular events, even small changes in lipid parameters could potentially impact long-term outcomes. It is important to note, however, that the duration of this study may not have been sufficient to fully assess the impact of T50 on cardiovascular risk factors or to observe any significant cardiovascular events.

Hypertension is another common comorbidity in T2DM, increasing the risk of macrovascular complications such as stroke, coronary heart disease, and heart failure (Climie et al. 2019). Participants with co-existing hypertension were already under treatment with antihypertensive medications at the time of enrolment. Throughout the study, no significant changes in blood pressure levels were observed, implying that T50 can be integrated into existing treatment regimens without adjusting antihypertensive therapy or raising concerns over adverse cardiovascular effects.

In recent, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) have been identified as crucial players in the regulation of glucose homeostasis, exerting significant effects on insulin secretion from pancreatic β cells in response to dietary glucose (de Mesquita et al. 2023). The figure 13 illustrates how GIP and GLP-1 act on pancreas,

bone, fat, GI tract, and brain (Asian Association for the Study of Diabetes and Blackwell Publishing Asia Pty Ltd 2010).

T50 comprises extracts from ten individual herbs: *Rhei Radix et Rhizoma*, *Rhizoma Coptidis*, *Paeoniae Radix Alba*, *Bupleuri Radix*, *Scutellariae Radix*, *Aurantii Fructus Immaturus*, *Pinelliae Rhizoma Praeparatum Cum Alumine*, *Crataegi Fructus*, *Mume Fructus*, and *Trichosanthis Radix*. Among these, *Rhei* and *Coptidis* are particularly notable.

Although data on the specific synergistic pathways of individual T50 ingredients remain limited, numerous experimental and clinical studies have reported hypoglycemic mechanisms of its active compounds. For instance, berberine which was derived from *Rhizoma Coptidis* exhibits anti-inflammatory and glucose-lowering effects (Baska et al., 2021). It enhances insulin sensitivity in peripheral tissues by upregulating GLUT-1, GLUT-4, and insulin receptor (InsR) activity, stimulating glycolysis, and reducing insulin resistance through mechanisms such as macrophage polarization, increased lipolysis, and enhanced energy expenditure. In the gut, berberine inhibits α-glucosidase, thereby reducing glucose absorption, modulates gut microbiota, lowers monosaccharide levels, and mitigates diabetes-related complications (Pang et al., 2021).

Similarly, rhein as an active compound in rhubarb (*Da Huang*) has been shown to improve insulin resistance independently of insulin secretion (Ji & Gu, 2021; Choi et al., 2006).

These findings suggest that T50 may exert its antidiabetic effects primarily by enhancing insulin sensitivity and modulating intestinal glucose absorption through the combined actions of its active components.

As the major component of T50, Rhizoma Coptidis, effectively controlled diabetes through and anti-inflammation and regulating gastrointestinal function, the effect of T50 on gut hormone secretion was measured in this study. It has been found that the increasing GIP levels in participants treated with the T50 herbal formulation. Although these increases were not statistically significant, they suggest a potential mechanism through which T50 improved glycaemic control possibly through enhancing insulin sensitivity associated with increased GIP secretion. The Figure 13 illustrates how GIP and GLP-1 act on pancreas, bone, fat, GI tract, and brain (Asian Association for the Study of Diabetes and Blackwell Publishing Asia Pty Ltd 2010).

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Seino, Y., Fukushima, M., & Yabe, D. (2010). GIP and GLP-1, the two incretin hormones: Similarities and differences. *Journal of Diabetes Investigation*, 1(1–2), 8–23. https://doi.org/10.1111/j.2040-1124.2010.00022.x

Figure 13. GIP action. GIP acts directly on the endocrine pancreas, bone, fat, gastrointestinal (GI) tract and brain. GLP-1 acts directly on the endocrine pancreas, GI tract, heart, and brain. Copyright © 2010 Asian Association for the Study of Diabetes and Blackwell Publishing Asia Pty Ltd). The recent development and release of the drug Tirzepatide (Mounjaro®) underscores the therapeutic potential of targeting GIP alongside GLP-1 to enhance glycaemic control in T2DM patients (ed Mesquita et al. 2023). Unlike other anti-diabetic agents, this classification of medication potentially leads to substantial weight reduction (de Mesquita et al. 2023).

This study has several limitations. Firstly, this trial had a small sample size and a relatively short treatment duration. Additionally, there was a lack of significant changes in GIP levels. The mechanism of T50 nor the GIP role in diabetes management can be determined.

CHATPER 4

Safety Measurements and Tolerability observation of T50 Product in Participants of Clinical Trial

Chapter 4: Safety Measurements and Tolerability observation of T50 Product in Participants of Clinical Trial

4.1 Introduction

An important issue of any clinical trial is to ensure the safety and well-being of participants (FDA. 2020. Guidance for Industry: E9 Statistical Principles for Clinical Trials). This involves monitoring and minimizing adverse effects that could cause harm to vital function of the participants. Tolerability refers to how well patients can endure the side effects of a new therapeutic agent alone, or combination with other medication.

Previous animal experiments and clinical studies have demonstrated that the T50 formulation does not cause harm to vital organs, such as the heart, liver, and kidneys, and only induces mild gastrointestinal discomfort in participants (Tong et al. 2013). As reported by Tong et al. (2013), no serious or moderate adverse events occurred during the 12-week trial. The TM81 group experienced 24 mild adverse events (6.69%) compared to 7 (5.83%) in the placebo group ($p = 0.743$). One participant experienced abdominal discomfort that resolved without intervention. Transient elevations in liver enzymes were observed in a few participants but were not clinically significant. No renal impairment or ECG abnormalities were noted.

Preclinical studies have also evaluated the safety and efficacy of T50. Acute and long-term toxicity assessments in rodents revealed no significant toxic effects at therapeutic doses. In STZ and high-fat diet-induced diabetic rats, oral administration of T50 (5 g/kg/day for 2 weeks) significantly reduced fasting glucose and improved insulin sensitivity.

Additionally, T50 exhibited antioxidant properties by enhancing the activity of superoxide dismutase (SOD) and catalase (CAT), and reducing malondialdehyde (MDA) levels, indicating protection against oxidative stress, a known contributor to diabetic complications.

However, T50 pills have not been used in combination with other pharmaceutical drugs. This clinical study critically assesses the safety and tolerability of T50 in combination with metformin for managing T2DM. Evaluating the safety and tolerability is paramount to ensuring that the intervention's benefits outweigh its risks.

The study's comprehensive approach encompasses a broad spectrum of safety measurements, including vital signs, hematologic and biochemical profiles, alongside detailed accounts of adverse and serious adverse events, to construct a safety profile of T50. Concurrently, tolerability observations were recorded through adverse event diary cards. This dual focus underscores the ethical significance of clinical trials and provides healthcare providers with essential insights for informed treatment decisions, aiming to enrich clinical practices with a well-tolerated TCM option.

4.2 Measurements on Safety and Tolerability

4.2.1 Effect of T50 on Liver Function Test

Liver function tests were conducted to monitor the hepatic effects of the herbal medicines. The enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which serve as main indicators of liver health, were measured at baseline and at 12-week period.

The ALT level was 27.43 ± 4.04 U/L in the placebo group at week 0 and 25.86 ± 2.22 U/L at week 12. In the treatment group, the ALT level was 39.47 ± 6.02 U/L at baseline and 32.53 ± 3.8 U/L at week 12. The result indicates a decrease in ALT level in T50 group ($p < 0.05$,

baseline vs. week 12 result). At baseline, the AST level was 20.86 ± 1.34 U/L in the placebo group and became 16.71 ± 2.01 U/L after 12 weeks. In the treatment group, the baseline was 26.67 ± 2.2 U/L, and after 12 weeks' intervention the AST level was 24.1 ± 1.5 U/L.

The results indicated a slight decrease in both AST levels in the placebo group and treatment group, while a significant reduction of ALT levels in T50 group. This finding suggests T50 treatment was not associated with adverse hepatic effect and may improve fatty liver caused elevation of ALT in patients with T2DM.

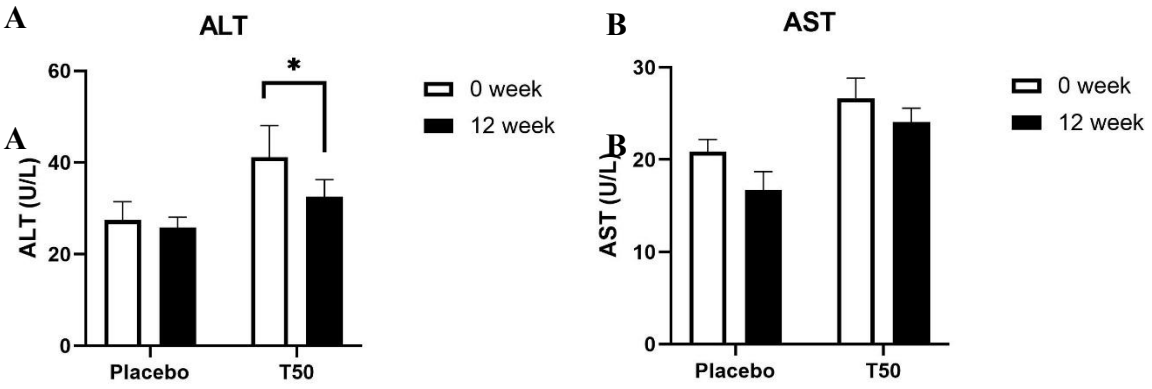

Figure 14. Safety observation on liver function test. Left panel A: ALT (U/L) between placebo group and aT50 treatment group at week 0 and week 12, *p = 0.0043; Right panel B: AST (U/L) between placebo group and T50 treatment group at week 0 and week 12. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase. Data were presented as Mean ± SE.

4.2.2 Effect of T50 on Kidney Function Test

Kidney function tests were monitored, focusing on serum creatinine levels, and estimated Glomerular Filtration Rate (eGFR), which are key indicators of renal health. Creatinine is a

waste product generated from the digestion of dietary protein and the natural break down of muscle tissue. It is filtered out of blood by the kidney (Hosten 1990).

In placebo group, the creatinine level was 72.86 ± 6.53 umol/L at baseline and became 75.00 ± 6.73 umol/L at week 12. The eGFR was 80.57 ± 4.08 mL/min/1.73m^2 at week 0 and was 75 ± 6.73 mL/min/1.73m^2 at week 12.

In treatment group, the creatinine level was 73.00 ± 3.39 umol/L at baseline and became 72.31 ± 3.45 umol/L at week 12. The eGFR was 85.30 ± 1.59 mL/min/1.73m^2 at week 0 and was 85.53±1.70 mL/min/1.73m^2 at week 12.

Creatinine levels remained stable, suggesting that T50 does not impair the kidney's ability to filter waste. Similarly, eGFR rates stayed within normal limits, further indicating that renal filtration capacity was not compromised. The comparative results from baseline to the 12-week endpoint indicated no significant changes in either parameter for participants receiving T50 versus those on the placebo. These findings suggest that T50 is safe for kidney function and does not induce nephrotoxicity in the study population.

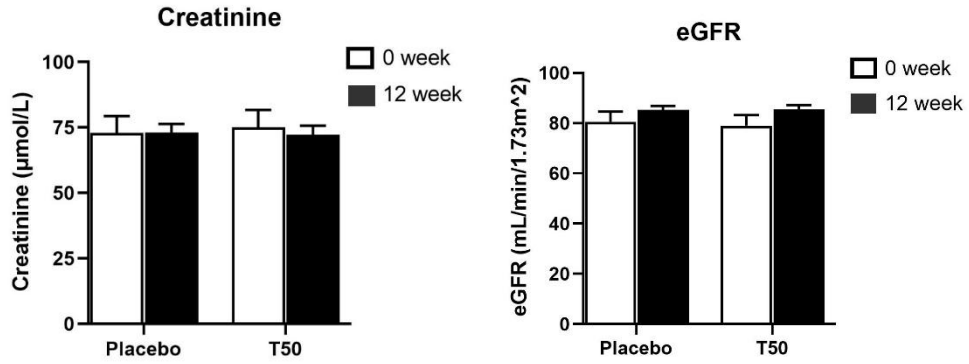

Figure 15. Safety observation on kidney function test. Left: Mean creatinine (µmol/L) between a placebo group and a T50 treatment group over a period o to 12 weeks; right: Mean eGFR (mLl/min/1,73m^2) between a placebo group and a T50 treatment group over a period o to 12 weeks. Abbreviation: eGFR, estimated glomerular rate. Data were presented as Mean ± SE.

4.2.3 T50 treatment on Adverse Event Frequency

In the clinical evaluation of T50, an herbal medicine containing ten ingredients with varying taste profiles, most participants reported acceptable tolerability, despite some ingredients possessing a naturally bitter taste. Out of the study cohort, six participants complained the bitterness of T50, with one individual opting to withdraw from the trial due to this issue. Gastrointestinal tolerance was closely monitored, revealing that one participant in the T50 group and one in the placebo group experienced mild to moderate diarrhea at the onset of treatment. However, these symptoms were transient, diminishing substantially by the fifth week of the trial. One participant in treatment group discontinued due to gastrointestinal discomfort. Furthermore, there were no reports of dizziness, skin rashes, heart palpitations, or urinary tract infections, suggesting a safety profile for T50 with no significant adverse effects impacting patient well-being or continuation in the study.

4.3 Discussion

Safety is a critical aspect of evaluating any therapeutic intervention. In this study, the safety profile of T50 as an add-on therapy to metformin was assessed by monitoring adverse events (AEs) and serious adverse events (SAEs), drug to drug interaction, liver function, kindly function and overall tolerability of the treatment regimen.

4.3.1 Adverse Events (AEs)

The comparative analysis of the safety profiles between the T50 plus metformin and placebo plus metformin groups provided crucial insights into the tolerability and safety of the T50 treatment. Throughout the study duration, both groups reported adverse events. 1 patient from the treatment reported diarrhoea and 1 patient from the placebo group reported diarrhoea. The incidence of adverse events in the T50 group is the same as the placebo group, suggesting that

the addition of T50 to a metformin regimen does not markedly increase the risk of adverse effects. Notably, T50 did not increase the chance of gastrointestinal complaints, which are a common concern associated with metformin use. In a 71 clinical trials meta-analysis, the results indicated that the use of metformin is associated with higher risk of abdominal pain, diarrhoea and nausea, and the risks of abdominal pain and nausea were highest comparing to placebo. It was highlighted that gastrointestinal adverse events affected up to 20% of patients with the use of metformin (Nabrdalik et al. 2022). The conclusion was that T2DM patients treated with metformin experienced a higher risk of gastrointestinal adverse events such as abdominal pain, nausea and diarrhea is higher compared to other antidiabetic drugs (Nabrdalik et al. 2022).

Common side effects experienced in other anti-diabetic therapies such as weight gain were not reported in this study. In fact, this study results showed a deduction on body weight, BMI and waist circumference. Serious adverse events (SAEs) such as hypoglycaemia, UTI, cardiovascular events, death were not found across both study arms, and no specific safety concerns attributable to T50 were identified. This safety profile, combined with the observed efficacy, supports the potential of T50 as a safe add-on therapy for T2DM patients, warranting further study in larger and more diverse populations.

4.3.2 Drug to Drug Interactions

Firstly, T50 enhances insulin sensitivity and reduces may through its anti-inflammatory effects. The mechanism of T50 seems do not conflict with metformin. The primary glucose-lowering effect of metformin is achieved by inhibiting hepatic gluconeogenesis and opposing the action of glucagon. This inhibition of mitochondrial complex I leads to impaired cAMP and protein kinase A signalling in response to glucagon. Although the stimulation of 5'-AMP-activated protein kinase (AMPK) is not essential for metformin's glucose-lowering effect, it enhances insulin sensitivity, primarily by regulating lipid metabolism (Pernicova & Korbonits 2014).

The Tangminling pill is a combination of ten traditional Chinese herbs containing Rhizoma coptidis, Scutellaria Baicalensis Georgi, Radix Paeoniae Alba, Rheum officinale Baill, Citrus aurantium L, Pinellia ternata, Crataegus pinnatifida Bunge, Smoked plum (Fructus mume), Radix Trichosanthis, and Bupleurum Chinese DC. Among these herbs, Rhizoma coptidis contains mainly three bioactive alkaloids including berberine, coptisine, and jatrorrhizine. Among these alkaloids, berberine has various pharmacological effects, including anti-diabetic, anti-inflammatory, antioxidant and cardioprotective properties (Cheng et al. 2019). Previous research found that it enhances glucose uptake in target tissues such as adipocytes and skeletal muscles and involves a serious signal transduction pathway. The pathway begins at the insulin receptor (InsR) and proceed through insulin receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI-3K), ultimately leading to the translocation of the glucose transporter GLUT4 (Wang et al. 2014).

Other than Rhizoma coptidis, Scutellaria baicalensis Georgi was found to have anti-diabetic effect as well. In Scutellaria baicalensis Georgi, there are two active ingredients: baicalin, and wogonin. Baicalin reduces renal fibrosis by down-regulating the transforming growth factor-β/Smad pathway and improves islet β-cell function in T2DM. Wogonin helps alleviate diabetic cardiomyopathy, cognitive deficits related to diabetes, and metabolic syndrome by increasing PPAR-α activity (Cheng et al. 2019).

Secondly, the study did not find evidence of drug-to-drug interactions between T50 and metformin. The AE and SAE rates were the same in treatment group and placebo group. Thirdly, as T50 and metformin both have glucose lowering effects, and as an add-on therapy, the HbA1c level was decreasing more compared with metformin monotherapy. The consistent reduction in HbA1c levels and stable metabolic parameters in the treatment group further support the

absence of drug-to-drug interactions. This was an important finding which ensures the therapeutic efficacy of metformin remained while benefiting from the additional effects of T50.

4.3.3 Liver Toxicity and Kidney Function

The results indicated a decrease in both AST levels in the placebo group and treatment group. A 6 RCTs meta-analysis study focusing on the effect of metformin administration on liver enzymes was conducted by Jalali et al. (2020). The results revealed that metformin can decrease AST levels (Jalali et al. 2020). This study finding suggested that T50 as an add-on therapy presented a significant reduction of ALT levels compared with placebo plus metformin, indicating that T50 was not associated with adverse hepatic effects but beneficial effects on fatty liver caused elevation of ALT in patients with T2DM.

It was found that the eGFR in treatment group was lower than the treatment group at the beginning of the study. A clinical trial regarding distribution of eGFR and determinants of its age loss was conducted by Waas et al. (2021). The study recruited 12,381 German patients and followed up on eGFR for 5 years. The result confirmed that eGFR normally declines with age by approximately 1 mL/min/m2 per year beginning in the third decade of life (Waas et al. 2021). It correlates with the age characters that the participants in treatment group were 10 years younger than the placebo group (refer Chapter 3.3.1). According to the chronic kidney disease staging system developed by the US National Kidney Foundation (NKF) in 2002, all participants fall into the stage 2 classification which means the kidneys have mild damage but still work well. During the study period, in the placebo group which the participants took metformin monotherapy, the creatinine level increased and eGFR decreased. The conclusion of Hsu's study was that metformin may have adverse effects on renal function in T2DM patients. In T50 clinical trial, after 12-week intervention, the creatinine level decreased in T50 group

and eGFR rate increased. These results indicate that T50 compensated the adverse effects from metformin and potentially has benefits on kidney function.

4.3.4 Cardiovascular effects

Cardiovascular disease (CVD) describes a range of conditions that impact the heart and blood vessels throughout the body, leading to stroke, high blood pressure, kidney disease, impaired blood supply to the legs and other related disorders (Raghavan et al. 2019). CVD is a leading cause of death among people with diabetes (Einarson et al. 2018). Einarson et al. (2018) analysed data from 57 research papers with 4,54,9,481 persons with T2DM and concluded that CVD affected 32.2% of person; 21.2% had coronary heart disease; 14.9% had heart failure; 10% had myocardial infarction (MI); 7.6% had stroke. It was found CVD is a leading cause of death among T2DM patients, accounting for about half of all deaths during the study period (Einarson et al. 2018). This highlights an important public health finding: controlling blood sugar alone is not enough for T2DM management. Previous study results revealed positive relationship between control of CVD risk factors and the reduced incidents for cardiovascular events (Ali et al. 2013). Consequently, there are two primary goals for T2DM. The first one is to improve glycaemic control and reduce the chances of diabetic complications and the second one is to address the risk factors related to the CVD (Raghavan et al. 2019).

In T50 formula, among 10 herbal ingredients, research showed that Crataegus pinnatifida Bunge (one of the ingredients in T50) has anti-inflammatory effects and reduces the risk of cardiovascular diseases by preventing inflammation caused blood vessel damage. Furthermore, Fructus mume in T50 formula offers neuroprotective benefits by down-regulating inflammation and inhibiting the TLR4 and p38 MAPK signalling pathways (Cheng et al. 2019).

Hypertension is a common co-morbidity in patients with T2DM. The consensus BP goal in diabetic persons with hypertension is less than 130/80 mmHg (Chowdhury, Moaddab & McFarlane 2023). Our study results showed the participants in the treatment group achieved the treatment goal on blood pressure control (systolic BP <130 mmHg) and provided further evidence that T50 as an additional therapy to metformin brings potential benefits on cardiovascular conditions. In addition, T50 as an add-on therapy to metformin did not cause weight gain and helped to achieve goals to control blood pressure, the results indicated that T50 may have potential benefits on cardiovascular system.

The T50 formulation was originally developed and investigated for T2DM. Existing evidence demonstrates its efficacy in improving glycaemic control in newly diagnosed T2DM. This pilot study aimed to evaluate the synergistic effect of T50 as an add-on to metformin in patients with long-standing T2D and suboptimal glycaemic control. The findings indicate that T50 exerts a moderate glucose-lowering effect by enhancing whole-body insulin sensitivity.

A large-scale clinical trial is warranted to further assess T50 as an adjunct to metformin therapy. In this study, the T50 group also achieved the target systolic blood pressure (<130 mmHg), suggesting potential cardiovascular benefits. Additionally, T50 did not induce weight gain or exacerbate gastrointestinal side effects commonly associated with metformin, indicating good tolerability.

T50 appears to be a safe and well-tolerated adjunct to metformin, with potential benefits for cardiovascular health and metabolic syndrome. Further research is needed to confirm these findings.

To sum up, the safety and tolerability of T50 as an add-on therapy to metformin for T2DM management was evaluated. The findings indicate that T50 did not pose significant safety risks

to liver and kidney function as evidenced by improving ALT and AST levels and decreasing creatinine level and improved eGFR level. Compared to the placebo group, the incident rate of adverse events in the T50 group was the same. It means that the common side effects associate with metformin: gastrointestinal complaints did not aggravate when taking T50 alongside with metformin. Moreover, T50 seemed have no drug-to-drug interactions with metformin. Furthermore, T50 did not contribute to weight again and appeared to be helpful for cardiovascular health by controlling blood pressure and maintaining lipid profiles. Further studies should be conducted to confirm these findings.

CHAPTER 5

Conclusion and Further Direction

Chapter 5: Conclusion and Further Direction

5.1 Summary of Findings

This study aimed to evaluate the effectiveness and safety of T50 as an adjunct therapy to metformin in the management of type 2 diabetes mellitus (T2DM). The research was conducted as a 12-week, randomized, double-blind, placebo-controlled clinical trial, involving 42 participants who had been on metformin for at least one month and exhibited unsatisfactory glycaemic control (HbA1c ≥7.0%).

The primary outcome measure was the change in HbA1c levels, while secondary outcomes included changes in fasting plasma glucose, insulin levels, insulin sensitivity (HOMA-IR), β-cell function (HOMA-β), lipid profiles, body weight, BMI, and waist circumference. The observed HbA1c reduction with T50 is below the 0.5% threshold considered clinically significant by the National Glycohemoglobin Standardization Program. While the reduction suggests potential glycaemic benefits, it is not yet conclusive. Larger-scale studies with extended treatment duration are needed to determine whether this effect can be sustained and reach clinical significance. Although improvements in insulin resistance were observed, there were no significant changes in β-cell function. Mild reductions were noted in body weight, BMI, and waist circumference, but no significant alterations were found in lipid profiles or blood pressure. This lack of significant change may be attributed to the fact that most participants were already using pharmaceutical medications to manage hyperlipidaemia and hypertension.

The study also demonstrated the safety and tolerability of adding T50 to metformin, as evidenced by the absence of alterations in liver and kidney function. No severe adverse

reactions were reported by participants, although one participant withdrew from the study due to gastric discomfort after taking T50 pills.

5.2 Clinical Implications

The modest reduction in HbA1c levels observed in the T50 group suggests that T50 may improve the glycaemic control when used as an add-on therapy to metformin. Its impact on HbA1c levels was limited, but still can address its potential benefits on improving insulin sensitivity. Unlike many other anti-diabetic medications, T50 did not cause weight gain or damage in kidney and liver function, indicating the treatment with T50 was generally safe and well tolerated. Thus, T50 may be optimal option for patients with T2DM who prefer natural therapies.

5.3 Limitations of the Current Study

The study presented in this thesis was a 12-week randomized, double-blind, placebo-controlled pilot clinical trial. The relatively small sample size limits the generalizability of the findings, necessitating larger-scale studies to confirm the results and establish the efficacy and safety of T50 across a broader range of populations.

While T50 formulation contains multi-herbal ingredients, several of its constituents with established pharmacological activity are listed in Table 2 (Chapter 1, page 16). These compounds, such as berberine and paeoniflorin, may serve as marker compounds for future PK assessments. Currently, no comprehensive PK data exists for T50 as a combined formulation, representing a notable research gap.

Additionally, based on Tukey's test and the observed variation in responsiveness to T50 treatment according to baseline HbA1c levels, extending the trial beyond 12 weeks may

provide sufficient time to fully evaluate the therapeutic contribution of T50 to glycaemic control.

5.4 Further Direction

In conclusion, T50 improved HbA1C levels in patients with T2DM who have inadequate glycaemic control on metformin alone, offering potential benefits in improving glycaemic control and reducing insulin resistance. T50 was generally safe and well-tolerated.

Given the limitations of this study, future research should focus on larger, multicentre trials with more diverse populations to validate the efficacy and safety of T50. Longer-term studies are also necessary to assess its impact on diabetes control and related complications. Nevertheless, this study contributes to the growing body of evidence supporting the use of TCM in the management of T2DM and underscores the importance of exploring alternative therapies to enhance patient care.

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APPENDIXES

Appendix A. Procedure of Measurements

1. Method of Physical Examinations and Vital Signs

Physical examination included height, body weight, BMI, and waist circumference.

Vital signs included heart rate and blood pressure.

Height

Height was measured at the screening visit using a wall mounted stadiometer accurate to 0.5 cm.

The participant was in the standing position, shoes removed, with their heels, buttocks and shoulder blades in contact with the vertical surface of the stadiometer. The moveable headboard was lowered gently until it touches the crown of the head. Height was recorded in centimetres to the nearest centimetre.

Body Weight

At each visit body weight was measured on calibrated scales shortly after the participants empties their bladder. The participant was to be in light indoor clothing with pockets empty and without shoes, belts, jewellery, or other accessories. Body weight was recorded in kilograms to the nearest 0.1 kilogram and measured at every site visit.

Body Mass Index (BMI)

BMI was calculated from the participant's recorded weight and height using the following equation: weight (kg) / height $(m)^2$

This was calculated to the nearest one-tenth unit to determine their eligibility for the trial at screening.

BMI values for the cut-off points for classification of overweight and obesity vary within different countries. According to the World Health Organization (WHO) Guidelines, a BMI of 18.5 to 24.9 kg/m^2 is normal, 25 to 29.9 kg/m^2 is classified as overweight or pre-obese, over 30 kg/m^2 is classified as obese, which includes obese class I (30 to 34.9 kg/m^2), obese class II (35 to 39.9 kg/m^2) and obese class III (>40.0 kg/m^2). A BMI of less than 18.5 kg/m^2 is classed as underweight. This classification is based on standards for adults of European descent.

Waist Circumference

Waist circumference was measured with a flexible tape measure with the participant wearing light clothing, at the mid-point between the highest point of the iliac crest and lowest part of the costal margin in the mid-axillary line in expiration. Measurements was collected at each clinic visits and recorded in centimetres to the nearest 0.5 centimetres.

Blood Pressure

Systolic blood pressure and diastolic blood pressure were measured every visit using a digital sphygmomanometer. The procedure for measurements is:

1. A quiet room with minimal extraneous activity and temperature fluctuation was used.

2. Participant emptied his/her bladder prior to blood pressure measurements.

3. Participant was seated in a chair with back supported and arms bared and supported at heart level.

4. Measurement began after at least 5 minutes of rest.

5. The appropriate cuff size must be used to ensure accurate measurements. The upper arm should be wholly encircled by at least 80% of the total length of the cuff. The appropriate cuff is to be applied around the upper arm so that the midpoint of the length of the cuff lies over the brachial artery and mid-height of the cuff is at heart level. The lower edge of the cuff should be across the natural crease of the inner aspect of the elbow.

6. Blood pressure will be measured in the same arm each time except at the screening visit when the blood pressure will be measured in both arms. A second reading will then be taken in the arm with the highest reading. If a difference of >10 mmHg is found between the first and second reading in systolic or diastolic blood pressure, a third reading will be taken.

7. Blood pressure will be measured twice, the measurements separated by at least two minutes.

8. Both systolic and diastolic blood pressure will be recorded, as well as heart rate using the same digital sphygmomanometer. The mean of the measurements will be used as the basis of evaluation.

Heart Rate

Heart rate will be measured every visit, concurrently with blood pressure, using the same digital sphygmomanometer.

2. 12-Lead ECG

A 12-lead resting ECG was obtained at the screening visit to check for any recent onset of cardiovascular disease that is not known. The ECG was traced prior to the baseline visit and the last visit of treatment by a suitably qualified person at the site. The ECG was recorded by a Laverty Pathology technician, then read and reported by a cardiologist at Laverty Pathology at study site (Central Laboratory).

3. Laboratory Safety Testing

Fasting blood samples were collected from all participants at screening, Week 4, 8, 12 and post-study follow-up visit. Please refer to table 3. for an exact description of the sample collected at each visit. Blood samples were sent to the Laverty Pathology for commercial analysis. A small amount of blood plasma (approximately 10 mL) will also be stored in cryotubes in a -80ºC freezer and used later for gut hormones. These analyses were performed at the laboratory in University of Technology Sydney.