THE EFFECT OF MULBERRY LEAF TEA ON POSTPRANDIAL GLYCEMIC CONTROL AND INSULIN SENSITIVITY IN PRE-DIABETIC AND NON-DIABETIC SUBJECTS

PARIVAT SUKRIKET

MASTER OF SCIENCE
IN
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SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
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ABSTRACT

Leaves of mulberry (*Morus alba*, L.) have been one of the prominent herbs widely used by traditional medicine for the treatment of diabetes mellitus. The purpose of this study is to evaluate the influence of mulberry leaf tea on postprandial plasma glucose level and insulin sensitivity. This study was conducted on 14 subjects, with a crossover design. Subjects were initially screened for non-diabetic status (FBS: < 126 mg/dL). Subjects were randomized to drink either 100ml of mulberry leaf tea or water 30 minutes prior to 75 grams sucrose solution. Venous blood samples were collected before sucrose ingestion (time point 0) and at 30, 60, 90, 120, and 150 minutes after. After OGTT, participants kept a diary of severity of abdominal and other symptoms score scales. The author found that the mulberry sample group tends to have a lower level of plasma glucose, serum insulin concentrations and $AUC_{0\text{-}150}$ compared to the controlled group, but without significant difference. The mean difference of the incremental glucose level at each time point also tends to be lower for the mulberry group, with significant difference at 30-min time point (P = 0.04). To conclude, in non-diabetic subjects, consumption of mulberry leaf tea may aid in postprandial glycemic control during the first 30 minutes after meal. However, it does not show blood glucose lowering effect in general. Mulberry leaf tea does not influence the physiological insulin sensitivity and does not cause significant adverse events.

Keywords: Antihyperglycemic/Insulin sensitivity/Mulberry leaf tea/OGTT/Pre-diabetes/ Non-diabetes

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CHAPTER 1

INTRODUCTION

1.1 Background

Recent epidemiology study has estimated that the prevalence of diabetes is increasing at an extremely dramatic rate. In 2000, the global diabetes prevalence was 171 million people, and it was estimated in 2006 that the number will rise to approximately 366 million people by the year 2030. However, the actual growing number is much more advanced, with 346 million diabetic patients reported in August 2011 (American Diabetes Association, 2003; Wild, Roglic, Green, Sicree, & King, 2004; World Health Organization, 2006, 2011). The national statistic for Thai diabetic patients has also shown a similar pattern of escalation. With diabetes, endocrine, and other metabolic disorders grouped together as a category, the population of this group was 1.58 million people in 2003; the number rose to 2.24 million in 2009 (National Statistical Office, 2003, 2009).

Diabetes and its complications have many negative health impacts. Diabetes is a disease in which the body either does not produce or cannot properly use the pancreatic hormone insulin (Balch, 2006b). This results in plasma glucose building up in the bloodstream instead of being taken into and used by the cells, leading to hyperglycemia. If this situation is not properly controlled, diabetes can lead to microvascular damages, including retinopathy, nephropathy, neuropathy, giving rise to macrovascular complications, including ischaemic heart disease, stroke, and peripheral vascular diseases. (World Health Organization Department of Noncommunicable Disease Surveillance, 1999)

Diabetes does not only have impact against quality of life and physical well being; it also affects patients and their relatives economically. In 2006, the American Diabetes Association, abbreviated as ADA, has made estimation for the national costs of diabetes merely in the USA in year 2002 to be 132 billion US dollars, and it is projected to increase to 192 billion US dollars in year 2020 (American Diabetes Association, 2003).

Preventions and treatments of diabetes are not only available with conventional medicine nut also with traditional medicine. For centuries, local traditional medicines were implemented in countries around the world as natural remedies against illness as well as for tonic and prophylaxis propose. One of the most well known oriental herbs is mulberry leaf, which has been shown to be efficacious for the treatment and prevention of diabetes (Grover, Yadav, & Vats, 2002; Gulubova & Boiadzhiev, 1975; Hikino, Mizuno, Oshima, & Konno, 1985; Hosseinzadeh & Sadeghi, 1999; Jang et al., 2002; Kimura et al., 1995; Lemus, Garcia, Delvillar, & Knop, 1999; Litthilert, 2001; Sharaf & Mansour, 1964). Decoction and oral administration of mulberry leaves have been used in Thailand (Chao, 1979; Theppanom Muengman, Poranee Wangdamrongwong, Orasa Sudtienkul, Waranya Saengpetchsong & Romsai Glasoontorn, 1990; Sangiam Pongboonrod, 1979;

Komol Siwabawon, 1980; Payorm Tantiwiwat, 1978; Wuth, 1997), Jordan (Al-Khalil, 1995), The Island of Rodrigues (Gurib-Fakim, Sewraj, Gueho, & Dulloo, 1996), Yugoslavia, Peru (Tucakov, 1978), and Spain (Ramirez et al., 1988) for treatment of a number of illnesses.

Several prospective studies, *in vivo* and *in vitro* experiments have been done regarding mulberry's chemical properties and its pharmacological potentials. The most strongly supported mechanism of mulberry leaf extract is its ability to cause carbohydrate maldigestion and malabsorption (Hansawasdi & Kawabata, 2006; Kimura et al., 2007; Kwon, Chung, Kim & Kwon, 2011; Mudra, Ercan-Fang, Zhong, Furne & Levitt, 2007; Yatsunami, Ichida & Onodera, 2008; Zhong, Furne & Levitt, 2006). There has also been recent studies which bring into evident the ability of mulberry leaf extract to regulate the genetic expression of hepatic and pancreatic enzymes and hormones, aiding the treatment of diabetes (Li et al., 2011). Other than the antihyperglycemic activities, mulberry leaf extract also benefit diabetic subjects through its antioxidant and antiglycation activities (Jarinyaporn Naowaboot, Patchareewan Pannangpetch, Veerapol Kukongviriyapan, Bunkerd Kongyingyoes & Upa Kukongviriyapan, 2009). Other than studies in rats, a few studies have also been done in human (Kimura, et al., 2007; Mudra, et al., 2007; Somsak Sinsatienporn et al., 2006). In one study, 27 Thai diabetic type 2 patients from Pathumthani Hospital were given extract of mulberry leaf for 8 weeks; there was no significant change in glucose level (Somsak Sinsatienporn et al., 2006). In another study, healthy and diabetic participants were subjected to oral glucose tolerance test with mulberry leaf extract administered prior to the test; significant reduction of blood glucose fluctuation was observed (Mudra, et al., 2007).

In the aspect of prophylaxis, functional food is a viable option for health-concern individuals. Mulberry leaf tea has been used for the prevention of diabetes in traditional medicine for a long time (Chao, 1979; Theppanom Muengman, et al., 1990; Sangiam Pongboonrod, 1979; Komol Siwabawon, 1980; Payorm Tantiwiwat, 1978; Wuth, 1997). Nevertheless, little scientific research regarding this health beverage has been done. A clinical trial on mulberry leaf tea was done focusing on the breath hydrogen and carbon dioxide, which reflects the level of carbohydrate malabsorption; however, the levels of glucose or insulin were not measured (Zhong, et al., 2006).

Clearly, it is imperative for members of the medical research throughout the globe to converge their attention on developing new interventions as well as improving existing treatments that can effectively diagnose diabetes, alleviate its symptoms, and most importantly, prevent the evitable. With mulberry leaf tea as the potential supportive and preventive option for diabetes and pre-diabetes, this study aimed to determine its effect in non-diabetic subjects on levels of plasma glucose, insulin, and the corresponding insulin sensitivity index. In addition, this study determined the correlation of mulberry leaf tea co-ingestion with a meal and the symptoms afterward.

1.2 Research Questions

1.2.1 Primary Question

This study attempted to determine whether co-ingestion of mulberry leaf tea by non-diabetic subjects with 75 grams sucrose solution reduces blood glucose fluctuation and improves the whole body physiological insulin sensitivity.

1.2.2 Secondary Question

Since mulberry leaf has been shown to induce carbohydrate malabsorption, the study also determined whether co-ingestion of mulberry leaf tea with sucrose solution causes abdominal or other unwanted symptoms.

1.3 Research Objectives

1.3.1 To evaluate the influence of mulberry leaf tea on the blood glucose and whole body physiological insulin sensitivity response to ingestion of sucrose solution by non-diabetic subjects.

1.3.2 To evaluate the severity of abdominal and other symptoms of the subjects after co-ingestion of mulberry leaf tea with sucrose solution.

1.4 Hypothesis

Co-ingestion of mulberry leaf tea with 75 grams sucrose solution by non-diabetic subjects can reduce blood glucose fluctuation as well as improving the whole body physiological insulin sensitivity without causing significant abdominal or other symptoms.

1.5 Conceptual Framework

The presumed antidiabetic property of mulberry leaf tea consists of a number of mechanisms of actions. The following diagram illustrates three primary concepts of action in which mulberry leaf tea accounts for.

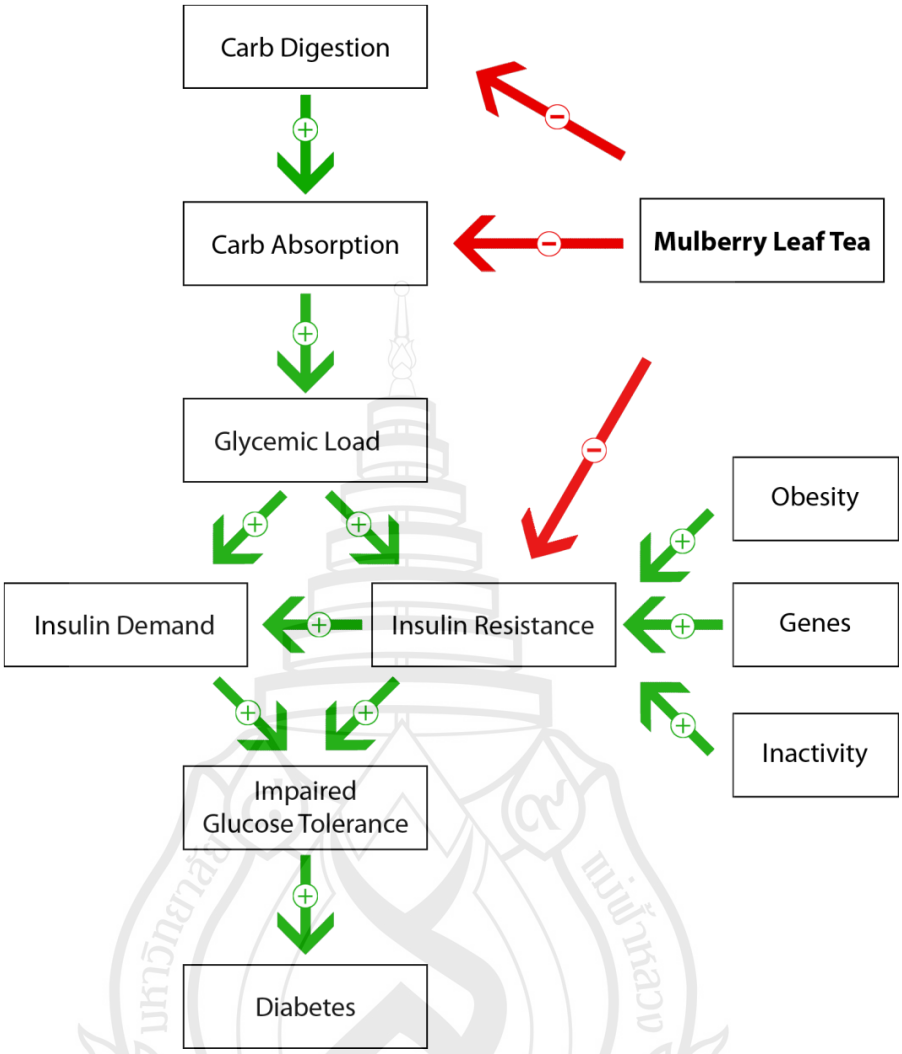


Figure 1.1 Conceptual Framework Diagram

Figure 1.1 illustrates a pathway of causal relationships which lead to diabetes. The diagram starts from carbohydrate digestion, which requires the action of amylase and glucosidase enzymes throughout the gastrointestinal tract. After proper digestion, complex carbohydrate molecules are converted into smaller units of monosaccharide which can then be absorbed at the intestinal epithelium.

In normal circumstances, plasma glucose will rise after ingestion of a meal that contains carbohydrate or protein. The increase in plasma glucose triggers the demand for insulin to down-regulate the level. Plasma glucose level rises at the rate depending on the type of meal ingested. Particularly for high glycemic index type of food, the resulting glycemic load shoots up after a meal. Routinely high glycemic load reduces the body's insulin sensitivity, leading to insulin resistance. As a vicious cycle, insulin resistance leads to more insulin demand for the same level of blood sugar. The constant need for higher level of insulin and deteriorated insulin sensitivity together bring the body into the

state of impaired glucose tolerance, which is pre-diabetes. As time goes on, the pre-diabetic person is likely to develop into diabetes.

From the explanation, mulberry leaf tea intervenes with three of the steps in the diagram. Mulberry leaf is notable for its inhibitory effect of carbohydrate digestive enzymes. Thus, it is hypothesized that the mulberry leaf tea would cause carbohydrate malabsorption. Also, there is a study that polysaccharides from mulberry leaf aqueous extract compete against glucose at the glucose transport sites, reducing glucose uptake in the intestine (Kwon, et al., 2011). Mulberry leaf tea is thus expected to cause carbohydrate malabsorption. In addition, mulberry leaf extract has been shown to increase peripheral glucose uptake at adipose and muscle cells. It is assumed that mulberry leaf tea would thereby increase insulin sensitivity.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus

2.1.1 Overview of Diabetes

Diabetes Mellitus, often shorten as diabetes or DM, is a disease in which the body either does not produce or cannot properly use the pancreatic hormone insulin (Balch, 2006b). Glucose, the type of sugar that is most abundant and usable by our body, is the main source of energy for every cell in our body. The levels of glucose in the blood are controlled by insulin, regulating the rate at which glucose is absorbed into the cells.

In normal circumstances, blood glucose will rise after we eat a meal that contains carbohydrate or protein. The increase in blood glucose triggers a release of insulin from cells in the pancreas, known as beta-cells. Insulin in turn stimulates cells throughout the body to allow glucose uptake. This results in the blood glucose levels falling back toward normal. The release of insulin then tapers off until the next time protein or carbohydrates are ingested.

For patients with diabetes, glucose builds up in the bloodstream instead of being taken into and used by the cells, leading to hyperglycemia. If this situation is not properly controlled, diabetes can lead to heart disease, kidney disease, edema, nerve damage, and infections of the mouth, gums, lungs, skin, feet, bladder, and genital areas. Skin sores may develop and fail to heal properly.

Diabetes is a condition primarily defined by the level of hyperglycemia, giving rise to risks of microvascular damage (World Health Organization Department of Noncommunicable Disease Surveillance, 1999). This includes retinopathy, damage to the eyes, nephropathy, damage to the kidney, and neuropathy, damage to the nervous system. Diabetes is associated with diminished quality of life, as well as reduced life expectancy. It is also associated with significant morbidity due to specific diabetes-related microvascular complications and increased risk of macro vascular complications, including ischaemic heart disease, stroke, and peripheral vascular diseases.

2.1.2 Global and National Prevalence of Diabetes

Recent epidemiology study has estimated that the prevalence of diabetes is increasing rapidly. The global prevalence of diabetes was 171 million people in the year 2000, and it is projected to increase to 366 million by 2030, doubling within less than 30 years (World Health Organization, 2006). However, the current number of diabetic patients worldwide has reached 346 million in August 2011, skyrocketing to almost twice the number a decade ago (World Health Organization, 2011). Diabetes epidemic is estimated to continuously grow even if the levels of obesity remain constant (Wild, et al.,

2004). In 2006, the American Diabetes Association, abbreviated as ADA, has made estimation for the national costs of diabetes merely in the USA in year 2002 to be 132 billion US dollars, and it is projected to increase to 192 billion US dollars in year 2020 (American Diabetes Association, 2003).

The national statistical office of Thailand has also reported an increasing trend of diabetes population. In 2003, the total number of Thai population with chronic diseases was about 10.1 million. Among the ten millions, 1.58 million patients were categorized into the group Diabetes/Endocrine system and metabolic disorders (National Statistical Office, 2003). In 2009, the total number of Thai population with chronic diseases was about 10.5 million, which is insignificantly different from six years earlier. However, the number of patients that were categorized into the group Diabetes/Endocrine system and metabolic disorders was 2.24 million people, which is approximately 42 percent larger than the previous data (National Statistical Office, 2009).

Table 2.1 The 2003 and 2009 Statistic of Thai Population with Chronic Diseases

Group of Chronic Diseases	Year 2003	Year 2009
Cardiovascular diseases	2,072,358	4,044,260
Musculo-skeletal disorders	1,742,736	1,375,210
Diseases of the digestive system	1,015,666	397,514
Diseases of the respiratory system	923,381	1,721,809
Diseases of the nervous system and mental disorder	602,846	453,665
Diseases of urinary system	210,847	303,596
Infectious diseases	86,241	81,903
Diabetes/Endocrine system and metabolic disorders	1,578,446	2,236,593
Total	**10,170,660**	**10,519,386**

From National Statistical Office. (2003). **The 2003 health and welfare survey**. Retrieved 9 September, 2011, from http://service.nso.go.th/nso/nso_center/project/table/files/S-health-welfare/2546/000/00_S-health-welfare_2546_000_000000_02300.xls; National Statistical Office. (2009). **The 2009 survey on health, welfare and food consumption behavior**. Retrieved 9 September, 2011, from http://service.nso.go.th/nso/nso_center/project/table/files/S-health-welfare/2552/000/00_S-health-welfare_2552_000_010000_00900.xls

The latter figures implicate the correlated global trend of increasing diabetes prevalence. The number of people around the world suffering from diabetes has clearly skyrocketed in the last few decades, claiming millions of lives and severely taxing the ability of health care systems to deal with the epidemic. It is therefore imperative for members of the medical research throughout the globe to converge their attention on developing new interventions as well as improving existing treatments that can effectively diagnose diabetes, alleviate its symptoms, and most importantly, prevent the evitable.

2.1.3 Pathophysiology and Classification of Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin, action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. (American Diabetes Association, 2008)

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the beta cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories, with other categories of abnormal glucose regulation often assigned according to the circumstances present at the time of diagnosis. Many diabetic individuals do not easily fit into a single class.

2.1.3.1 Type 1 Diabetes

Type 1 diabetes affects 5 to 10 percent of people with diabetes and usually starts at an early age. It is referred to by several names, including insulin-dependent diabetes mellitus (IDDM), DM type 1, and juvenile diabetes. This type of diabetes results from the body's failure to produce insulin, thus constantly requiring exogenous insulin administration. It is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type of diabetes can be further classified as either immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, where beta cells are attacked by self immune T-cell (autoimmune) (Rother, 2007). There is no known preventive measure against type 1 diabetes. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed juvenile because it represents a majority of the diabetes cases in children.

2.1.3.2 Type 2 Diabetes

Type 2 diabetes, by far the most common form of diabetes, affects 90 to 95 percent of diabetes sufferers. It is formerly referred to as non-insulin-dependent diabetes mellitus (NIDDM) and adult-onset diabetes. In type 2 diabetes, the pancreas does produce insulin in small quantities, but not enough to fuel the cells. The cells may also become

resistant to the effects of what little insulin there remains in the bloodstream. This type of diabetes is therefore characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin may involve the insulin receptor. In the early stage of type 2 diabetes, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce the production of glucose by the liver.

2.1.3.3 Gestational Diabetes

Gestational diabetes mellitus, GDM for short, is when pregnant women, who have never had diabetes before, have a high blood sugar level during pregnancy. GDM resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. Hormonal changes during pregnancy can affect the body's resistance to insulin. It occurs in about 2-5% of all pregnancies and may improve or disappear after delivery, but it seems to be a sign that the woman is more likely to develop type 2 diabetes in later life. Gestational diabetes is fully treatable, though it requires careful medical supervision throughout the pregnancy.

Although it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant destruction. In severe cases, perinatal death may occur. Labor induction may be indicated with decreased placental function.

The American Diabetes Association has defined GDM as "type 3 diabetes" (American Diabetes Association, 2005).

2.1.4 Diabetes Signs and Symptoms

The classical symptoms of diabetes are frequent urination (polyuria), increased thirst (polydipsia), and increased hunger (polyphagia) (Cooke & Plotnick, 2008). Symptoms may develop rapidly in type 1, perhaps weeks or months. On the other hand, symptoms usually develop much more slowly and may be subtle or absent for type 2 diabetes.

For the eyes, prolonged high blood sugar will lead to excess glucose absorption, resulting in changes in shape of the lenses, causing changes in vision. Glucose level management has to be sustained, and the lens would usually return to its original shape. Blurred vision is a common complaint leading to a diabetes diagnosis.

Diabetic Ketoacidosis, shortly called DKA, may also be present at a later state. It is a condition of metabolic dysregulation characterized by the smell of acetone; a rapid, deep breathing known as Kussmaul Breathing, nausea, vomiting, and abdominal pain (Kussmaul, 1874).

Hyperosmolar nonketotic state is similar to the latter condition except it is not resulted from excess ketone bodies. This condition is more common in type 2 diabetes and is mainly the result of dehydration. Diabetes patients often drink extreme amount of sugar-containing drinks, leading to a vicious cycle in regard to the loss of water (Cirasino, Thiella, Invernizzi, Silvani, & Ragaini, 1992).

A number of skin rashes can occur in diabetes that are collectively known as diabetic dermadromes (James & Berger, 2006).

2.1.5 Current Criteria for the Diagnosis of Diabetes

Previously, the Department of Noncommunicable Disease Surveillance, World Health Organization, had provided the definition and diagnosis criteria of diabetes with two tests; the fasting plasma glucose, and 2-h glucose test. In 2010, the American Diabetes Association Standards of Medical Care in Diabetes added the glycohemoglobin ≥ 6.5% (≥ 48 mmol/mol) as another criterion for the diagnosis of diabetes. In short, diabetes mellitus is characterized by recurrent or persistent hyperglycemia. It is diagnosed by demonstrating any one of the conditions in the following table.

Table 2.2 Current Criteria for the Diagnosis of Diabetes-2010

Type of Blood Test	Level of Plasma Concentration for Diabetes
Glycohemoglobin (HbA1C)	≥ 6.5% (48 mmol/mol)
Fasting Blood Sugar (FBS, FPG)	≥ 126 mg/dl (7.0 mmol/l)
2-h or Random Plasma Glucose	≥ 200 mg/dl (11.1 mmol/l)

From Executive summary: Standards of medical care in diabetes. (2010). **Diabetes Care, 33**(Suppl 1), S4-S10

2.1.6 Medications used in Diabetes

Type 2 diabetes may be managed with any number of different drugs. The following table is a summary of the major medications currently used for diabetes management together with how they act in the body, generic and brand names, and general description.

2.1.6.1 Oral Antidiabetic Agents

Orally administered drugs available for the treatment of type 2 diabetes include the sulfonylureas, the biguanide metformin, the alpha-glucosidase inhibitor, and the thiazolidinedione. These agents reduce blood glucose levels by different mechanism.

1. Sulfonylureas

Sulfonylureas (SFU) are the first oral agents available for the treatment of type 2 diabetic subjects. Sulfonylureas promote insulin secretion through direct stimulation of pancreatic beta cells. They bind to ATP-sensitive potassium channels and inhibit potassium ion efflux. The resulting depolarization allows influx of calcium ions through the voltage-dependent calcium channels. The release of insulin is triggered by the rise of intracytosolic calcium ion concentration. Secondary failure of sulfonylureas-induced insulin secretion results from beta cell "exhaustion", which occurs in approximately 10% of patients per year. After 10 years of treatment by sulfonylureas, approximately 50% of the treated patients become non-responder (Oiknine & Mooradian, 2003).

All sulfonylureas are able to augment insulin secretion in patients with type 2 diabetes who still have pancreatic insulin reserve. These drugs have different durations of action, dose responses, and side effects. Sulfonylureas can reduce the level of fasting plasma glucose by 20-30%. They have a substantial primary and secondary failure

rate. Potential side effects of sulfonylureas include hypoglycemia, weight gain, and hyperinsulinemia. Thus, it is relatively uncommon for patients treated with sulfonylureas alone to achieve normoglycemia. Sulfonylureas may be used in combination with other oral agents or insulin. Although this approach has been shown to decrease insulin requirements in some patients with type 2 diabetes, it is not effective in all patients. In addition, these patients may encounter episodes of hypoglycemia (Lipkin, 1999).

2. Meglitinides

These agents are non-sulfonylurea secretagogues with a sulfonylurea-like mechanism of action. They target a different binding site on pancreatic beta cells, leading to a similar cascade of events triggering insulin release. Repaglinide, a benzoic acid derivative, and the new agent, nateglinide, a phenylalanine derivative, are examples of drugs belonging to this class of agents. Their pharmacokinetic profile is favorable in terms of targeting postprandial hyperglycemia.

Repaglinide is completely metabolized by the liver and eliminated mostly by biliary route (90%). The drug elimination kinetics is widely variable; its half-life ranges between 0.5 hours and 8 hours. In elderly patients with type 2 diabetes mellitus who have reduced creatinine clearance, the mean diurnal plasma concentration was found to be significantly higher than healthy controls. Similar results were noticed in patients with moderate to severe liver disease. Nateglinide should also be used cautiously in the context of renal and/or hepatic impairment. Studies have proved repaglinide as efficacious as sulfonylureas. Nateglinide, however, appears to be less potent than sulfonylureas. Another drawback in using these agents is the frequent pre-meal dosing that may affect compliance. Although it was hoped that weight gain and hypoglycemia would occur less often with Meglitinides than with sulfonylureas, a recent clinical trial found no major clinical advantage when repaglinide was compared to glipizide (Lipkin, 1999).

3. Biguanides

Metformin is the biguanide that is most prescribed worldwide. It is much safer than earlier biguanides, phenformin and buformin. The latter agents were withdrawn from the market because of their tendency to cause lactic acidosis. Its principal action is to reduce hepatic gluconeogenesis. Excessive hepatic glucose production is then diminished at any given level of plasma insulin. To a lesser degree, possibly an indirect effect, metformin increases insulin mediated glucose uptake and utilization in peripheral tissues, especially in the muscle. Metformin is therefore an antihyperglycemic agent preferred in obese patients with insulin resistance. Metformin is not metabolized and mostly renally excreted (90%) in 12 hours. Its bioavailability is approximately 50 to 60 percent, being absorbed mainly from the small intestine. The estimated plasma half-life is about 1.5 to 4.9 hours. Metformin is found to rarely bind to plasma proteins. It is beneficial at a low dose of 500 mg per day with meals, demonstrating a progressive response to doses up to 2000 mg per day. Although metformin is approved for up to 2550 mg per day, little additional benefit is seen beyond 2000 mg dose. It is not associated with weight gain and reduced adiposity. Metformin also has a modest antihypertensive and antihyperlipidemic effect, with slight increase in HDL cholesterols and decrease in triglycerides and LDL cholesterols (Lipkin, 1999).

As monotherapy, it does not cause hypoglycemia. It can be used as a single agent with efficacy equivalent to that of sulfonylurea; otherwise both agents in combination are also feasible. This combination has the potential of achieving

normoglycemia in patients with type 2 diabetes without concomitant weight gain and hyperinsulinemia. Metformin may also be used with alpha-glucosidase inhibitor or insulin. Of the newer drugs, studies indicate that metformin is best for normalizing abnormal blood lipids. Contraindications to the use of metformin include kidney disease, having serum creatinine level greater than 130 micromol/l in men and 120 micromol/l in women, diabetic ketoacidosis, and pregnancy (Lipkin, 1999).

4. Thiazolidinediones

This class of agents is relatively new. It is the most expensive class of antidiabetic agents. Troglitazone, the first compound in this class, was withdrawn from the market because of its association with unpredictable fulminant hepatic failure. The two currently available agents, rosiglitazone and pioglitazone, do not appear to have the same tendency to cause hepatotoxicity.

Thiazolidinediones enhance glucose uptake and utilization in peripheral tissues, mainly skeletal muscle. Thiazolidinediones bind to the peroxisomal proliferator-activated receptor (PPAR-gamma). The nuclear receptor most highly expressed not only in adipose tissue, but also found in skeletal muscle, liver, intestine, kidney, vascular smooth muscle, heart, and macrophages. Once activated, PPAR-gamma receptor binds to DNA, leading to transcriptional modulation of genes involved in carbohydrate and lipid metabolism. Thiazolidinediones decrease insulin resistance in peripheral tissues with only a minor effect on hepatic glucose production at high doses. Given the abundance of PPAR-gamma receptors in adipocytes compared to myocytes, the predominant action of thiazolidinediones on skeletal muscle may be through an indirect interaction between fat and muscle cells.

Thiazolidinediones do not cause hypoglycemia when used alone. Rosiglitazone and pioglitazone have a half-life ranging between 3 to 7 hours. Some active metabolites of pioglitazone have longer half-lives. These agents are 99 percent protein bound. Rosiglitazone and pioglitazone have minimal drug-drug interaction. While pioglitazone partially induce the cytochrome P450 system, metabolized through the CYP2C8 isoenzyme and CYP3A1, rosiglitazone is metabolized through the CYP2C8 isoenzyme only and does not induce the cytochrome P450 system. Thiazolidinediones are hepatically metabolized and eliminated by biliary route. The dose of glitazones does not need to be adjusted for renal insufficiency. But in the context of hepatic failure, thiazolidinediones must be discontinued. Nevertheless, rosiglitazone and pioglitazone have not demonstrated an increased rate of liver enzyme abnormality so far. Based on the troglitazone experience, the current recommendation is still to monitor liver enzymes at baseline every other month in the first year and periodically afterward.

Thiazolidinediones also have non-hypoglycemic effect. Both rosiglitazone and pioglitazone slightly raise HDL cholesterol levels. LDL cholesterol levels are increased with both agents although more so with rosiglitazone. Pioglitazone, but not rosiglitazone, may have a triglyceride lowering effect possibly because of its additional PPAR-gamma activity. Thiazolidinediones also slightly reduce blood pressure, enhance fibrinolysis, improve endothelial function, and decrease *in vitro* vascular inflammation (Lipkin, 1999).

5. Alpha-Glucosidase Inhibitors

There are three agents in this category that are currently marketed worldwide. These include acarbose, miglitol, and voglibose. These agents primarily target

postprandial hyperglycemia. Postprandial hyperglycemia has been linked with cardiovascular mortality.

Alpha-glucosidase inhibitors have a unique mechanism of action. These agents competitively inhibit alpha-glucosidases, the brush border enzymes of the proximal small intestinal epithelium. This reversible inhibition delays hydrolysis of polysaccharides into absorbable monosaccharides, such as glucose. Carbohydrate absorption occurs over a greater portion of the small intestine, blunting postprandial glucose excursions.

Hypoglycemia may occur when alpha-glucosidase inhibitors are used in combination therapy with either insulin or sulfonylureas. In that context, glucose rather than sucrose or complex carbohydrate should be used. Glucose is readily absorbed and will promptly correct hypoglycemia.

Alpha-glucosidase inhibitors are safe and may be suitable agents in the elderly diabetic patients, either as monotherapy or in combination therapy. The need for multiple pre-meal dosing, gastrointestinal side effects, and cost may however limit their use (Lipkin, 1999).

In general, when proper glycemic control is not achieved despite diet, exercise, and the use of other oral antidiabetic agents, insulin therapy is often necessary.

Current clinical uses of diabetes medication include all of the mentioned antidiabetic drugs, which cause certain adverse drug reactions. Some of them are of high costs, leaving many patients with suboptimal treatments. In present, remedies of natural source illustrate considerable potentials, claiming to be capable of reducing elevated blood glucose levels. These remedies, including *M. alba* L., may be developed in search of a better anti-diabetic agent with cost-effectiveness, efficacy, and safety.

Table 2.3 Oral Medications for Type 2 Diabetes

Action in the Body	Generic Name	Brand Name (s)	Descriptions
1. Sulfonylureas Stimulates beta cells to release more insulin	Chlropropamide	Diabinese	Take one or two times daily before meals. First-generation drug
	Glipizide	Glucotrol	Second-generation drug. Used in smaller doses than first-generation agents.
	Glyburide	DiaBeta Micronase Glynase	Second-generation drug. Smaller doses.
	Glimepiride	Amaryl	Second-generation drug. Smaller doses.
2. Meglitinides Stimulate the pancreas to release more insulin	Repaglinide	Prandin	Taken before each of three meals.
3. Nateglinides Works similarly to sulfonylureas	Nateglinide	Starlix	Taken before each of three meals.

Table 2.3 (Continued)

Action in the Body	Generic Name	Brand Name (s)	Descriptions
4. Biguanides			
Sensitizes the body to insulin already present	Metformin Metformin Extended Release Metformin with Glyburide	Glucophage Glucophage XR Glucovance	Taken two times daily with food for best results.
5. Thiazolidinediones (Glitazones)			
Help insulin work better in muscle and fat and lowers insulin resistance	Rosiglitazone Pioglitazone	Avandia Actos	Taken once or twice daily with food. Very rare but serious side effects on the liver are possible
6. Alpha-Glucosidase Inhibitors			
Slows or blocks breakdown of starches and sugars; action slows rise in blood sugar after eating	Acarbose Miglitol Voglibose	Precose Glyset Basen	Should be taken with first bite of meals.

From Balch, P. A. (2006c). Part 2 - the Disorders (Diabetes) **Prescription for Nutritional Healing: A Practical A-to Z- Reference to Drug-Free Remedies Using Vitamins, Minerals, Herbs & Food Supplements** (4 ed., pp. 366). New York: AVERY, a member of Penguin Group (USA) Inc.

Type 1 diabetes is managed with injections for the hormone insulin, as well as with close attention to diet and other lifestyle factors. Not all insulins are the same, however, and it may take some trial and error to find the one or ones that are right for any given individual. The principal differences are related to the speed with which they take effect, and the period of time they remain active in the body. The following table shows the major types of insulin that are commonly available and used in the present.

Table 2.4 Insulin Preparations for Type 1 Diabetes

Insulin Type	Onset of Action	Peak of Action	Duration of Action
1. Long-Acting Insulin			
Humalog (Lispro)	15 minutes	30-90 minutes	3-5 hours
NovoLog (Aspart)	15 minutes	40-50 minutes	3-5 hours
2. Short-Acting Insulin (Regular)			
Humulin R	30-60 minutes	50-120 minutes	5-8 hours
Novolin R	30-60 minutes	50-120 minutes	5-8 hours

Table 2.4 (Continue)

Insulin Type	Onset of Action	Peak of Action	Duration of Action
3. Intermediate-Acting Insulin (NPH)			
Humulin L	1-2.5 hours	7-15 hours	18-24 hours
Humulin N	1-3 hours	8 hours	20 hours
Novolin N	1-3 hours	8 hours	20 hours
4. Intermediate- and Short-Acting Insulin Mixtures			
Humulin 50/50 Humulin 70/30 Humulin Mix 75/25 Humulin Mix 50/50 Novolin 70/30 Novolog Mix 70/30	The onset, peak, and duration of action of these mixtures reflect a composite of the intermediate- and short- or rapid-acting components, with one peak of action.		
5. Long-Acting Insulin			
Ultralente	4-8 hours	8-12 hours	36 hours
Lantus (Glargine)	1 hour	None	24 hours

From Balch, P. A. (2006a). Part 2 - the Diseases (Diabetes) **Prescription for Nutritional Healing: A Practical A-to Z- Reference to Drug-Free Remedies Using Vitamins, Minerals, Herbs & Food Supplements** (4 ed., pp. 369). New York: AVERY, a member of Penguin Group (USA) Inc.

2.1.7 Medicinal Herbs with Anti-diabetic Activities

For many centuries, local traditional medicines were implemented in countries around the world as natural remedies against illness. After the development of the modern conventional medicine, recent uses of these traditional herbs still continue for several reasons, including the local belief of efficacy and, more importantly, safety as opposed to the modern drugs that inevitably come along with adverse drug reactions that may occur after prolonged usage.

Therapeutic properties of herbal remedies are versatile. One of them includes anti-diabetes, which involve the use of several plants with multiple parts. Other than *M. alba* L., which is the plant of this study's concern, the following table displays a list of plants that have been used for its anti-diabetic properties.

Table 2.5 List of plants with Anti-Diabetic Activity

Botanical name	Family	Parts used	Mechanism of action	Chemical constituents
Abies pindrow Royle	Pinaceae	Entire plant	Insulin secretagogue activity	Volatile oil
Abroma augusta Linn	Sterculiaceae	Roots & Leaves	Lowering blood sugar	Fixed oil, Alkaloid
Acacia arabica Wild	Leguminosae	Seed	Initiates release of insulin	Arabin

Table 2.5 (Continue)

Botanical name	Family	Parts used	Mechanism of action	Chemical constituents
Achyranthus aspera L		Entire plant	Decreases blood sugar	
Agrimony eupatoria L.	Rosaceae	Leaves	Insulin releasing & insulin like activity	
Ajauga iva Wall. ex. Benth	Labiatae	Entire plant	Decreases plasma glucose level	
Allium sativum Linn.	Liliaceae	Roots	Antihyperglycemic and antinociceptive effect	Volatile oil, Allin, Allicin
Allium cepa Linn.	Liliaceae	Bulb	Stimulating effects on glucose utilization and antioxidant enzyme	Protein, carbohydrate, vitamin A, B, C, Allyl propyldisulphide
Aloe vera Tourn. ex. Linn.	Liliaceae	Entire plant		Aloin glycoside
Aloe barbadensis Miller	Liliaceae	Leaves	Stimulating synthesis and/or release of insulin	Barbaloin, isobarbaloin, resin
Amaranthus spinosus Linn.	Amaranthaceae	Stem		
Anacardium occidentale Linn	Anacardiaceae	Entire plant		Flavonols, terpenoid, caumarin, phenolic compound,essential oil
Andrographis paniculata Nees	Acanthaceae	Entire plant	Increases glucose metabolism	Diterpenoid lactone andrographoloid
Annona squamosa	Annonaceae	Leaves	Hypoglycemic and anti-hyperglycemic activities	Acetogenins-squamosin B, squamosamide, reticulatain-2,isosquamosin
Artemisia pallens Wall	Compositae	Aerial parts	Leaf-extract, increased plasma insulin level, hypoglycemic, increases peripheral glucose utilization or inhibits glucose reabsorption	Essential oil, davanone
Averrhoa bilimbi	Oxalidaceae	Leaves	Increases serum insulin level	
Azadirachta indica A.juss.	Meliaceae	Leaves	Glycogenolytic effect due to epinephrine action was blocked	Nimbidin, nimbin, nimbidol, nimbosterol
Beta vulgaris Linn	Chenopodiaceae		Reduces blood glucose level by regeneration of beta cells	

Table 2.5 (Continued)

Botanical name	Family	Parts used	Mechanism of action	Chemical constituents
Bidens pilosa	compositae	Aerial parts		Polyacetylenic glucoside
Bixa orellana L.	Bixaceae	Entire plant	Increases plasma insulin concentration & increases insulin binding on insulin receptor	Oleo-resin
Boerhaavia diffusa L.	Nyctaginaceae	Leaves & Entire plant	Increases in hexokinase activity, decreases in glucose-6-phosphatase	Alkaloid punarnavaine, punarnavoside
Brassica juncea L.	Cruciferae		Food adjuvants for diabetic patients	Isothiocyanate glycoside singrin, protein, fixed oil
Caesalpinia bonducella Flem.	Leguminose	Seed kernels	Free radical scavenging	Fatty oil
Camellia sinensis	Theaceae	Leaves	Increases insulin secretion	Polyphenolic constituents (EGCG)
Capparis deciduas Edgew	Capparidaceae	Powder	Hypoglycemic, antioxidant, hypolipidaemic	
Capsicum frutescens Linn.	Solanaceae		Increases insulin secretion & reduction of insulin binding on insulin receptor	
Carum carvi Linn.	Umbelliferae	Fruits		Volatile oil, resin, carvone, fixed oil
Cassia alata	Caesalpiniaceae	Leaves		
Cassia auriculata	Caesalpiniaceae	Flower	Increases utilization of glucose through increase glycolysis	
Catharanthus roseus G.Don	Apocynaceae	Leaves, twig & flower	Increases metabolisation of glucose	Indole alkaloid, vincristine vinblastin
Cinnamomum zeylanicum Nees	Lauraceae	Bark	Elevation in plasma insulin	Volatile oil, tannin, mannitol, calcium oxalate
Clausena anisata Burm.f.	Rutaceae	Roots	Stimulates secretion of insulin	
Coriandrum sativum Linn.	Umbelliferae	Seed		Volatile oil, fixed oil, protein
Coscinium fenestratum Calebr	Menispermaceae	Stem	Increases enzymatic antioxidants	Barberine , glycoside, saponin
Croton cajucara Benth	Euphorbiaceae	Bark		Fixed oil
Cryptolepis sanguinolenta R.	Asclepidaceae	Entire plant	Increases glucose uptake by 3T3-L1 cells	Cryptolepine

Table 2.5 (Continued)

Botanical name	Family	Parts used	Mechanism of action	Chemical constituents
Eclipta alba Linn.	Compositae	Leaves	Decreases activity of glucose-6-phosphatase & fructose-1-6,bisphasphatase	Ecliptin alkaloid
Embellica officinalis Gaertn.	Euphorbiaceae	Fruits	Reduces 5-hydroxymethylfurfural, creatinine albumin level	Vitamin C, tannin
Enicostemma littorale Blume	Gentianaceae	Entire plant	Decreases glycosylated Hb & glucose 6 phosphatase	Swertiamarine glycoside
Eugenia jambolana Lam.	Myrtaceae	Seed, fruit, leaves, kernel	Lowers plasma glucose level	
Eucalyptus globulus Labill.	Myrtaceae	Leaves	Increases insulin secretion from clonal pancreatic beta line (BRIN-BD 11)	Essential oil , cineol
Euphrasia officinale	Scrophulariaceae	Leaves		
Ficus religiosa Linn.	Moraceae	Entire plant	Initiating release of insulin	Tannin
Ficus bengalensis Linn.	Moraceae	Bark	Rising serum insulin	Tannin
Ficus carica	Moraceae	Leaves		
Gymnema montanum Hook f.	Asclepiadaceae	Leaves	Antioxidant & antiperoxidative	
Gymnema sylvestre R.	Asclepiadaceae	Leaves	Lowers plasma glucose level	Gymnemic acid, quercital
Gentiana olivier Griseb.	Gentianaceae	Flowers	Lowers plasma glucose level	Iso-orientin C-glycoside
Glycerrhiza glabra Linn.	Leguminosae	Root	Lowers plasma glucose level	Triterpenoid,saponin, glycerrhizin
Gynura procumbens	Compositae	Leaves	Lowers plasma glucose level	
Hibiscus rosa sinensis Linn.	Malvaceae	Entire plant	Stimulates insulin secretion from beta cells	Vitamin B, C, fat
Helicteres isora Linn.	Sterculiaceae	Root	Decreases plasma triglyceride level & insulin sensitizing activity	Saponin, tannin, lignin
Hordeum vulgare	Graminaeae			
Hovenia dulcis Thunb	Rhamnaceae	Entire plant		Flavonoids
Ipomoea aquatica Forsk.	Convolvulaceae	Leaves	Reduces fasting blood sugar level & serum glucose level	Carotene

Table 2.5 (Continued)

Botanical name	Family	Parts used	Mechanism of action	Chemical constituents
Ipomoea batata Linn.	Convolvulaceae	Tubers	Reduces insulin resistance & blood glucose level	
Juniperus communis Linn.	Pinaceae	Fruits	Increases peripheral glucose consumption & induce insulin secretion	
Lupinus albus Linn.	Fabaceae	Seed	Lowering serum glucose level	Alkaloid, fatty oil, asparagines
Luffa aegyptiaca Mill.	Cucurbitaceae	Seed	Lactigogue activity	Fatty oil
Leucas lavandulaefolia Rees	Labiatae	Entire plant	Reduces blood glucose level	
Lagerstronemia speciosa	Lythraceae	Leaves		
Lepidium sativum	Cruciferae	Seeds		
Mangifera indica Linn.	Anacardiaceae	Leaves	Reduction of intestinal absorption of glucose	Mangiferin
Myrtus communis L.	Myrtaceae	Leaves	Lowering blood glucose level	Volatile oil, mirtii oleum
Memecylon umbellatum Burm	Melastomataceae	Leaves	Lowering serum glucose	
Momordica cymbalania Fenzl ex naud	Cucurbitaceae	Fruit powder	Reduces blood glucose level	
Mucuna pruriens L.	Leguminosae	Seed	Reduces blood glucose level	
Musa sapientum Linn.	Musaceae	Flower	Reduces blood glucose & glycosylated Hb	
Momordica charantia Linn.	Cucurbitaceae	Fruit	Reduces blood glucose level	Momordicine alkaloid, ascorbic acid
M. indica L.	Moraceae	Leaves	Increases glucose uptake	
Murraya koeingii (L) spreng.	Rutaceae	Leaves	Increases glycogenesis , decrease glycogenolysis & gluconeogenesis	
Nelumbo nucifera Gaertn.	Nymphaeaceae	Rhizome	Reduces blood sugar level	Nuciferin, nornuciferin
Ocimum sanctum Linn.	Labiatae	Leaves	Lowering blood sugar level	Volatile oil, phenol, aldehyde, fixed oil, alkaloid, tannin, ascorbic acid
Olea europia Linn.	Oleaceae	Leaves	Potentiation of glucose , induced insulin released , & increase peripheral uptake of glucose	Oleuropeoside

Table 2.5 (Continued)

Botanical name	Family	Parts used	Mechanism of action	Chemical constituents
Opuntia ficus-indica Mill	Cactaceae	Stem		
Pandanus odorus Linn.	Pandanaceae	Root	Decreases plasma glucose level	Essential oil
Panax ginseng Mey.	Araliaceae	Root & entire plant	Lowering blood sugar level	Glycans, panaxans I,J,K & L
Punica granatum Linn	Punicaceae	Seed	Reduces blood sugar level	Vitamin C, protein, tannin, gallic acid, pelletierine
Picrorrhiza kurroa Royle ex. Benth	Scrophulariaceae	Entire plant	Decreases serum glucose	Picrorrhizin, kutkin
Phyllanthus amarus	Euphorbiaceae	Entire plant	Decreases blood glucose level	Alkaloids
Phaseolus vulgaris	Papilionaceae	Pod, seed, whole plant	Hypoglycemic, hypolipidemic, inhibit alpha amylase activity, antioxidant.	
Salacia oblonga	Celastraceae	Root	Inhibition of alpha glucosidase activity	
Salacia reticulata Wight.	Celastraceae	Stem & root	Inhibition of alpha glucosidase activity	
Swertia chirayata Roxb.ex.Flem	Gentianaceae	Entire plant	Stimulates insulin release from islets	Zanthone mangiferin, gentianine, swerchirin
Syzygium cumini Linn	Myrtaceae	Seed	Decreases blood glucose level	
Scoparia dulcis Linn.	Scrophulariaceae	Leaves	Decreases glycosylated Hb & Increases total Hb, Insulin-secretagogue activity	
Trigonella foenum graceum	Leguminosae	Seed	Decreases blood glucose concentration	Protein, fat, volatile oil, fixed oil, carbohydrate
Tribulus terrestris Linn.	Zygophyllaceae	Saponin	Decreases serum glucose	Harmine
Tinospora crispa Linn.	Menispermaceae	Stem	Anti-hyperglycemic, stimulates insulin release from islets	
Tinospora cardifolia Wild.	Menispermaceae	Root	Decreases blood glucose & brain lipid	Berberine, starch
Tamarindus indica Linn.	Caesalpimiaceae	Seed		
Teramnus labialis (Roxb) Benth	Fabaceae	Aerial parts		Caumarin - fraxidin
Urtifca dioica Linn.	Urticaceae	Leaves	Increases insulin secretion	Fatty oil

Table 2.5 (Continued)

Botanical name	Family	Parts used	Mechanism of action	Chemical constituents
Viscum album Linn.	Loranthaceae	Entire plant	Alpha glucosidase inhibitor	
Vinca rosea	Apocynaceae	Leaves	Beta cell rejuvenation, regeneration, & stimulation	Vincristine , vinblastine
Withania somnifera Dunal	Solanaceae	Root	Decrease blood sugar level	Withanine, somnine, withaferine, withanolides
Xanthium strumarium	Compositae	Fruits	Increases glucose utilization	Phenolic compound , caffeic acid
Zingiber officinale Roscoe	Zingiberaceae	Rhizome	Increases insulin level & decrease fasting glucose level	Sesquiterpene
Zizyphus sativa Gaertn	Rhamnaceae	Leaves	Dose dependent reduction in blood glucose level	Tannin

From Mishra, S. B., Rao, C. V., Ojha, S. K., Vijayakumar, M. & Verma, A. (2010). An analytical review of plants for antdiabetic activity with their phytonutrient & mechanism of action. **International Journal of Pharmaceutical Sciences and Research, 1**(1), 30-48.

2.2 Pre-Diabetes

2.2.1 Overview of Pre-Diabetes

Before people develop type 2 diabetes, they typically have to go through the phase of being "Pre-diabetic," the condition that the levels of fasting plasma glucose is below the overt excess threshold which is used to diagnose diabetes, yet significantly higher than the normoglycemia baseline. There were 41 million people in the United States who have pre-diabetes in 2006 (American Diabetes Association, 2006). As of 2011, the number has dramatically risen to 79 million people (American Diabetes Association, 2011b), practically double within merely 5 years. Recent research has shown that some long-term damage to the body, especially the heart and the circulatory system, may have already been done during the pre-diabetic period. Research has also shown that if actions are taken to manage the blood glucose level during pre-diabetes, it is possible to delay or even prevent type 2 diabetes from ever developing. People with pre-diabetes can expect to benefit from much of the same advice good for nutrition and physical activity.

Impaired glucose tolerance, abbreviated as IGT, is a degree of hyperglycemia which may precede type 2 diabetes. IGT is formerly known as "Borderline Diabetes." Another similar terminology is IFG, standing for impaired fasting glucose. Both conditions are considered a pre-diabetic state, associated with insulin resistance and increased risk of cardiovascular pathology, although IFG is of lesser risk than IGT (Nicholas, Hillier, & Brown, 2007).

2.2.2 Criteria for the Diagnosis of Pre-Diabetes

While diabetes and pre-diabetes occur in people of all ages and races, some groups have a higher risk for developing the disease than others. Diabetes is more common in African Americans, Latinos, Native Americans, and Asian Americans/Pacific Islanders, as well as the aged population. This means they are also at increased risk for developing pre-diabetes.

There are three different tests that can be used to determine whether a person has pre-diabetes (American Diabetes Association, 2011a).

1. *Glycated Hemoglobin*: Medical communities of different areas around the world have several synonyms for this term, including hemoglobin A1C, glycohemoglobin, HbA1C, A1C, or Hb1c. The measurement primarily aids in identifying the average plasma glucose concentration over prolonged periods of time.

2. *Fasting Plasma Glucose*: Fasting plasma glucose is abbreviated as FPG and is also called Fasting Blood Sugar (FBS) in some countries, including Thailand. Overt value of FPG reflects the condition of impaired fasting glucose (IFG).

3. *Oral Glucose Tolerance Test (OGTT)*: This is a medical test in which glucose is given, then blood samples are collected continuously 2 hours afterward to determine how quickly the glucose levels return to normal. Overt value of plasma glucose after OGTT reflects the condition of impaired glucose tolerance (IGT).

Table 2.6 Current Criteria for the Diagnosis of Pre-Diabetes-2010

Type of Blood Test	Level of Plasma Concentration for Pre-Diabetes
Glycohemoglobin (HbA1C)	≥ 5.7% (39 mmol/mol)
Fasting Blood Sugar (FBS, FPG)	≥ 100 mg/dl (5.6 mmol/l)
2-h or Random Plasma Glucose	≥ 140 mg/dl (7.8 mmol/l)

From Executive summary: Standards of medical care in diabetes. (2010). Diabetes **Care, 33**(Suppl 1), S4-S10.

2.2.3 Prophylaxis of Diabetes

The plasma glucose levels of a person, as well as his or her insulin demand, will rise more rapidly after having a meal with high glycemic load. The high level of plasma glucose and excessive insulin secretion had been shown to contribute to the loss of insulin-secreting function of the pancreatic beta-cells that leads to irreversible diabetes

(Willett, Manson, & Liu, 2002). Several large prospective studies have shown that high dietary glycemic loads are associated with an increased risk of type 2 diabetes development (Gross, Li, Ford, & Liu, 2004; Krishnan et al., 2007; Salmerón, Ascherio, et al., 1997; Salmerón, Manson, et al., 1997; Schulze et al., 2004; Villegas et al., 2007).

2.3 *Morus alba L.*

2.3.1 Scientific Classification and Botanical Nomenclature

The nomenclature of this plant includes various synonyms, including *M. alba* L., *M. indica* L., *M. atropurpurea* Roxb., *M. merettiana* Jacq. Ex Burr (Plant Resources of South-East Asia, 1999). The common names of this plant include white mulberry, Russian mulberry, silkworm mulberry, and China mulberry.

Table 2.7 *M. alba* L. Basic Information

Scientific Classification	
Kingdom	Plantae
(unranked)	Angiospermae
(unranked)	Eudicots
(unranked)	Rosids
Order	Rosales
Family	Moraceae
Tribe	Moreae
Genus	Morus
Species	Morus alba
Binomial Nomenclature	*Morus alba* Linn.

From Plant Resources of South-East Asia. (1999). In L. S. de Padua, N. Bunyapraphatsara & R. H. M. J. Lemmens (Eds.), **Medicinal and poisonous plants 1** (Vol. 12, pp. 362-363). Leiden, the Netherlands: Backhuys Publisher.

2.3.2 Mulberry Leaf Chemical Constituents

The chemical compounds presented in the leaf of *M. alba* Linn. are:

2.3.2.1 Alkaloid compounds (Kusano et al., 2002)

1. 1-deoxynojirimycin
2. N-methyl-1-deoxynojirimycin
3. 2-O-alpha-D-galactopyranosyl (GAL-DNJ)
4. Fagomine
5. 1,4-dideoxy-1,4-imino-D-arabinitol
6. Calystegin B2

2.3.2.2 Flavonoid compounds (Takasugi, Nagao, Masamune, & Shirata, 1980; Kim et al., 1999; (Kim, Gao, & Kang, 2000)

1. Chalcomoracin
2. Quercetin
3. Quercetin-3
4. O-beta-D-glucopyranoside
5. Quercetin-3-O-(6”-O-acetyl)beta-D-glucopyranoside
6. Quercetin-3-O-beta-D-glucopyranosyl-(1-6)-beta-D-glycopyranoside
7. Quercetin-3-O-alpha-L-rhamnopyranosyl-(1-6)-beta-D-glycopyranoside (rutin)
8. Quercetin-3
9. 7-di-O-beta-D-glucopyranoside
10. Isoquercitin
11. Astragalin (Kaempferol-3-O-beta-D-glucopyranoside)
12. Kaempferol-3-O-(6”-O-acetyl)-beta-D-glucopyranoside
13. Kaempferol-3-O-alpha-L-rhamnopyranosyl-(1-6)-beta-D-glucopyranoside
14. Moracetin
15. Mulberroside F

2.3.2.3 Other compounds (Hirayama, Sugimura, Saito & Nakamura, 2000; Doi, Kojima, Makino, Kimura & Fujimoto, 2001; Watanabe, 1958)

1. Protein (Urease)
2. Coumarin (Scopolin, Skimmin)
3. Sesquiterpene (Roseoside II)
4. Alcohol (3,5-dinitobenzoates, n-butanol, beta-gamma-hexanol)
5. Acid (rho-phenyl phenacyl esters, 5-hydroxypipecolic acid)
6. Aldehyde and Ketone (Methyl-ethyl ketone, 2,4-dinitrophenylhydrazones, n-butylaldehyde isobutylaldehyde, n-valeraldehyde)

Furthermore, *M. alba* Linn. leaf contains a high level of crude proteins, amino acids, copper, manganese, vitamin D, ascorbic acid, vitamin B1, and folic acid (Sharaf, 1964).

2.3.3 Ethnobotanical and Pharmacological actions

The leaf of *M. alba* Linn., or mulberry leaf, is one of the most well known oriental medicinal herbs. Traditional medications involving the usage of mulberry leaf have been found to be efficacious for the treatment of hypoglycemic (Jang, et al., 2002), astringent, diuretic, antihypertensive, and antispasmodic (Ríos, Recioa, & Villar, 1987).

Not only Thailand, plenty of countries around the world have used mulberry leaf in their herbal remedies for treatment of several disorders. In Thailand, the decoction and oral administration of mulberry leaves have been used for cough suppressant, antipyretic, relief of sore throat, thirst quenching, sedative, relief of redden eyes, and diaphoretic (Chao, 1979; Theppanom Muengman, et al., 1990; Sangiam Pongboonrod, 1979; Komol Siwabawon, 1980; Payorm Tantiwiwat, 1978; Wuth, 1997). In Jordan, decoction of mulberry leaf is used as diuretic and vermifuge (Al-Khalil, 1995). In The island of Rodrigues, decoction of mulberry leaf is used in case of hypertension and hernia (Gurib-Fakim, et al., 1996). In Yugoslavia and Peru, hot water extract of mulberry leaves is used

to treat diabetes patients (Tucakov, 1978). In Spain, hot water extract of mulberry leaves is used as astringent and hypoglycemic patients (Ramirez, et al., 1988).

There are a number of studies which put several pharmacological effects of mulberry leaf into evident. The following are some of the prominent effects of mulberry leaf.

1. Antidiabetic activity (Grover, et al., 2002; Gulubova & Boiadzhiev, 1975; Hikino, et al., 1985; Hosseinzadeh & Sadeghi, 1999; Jang, et al., 2002; Kimura, et al., 1995; Lemus, et al., 1999; Litthilert, 2001; Sharaf & Mansour, 1964).
2. Antifungal activity (Du et al., 2003; Kyuk & Yum, 1963; Nomura & Fukai, 1978; Shirata et al., 1983; Singh, Dubey & Tripathi, 1994);
3. Anti-inflammatory activity, Analgesic and antipyretic activity (Gaoat, Medeldorf, & Mary, 1997; Hong, Hur, Kim, Nam, & Lee, 2002; Yamatake, Shibata, & Nagai, 1976).
4. Antimicrobial activity (Hirohisa, Yukai & Hidenhiko, n.d.; Nomura & Fukai, 1978); Ohsugi et al., 1999; Park, Lee & Yang, 1990; Park et al., 2003; Ríos, et al., 1987).
5. Antioxidant activity (Doi, Kojima & Fujimoto, 2000; Kim, et al., 2000; Oh et al., 2002; Ohsugi, et al., 1999).
6. Antitumor activity (Kim, et al., 2000; Moon et al., 1983; Nomura, Hano, & Alda, 1996).
7. Cardiovascular and central nervous system activity (Doi, et al., 2000; Sharaf, 1964; Ikuta, Fukai & Ueda, 1986; Inokuchi, Okabe, Yamanuchi & Nagamatsu, 1984; Iwami, 1960); Kondo & Takemoto, 1973; Nomura & Fukai, 1980, 1981; Nomura, Fukai & Kuwanon, 1980; Nomura, Fukai, Hano & Uzawa, 1981, 1982; Oshima, Konno & Hikino, 1980; Oshima, Konno, Hikino & Matsushita, 1980; Sunnta Ratanapo, Wayakorn Ngamjunyaporn & Montri Chulavatnatol, 1998; Yamatake, et al., 1976).
8. Cytotoxic activity and antimutagenic effect (Alekperov, 2002; Nam et al., 2002; Oh, et al., 2002)
9. Gastrointestinal tract activity (Chen et al., 1995; Sharaf & Mansour, 1964; Yamatake, et al., 1976)
10. Immunomodulating activity (Shin et al., 1998; Wang, Y. & Hu, 1995)

2.3.4 Toxicity Assessment

The acute and subchronic toxicity of *M. alba* Linn. leaf extract had been established. In the acute toxicity study, *M. alba* Linn. leaf extracts were administered intraperitoneally (i.p.) and orally into mice and Wistar rats. Following a single i.p. dose, the LD_{50} value of *M. alba* Linn. leaf extracts in the mice and Wistar rats were approximately 4 and 5 g/kg, respectively. However, when *M. alba* Linn. leaf extracts were administered orally, doses as high as 5 g/kg did not cause any significant toxic effects. There were also no deaths in these groups of animals. The only abnormal signs and symptoms were CNS depression and respiratory depression. Furthermore, all tested animals recovered within 15-30 minutes.

In the subchronic toxicity study, the *M. alba* Linn. leaf extracts were administered orally into Wistar rats for 60 days at doses 1, 2, and 3 g/kg/day. Almost all doses of *M. alba* Linn. leaf extracts did not significantly affect the blood chemistry and hematologic values comparing to the control group. Only the dose of 3g/kg/day increased the blood

glucose levels significantly in the female group. Microscopic examination of major organs indicated no significant histopathological abnormalities (Trabsung, 2004).

The LD_{50} value of the ethanol/water (1:1) extracts of the entire plant of mulberry administered intraperitoneally to mice was more than 1.0 g/kg (Bhakuni, Dhar, Dhawa, & Mehrotra, 1969)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Type

This research is an experimental study, designed to be a randomized, opened-label, controlled, crossover clinical trial.

3.2 Research Design

Subject selection: 14 participants were selected according to the designed criteria to join the experiment. They were randomized into 2 groups; the treatment group and control group
↓
Standard Intervention: Each participant from both groups ingested 75 grams of sucrose solution with a prepared beverage. The beverage given to the treatment group was mulberry leaf tea while the control group was given warm water.
↓
Result Measurements: Venous blood samples were collected from all participants for measurement of their blood glucose and insulin levels right before the ingestion and every 30 minutes afterward for a total of 6 times. (0, 30, 60, 90, 120, 150)
↓
Adverse Event Monitoring: After the experiment, all participants kept a diary of severity of abdominal and other symptoms rated on a linear scale to monitor the effect of the beverages they received.
↓
Crossover Intervention: After a washout period of 1 week, all participants repeated the test with the opposite treatment. This included result measurements and monitoring of adverse events.

Figure 3.1 Research Design

3.3 Research Participants

3.3.1 Inclusion Criteria

3.3.1.1 Male or female subjects aged between 30-60 years old

3.3.1.2 No current active diseases of gastrointestinal tract, liver, kidney, allergy, and diabetes.

3.3.1.3 Participants were subjected to pass blood test of Blood Urea Nitrogen (BUN), serum creatinine, AST, ALT, ALP, fasting blood glucose, and complete blood count, physical examination and medical history record screening.

3.3.1.4 All blood test results, except fasting blood glucose, should display value within the normal reference range. The fasting blood glucose has to be less than the diagnostic diabetic level (126 mg/dL).

3.3.1.5 Subjects voluntarily participated in the study and signed their names in consent forms

3.3.2 Exclusion Criteria

3.3.2.1 History of being allergic or intolerant to mulberry products or derivatives

3.3.2.2 History of diseases of gastrointestinal tract, liver, kidney, allergy, and other diseases which may influence the bioavailability of mulberry's active compounds

3.3.2.3 History of regular alcohol consumption or drug abuse

3.3.2.4 History of regular smoking

3.3.2.5 Subjects were under other medications a month prior to the study, particularly medications which alter the liver enzymes metabolism

3.3.2.6 Subjects have participated in other clinical trial a month prior to the study

3.3.3 Discontinuation Criteria

3.3.3.1 Subjects showed signs or symptoms of adverse reactions in which physician sees fit to discontinue from the study

3.3.3.2 Subjects did not follow the regulations of the study

3.3.3.3 Subjects would like to be discontinued from the study

3.3.4 Sample Size

Adequate sample size is defined by the following formula;

$$N = \frac{2(Z_{\frac{\alpha}{2}} + Z_{\beta})^2 \delta^2}{(x_1 - x_2)^2}$$

Providing that:

X_1	=	mean of group 1
X_2	=	mean of group 2
δ^2	=	pooled variance

$$\delta^2 = \frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2}$$

Given that: $\alpha = 0.05$; $Z_{\frac{\alpha}{2}} = 1.96$

$\beta = 0.10$; $Z_{\beta} = 1.28$

The calculation refers to the study of Joanna Hlebowicz, M.D., Gassan Darwiche, M.D., Ph.D., Ola Bjorgell, M.D., Ph.D., and Lars-Olof Almer, M.D., Ph.D., which is a randomized crossover controlled trial on muesli with 4 g oat beta-glucan effect on postprandial plasma glucose by the use of Oral Glucose Tolerance Test (OGTT).

$n_1 = 12$ $n_2 = 12$

$x_1 = 39.4$ $x_2 = 54.8$

$S_1 = 7.8$ $S_2 = 14.9$

Evaluate the pooled variance $= \frac{(12-1)7.8^2+(12-1)14.9^2}{12+12-2} = 141.425$

$$N = \frac{2(1.96+1.28)^2 141.425}{(39.4-54.8)^2} = 12.52$$

According to the calculation, the adequate sample size for this research is at least 13. Therefore, this study employed 14 subjects.

3.4 Research Tools

The materials that were used in this study include the following:

3.4.1 Chemicals

3.4.1.1 Dried mulberry leaf tea powder (*M. alba* L., Khun Pai) was provided by The Queen Sirikit Department of Sericulture, Ministry of Agriculture and Co-Operatives, Bangkok, Thailand, via support of C.K.Industry (2000) Co.,Ltd.

3.4.1.2 75 grams of sucrose solution in 150 milliliters of water

3.4.2 Equipment

3.4.2.1 5 ml NaF plastic tube for blood plasma sample collection (For glucose measurement)

3.4.2.2 5 ml plastic tube for blood serum sample collection (For insulin measurement)

3.5 Research Procedures

3.5.1 Participants Preparation

3.5.1.1 Volunteers were screened for non-diabetic condition according to the inclusion and exclusion criteria.

3.5.1.2 Participants provided their personal profile.

3.5.1.3 Participants were explained about the detail and procedures of the study.

3.5.1.4 Participants willingly signed the informed consent form for clinical trial.

3.5.1.5 Participants were prohibited from consumptions of beverages containing alcohol and caffeine, smoking, and any food or beverage that may contain mulberry compounds a week prior to the test day.

3.5.1.6 Participants were required to fast after midnight prior to the test day.

3.5.2 Experiment Procedures

3.5.2.1 After at least 8 hours of fasting, participants were randomly divided into two groups by quota sampling.

3.5.2.2 Each participant from both groups ingested 75 grams of sucrose solution with a prepared beverage. The beverage given to the treatment group was mulberry leaf tea while the control group was given warm water.

3.5.2.3 Blood samples were collected from all participants for measurement of their blood glucose and insulin levels right before the ingestion and 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 150 minutes after the ingestion. NaF plastic tubes were used to collect blood sample for glucose levels measurement. Clotted blood, which was later separated into serum, was collected for insulin level measurement. The blood sample collection technique was venopuncture.

3.5.2.4 After the experiment, all participants kept a diary of severity of abdominal and other symptoms rated on a linear scale (0 = none through 4 = severe) to monitor the effect of the beverages they received.

3.5.2.5 After a washout period of 1 week, all participants repeated the test with the opposite treatment. This included result measurements and monitoring of adverse events.

3.5.3 Results Evaluation and Analysis

Blood glucose and insulin levels at different time points were compared between the treatment group and the control group. The area under the curve (AUC) was calculated for glucose and insulin for each subject. From the model of Oral Glucose Tolerance Test (OGTT), the whole body physiological insulin sensitivity was obtained (Matsuda & DeFronzo, 1999). This composite insulin sensitivity index (ISI-comp) is also referred to as the Matsuda Index.

Descriptive statistics were run on all measures, and the results are given as means ± SEM. All statistical calculations were performed using SPSS for Windows software (version 20.0.0, 2011). Statistics were verified for having normal distribution using One-Sample Kolmogorov-Smirnov test. Differences in blood glucose and insulin levels were evaluated with independent-samples t test.

After an 8-hour period after the ingestion of the mulberry leaf tea or warm water, subjects ketp a record of their symptoms that might happen during the period. The symptoms were rated on a linear scale of 0 (none) to 4 (severe). The included symptoms are as followed:

1. Headache
2. Fullness
3. Itching
4. Incomplete evacuation
5. Nausea
6. Excessive rectal gas
7. Fatigue
8. Bloating
9. Abdominal pain

The symptoms were compared between ingestion of mulberry leaf tea and warm water. The comparisons were calculated for statistical significance with Pearson Chi-Square test. This study, employing 14 pre-diabetic subjects, set significance at $P < 0.05$

CHAPTER 4

RESULTS

Baseline Demographic Data

There were 14 subjects participated in the study with ages ranging from 30 to 60 years old (Mean ± SD: 51.21 ± 9.45), as shown in Table 4.1. The gender was equally distributed, with 7 males and 7 females. The average body mass index of the subjects reflects a slightly overweight group of individuals (Mean ± SD: 26.10 ± 3.67). Blood pressure, heart rate, body temperature, complete blood counts, and other blood chemistry profiles were all within normal ranges.

Table 4.1 Characteristics of Study Subjects at Baseline

Parameter	Value
Total Number, M/F (n/n)	14.00 (7/7)
Age (years)	51.21 ± 9.45
BMI (kg/m^2)	26.10 ± 3.67
Systolic BP (mmHg)	126.50 ± 13.20
Diastolic BP (mmHg)	77.57 ± 10.69
Heart rate (BPM)	72.57 ± 9.15
Temperature (°C)	36.18 ± 0.42
CBC	
Hematocrit (%)	40.79 ± 4.66
Hemoglobin (g/dL)	13.47 ± 1.89
RBC ($10^6/mm^3$)	4.95 ± 0.58
WBC ($10^3/mm^3$)	5.97 ± 1.31
Platelet ($10^3/mm^3$)	252.50 ± 66.37
Blood chemistry	
Fasting glucose (mg/dL)	107.36 ± 15.99
BUN (mg/dL)	12.96 ± 4.05
Creatinine (mg/dL)	0.80 ± 0.18
AST (U/L)	19.07 ± 4.60
ALT (U/L)	21.71 ± 8.27
ALP (U/L)	70.43 ± 17.64

Table 4.1 (Continue)

Parameter	Value
Educational level	
Elementary school	1 (7.14%)
High school	7 (50%)
Bachelor's degree	1 (7.14%)
Master degree or higher	5 (35.71%)
Marital status	
Single	4 (28.58%)
Married	8 (54.14%)
Divorced	1 (7.14%)
Widowed	1 (7.14%)
Allergic history	
Yes	1 (7.14%)
No	13 (92.86%)
Having underlying diseases	
Yes	3 (21.43%)
No	11 (78.57%)
Having medication history	
Yes	3 (21.43%)
No	11 (78.57%)
Family DM history	
Yes	7 (50%)
No	7 (50%)
Having gestational DM history	
Yes	0 (0%)
No	14 (100%)
Having impaired fasting glucose history	
Yes	10 (71.43%)
No	4 (28.57%)
Smoking	
None	14 (100%)
Rarely	0 (0%)
Sometimes	0 (0%)
Frequently	0 (0%)
Alcohol intake	
None	11 (78.57%)
Rarely	2 (14.29%)
Sometimes	1 (7.14%)
Frequently	0 (0%)
Soft drink intake	
None	9 (64.29%)
Rarely	3 (21.43%)

Table 4.1 (Continue)

Parameter	Value
Sometimes	0 (0%)
Frequently	2 (14.29%)
Physical activity (Exercise)	
None	1 (7.14%)
Rarely	2 (14.29%)
Sometimes	8 (57.14%)
Frequently	3 (21.43%)

Note. Values are shown as mean ± SD

Table 4.1 displays an array of characteristic of the participants recorded at baseline. Most subjects do not have underlying diseases (78.57%), but half of them have familial history of diabetes. All of the subjects do not smoke, and most of them do not drink alcohol. Most subjects reported regular and frequent exercise.

1. Postprandial Glucose and Insulin Response

After oral glucose tolerance test, the sample group which received mulberry leaf tea prior to the tolerance test tends to have a lower level of plasma glucose, serum insulin concentrations and $AUC_{0\text{-}150}$ compared to the controlled group, but without significant difference. The mean difference of the incremental glucose level at each time point also tends to be lower for the mulberry group, with significant difference at 30-min time point (P = 0.04), shown in Table 4.3 and Figure 4.2. The physiological insulin sensitivity, measured by Matsuda index and HOMA-IR, are not different, shown in Table 4.20

2. Levels, Mean Difference, and AUC of Glucose and Insulin of all Subjects

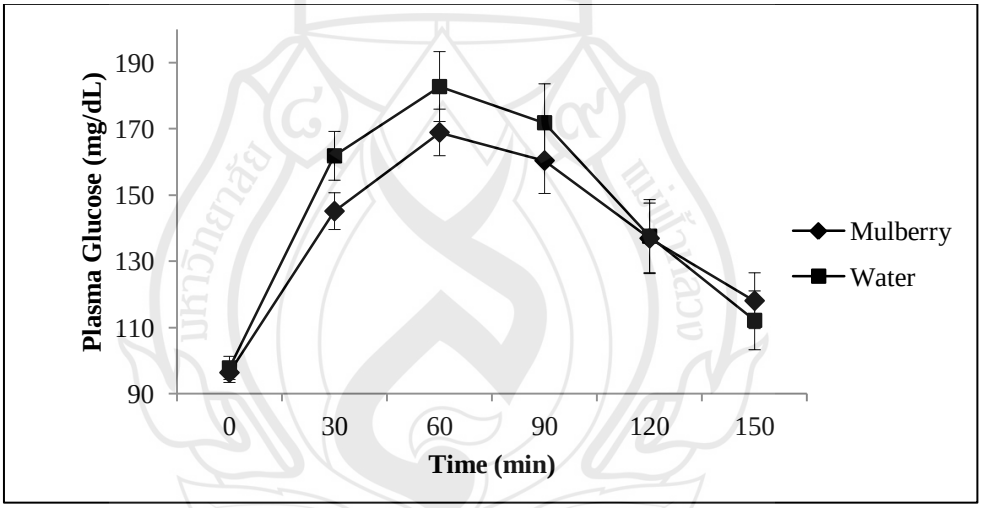
Table 4.2 and Figure 4.1 illustrate the effect of mulberry leaf tea versus warm water on the concentration of plasma glucose in non-diabetic subjects. It can be seen that plasma glucose levels for the mulberry leaf tea sample group tends to be lower than the warm water group, with less steep rise and fall (fluctuation). However, the difference is not significant; only at T30 is the different slightly larger, yet not large enough (P = 0.08).

Table 4.3 and Figure 4.2 show the mean difference value for the incremental concentration of plasma glucose compared to baseline (T0). This data represents the change in blood glucose level over time. It can be seen that the change in blood glucose for the warm water group is greater, with significant difference during the first 30 minutes of the test (P = 0.04). During the first 30 minutes of OGTT, the glucose levels of the mulberry leaf tea sample group raised 48.79 ± 4.60 mg/dL on average, as compared to a rise of 64.07 ± 5.54 mg/dL for the warm water sample group.

Table 4.2 Plasma Glucose Levels of the Subjects During Oral Glucose Tolerance Test after Ingesting Either Mulberry Leaf Tea or Warm Water

Time point (min)	Plasma glucose level (mg/dL)		*P*-value
	Mulberry leaf tea	Warm water	Between group
T0	96.29 ± 2.98	97.71 ± 3.50	0.76
T30	145.07 ± 5.55	161.79 ± 7.37	0.08
T60	168.86 ± 7.04	182.71 ± 10.56	0.29
T90	160.36 ± 9.95	171.79 ± 11.74	0.46
T120	136.86 ± 10.66	137.50 ± 11.06	0.97
T150	118.00 ± 8.43	112.07 ± 8.88	0.63

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



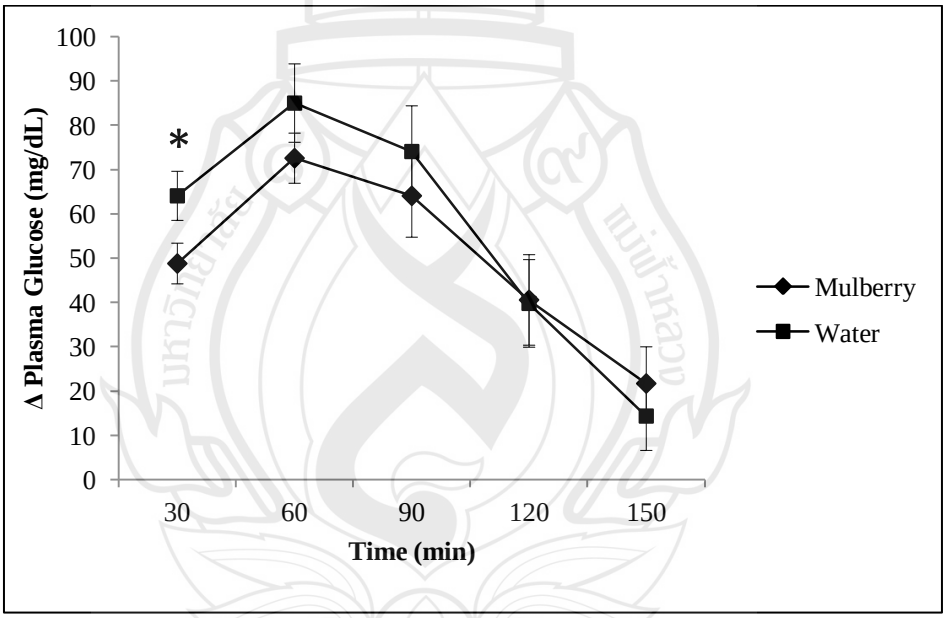
Note. Data corresponds with Table 4.2, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 4.1 Plasma Glucose Levels of the Subjects During Oral Glucose Tolerance Test after Ingesting Either Mulberry Leaf Tea or Warm Water

Table 4.3 Mean Difference of Incremental Plasma Glucose

Time point (min)	Mean difference of plasma glucose level (mg/dL)		*P*-value Between group
	Mulberry leaf tea	Warm water	
T0-30	48.79 ± 4.60 *	64.07 ± 5.54 *	0.04 *
T0-60	72.57 ± 5.66	85.00 ± 8.84	0.25
T0-90	64.07 ± 9.33	74.07 ± 10.31	0.48
T0-120	40.57 ± 10.21	39.79 ± 9.88	0.96
T0-150	21.71 ± 8.27	14.36 ± 7.76	0.52

Note. Values are shown as mean ± SEM. * Significant difference between the drink according to independent-samples t test ($P = 0.04$)



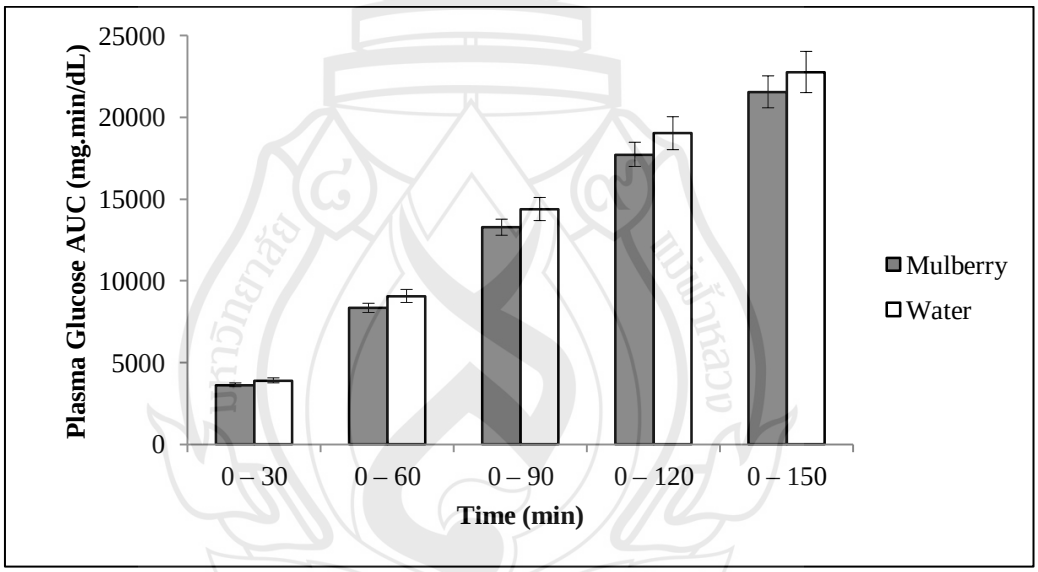
Note. Data corresponds with Table 4.3, showing mean and SEM value. * Significant difference between the drink according to independent-samples t test ($P = 0.04$)

Figure 4.2 Mean Difference of Incremental Plasma Glucose Levels

Table 4.4 Incremental AUC of Plasma Glucose Levels

Time interval	Incremental glucose AUC (mg.min/dL)		% difference	*P*-value Between group
	Mulberry leaf tea	Warm water		
0-30	3620.36 ± 114.35	3892.50 ± 151.86	6.99	0.16
0-60	8328.75 ± 283.79	9059.46 ± 399.92	8.07	0.15
0-90	13264.82 ± 492.30	14376.43 ± 706.86	7.73	0.21
0-120	17721.43 ± 741.49	19015.71 ± 1008.34	6.81	0.31
0-150	21544.29 ± 976.80	22759.29 ± 1261.23	5.34	0.45

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



Note. Data corresponds with Table 4.4, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 4.3 Incremental AUC of Plasma Glucose Levels

Table 4.4 and Figure 4.3 display the incremental area under the curve of plasma glucose levels of the subjects. Glucose incremental AUC can reflect the glycemic index of the meal when doing OGTT comparing a test meal with a reference meal (Josic et. al., 2010). It can be seen from the data and the graph that the sample group which took mulberry leaf tea prior to OGTT has a slightly lower glucose AUC, with 8.07% less glucose AUC value during the first 60 minutes of the test. However, the difference is not significant ($P > 0.05$).

Table 4.5 and Figure 4.4 illustrate the effect of mulberry leaf tea versus warm water on the concentration of serum insulin in non-diabetic subjects. It can be seen that serum insulin levels for the mulberry leaf tea sample group tends to be lower than the warm water group, with less steep rise and fall (fluctuation). However, the difference is not significant.

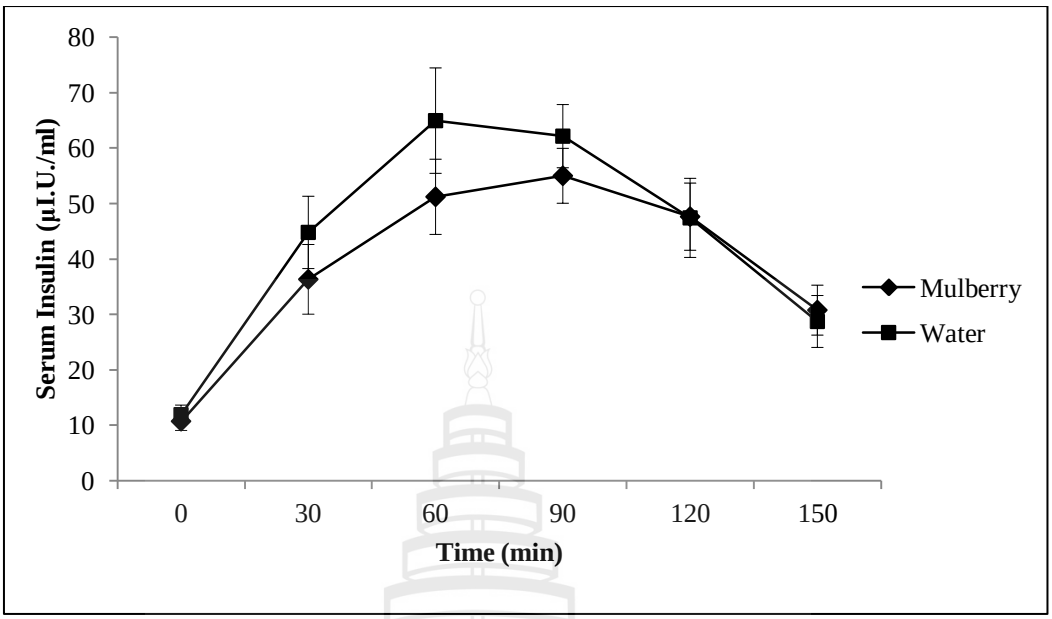
Table 4.6 and Figure 4.5 show the mean difference value for the incremental concentration of serum insulin compared to baseline (T0). This data represents the change in blood insulin level over time. It can be seen that serum insulin levels for the mulberry leaf tea sample group tends to be lower than the warm water group, but without significant difference.

Table 4.7 and Figure 4.6 display the incremental area under the curve of serum insulin levels of the subjects. It can be seen from the data and the graph that the sample group which took mulberry leaf tea prior to OGTT has a slightly lower insulin AUC, with 19.04% less insulin AUC value during the first 60 minutes of the test. However, the difference is not significant ($P > 0.05$).

Table 4.5 Serum Insulin Levels of the Subjects During Oral Glucose Tolerance Test after Ingesting Either Mulberry Leaf Tea or Warm Water

Time point	**Serum insulin level (μI.U./ml)**		***P*-value**
(min)	**Mulberry leaf tea**	**Warm water**	**Between group**
T0	10.74 ± 1.66	11.93 ± 1.73	0.63
T30	36.33 ± 6.28	44.79 ± 6.52	0.36
T60	51.21 ± 6.78	64.94 ± 9.50	0.25
T90	55.00 ± 4.95	62.14 ± 5.70	0.35
T120	47.63 ± 6.06	47.41 ± 7.13	0.98
T150	30.78 ± 4.50	28.73 ± 4.68	0.75

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



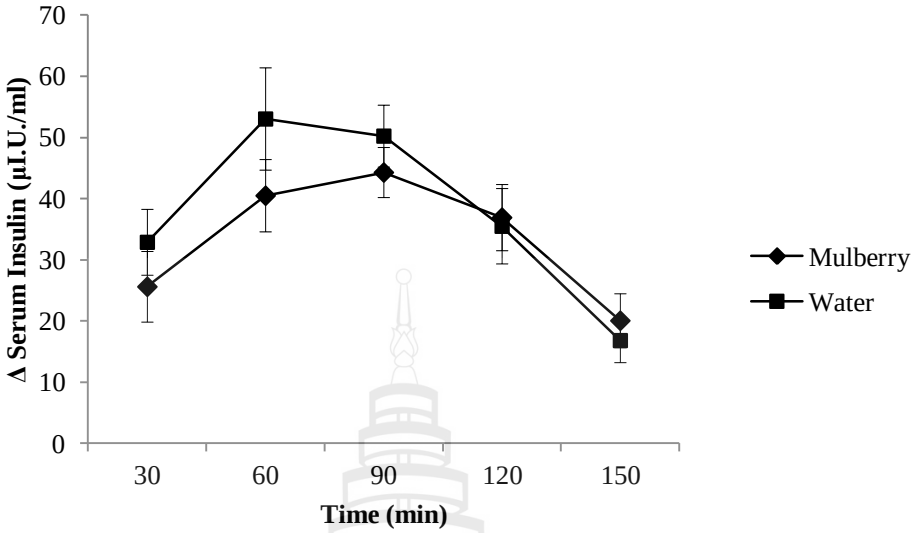
Note. Data corresponds with Table 4.5, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 4.4 Serum Insulin Levels of the Subjects During Oral Glucose Tolerance Test after Ingesting Either Mulberry Leaf Tea or Warm Water

Table 4.6 Mean difference of Incremental Serum Insulin Levels

Time point (min)	Mean difference of serum insulin level (µI.U./ml)		*P*-value Between group
	Mulberry leaf tea	Warm water	
T30	25.59 ± 5.77	32.86 ± 5.38	0.37
T60	40.47 ± 5.91	53.01 ± 8.35	0.23
T90	44.26 ± 4.09	50.21 ± 5.06	0.37
T120	36.89 ± 5.41	35.48 ± 6.16	0.87
T150	20.04 ± 4.40	16.80 ± 3.61	0.57

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



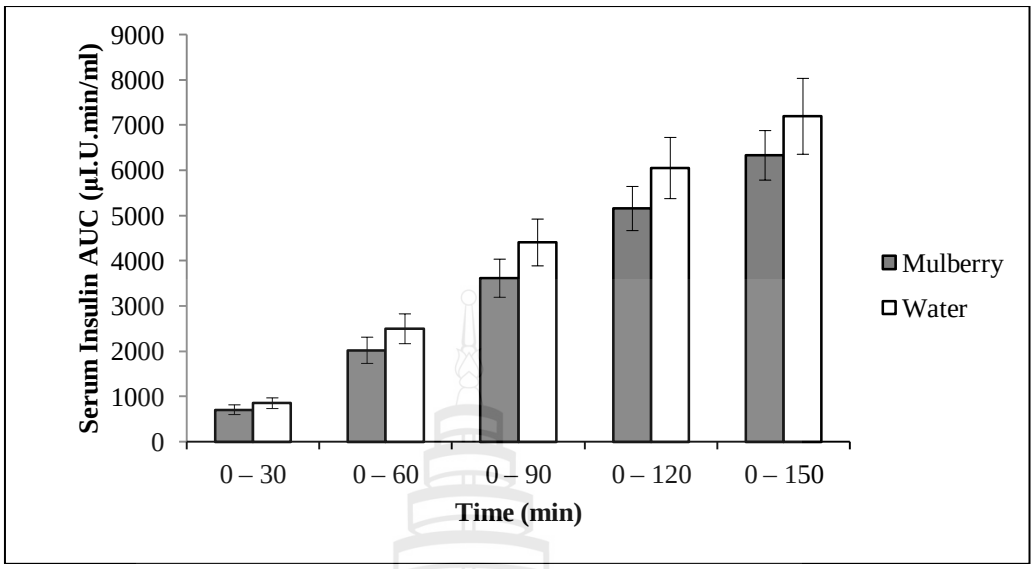
Note. Data corresponds with Table 4.6, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 4.5 Mean difference of Incremental Serum Insulin Levels

Table 4.7 Incremental AUC of Serum Insulin Levels

Time interval	Incremental insulin AUC (µI.U.min/ml)		% difference	*P*-value Between group
	Mulberry leaf tea	Warm water		
0-30	706.60 ± 107.32	850.04 ± 117.66	16.87	0.38
0-60	2020.29 ± 290.39	2495.44 ± 328.79	19.04	0.29
0-90	3614.00 ± 421.42	4403.69 ± 517.88	17.93	0.25
0-120	5154.09 ± 488.21	6049.37 ± 676.70	14.80	0.29
0-150	6330.23 ± 546.74	7192.54 ± 838.76	11.99	0.40

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



Note. Data corresponds with Table 4.7, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 4.6 Incremental AUC of Serum Insulin Levels

3. Physiological insulin sensitivity and resistance indices

Values of blood glucose and insulin levels at different time point during an OGTT were put into the Matsuda Index formula, which provides a parameter of composite insulin sensitivity index (ISI-Comp). From the study, the Matsuda Index of both sample groups is not different, as shown in Table 4.8, although the mulberry leaf tea sample group shows a slightly greater value, indicating higher insulin sensitivity.

Another common parameter that is used to measure insulin resistance is HOMA-IR (Homeostasis Model Assessment of Insulin Resistance), which takes into account the levels of fasting blood glucose and insulin, which in this study are the baseline (T0) values. No significant difference is found, despite the lower value in the mulberry leaf tea sample group, which indicates less insulin resistance.

4. Post Experiment Adverse Events

Test subjects kept a diary of severity of symptoms score scales. Symptoms include headache, fullness, itching, incomplete evacuation, nausea, excessive rectal gas, fatigue, bloating, and abdominal pain. There was no significant difference in severity for any symptoms between mulberry and warm water sample group. Details are elaborated in Table 4.9.

Table 4.8 Physiological Insulin Sensitivity and Resistance Indices of All Subjects

Parameter	Insulin Sensitivity/Resistance Index		*P*-value
	Mulberry leaf tea	Warm water	Between group
Matsuda Index (ISI)	4.56 ± 0.39	4.38 ± 0.67	0.82
HOMA-IR	2.55 ± 0.39	2.94 ± 0.49	0.54

Note. No significant difference is found between the 2 groups ($P > 0.05$).

Table 4.9 Adverse Events 8 Hours Post Experiment, Reported by Subjects

Symptom		Mulberry leaf tea group	Warm water group	*P*-value Between group
1. Headache	None	13 (92.90 %)	14 (100 %)	0.31
	Mild	1 (7.10 %)	0 (0.00%)	
2. Fullness	None	10 (71.40 %)	11 (78.60%)	0.66
	Mild	4 (28.60 %)	3 (21.40%)	
3. Itching	None	14 (100 %)	13 (92.90%)	0.31
	Mild	0 (0.00 %)	1 (7.10%)	
4. Incomplete evacuation	None	13 (92.90%)	14 (100 %)	0.31
	Moderate	1 (7.10%)	0 (0.00%)	
5. Nausea	None	13 (92.90 %)	13 (92.90 %)	1.00
	Mild	1 (7.10%)	1 (7.10%)	
6. Excessive rectal gas	None	12 (85.70 %)	12 (85.70 %)	1.00
	Mild	2 (14.30 %)	2 (14.30 %)	
7. Fatigue	None	12 (85.70 %)	12 (85.70 %)	1.00
	Mild	1 (7.14%)	1 (7.14%)	
	Moderate	1 (7.14%)	1 (7.14%)	
8. Bloating	None	12 (85.70 %)	13 (92.90%)	0.39
	Mild	1 (7.14%)	0 (0.00%)	
	Moderate	1 (7.14%)	0 (0.00%)	
	Severe	0 (0.00%)	1 (7.10%)	
9. Abdominal pain	None	13 (92.90%)	13 (92.90%)	1.00
	Mild	1 (7.10%)	1 (7.10%)	

CHAPTER 5

DISCUSSION, CONCLUSION AND SUGGESTIONS

5.1 Discussion

The primary endpoint in this study was the effect of mulberry leaf tea on postprandial glucose and insulin levels in non-diabetic subjects. The author's hypothesis was that mulberry leaf tea could lower postprandial glucose and insulin level, as well as improving the whole body physiological insulin sensitivity in non-diabetic subjects. Several prospective studies, *in vivo* and *in vitro* experiments have been done regarding mulberry's chemical properties and its pharmacological potentials. The most strongly supported mechanism of mulberry leaf extract is its ability to cause carbohydrate maldigestion and malabsorption (Hansawasdi & Kawabata, 2006; Kimura, et al., 2007; Kwon, et al., 2011; Mudra, et al., 2007; Yatsunami, et al., 2008; Zhong, et al., 2006). There has also been recent studies which bring into evident the ability of mulberry leaf extract to regulate the genetic expression of hepatic and pancreatic enzymes and hormones, aiding the treatment of diabetes (Li, et al., 2011). In another study, healthy and diabetic participants were subjected to OGTT with mulberry leaf extract administered prior to the test; significant reduction of blood glucose fluctuation was observed (Mudra, et al., 2007).

Based on Table 4.2 and Table 4.5, the author did not observe significant difference in glucose and insulin levels. Although the trends of both parameters seem to be lower for the mulberry leaf tea group, the difference is subtle. A similar observation has been reported by Somsak Sinsatienporn et al. (2006). They suggested that the hypoglycemic effect of higher dose may be needed. However, the study by Somsak Sinsatienporn et al. (2006) was open-labeled without concurrent controls, and diet control instructions were not provided to the subjects.

Even though the actual values of blood glucose and insulin concentration between the groups are not different, the mean differences of incremental glucose values compared to baseline are different, with statistical significance during the first 30 minutes of the test (P = 0.04), shown in Table 4.3. During the first 30 minutes of OGTT, the glucose levels of the mulberry leaf tea sample group raised 48.79 ± 4.60 mg/dL on average, as compared to a rise of 64.07 ± 5.54 mg/dL for the warm water sample group. This statistic implies that consumption of mulberry leaf tea may aid in postprandial glycemic control during the first 30 minutes after meal.

Improvement of postprandial glucose fluctuation over time is also seen in a study conducted by Mudra and other (2007). In that study, 10 healthy subjects and 10 subjects with type 2 diabetes participated in a similar setting of research, with OGTT after co-ingestion of mulberry extract and 75 grams sucrose solution. From the results, it was concluded that the co-ingestion of mulberry extract with 75 grams sucrose solution

significantly reduced the increase in blood glucose levels in both healthy and diabetic subjects. Mudra's conclusion is consistent with this study results, having a significantly lower changes of glucose levels over time. The difference is that Mudra's results displayed an overall improvement of blood glucose fluctuation, while this study shows a possible glycemic controlling effect only during the first 30 minutes of the test.

The author assumed that Mudra's experiment yielded a better, more significant result due to several factors. For one, this study used mulberry leaf tea for the intervention, which is a product of crude quality, while Mudra et al. used mulberry leaf extract, which probably has a higher standardization of active compound and thus greater potency.

Glucose incremental AUC can reflect the glycemic index of the meal when doing OGTT comparing a test meal with a reference meal (Josic, Olsson, Wickeberg, Lindstedt, & Hlebowicz, 2010). It can be seen from the data and the graph that the sample group which took mulberry leaf tea prior to OGTT has a slightly lower glucose AUC, with 8.07% less glucose AUC value during the first 60 minutes of the test. However, the difference is not significant ($P > 0.05$), as shown in Table 4.4.

The effect of mulberry leaf tea versus warm water on the concentration of serum insulin in non-diabetic subjects are reflected from the actual values, the mean difference of the incremental concentration, and the AUC values, displayed in Table 4.5, Table 4.6, and Table 4.7. There is a trend for a lower level of serum insulin in all of these parameters, yet the differences are quite small and not significant. From this statistic, it can be inferred that mulberry leaf tea may not affect the serum insulin concentration.

This is contrary to a previous study done by Kimura et al. (2007). They measured the level of plasma glucose and serum insulin after co-ingestion of mulberry powder and 50 grams sucrose solution, discovering significant differences in serum insulin levels at 60-min time point. However, the study used 1-deoxynojirimycin(DNJ)-enriched powder, which is recognized as a promising antihyperglycemic compound. Significant glucose and insulin lowering effects were observed for administration of 0.8 grams or higher DNJ-enriched mulberry powder, whereas 0.4 gram DNJ-enriched powder did not display significant results. This could suggest that mulberry leaf tea that was used in this study might have inadequate potency due to insufficient level of DNJ.

Despite the insignificant difference of most parameters between mulberry sample group and the control group, the result of each individual subject shows that the mulberry sample group has a better glycemic control compared to the control group. This may be due to the large value of standard deviation from the wide range of baseline fasting glucose levels. Thus, the author tried to repeat the statistical analysis with segmentation; those who have levels of fasting blood glucose less than 100 mg/dL are grouped into normal fasting glucose (NFG), and those who have levels of fasting blood glucose more than or equal to 100 mg/dL are grouped into impaired fasting glucose (IFG).

Therefore, the dataset is further segmented based on fasting glucose levels of the subject during OGTT (Time point 0, before ingestion of sucrose solution). Although all subjects' baseline fasting glucose levels were between 100 and 125 mg/dL, their fasting glucose levels during the test varied, with some samples having fasting plasma glucose lower than 100 mg/dL, and some higher. For samples with fasting plasma glucose levels more than or equal to 100 mg/dL, but not higher than 125 mg/dL, they are categorized to the impaired fasting glucose group (IFG). Samples with fasting plasma glucose levels lower than 100 mg/dL are categorized to the normal fasting glucose group (NFG). Within

its own group (IFG/NFG), statistics comparing the difference between the samples taking mulberry leaf tea and the one taking plain warm water are analyzed.

The total sample size of this study is 14 subjects. Segmenting into NFG and IFG categories, the mulberry leaf tea sample group consisted of 8 NFG subjects and 6 IFG subjects, whereas the warm water sample group consisted of 9 NFG subjects and 5 IFG subjects.

Table 5.1 and Figure 5.1 illustrate the effect of mulberry leaf tea versus warm water on the concentration of plasma glucose in IFG subjects. The plasma glucose levels for the mulberry leaf tea sample group are lower than the warm water group, with less fluctuation of the values. Significant difference is found at time point 30 (P = 0.02). At time point 30, the average plasma glucose level of the mulberry leaf tea sample group is 156.00 ± 7.13, while the corresponding value of the warm water sample group is 184.40 ± 7.22.

Table 5.2 and Figure 5.2 show the mean difference value for the incremental concentration of plasma glucose compared to baseline (T0) of IFG subjects. It can be seen that the change in blood glucose for the warm water group is greater, with significant difference during the first 30 minutes of the test (P = 0.03). During the first 30 minutes of OGTT, the glucose levels of the mulberry leaf tea sample group raised 48.17 ±7.27 mg/dL on average, as compared to a rise of 72.60 ± 4.91 mg/dL for the warm water sample group.

Table 5.3 and Figure 5.3 illustrate the effect of mulberry leaf tea versus warm water on the concentration of plasma glucose in NFG subjects. The difference is not significant.

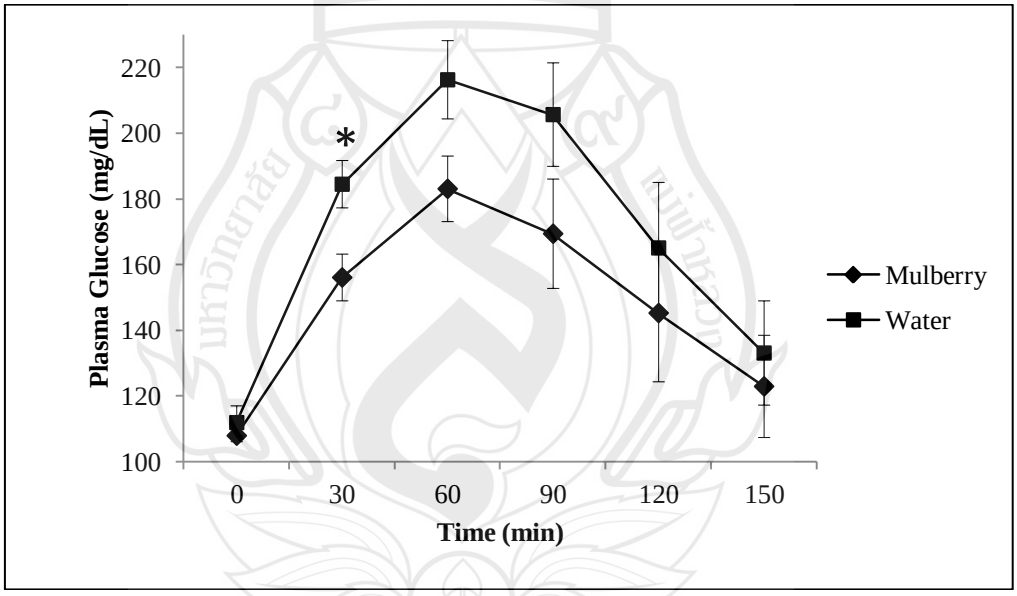
Table 5.4 and Figure 5.4 show the mean difference value for the incremental concentration of plasma glucose compared to baseline (T0) of NFG subjects. The change in blood glucose for the warm water group is slightly greater, but without significant difference.

Table 5.5 and Figure 5.5 display the incremental area under the curve of plasma glucose levels of IFG subjects. It can be seen from the data and the graph that the sample group which took mulberry leaf tea prior to OGTT has a significantly lower glucose AUC levels at the first 30 minutes, 60 minutes, and 90 minutes compared to baseline. Glucose AUC of the mulberry leaf tea sample group are 3957.50 ± 111.94, 9042.50 ± 312.84, and 14325.00 ± 606.05 mg.min/dL, while the values for the warm water sample group are 4443.00 ± 172.43, 10452.00 ± 383.67, and 16779.00 ± 718.13 mg.min/dL for the first 30, 60, and 90 minutes, respectively.

Table 5.1 Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Time point (min)	Plasma glucose level (mg/dL) – IFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
T0	107.83 ± 1.85	111.80 ± 5.09	0.45
T30	156.00 ± 7.13 *	184.40 ± 7.22 *	0.02 *
T60	183.00 ± 9.99	216.20 ± 11.92	0.06
T90	169.33 ± 16.66	205.60 ± 15.75	0.15
T120	145.17 ± 20.95	165.00 ± 19.95	0.52
T150	122.83 ± 15.57	133.00 ± 15.86	0.66

Note. Values are shown as mean ± SEM. * Significant difference between the drink according to independent-samples t test ($P = 0.02$)



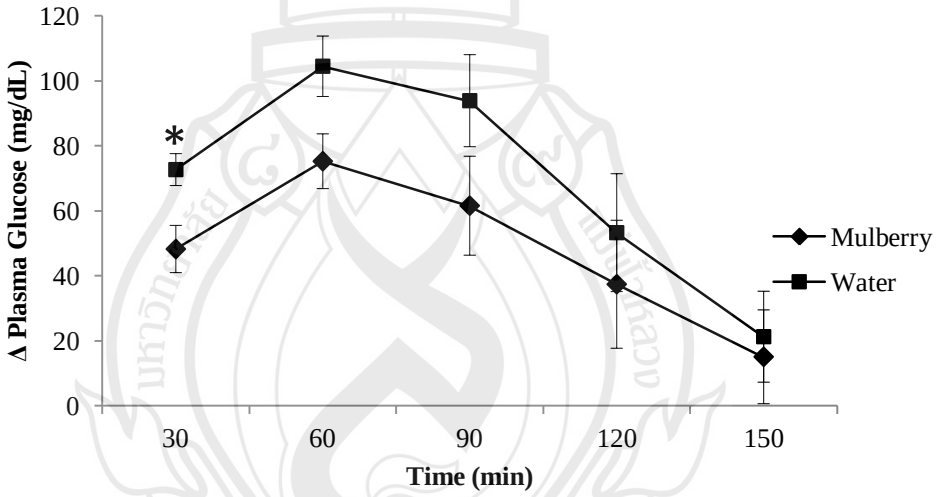
Note. Data corresponds with Table 5.1, showing mean and SEM value. * Significant difference between the drink according to independent-samples t test ($P = 0.02$)

Figure 5.1 Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Table 5.2 Mean Difference Of Incremental Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Time point (min)	Mean difference of plasma glucose level (mg/dL) - IFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
T30	48.17 ± 7.27 *	72.60 ± 4.91 *	0.03 *
T60	75.17 ± 8.42	104.40 ± 9.30	0.05
T90	61.50 ± 15.22	93.80 ± 14.16	0.16
T120	37.33 ± 19.69	53.20 ± 18.13	0.58
T150	15.00 ± 14.43	21.20 ± 13.99	0.77

Note. Values are shown as mean ± SEM. * Significant difference between the drink according to independent-samples t test ($P = 0.03$)



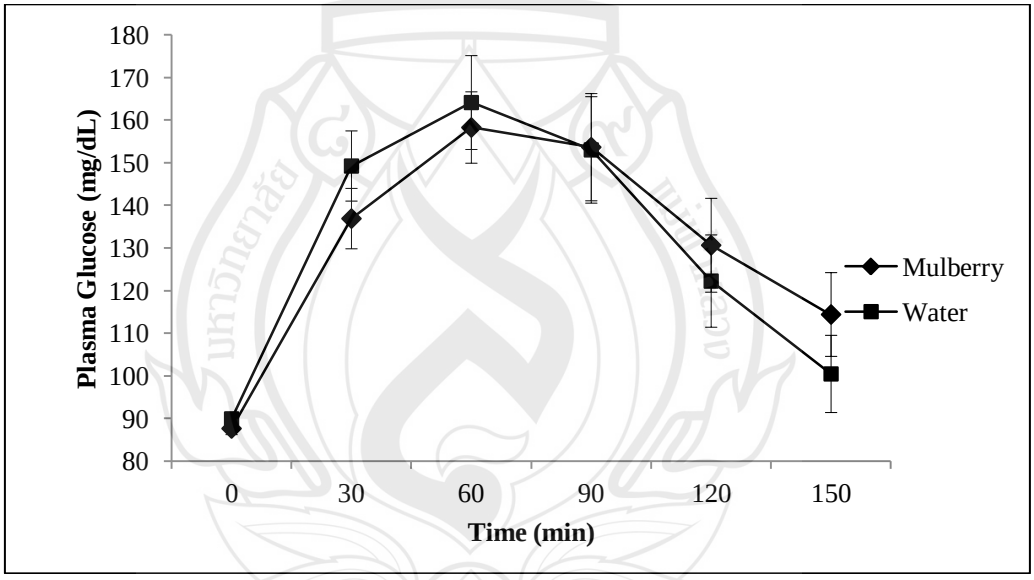
Note. Data corresponds with Table 5.2, showing mean and SEM value. * Significant difference between the drink according to independent-samples t test ($P = 0.03$)

Figure 5.2 Mean Difference of Incremental Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Table 5.3 Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Time point (min)	Plasma glucose level (mg/dL) - NFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
T0	87.63 ± 1.43	89.89 ± 1.55	0.30
T30	136.88 ± 7.09	149.22 ± 8.22	0.28
T60	158.25 ± 8.37	164.11 ± 11.01	0.68
T90	153.63 ± 12.58	153.00 ± 12.48	0.97
T120	130.63 ± 11.01	122.22 ± 10.82	0.60
T150	114.38 ± 9.82	100.44 ± 9.06	0.31

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



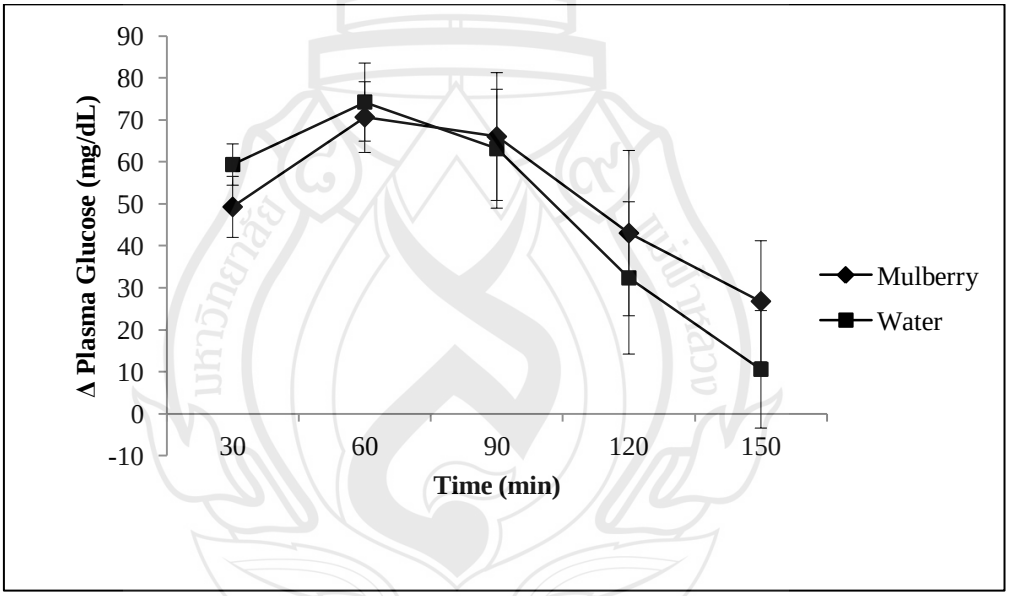
Note. Data corresponds with Table 5.3, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 5.3 Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Table 5.4 Mean Difference of Incremental Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Time point (min)	Mean difference of plasma glucose level - NFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
T30	49.25 ± 6.36	59.33 ± 7.94	0.35
T60	70.63 ± 8.06	74.22 ± 11.53	0.81
T90	66.00 ± 12.56	63.11 ± 13.07	0.88
T120	43.00 ± 11.35	32.33 ± 11.65	0.52
T150	26.75 ± 10.11	10.56 ± 9.63	0.26

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



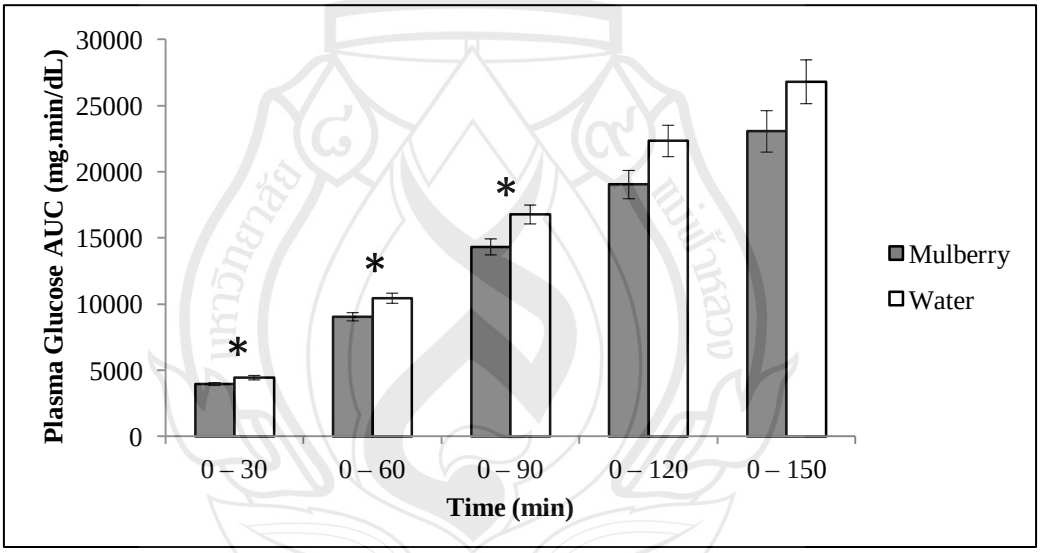
Note. Data corresponds with Table 5.4, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 5.4 Mean Difference of Incremental Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Table 5.5 Incremental AUC of Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Time interval	Incremental glucose AUC (mg.min/dL) - IFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
0-30	3957.50 ± 111.94 *	4443.00 ± 172.43 *	0.04 *
0-60	9042.50 ± 312.84 *	10452.00 ± 383.67 *	0.02 *
0-90	14325.00 ± 606.05 *	16779.00 ± 718.13 *	0.03 *
0-120	19040.00 ± 1073.14	22338.00 ± 1189.15	0.07
0-150	23060.00 ± 1563.10	26808.00 ± 1657.04	0.14

Note. Values are shown as mean ± SEM. * Significant differences between the drink according to independent-samples t test ($P < 0.05$) at time interval 0 – 30, 0 – 60, and 0 – 90.



Note. Data corresponds with Table 5.5, showing mean and SEM value. * Significant differences between the drink according to independent-samples t test ($P < 0.05$) at time interval 0 – 30, 0 – 60, and 0 – 90.

Figure 5.5 Incremental AUC of Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Table 5.6 and Figure 5.6 display the incremental area under the curve of plasma glucose levels of NFG subjects. It can be seen from the data and the graph that the sample group which took mulberry leaf tea prior to OGTT has a slightly lower glucose AUC. However, the difference is not significant ($P > 0.05$).

Table 5.7 and Figure 5.7 illustrate the effect of mulberry leaf tea versus warm water on the concentration of serum insulin in IFG subjects. It can be seen that serum insulin levels for the mulberry leaf tea sample group tends to be lower than the warm water group, with less steep rise and fall. However, the difference is not significant.

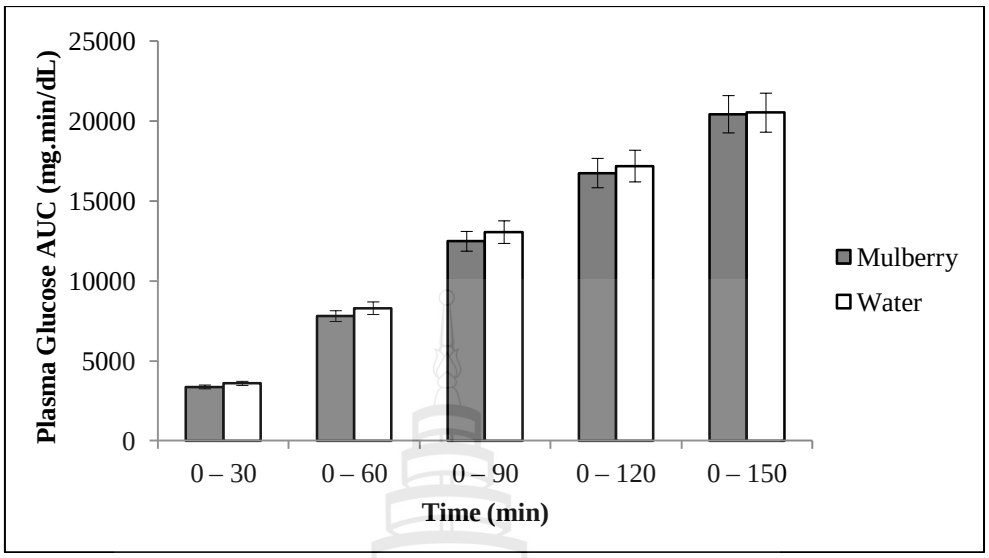
Table 5.8 and Figure 5.8 illustrate the effect of mulberry leaf tea versus warm water on the concentration of serum insulin in NFG subjects. It can be seen that serum insulin levels for the mulberry leaf tea sample group tends to be lower than the warm water group, with less fluctuation. However, the difference is not significant.

Table 5.9 and Figure 5.9 show the mean difference value for the incremental concentration of serum insulin compared to baseline (T0) of IFG subjects. This data represents the change in blood insulin level over time. It can be seen that serum insulin levels for the mulberry leaf tea sample group tends to be lower than the warm water group, but without significant difference.

Table 5.6 Incremental AUC of Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Time interval	Incremental glucose AUC (mg.min/dL) - NFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
0-30	3367.50 ± 120.30	3586.67 ± 131.52	0.24
0-60	7793.44 ± 337.34	8285.83 ± 392.32	0.36
0-90	12469.69 ± 616.57	13041.67 ± 704.41	0.56
0-120	16732.50 ± 918.67	17170.00 ± 992.47	0.75
0-150	20407.50 ± 1166.75	20510.00 ± 1219.61	0.95

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



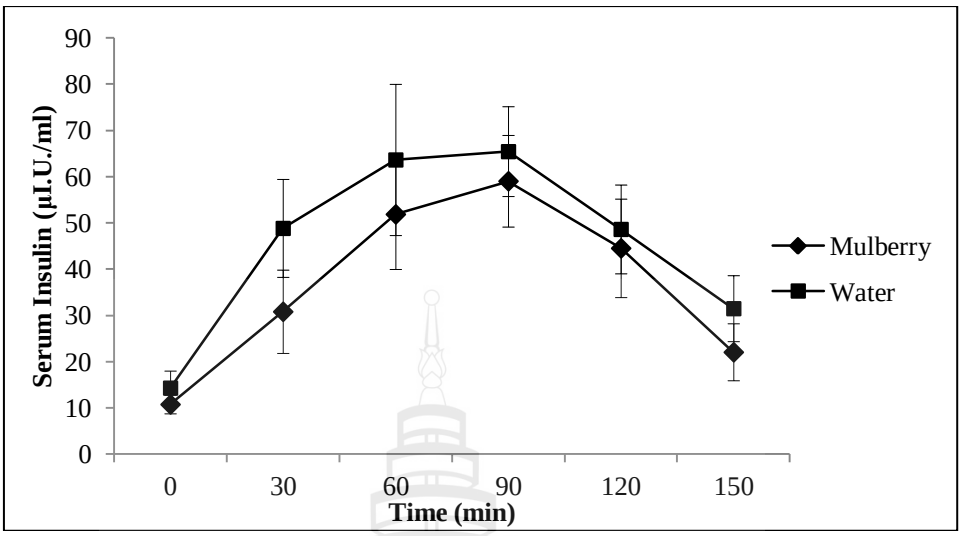
Note. Data corresponds with Table 5.6, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 5.6 Incremental AUC of Plasma Glucose Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Table 5.7 Serum Insulin Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Time point (min)	Serum insulin level - IFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
T0	10.72 ± 2.03	14.26 ± 3.69	0.40
T30	30.77 ± 9.01	48.80 ± 10.59	0.22
T60	51.83 ± 11.91	63.60 ± 16.35	0.57
T90	59.00 ± 9.90	65.40 ± 9.71	0.66
T120	44.49 ± 10.65	48.58 ± 9.61	0.79
T150	22.01 ± 6.16	31.44 ± 7.13	0.34

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



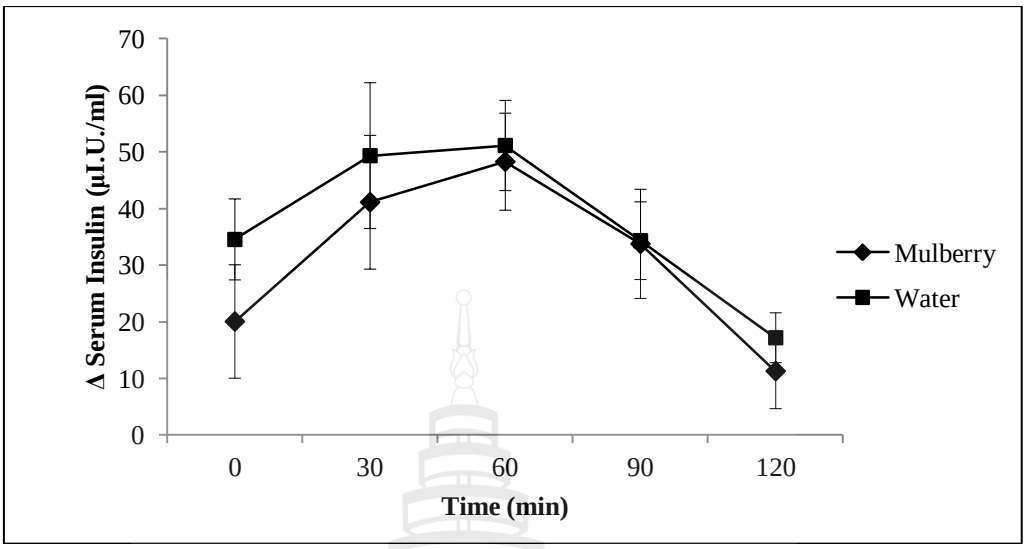
Note. Data corresponds with Table 5.7, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 5.7 Serum Insulin Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Table 5.8 Mean Difference of Incremental Serum Insulin Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Time point (min)	Mean difference of serum insulin level - IFG		*P*-value Between group
	Mulberry leaf tea	Warm water	
T30	20.05 ± 10.01	34.54 ± 7.15	0.29
T60	41.12 ± 11.81	49.34 ± 12.88	0.65
T90	48.28 ± 8.57	51.14 ± 7.97	0.82
T120	33.77 ± 9.64	34.32 ± 6.86	0.97
T150	11.29 ± 6.63	17.18 ± 4.40	0.50

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



Note. Data corresponds with Table 5.8, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 5.8 Mean Difference of Incremental Serum Insulin Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Table 5.10 and Figure 5.10 show the mean difference value for the incremental concentration of serum insulin compared to baseline (T0) of NFG subjects. It can be seen that serum insulin levels for the mulberry leaf tea sample group tends to be lower than the warm water group, but without significant difference.

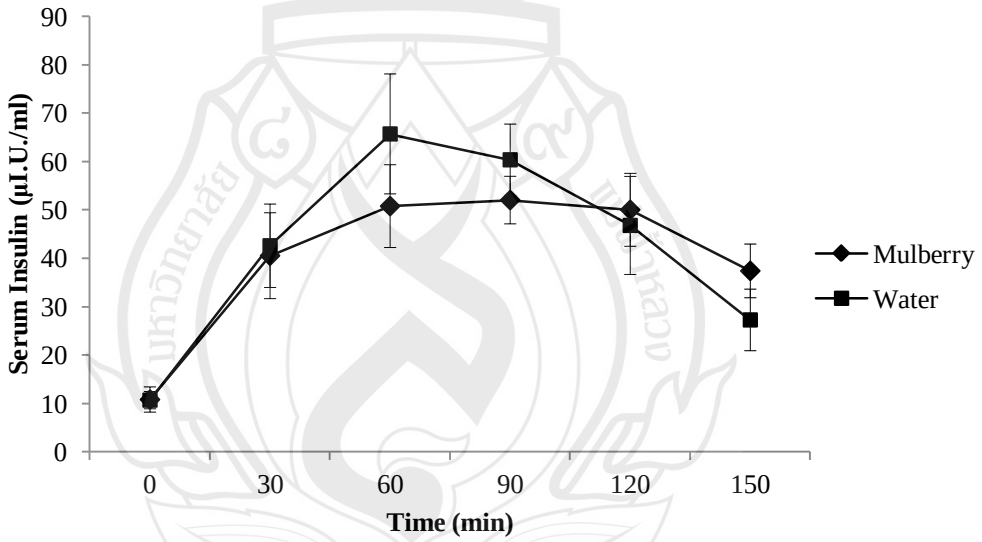
Table 5.11 and Figure 5.11 display the incremental area under the curve of serum insulin levels of IFG subjects. It can be seen from the data and the graph that the sample group which took mulberry leaf tea prior to OGTT has slightly lower insulin AUC. However, the difference is not significant.

Table 5.12 and Figure 5.12 display the incremental area under the curve of serum insulin levels of NFG subjects. It can be seen from the data and the graph that the sample group which took mulberry leaf tea prior to OGTT has slightly lower insulin AUC, but without significant difference.

Table 5.9 Serum Insulin Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Time point (min)	Serum insulin level - NFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
T0	10.76 ± 2.60	10.63 ± 1.79	0.97
T30	40.50 ± 8.88	42.56 ± 8.63	0.87
T60	50.75 ± 8.56	65.68 ± 12.40	0.35
T90	52.00 ± 4.92	60.33 ± 7.40	0.38
T120	49.99 ± 7.54	46.76 ± 10.14	0.81
T150	37.36 ± 5.56	27.22 ± 6.37	0.25

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



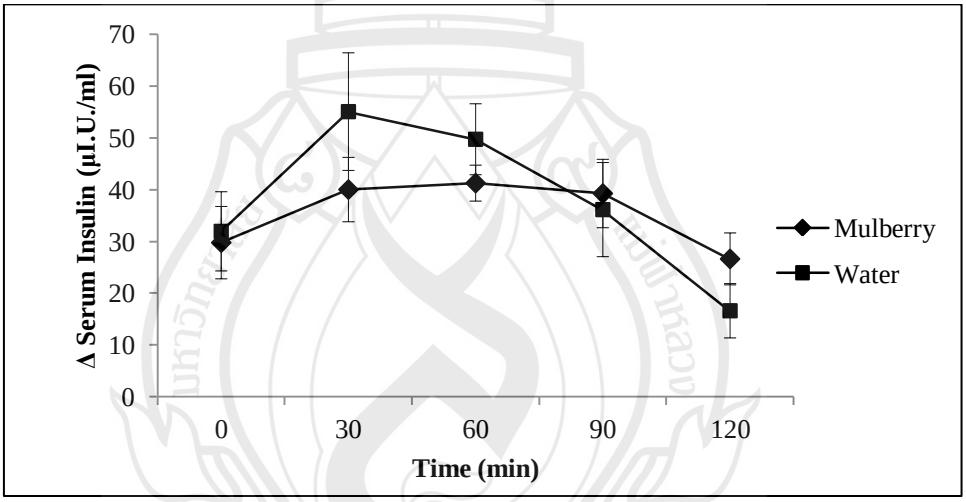
Note. Data corresponds with Table 5.9, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 5.9 Mean Difference of Incremental Serum Insulin Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Table 5.10 Mean Difference of Incremental Serum Insulin Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Time point (min)	Mean difference of serum insulin level - NFG		*P*-value Between group
	Mulberry leaf tea	Warm water	
T30	29.74 ± 6.99	31.92 ± 7.65	0.84
T60	39.99 ± 6.22	55.04 ± 11.35	0.28
T90	41.24 ± 3.47	49.70 ± 6.85	0.31
T120	39.23 ± 6.61	36.12 ± 9.09	0.79
T150	26.60 ± 5.01	16.59 ± 5.25	0.19

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



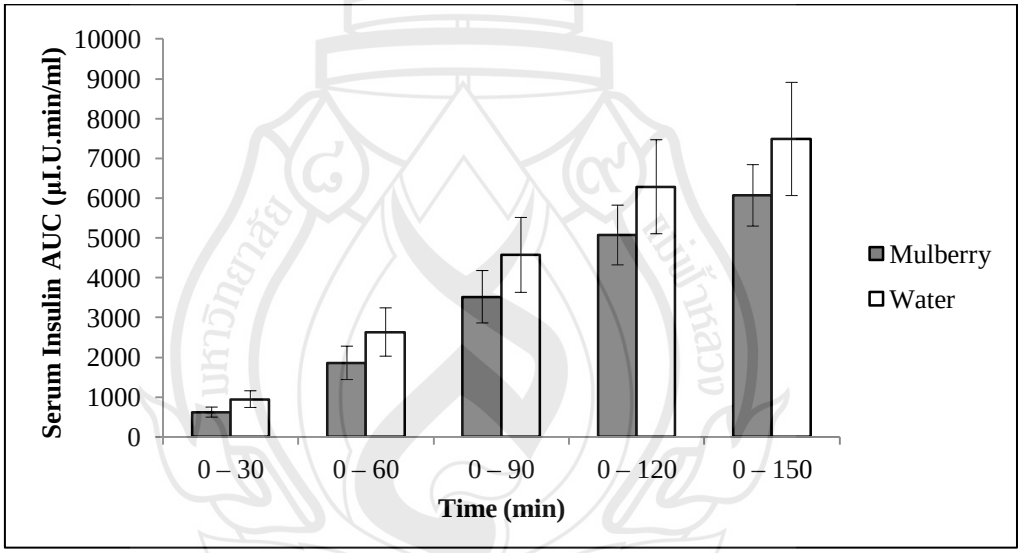
Note. Data corresponds with Table 5.10, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 05.10 Mean Difference of Incremental Serum Insulin Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Table 5.11 Incremental AUC of Serum Insulin Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Time interval	Insulin AUC - IFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
0-30	621.55 ± 126.74	946.32 ± 209.32	0.20
0-60	1858.28 ± 420.06	2633.25 ± 606.32	0.31
0-90	3518.50 ± 658.67	4571.01 ± 941.50	0.37
0-120	5070.90 ± 751.93	6283.14 ± 1181.43	0.39
0-150	6068.05 ± 771.37	7484.79 ± 1422.56	0.38

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups ($P > 0.05$).



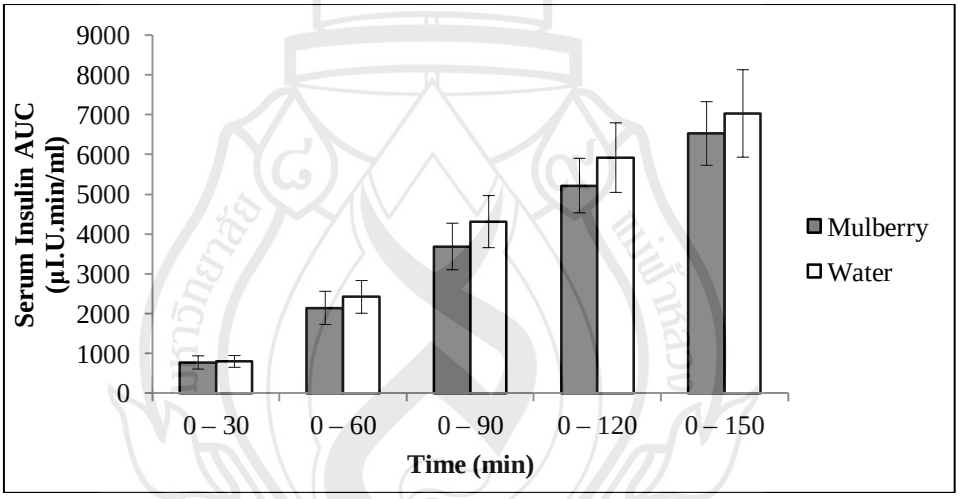
Note. Data corresponds with Table 5.11, showing mean and SEM value. No significant difference is found between the 2 groups ($P > 0.05$).

Figure 5.11 Incremental AUC of Serum Insulin Levels of the Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Table 5.12 Incremental AUC of Serum Insulin Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Time interval	Insulin AUC - NFG		*P*-value Between group
	Mulberry leaf tea	Warm water	
0 – 30	770.38 ± 165.74	796.55 ± 147.72	0.91
0 – 60	2141.79 ± 417.87	2418.88 ± 411.20	0.64
0 – 90	3685.63 ± 585.72	4310.73 ± 654.75	0.49
0 – 120	5216.48 ± 685.88	5919.50 ± 874.02	0.54
0 – 150	6526.86 ± 799.92	7030.18 ± 1098.63	0.72

Note. Values are shown as mean ± SEM. No significant difference is found between the 2 groups (P > 0.05).



Note. Data corresponds with Table 5.12, showing mean and SEM value. No significant difference is found between the 2 groups (*P* > 0.05).

Figure 5.12 Incremental AUC of Serum Insulin Levels of the Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Both NFG and IFG categories are evaluated for their insulin sensitivity and resistance indices. Similar to the uncategorized data, ISI-Comp and HOMA-IR parameters are not different between the mulberry leaf tea sample group and the warm water sample group for both IFG and NFG datasets, as shown in Table 5.13 and 5.14

Table 5.13 Physiological Insulin Sensitivity and Resistance Indices of Subjects with Fasting Plasma Glucose > 100 mg/dL (Impaired Fasting Glucose)

Parameter	Insulin Sensitivity/Resistance Index - IFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
Matsuda Index (ISI)	4.12 ± 0.61	3.59 ± 1.29	0.70
HOMA-IR	2.87 ± 0.59	4.03 ± 1.07	0.35

Note. No significant difference is found between the 2 groups ($P > 0.05$).

Table 5.14 Physiological Insulin Sensitivity and Resistance Indices of Subjects with Fasting Plasma Glucose < 100 mg/dL (Normal Fasting Glucose)

Parameter	Insulin Sensitivity/Resistance Index - NFG		*P*-value
	Mulberry leaf tea	Warm water	Between group
Matsuda Index (ISI)	4.88 ± 0.51	4.81 ± 0.77	0.94
HOMA-IR	2.30 ± 0.54	2.33 ± 0.38	0.97

Note. No significant difference is found between the 2 groups ($P > 0.05$).

In this study, subjects' physiological insulin sensitivity was measured using Matsuda index and HOMA-IR. The author found that mulberry leaf tea does not influence the physiological insulin sensitivity. This is contrary to a previous study done by Kimura et al. (2007). They measured the level of plasma glucose and serum insulin after co-ingestion of mulberry powder and 50 grams sucrose solution, discovering significant differences in serum insulin levels at 60-min time point. However, the study used 1-deoxynojirimycin(DNJ)-enriched powder, which is recognized as a promising antihyperglycemic compound. Significant glucose and insulin lowering effects were observed for administration of 0.8 grams or higher DNJ-enriched mulberry powder, whereas 0.4 gram DNJ-enriched powder did not display significant results. This could suggest that mulberry leaf tea that was used in this study might have inadequate potency due to insufficient level of DNJ.

Nevertheless, the controversy of potency of pure DNJ compound versus aqueous mulberry leaf extract still exists. The study of Kwon et al. (2011) compared the postprandial hypoglycemic effects of pure DNJ compound with aqueous mulberry leaf extract. The study postulated that an array of active components in mulberry leaf extract may provide higher potency in inhibiting intestinal glucose absorption compared to the single component DNJ. The author suggests that aqueous mulberry leaf extract, including mulberry leaf tea, may provide a greater benefit over single component DNJ compound, though the potency of the extract could be optimized via standardization of DNJ

concentration determination. Further study regarding mulberry leaf tea could be done, adding the standardization of DNJ levels for possible improvement.

In summary, the sub analysis of the segmented data shows that for IFG subjects, there are significant differences for their blood glucose levels, Table 5.1, the mean difference of the incremental glucose levels, Table 5.2, and the AUC of glucose levels, Table 5.5. There are no significant differences for the insulin parameters, and the parameters of NFG subjects show no significant change in any parameters.

From the segmented statistic, it is promising that mulberry leaf tea may aid in glycemic control for pre-diabetic subjects, having impaired fasting glucose level. Nevertheless, further studies are required to warrant the efficacy of mulberry leaf tea on postprandial glycemic control for pre-diabetic subjects, employing a larger sample size. The adequate sample size for this study was calculated to be 13, so the author chose to recruit a total of 14 subjects. However, for the sake of sub analysis of the results, dataset with IFG/NFG segmentation was created. Segmenting into NFG and IFG categories, the mulberry leaf tea sample group consisted of 8 NFG subjects and 6 IFG subjects, whereas the warm water sample group consisted of 9 NFG subjects and 5 IFG subjects. The author must admit that the interpretation from this dataset is very limited, because the size of each sample group is too small to represent sufficiently reliable answers to the existing questions.

5.2 Conclusion

In non-diabetic subjects, consumption of mulberry leaf tea may aid in postprandial glycemic control during the first 30 minutes after meal. However, it does not show blood glucose lowering effect in general. Mulberry leaf tea does not influence the physiological insulin sensitivity and does not cause significant adverse events.

5.3 Suggestions

In the author's opinion, the effect of mulberry leaf tea on glycemic control and insulin sensitivity is still inconclusive. Although the conclusion from the experiment in this study is negative, there are still a number of studies which have been conducted properly and had produced positive conclusions.

There are a few factors that the author believes can be adjusted for a better study setting for further research.

5.3.1 Segmentation of subjects based on their fasting plasma glucose:

Even though this study has already attempted a sub analysis, the available dataset is too limited to make a reliable conclusion. However, the trend that can be seen here is the possibility of significant benefits mulberry leaf tea could provide to people with IFG condition. With adequate sample size for each group of subjects, the author suggests that a new research can be done, examining the effect of mulberry leaf tea on postprandial

glycemic control and insulin sensitivity on health subjects with normal fasting glucose, pre-diabetic subjects with impaired fasting glucose, and if possible, diabetic subjects with overt fasting glucose levels.

5.3.2 Standardization of mulberry leaf tea active compound:

Prior to the start of this study, the author has researched on a variety of reliable methods that can determine the concentration of the active compound, one of which is DNJ. Candidate methods include Liquid Chromatography-Mass Spectrometry (LCMS/MS). This method was done to analyze the levels of active compounds in different height of leaves of different mulberry (*Morus* spp.) varieties (Ingkaninan et al., 2008). If the amount of active compounds, particularly 1-DNJ, that would be administered to the test subjects could be determined beforehand, it is possible to find a more precise conclusion that can address the optimal dose for the mulberry leaf tea to control the postprandial glycemia of healthy, pre-diabetic, and diabetic subjects through clinical trials. Regretfully, this study's research method was limited due to the author's financial constraints, thus the determination of these compounds was not done.

5.3.3 Clinical trial with long length of monitoring:

There have been a number of researches which were conducted using mulberry leaf extract on healthy and diabetic subjects. However, the author did not find a study which used mulberry leaf tea as the interventional tool. It is interesting to track long-term parameters which involve risks of diabetes and diabetes management, including fasting plasma glucose, fasting insulin, glycated hemoglobin levels (HbA1C), triglycerides, and etc.

5.3.4 Comparison of mulberry leaf tea and antidiabetic medicine:

For the purpose of treatment and prophylaxis of diabetes, it is essential to evaluate the advantages and disadvantages of choosing between mulberry leaf tea or extract and other antidiabetic drugs with similar mechanism of actions, including glybutamide, metformin, acarbose, voglibose, and etc. Potency and severity of adverse events should be compared for the optimal benefits of diabetes treatment and prevention.

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APPENDICES

APPENDIX A

INFORMED CONSENT FORM

หนังสือยินยอมเข้าร่วมโครงการวิจัย

วันที่ ..... เดือน ..... พ.ศ. ....

ข้าพเจ้า นาย/นางสาว/นาง ..... อายุ ..... ปี อยู่บ้านเลขที่ ..... หมู่ที่ .....

ถนน ..... ตำบล ..... อำเภอ ..... จังหวัด .....รหัสไปรษณีย์.....

โทรศัพท์ .....

ขอทำหนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัยเพื่อเป็นหลักฐานแสดงว่า

1. ข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยของ (หัวหน้าโครงการ) นายปริวรรต ศุกรีเขตร เรื่อง “ผลของชาใบหม่อนต่อการควบคุมระดับน้ำตาลในเลือด และความไวต่ออินซูลินในผู้ที่มีระดับน้ำตาลในเลือดสูง” ด้วยความสมัครใจ โดยมิได้มีการบังคับ หลอกลวงแต่ประการใด และพร้อมจะให้ความร่วมมือในการวิจัย

2. ข้าพเจ้าได้รับการอธิบายและตอบข้อสงสัยจากผู้วิจัยเกี่ยวกับวัตถุประสงค์การวิจัย วิธีการวิจัย ความปลอดภัย อาการ หรืออันตรายที่อาจเกิดขึ้น รวมทั้งประโยชน์ที่จะได้รับจากการวิจัย โดยละเอียดแล้วตามเอกสารชี้แจงผู้เข้าร่วมการวิจัยแนบท้าย

3. ข้าพเจ้าได้รับการรับรองจากผู้วิจัยว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ จะเปิดเผยได้เฉพาะในรูปแบบของการสรุปผลการวิจัยเท่านั้น

4. ข้าพเจ้าได้รับทราบจากผู้วิจัยแล้วว่า หากเกิดอันตรายใด ๆ จากการวิจัย ผู้วิจัยจะรับผิดชอบค่ารักษาพยาบาลที่เป็นผลสืบเนื่องจากการวิจัยนี้

5. ข้าพเจ้าได้รับทราบว่า ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากการวิจัยครั้งนี้เมื่อใดก็ได้ โดยไม่มีผลกระทบใด ๆ ต่อการรักษาพยาบาลตามสิทธิ์ที่ข้าพเจ้าควรได้รับ

ข้าพเจ้าได้อ่านและเข้าใจข้อความตามหนังสือนี้แล้ว จึงได้ลงลายมือชื่อไว้เป็นสำคัญ พร้อมกับหัวหน้าโครงการวิจัยและพยาน

ลงชื่อ ..... ผู้ยินยอม

(.....)

ลงชื่อ ..... หัวหน้าโครงการ

(.....)

ลงชื่อ ..... พยาน

(.....)

ลงชื่อ ..... พยาน

(.....)

APPENDIX B

DUE TO THE RESEARCH PARTICIPANT

เอกสารชี้แจงโครงการวิจัยแก่ผู้เข้าร่วมการวิจัย

ในเอกสารนี้อาจมีข้อความที่ท่านอ่านแล้วยังไม่เข้าใจ โปรดสอบถามหัวหน้าโครงการวิจัย ให้ช่วยอธิบายจนกว่าจะเข้าใจดี ท่านอาจจะขอเอกสารนี้กลับไปอ่านที่บ้านเพื่อปรึกษาหารือกับญาติพี่น้อง เพื่อนสนิท แพทย์ประจำตัวของท่าน หรือแพทย์ท่านอื่น เพื่อช่วยในการตัดสินใจเข้าร่วมการวิจัย

ชื่อโครงการ: ผลของชาใบหม่อนต่อการควบคุมระดับน้ำตาลในเลือด และความไวต่ออินซูลินในผู้ที่มีระดับน้ำตาลในเลือดสูง

ชื่อผู้วิจัย: นายปริวรรต ศุกรีเขตร

ท่านได้รับเชิญให้เข้าร่วมการศึกษาวิจัยเรื่อง “ผลของชาใบหม่อนต่อการควบคุมระดับน้ำตาลในผู้ที่มีระดับน้ำตาลในเลือดสูง” การศึกษาวิจัยนี้ทำโดย นายปริวรรต ศุกรีเขตร โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพมหานคร คาดไว้ว่าจะมีอาสาสมัครเข้าร่วมการศึกษาวิจัยในครั้งนี้จำนวน 13 ราย

วัตถุประสงค์ของโครงการวิจัย

ได้มีการใช้ใบหม่อนในทางยาตามแนวทางการแพทย์แผนโบราณ เพื่อใช้ในการป้องกันและบรรเทาโรคเบาหวาน ท่านได้รับเชิญให้เข้าร่วมการศึกษาวิจัยนี้เนื่องจากผู้วิจัยได้วินิจฉัยว่าท่านมีภาวะน้ำตาลในเลือดสูงกว่าปกติ ซึ่งอยู่ในกลุ่มเสี่ยงที่จะพัฒนาไปเป็นโรคเบาหวาน การศึกษาวิจัยนี้ทำขึ้นเพื่อวัดผลของ “ชาใบหม่อน” ในการลดระดับน้ำตาลในเลือดและเพิ่มประสิทธิภาพการใช้อินซูลินของร่างกาย

การศึกษานี้ใช้ระยะเวลาในการศึกษา 3 สัปดาห์ โดยท่านต้องเข้าพบผู้วิจัยจำนวน 3 ครั้ง ครั้งละ 1 วัน เว้นระยะเวลาระหว่างการเข้าพบแต่ละครั้ง 1 สัปดาห์ และท่านจำเป็นต้องอยู่ที่ศูนย์การศึกษาเป็นเวลาประมาณ 4.5 ชั่วโมงสำหรับการเข้าพบแต่ละครั้ง ท่านจะได้รับเครื่องดื่มที่ใช้ในการศึกษาวิจัย คือ “ชาใบหม่อน” หรือ “น้ำอุ่น” ในแต่ละครั้งที่เข้าร่วมการศึกษา และจะได้รับสารละลายน้ำตาลซูโครส (น้ำเชื่อม) ครึ่งชั่วโมงให้หลังจากการดื่มชา

สถานที่วิจัย สถานที่ทำงานและหมายเลขโทรศัพท์ที่ติดต่อได้ทั้งในและนอกเวลาราชการ

ศูนย์การแพทย์ คิว เมดิคอล เซ็นเตอร์ อโศก กรุงเทพมหานคร โทรศัพท์ 081 – 892 – 4298

สิ่งที่ท่านต้องปฏิบัติเมื่อเข้าร่วมโครงการวิจัยนี้

หากท่านสมัครใจที่จะเข้าร่วมการศึกษาวิจัยนี้ จะมีขั้นตอนที่จะต้องปฏิบัติดังนี้คือ:

1. ท่านจะถูกร้องขอให้อ่าน ลงนามและลงวันที่ในหนังสือแสดงความยินยอม หลังจากที่ท่านได้รับการอธิบาย และการตอบคำถามจากผู้วิจัยเป็นที่เรียบร้อยแล้ว

2. การเข้าพบครั้งที่ 1 (การคัดเลือก)

หากท่านยินยอมที่จะเข้าร่วมการศึกษาวิจัย และได้อ่านและลงนามในเอกสารชี้แจงผู้เข้าร่วมโครงการวิจัยและหนังสือให้ความยินยอมเป็นที่เรียบร้อยแล้ว ผู้วิจัยจะทำการตรวจเพื่อดูว่าท่านเหมาะสมที่จะเข้าร่วมการวิจัยได้หรือไม่ การตรวจวัดนี้รวมถึงการทบทวนประวัติทางการแพทย์ของท่าน การตรวจร่างกาย จะมีการเจาะเลือดประมาณ 15 มิลลิลิตร (1 ช้อนโต๊ะ) ทางหลอดเลือดดำที่แขนของท่าน เพื่อไปหาค่าตรวจทางห้องปฏิบัติการอีกด้วย

<u>ท่านจะไม่ได้รับอนุญาตให้ดื่มเครื่องดื่มที่มีคาเฟอีนเป็นองค์ประกอบ เช่น กาแฟ ชา เครื่องดื่มบำรุงกำลัง หรือแอลกอฮอล์ สูบบุหรี่ และรับประทานหรือดื่มอาหารใดๆที่มีส่วนประกอบของใบหม่อนเป็นระยะเวลา 1 สัปดาห์ก่อนวันทดสอบ คือการตรวจครั้งที่ 2</u>

<u>ท่านจะต้องทำการงดน้ำและอาหารหลังจากเวลาเที่ยงคืนในคืนก่อนวันทดสอบ คือการตรวจครั้งที่ 2</u>

3. การเข้าพบครั้งที่ 2

ในการตรวจครั้งนี้ ท่านจะได้รับการตรวจวัดน้ำหนัก อุณหภูมิร่างกาย ความดันเลือดและอัตราการเต้นของหัวใจ และหากท่านมีคุณสมบัติครบถ้วนตามเกณฑ์ ท่านจะถูกสุ่มเลือกว่าจะได้รับการทดสอบด้วยชาใบหม่อน หรือน้ำอุ่น จากนั้นผู้วิจัยจะขอให้ท่านดื่มเครื่องดื่มตามที่ท่านถูกสุ่มเลือกให้ได้รับ (ชาใบหม่อนหรือน้ำอุ่น) และหลังจากนั้นเป็นเวลา 30 นาที ท่านจะถูกขอให้ดื่มสารละลายน้ำตาลซูโครส (น้ำเชื่อม)

จะมีการเจาะเลือดทางหลอดเลือดดำที่แขนของท่านผ่านทางชุดอุปกรณ์ที่ใช้ป้องกันการแข็งตัวของเลือดที่เรียกว่า “เฮ็พพาริน ล็อค” ซึ่งมีลักษณะเป็นอุปกรณ์ขนาดเล็กที่ใส่คาหลอดเลือดดำไว้สำหรับเก็บเลือดทางหลอดเลือดดำเป็นระยะๆ จะมีการเก็บเลือดของท่านจำนวน 6 ครั้ง คือ ที่เวลาก่อนเริ่มดื่มสารละลายซูโครส (น้ำเชื่อม) และชาใบหม่อนหรือน้ำอุ่น และในนาทีที่ 30, 60, 90, 120 และ 150 หลังการดื่ม ปริมาณเลือดที่เก็บในแต่ละครั้งคือ 2 ซี.ซี.

หลังการเก็บเลือดในนาทีที่ 150 ท่านจะถูกขอให้อยู่ต่อในศูนย์วิจัยเป็นเวลาอย่างน้อยอีก 30 นาที เพื่อสังเกตอาการไม่พึงประสงค์ใดๆที่อาจจะเกิดขึ้น

ท่านจะถูกขอให้จดบันทึกอาการข้างเคียงที่อาจจะเกิดขึ้นในระบบทางเดินอาหารหรือระบบอื่นๆของร่างกายภายในช่วงเวลา 8 ชั่วโมงหลังการทดสอบ

ท่านจะถูกขอให้กลับมาที่ศูนย์วิจัยในอีก 7 วันข้างหน้า โดยท่านจำเป็นต้องงดน้ำและอาหารหลังเวลาเที่ยงคืนในคืนก่อนวันทดสอบ คือการเข้าพบครั้งที่ 3

ท่านจะไม่ได้รับอนุญาตให้ดื่มเครื่องดื่มที่มีคาเฟอีนเป็นองค์ประกอบ เช่น กาแฟ ชา เครื่องดื่มบำรุงกำลัง หรือแอลกอฮอล์ สูบบุหรี่ และรับประทานหรือดื่มอาหารใดๆที่มีส่วนประกอบของใบหม่อนเป็นระยะเวลา 1 สัปดาห์ก่อนวันทดสอบ คือการเข้าพบครั้งที 3

4. การเข้าพบครั้งที่ 3 (1 สัปดาห์หลังจากได้รับการรักษาครั้งแรก)

ในการตรวจครั้งนี้ ท่านจะได้รับการตรวจวัดน้ำหนัก อุณหภูมิร่างกาย ความดันเลือด และอัตราการเต้นของหัวใจ และการเจาะเลือดผ่านอุปกรณ์ “เฮ็พพาริน ล็อค” เช่นเดียวกับการทดสอบครั้งที่แล้ว

จะมีการสลับชนิดของเครื่องดื่มที่ท่านได้รับ คือหากครั้งที่แล้วท่านได้รับชาใบหม่อนการทดสอบในครั้งนี้ท่านจะได้รับน้ำอุ่น หรือในทางกลับกัน หลังจากนั้น 30 นาที ผู้วิจัยจะขอให้ท่านดื่มสารละลายน้ำตาลซูโครส (น้ำเชื่อม) เช่นเดิม แต่

หลังการเก็บเลือดในนาทีที่ 150 ท่านจะถูกขอให้อยู่ต่อในศูนย์วิจัยเป็นเวลาอย่างน้อยอีก 30 นาที เพื่อสังเกตอาการไม่พึงประสงค์ใดๆที่อาจจะเกิดขึ้น

ท่านจะถูกขอให้จดบันทึกอาการข้างเคียงที่อาจจะเกิดขึ้นในระบบทางเดินอาหารหรือระบบอื่น ๆ ของร่างกายภายในช่วงเวลา 8 ชั่วโมงหลังการทดสอบ และขอให้ท่านส่งแบบฟอร์มการจดบันทึกให้ผู้วิจัยทางไปรษณีย์ โดยใช้ซองจดหมายที่ทางผู้วิจัยเตรียมไว้ให้

การตรวจเมื่อมีการถอนตัวออกจากการศึกษาวิจัยก่อนกำหนด

หากแพทย์มีความเห็นถอนตัวท่านออกจากการศึกษาวิจัยก่อนกำหนดการสิ้นสุดการศึกษาวิจัย ท่านจะได้รับการร้องขอให้ทำการตรวจประเมินที่กำหนดไว้ในการตรวจครั้งที่ 2 เมื่อเวลาที่ถอนตัวออกจากการศึกษาวิจัย

ข้อจำกัดในการดำเนินชีวิตระหว่างการเข้าร่วมโครงการ

ท่านจะไม่ได้รับอนุญาตให้ดื่มเครื่องดื่มที่มีคาเฟอีนเป็นองค์ประกอบ เช่น กาแฟ ชา เครื่องดื่มบำรุงกำลัง หรือแอลกอฮอล์ สูบบุหรี่ และรับประทานหรือดื่มอาหารใดๆที่มีส่วนประกอบของใบหม่อนเป็นระยะเวลา 1 สัปดาห์ก่อนวันทดสอบทั้ง 2 ครั้ง และท่านจะได้รับการร้องขอให้กลับมาที่หน่วยการศึกษาสัปดาห์ละครั้ง ติดต่อกันเป็นระยะเวลา 2 สัปดาห์

ประโยชน์ที่อาจเกิดขึ้นเมื่อเข้าร่วมการศึกษาวิจัย

ไม่มีการรับประกันว่าท่านจะได้รับประโยชน์โดยตรงจากการศึกษาวิจัยนี้ ความรู้เพิ่มเติมที่ได้จากการศึกษาวิจัยในครั้งนี้อาจเป็นการให้ข้อมูลเกี่ยวกับการใช้ “ชาใบหม่อน” ในบุคคลทั่วไป

ความเสี่ยงและความไม่สบายที่อาจจะเกิดขึ้นเมื่อเข้าร่วมการศึกษาวิจัย

ขั้นตอนในการศึกษาวิจัยอาจมีผลเกี่ยวข้องในการทำให้เกิดความเสี่ยง และความไม่สบายบางอย่างได้ “ชาใบหม่อน” ที่ใช้ในการศึกษาวิจัยสามารถทำให้เกิดอาการข้างเคียงได้แม้ได้ใช้ตามที่แนะนำไว้ เพื่อประโยชน์สูงสุดของตัวท่าน แพทย์อาจสั่งจ่ายยาอื่นเพิ่มเติมจากยาที่ใช้ในการศึกษาวิจัย เพื่อบรรเทาอาการข้างเคียงของท่าน มีความเป็นไปได้ว่าอาจเกิดอาการแทรกซ้อนและอาการข้างเคียงที่ไม่เคยปรากฏมาก่อนในการศึกษาวิจัยครั้งนี้

“ชาใบหม่อน”

อาการข้างเคียงที่อาจเกิดขึ้นได้จากการดื่มชาใบหม่อน ได้แก่ อาการของระบบทางเดินอาหารและลำไส้ อาจทำให้ท้องเสียได้ อาจทำให้อุจจาระเหลวเมื่อได้รับคู่กับน้ำตาลในปริมาณมาก อาจเกิดเสียงในลำไส้หรือท้องอืดเหมือนกับการรับประทานพืชที่มีฝัก เช่นถั่วหรือผักสด อาจทำให้รู้สึกอิ่ม หรือแน่นท้อง ซึ่งหากเกิดอาการเหล่านี้ขึ้น อาการเหล่านี้จะลดลงและหายไปเองภายในช่วงสั้น ๆ สารแทนนินในชาอาจทำให้เกิดอาการท้องผูกได้

“การเจาะเลือด”

มีความเสี่ยงเล็กน้อยในการทำให้เกิดอาการปวด หรือช้ำ และติดเชื้อในบริเวณที่มีการเจาะเลือด

ข้อมูลที่ได้จากการศึกษาวิจัย

ในทุกแง่มุมของการศึกษาวิจัย รวมถึงผลที่ได้จากการศึกษาวิจัย จะถูกเก็บรักษาไว้เป็นความลับสุดยอด มีเพียงเฉพาะผู้วิจัยเท่านั้นที่จะสามารถเข้าถึงข้อมูลของผู้เข้าร่วมการศึกษาวิจัย รายงานการศึกษาวิจัยอาจตีพิมพ์ได้ แต่ข้อมูลของผู้เข้าร่วมการศึกษาวิจัยจะไม่สามารถถูกพิสูจน์ได้

ค่าใช้จ่ายในการเข้าร่วมการศึกษาวิจัย

ผู้วิจัยจะเป็นผู้ออกค่า “ชาใบหม่อน” ค่าตรวจทางห้องปฏิบัติการและค่าตรวจอื่นที่เกี่ยวข้องกับการศึกษาวิจัย สำหรับค่าใช้จ่ายอื่นๆที่เกิดขึ้นจากขั้นตอนการรักษาตามปกติ ท่านจะต้องเป็นผู้รับผิดชอบ

การจ่ายค่าตอบแทน

ท่านจะได้รับค่าเดินทางเพื่อการวิจัยต่อการเข้าพบแพทย์ตามเวลาที่กำหนดไว้ครั้งละ 200 บาท

ความช่วยเหลือที่ท่านจะได้รับหากได้รับอันตรายจากยา

หากท่านได้รับการเจ็บป่วยที่เกิดเนื่องจากชาใบหม่อนที่ใช้ในงานวิจัย หรือในกรณีที่เกิดภาวะแทรกซ้อน ท่านจะได้รับการดูแลรักษาที่เหมาะสมตามสมควรจะได้รับที่เกี่ยวข้องกับการบาดเจ็บนั้น โดยทางผู้วิจัยวิจัยจะเป็นผู้ออกค่าใช้จ่ายต่างๆ อันเกิดจากการเจ็บป่วยที่เป็นผลมาจากยาที่ใช้ในการศึกษาวิจัย แต่ท่านจะไม่ได้รับค่าชดเชยใดๆ หากท่านเชื่อว่าท่านได้รับอันตรายจากชาใบหม่อนที่ใช้ในงานวิจัย ท่านสามารถสอบถามข้อมูลเพิ่มเติมกับผู้วิจัยได้ทุกเมื่อ โดยกรุณาติดต่อ นายปริวรรต ศุกรีเขตร เบอร์โทรศัพท์ 081 – 892 – 4298 ได้ตลอด 24 ชั่วโมง

การตัดสินใจและสิทธิของท่าน

การเข้าร่วมการศึกษาในครั้งนี้เกิดขึ้นจากความสมัครใจโดยทั้งหมด ท่านจะไม่ถูกบังคับให้เข้าร่วมการศึกษาวิจัยในครั้งนี้ และเมื่อท่านเข้าร่วมการศึกษาแล้วท่านมีสิทธิที่จะถอนตัวออกจากการศึกษาเมื่อใดก็ได้ การตัดสินใจใดๆของท่านจะไม่ส่งผลกระทบต่อการรักษาของท่าน หรือความสัมพันธ์ต่อบุคลากรทางการแพทย์แต่ประการใด หากท่านเลือกที่จะไม่เข้าร่วม หรือถอนตัวออกจากการศึกษาวิจัย เรายังจะให้การรักษามาตรฐานที่ดีที่สุดกับท่านต่อไปแพทย์และผู้วิจัยมีสิทธิที่จะยุติการเข้าร่วมการศึกษาวิจัยนี้ของท่าน โดยไม่ต้องขอความยินยอมจากท่าน หากเห็นว่าเป็นการเหมาะสม ซึ่งรวมถึงการเกิดเหตุการณ์ไม่พึงประสงค์ การบาดเจ็บ หรือ อาการทางคลินิกของท่านอาจทำให้ท่านมีความเสี่ยงในการเกิดอาการแทรกซ้อนอย่างอื่น หรือด้วยเพราะเหตุผลอื่นๆ

ข้าพเจ้าได้อ่านและเข้าใจรายละเอียดในเอกสารนี้ครบถ้วนแล้ว

ลงชื่อ _____ วันที่_____

( )

APPENDIX C

GENERAL INFORMATION OF RESEARCH SUBJECT

เรื่อง: ผลของชาใบหม่อนต่อการควบคุมระดับน้ำตาลในเลือด และความไวต่ออินซูลินในผู้ที่มีระดับน้ำตาลในเลือดสูง

The effect of mulberry leaf tea on postprandial glycemic control and insulin sensitivity in pre-diabetic subjects

เลขที่แบบบันทึกข้อมูล_____ HN_____

วันที่_____

ข้อมูลส่วนตัว

ชื่อ_____นามสกุล_____

อายุ _____ ปี เพศ_____

อาชีพ_____

ที่อยู่_____

_____

เบอร์ติดต่อ_____

สถานภาพ ☐โสด ☐สมรส ☐หม้าย ☐หย่า

การศึกษา ☐มัธยม ☐ปวช/ปวส ☐ปริญญาตรี ☐สูงกว่าปริญญาตรี

ประวัติแพ้ ☐ไม่มี ☐มี ชื่อยา/อาหาร_____

อาการ_____

โรคประจำตัว ☐ไม่มี ☐มี ชื่อโรค_____

ได้รับการวินิจฉัยเมื่อ_____

การรักษา โดย_____

ยาใช้ประจำ ☐ไม่มี ☐มี ชื่อยา_____
ระยะเวลาการใช้_____
วิธีการใช้_____
ใช้ครั้งสุดท้ายเมื่อ_____

ประวัตครอบครัวที่ป่วยเป็นเบาหวาน ☐ไม่มี ☐มี

ประวัติโรคเบาหวานขณะตั้งครรภ์หรือมีประวัติคลอดลูกตัวโต (น้ำหนักตั้งแต่4กิโลกรัมขึ้นไป)
☐ไม่มี ☐มี

มีประวัติหรือเคยมีประวัติน้ำตาลในเลือดสูง (FPG 100-125 mg%หรือOGTT $\geq$ 140mg%)
☐ไม่มี ☐มี

ลักษณะการใช้ชีวิต และพฤติกรรมส่วนตัว

1. การรับประทานอาหาร
    1) จำนวนมื้อ / เวลา / ความตรงเวลา / ปริมาณ / มื้อพิเศษ
    _____
    _____
    2) ลักษณะใดที่ตรงกับพฤติกรรมการรับประทานอาหารของท่านมากที่สุด (คำนึงถึงสุขภาพเป็นหลัก / คำนึงถึงสุขภาพพอสมควร / ไม่คำนึงถึงสุขภาพเลย)
    _____
    3) ประเภทของอาหารที่รับประทานบ่อย
    _____
2. การดื่มน้ำและเครื่องดื่มแอลกอฮอล์
    1) น้ำเปล่า
    _____
    2) น้ำอัดลม
    _____
    3) เครื่องดื่มแอลกอฮอล์
    _____
    กรุณาระบุปริมาณต่อวัน หากเป็นน้ำอัดลมและเครื่องดื่มแอลกอฮอล์ให้ระบุความถี่ด้วย เช่น ดื่มน้ำอัดลม 1 ขวดต่อวัน ความถี่ประมาณ 4 วันต่อสัปดาห์

3. กิจกรรมทางกาย

1) การใช้ชีวิตทั่ว ๆ ไปของท่านที่ไม่รวมถึงการออกกำลังกายอย่างจริงจังนั้นตรงกับลักษณะใดมากที่สุด (ออกแรงมาก / ออกแรงปานกลาง / แทบจะไม่ออกแรง)

_____

2) การออกกำลังกาย

_____

กรุณาระบุ ความถี่ ระยะเวลา ในการออกกำลังกายด้วย เช่น วิ่ง (30 นาที 1 วันต่อสัปดาห์) และเล่นฟุตบอล (1 ชั่วโมง 1 วันต่อสัปดาห์) เป็นต้น

4. การสูบบุหรี่

1) ท่านสูบบุหรี่หรือไม่

_____

2) ท่านได้รับควันบุหรี่จากคนอื่นบ้างหรือไม่

_____

หากสูบบุหรี่ กรุณาระบุจำนวนมวนต่อวันด้วย

APPENDIX D

CASE RECORD FORM

The effect of mulberry leaf tea on postprandial glycemic control and insulin sensitivity in pre-diabetic and non-diabetic subjects

PATIENT DETAILS:

Patient Initials | | | |

Patient Medical Record No. | | | | | | | | - | | |

Date of Birth

Date of Birth		
Day	**Month**	**Year**

Date of Informed Consent		
Day	**Month**	**Year**

CO-MORBID CONDITIONS

Date of Assessment						
Date		Month			Year	

Has the patient had any history of the following events? (Please tick "✓")	No	Yes*	*If Yes, specify duration and details
Diabetes			
Hypertension			
Ischemic Heart Disease (IHD)			
Transient Ischemic Attack			
Cerebro vascular Accident			
Peripheral Vascular Disease			
Thrombosis of Vascular Access			
Liver disease			
Others, specify			

PHYSICAL EXAMINIATION

Date of Assessment							Temperature °C			Route Code*	*Route codes A = Axillary O = Oral T = Tympanic R = Rectal
Date		Month			Year						
										●	

Resting blood pressure and heart rate to be taken after 5 minute sitting and prior to taking blood.

Body weight (kg)			#Blood Pressure (mm/Hg)		#Heart Rate (beats/min)
			systolic	diastolic	
		●			

Were any clinically significant abnormalities found? (please tick "✓")			
	NO	**YES***	***If yes, specified**
Head and Neck			
Ears/Eyes/Nose/Throat			
Heart			
Lungs			
Abdomen			
Lymph. Nodes			
Extremities			
Neurologic			
Musculoskeletal			
Skin			
Other specify:			

COMPLETE BLOOD COUNT AT SCREENING

Item / Units \ Date of Assessment		Date	Month	Year
Hematocrit	%			
Hemoglobin	g/dL			
Red Blood Cell Count	X 10^6 /µL			
Platelets	X 10^3 /µL			
White Blood Cell Count	X 10^3 /µL			

SCREENING

BLOOD CHEMISTRY AT SCREENING

Item / Units \ Date of Assessment		Date		Month			Year	
Glucose	mg/dL							
AST	U/L							
ALT	U/L							
Alkaline Phosphatase	U/L							
Blood Urea Nitrogen	mg/dL							
Serum Creatinine	mg/dL							

INCLUSION/EXCLUSION

PATIENT ELIGIBITY – INCLUSION CRITERIA –

To be eligible for the study, patients must fulfill <u>ALL</u> of the following criteria:
(Please tick "✓")

	YES	**N**O
1) Male or female subjects aged between 30 – 60 years old		
2) No current active diseases of gastrointestinal tract, liver, kidney, allergy, diabetes, and AIDS.		
3) Participants are subjected to pass blood test of Blood Urea Nitrogen (BUN), serum creatinine, AST, ALT, ALP, fasting blood glucose, and complete blood count, physical examination and medical history record screening.		
4) All blood test results, except fasting blood glucose, should display value within the normal reference range. The fasting blood glucose has to less than the diagnostic diabetic level (126 mg/dL)		
5) Subjects voluntarily participate in the study and sign their names in consent forms		

INCLUSION / EXCLUSION

PATIENT ELIGIBILITY – EXCLUSION CRITERIA

Patients who fulfill ANY of the following criteria are not eligible for the study:
(Please tick "✓")

	YES	NO
1. History of being allergic or intolerant to mulberry products or derivatives		
2. History of diseases of gastrointestinal tract, liver, kidney, allergy, AIDS, and other diseases which may influence the bioavailability of mulberry's active compounds		
3. History of regular alcohol consumption or drug abuse		
4. History of regular smoking		
5. Subjects are under other medications a month prior to the study, particularly medications which alter the liver enzymes metabolism		
6. Subjects have participated in other clinical trial a month prior to the study		
7. Currently or previously (within 1 month) enrolled on other investigational drug trial(s).		

VISIT 1

TEMPERATURE

Date of Assessment							Temperature °C			Route Code*	*Route codes A = Axillary O = Oral T = Tympanic R = Rectal
Date		Month		Year							
										●	

BLOOD PRESSURE, HEART RATE AND BODY WEIGHT

Resting blood pressure and heart rate to be taken after 5 minute sitting.

Date of Assessment							Blood Pressure (mmHg)		Heart rate (beats/min)	Body weight (kg)			
Date		Month		Year			systolic	diastolic					
													●

TYPE OF BEVERAGE ADMINISTRATION

Date of Administration						
Date		Month			Year	

Type of Beverage		Volume Consumed
☐	Mulberry tea	
☐	Warm water	

GLUCOSE AND INSULIN LEVELS

Date of Administration						
Date		Month			Year	

Time point (min)	Glucose (mg/dL)	Insulin ( )
0		
30		
60		
90		
120		
150		

VISIT 2

TEMPERATURE

Date of Assessment							Temperature ° C			Route Code*	*Route codes A = Axillary O = Oral T = Tympanic R = Rectal
Date		Month			Year						
										●	

BLOOD PRESSURE, HEART RATE AND BODY WEIGHT

Resting blood pressure and heart rate to be taken after 5 minute sitting and prior to taking blood.

Date of Assessment							Blood Pressure (mmHg)		Heart rate (beats/min)	Body weight (kg)		
Date		Month			Year		systolic	diastolic				
												●

TYPE OF BEVERAGE ADMINISTRATION

Date of Administration						
Date		Month			Year	

Type of Beverage		Volume Consumed
☐	Mulberry tea	
☐	Warm water	

GLUCOSE AND INSULIN LEVELS

Date of Administration						
Date		Month			Year	

Time point (min)	Glucose (mg/dL)	Insulin ( )
0		
30		
60		
90		
120		
150		

END OF STUDY

Date of end of study:

Date		Month			Year	

Enter main reason for patient ending study: | | |

(see codes below)

01. Patient completed study according to the protocol
02. Intolerable adverse event (Record on Adverse Event page)
03. Withdrawal requested *
04. Unrelated medical condition *
05. Administrative / Investigator decision *
06. Loss to follow-up *
07. Death on study (please complete 'Death Summary' page)
08. Other *

* Give details:

I have reviewed this case report form and I confirm that it is a true and accurate of this patient's history while on study.

Investigator's Signature _____ Date _____

WITHDRAWAL

Date of withdrawal:	Date		Month			Year	

Enter main reason for patient withdrawal: | | |
(see codes below)

02. Intolerable adverse event (Record on Adverse Event page)
03. Withdrawal requested *
04. Unrelated medical condition *
05. Administrative / Investigator decision *
06. Loss to follow-up *
07. Death on study (please complete 'Death Summary' page)
08. Other *

* Give details on End of Study page

Investigator's signature: _____ **Date:** _____

VISIT 1

แบบฟอร์มประเมินอาการไม่พึงประสงค์

วันที่ประเมิน						
วัน		เดือน			ปี	

Has the patient had any of the following events during the 8-hour period after the visit?
Please provide score based on degree of severity (0 = none, 4 = severe) (Please tick "✓")

ในช่วงเวลา 8 ชั่วโมงหลังการทดสอบ ขอให้ท่านสังเกตอาการไม่พึงประสงค์ใดๆดังต่อไปนี้หรืออาการอื่นๆ ที่อาจจะเกิดขึ้น โดยให้ระดับความรุนแรงดังนี้

"0" ไม่มีอาการเลย

"1" มีอาการน้อย

"2" มีอาการอยู่บ้างแต่ไม่ส่งผลต่อการดำเนินชีวิตของท่าน

"3" มีอาการมาก โดยท่านสามารถทนต่ออาการนั้นได้ แต่ท่านรู้สึกว่าอาการนั้นส่งผลต่อการดำเนินชีวิตประจำวันของท่าน

"4" มีอาการชัดเจน โดยท่านไม่สามารถทนต่ออาการนั้นได้และทำให้ท่านไม่สามารถดำเนินชีวิตประจำวันได้ตามปกติ

กรุณาทำสัญลักษณ์ "✓" ในช่องให้คะแนน

	0	1	2	3	4	หมายเหตุ
ปวดศีรษะ						
รู้สึกอิ่ม						
มีอาการคัน						
รู้สึกถ่ายไม่สุด						
มีอาการคลื่นไส้						
รู้สึกมีลมมาก ผายลมบ่อย						
รู้สึกอ่อนล้า						
ท้องอืด						
ปวดท้อง						
อื่น ๆ (โปรดระบุรายละเอียด)						

VISIT 2

แบบฟอร์มประเมินอาการไม่พึงประสงค์

วันที่ประเมิน						
วัน		เดือน			ปี	

Has the patient had any of the following events during the 8-hour period after the visit?

Please provide score based on degree of severity (0 = none, 4 = severe) (Please tick "✓")

ในช่วงเวลา 8 ชั่วโมงหลังการทดสอบ ขอให้ท่านสังเกตอาการไม่พึงประสงค์ใดๆดังต่อไปนี้หรืออาการอื่นๆ ที่อาจจะเกิดขึ้น โดยให้ระดับความรุนแรงดังนี้

"0"ไม่มีอาการเลย

"1" มีอาการน้อย

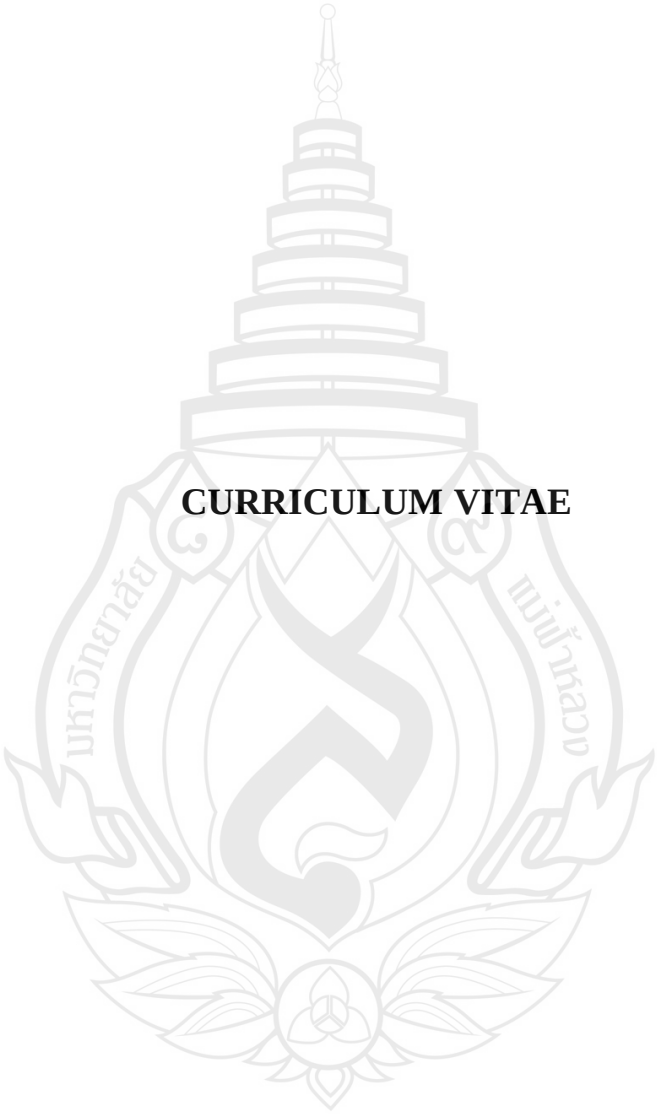
"2" มีอาการอยู่บ้างแต่ไม่ส่งผลต่อการดำเนินชีวิตของท่าน

"3" มีอาการมาก โดยท่านสามารถทนต่ออาการนั้นได้ แต่ท่านรู้สึกว่าอาการนั้นส่งผลต่อการดำเนินชีวิตประจำวันของท่าน

"4" มีอาการชัดเจน โดยท่านไม่สามารถทนต่ออาการนั้นได้และทำให้ท่านไม่สามารถดำเนินชีวิตประจำวันได้ตามปกติ

กรุณาทำสัญลักษณ์ "✓" ในช่องให้คะแนน

	0	1	2	3	4	หมายเหตุ
ปวดศีรษะ						
รู้สึกอิ่ม						
มีอาการคัน						
รู้สึกถ่ายไม่สุด						
มีอาการคลื่นไส้						
รู้สึกมีลมมาก ผายลมบ่อย						
รู้สึกอ่อนล้า						
ท้องอืด						
ปวดท้อง						
อื่น ๆ (โปรดระบุรายละเอียด)						



CURRICULUM VITAE

CURRICULUM VITAE

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