The Effect of Mulberry Leaf Tea on Postprandial Glycemic Control and Insulin Sensitivity: A Randomized, Placebo-Controlled Crossover Study

Parivat Sukriket[1], Surapong Lookhanumarnjao[1] and Akkarach Bumrungpert[2,*]

[1]*School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand*

[2]*Department of Nutrition, Faculty of Public Health, Mahidol University, Bangkok, Thailand*

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 objective of this study was to evaluate the effect of mulberry leaf tea on postprandial plasma glucose and insulin sensitivity after the ingestion of sucrose solution in non-diabetic subjects. 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 either mulberry leaf tea or water as control. The drink was consumed 30 minutes prior to oral administration of 75 grams sucrose solution. Blood samples were collected before (time point 0) and after sucrose ingestion at 30, 60, 90, 120, and 150 minutes. The treatment 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_{0-150} 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). No significant differences were found in insulin sensitivity. Therefore, the consumption of mulberry leaf tea may aid in postprandial glycemic control during the first 30 minutes after meal.

Keywords: Mulberry leaf tea, Blood glucose, Insulin Sensitivity, Oral glucose tolerance test, Diabetes.

INTRODUCTION

Recent epidemiology study has estimated that the prevalence of diabetes is increasing at an extremely dramatic rate. The national statistic for Thai diabetic patients has also shown a similar pattern of escalation. 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 [1-2]. 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 [3].

Preventions and treatments of diabetes are not only available with conventional medicine but 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 [4-12].

With mulberry leaf tea as the potential supportive and preventive option for diabetes and non-diabetes, this study aims to determine its effect in non-diabetic subjects on levels of plasma glucose, serum insulin, and the corresponding insulin sensitivity index. Therefore, the objective is to evaluate the effect of mulberry leaf tea on postprandial plasma glucose and insulin sensitivity after the ingestion of sucrose solution in non-diabetic subjects.

MATERIALS AND METHODS

Study Protocol

The study protocol was approved by the Ethics Committee for Human Research, Mae Fah Luang University, Thailand. Subjects signed the informed consent form before participation.

Participants included 14 volunteers who were initially screened for non-diabetic condition, with fasting plasma glucose below 126 mg/dL. Male or female subjects aged between 30 - 60 years old. Subjects had no diabetes, kidney or liver disease. Subjects who were smokers, pregnant or nursing, regular users of a pharmaceutical or food supplement that impacts glucose metabolism were excluded. Dried mulberry leaf

*Address correspondence to this author at the Department of Nutrition, Faculty of Public Health, Mahidol University, Bangkok 10400, Thailand; Tel: (+66) 2354-8539; Fax: (+66) 2640-9839; E-mail: abnutrition@yahoo.com

ISSN: 2223-3806 / E-ISSN: 1927-5951/16

tea powder (*Morus alba* L., Khun Pai) was provided by The Queen Sirikit Department of Sericulture, Ministry of Agriculture and Co-Operatives, Bangkok, Thailand.

On the date of experiment, subjects were asked to fast for at least 8 hours. Subjects were then randomly given a cup of mulberry leaf tea or warm water as controlled beverage in the morning. For mulberry leaf tea, 2 grams of mulberry leaf tea powder were mixed with 100 milliliters of boiling water, left still for 12 minutes, then asked to be ingested by the subjects. Warm water, which represented controlled beverage, was prepared in a similar manner, except the presence of the mulberry leaf tea powder. 30 minutes after the consumption of beverage, subjects were asked to ingest 75 grams of sucrose in 150 milliliters of warm water. Venous blood samples were collected before sucrose ingestion and at 30, 60, 90, 120, and 150 minutes thereafter through injection plug for blood glucose and insulin level analysis. Subjects then kept a diary of severity of abdominal and other symptoms within 8 hours after the experiment. The experiment was repeated in 1 week with the opposite treatment.

Statistical 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. The whole body physiological insulin sensitivity was obtained using the Matsuda Index calculation for composite insulin sensitivity index (ISI) and homeostasis model assessment-estimated insulin resistance (HOMA-IR), indicator of insulin resistance [18-19].

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, while differences in the questions regarding post-experiment adverse events were evaluated with Pearson Chi-Square test. The levels of significance were set to $p < 0.05$.

RESULTS

General Characteristic of Subjects

There were 14 subjects participated in the study with ages ranging from 30 to 60 years old (51.21 ± 9.45). The gender was equally distributed, with 7 males and 7 females. The average body mass index (BMI) of the subjects reflects a slightly obese group of individuals (26.10 ± 3.67). Blood pressure, heart rate, body temperature, complete blood counts, and other blood chemistry profiles were all within normal ranges (Table **1**).

Table 1: General Characteristics of Subjects

Parameter	Values
Total Number, Male/Female (n/n)	14 (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
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

Values are mean ± SD.

Postprandial Glucose and Insulin Response

After oral glucose tolerance test (OGTT), 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_{0-150} compared to the controlled group, but without significant difference. The mean difference of the incremental glucose level at each time point also tended to be lower for the mulberry group, with significant difference at 30-min time point (p = 0.04) (Figure **1**). The physiological insulin sensitivity, measured by Matsuda index and HOMA-IR, were not different (Table **2**).

Test subjects kept a diary of severity of symptoms score scales. Symptoms include headache, fullness, itching, incomplete evacuation, nausea, excessive

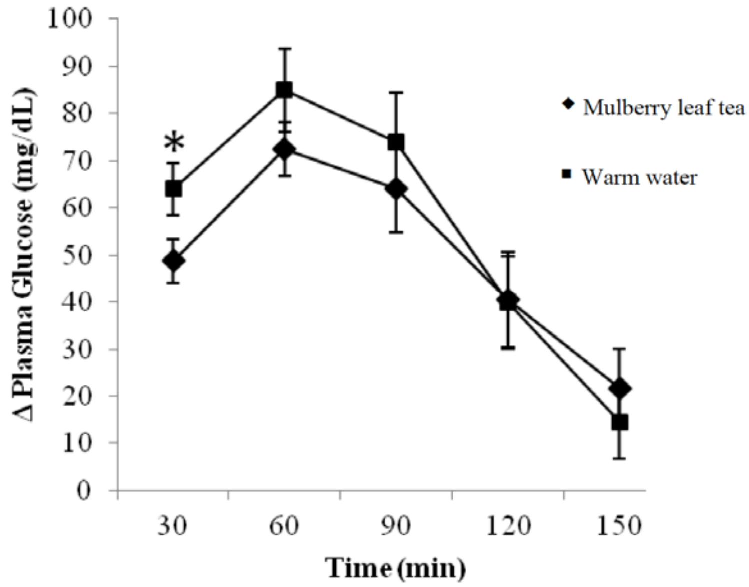

Figure 1: The effect of mulberry leaf tea consumption on incremental glucose levels after sucrose loading in non-diabetic subjects.

*Statistically significant difference between groups at $p < 0.05$.

Table 2: Physiological Insulin Sensitivity

Parameter	Mulberry leaf tea	Warm water	*p*
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

Values are mean ± SEM, Significant difference at $p < 0.05$.

rectal gas, fatigue, bloating, and abdominal pain. There was no significant difference in severity for any symptoms between mulberry and warm water sample group.

DISCUSSION

The primary endpoint in this study was the effect of mulberry leaf tea on postprandial glucose and insulin levels. 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. 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 [12-18]. 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 [19]. 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 [16].

The author observed no difference in glucose and insulin levels. Although the trend of both parameters seem to be lower for the mulberry group, the difference is not significant. A similar observation has been reported by Sinsatienporn, *et al.* [20]. They suggested that the hypoglycemic effect of higher dose may be needed. However, the study by Sinsatienporn, *et al*. was open-labeled without concurrent controls, and diet control instructions were not provided to the subjects.

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). The results show that for IFG subjects (n = 6), there are significant differences for their blood glucose levels, the mean difference of the incremental glucose levels, the AUC of glucose levels, and the mean difference of the incremental glucose AUC at 30-min and 60-min time points ($p < 0.05$). There are no significant differences for the insulin parameters, and the parameters of NFG subjects show no significant change in any parameters.

From the latter statistic, it is promising that mulberry leaf tea may aid in glycemic control for pre-diabetic subjects, having impaired fasting glucose level. However, 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.

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.* [13]. 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, Chung, Kim, & Kwon compared the postprandial hypoglycemic effects of pure DNJ compound with aqueous mulberry leaf extract [14]. 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.

Therefore, based on the results obtained, the consumption of mulberry leaf tea may aid in postprandial glycemic control during the first 30 minutes after meal in non-diabetic subjects. 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.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ACKNOWLEDGEMENT

The authors thank to The Queen Sirikit Department of Sericulture, Ministry of Agriculture and Co-Operatives, Bangkok, Thailand for providing dried mulberry leaf tea powder.

REFERENCES

[1] American Diabetes Association. Economic costs of diabetes in the US in 2002: Diabetes Care 2003; 26: 917-32. http://dx.doi.org/10.2337/diacare.26.3.917

[2] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-53. http://dx.doi.org/10.2337/diacare.27.5.1047

[3] Olokoba AB, Obateru OA, Olokoba LB. Type 2 Diabetes Mellitus: A Review of current trends. Oman Med J 2012; 27: 269-73. http://dx.doi.org/10.5001/omj.2012.68

[4] Grover J, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 2002; 81: 81-100. http://dx.doi.org/10.1016/S0378-8741(02)00059-4

[5] Gulubova R, Boiadzhiev T. Morphological changes in the endocrine pancreas of the rabbit after administration of a Morus alba extract. Eksp Med Morfol 1975; 14: 166-71.

[6] Hikino H, Mizuno T, Oshima Y, Konno C. Isolation and hypoglycemic activity of Moran A, a glycoprotein of Morus alba root barks. Planta Med 1985; 51: 159-160. http://dx.doi.org/10.1055/s-2007-969435

[7] Hosseinzadeh H, Sadeghi A. Antihyperglycemic effects of Morus nigra and Morus alba in mice. Pharm Pharmacol Lett 1999; 9: 63-5.

[8] Jang M-H, Kim H, Shin M-C, *et al.* Administration of Folium mori extract decreases nitric oxide syntase expression in the hypothalamus of streptozotocin-induced diabetic rats. J Pharmacol 2002; 90: 189-92.

[9] Nojima H, Kimura I, Chen FJ, *et al.* Antihyperglycemic effects of N-containing sugars from Xanthocercis zambesiaca, Morus bombycis, Aglaonema treubii, and Castanospermum australe in streptozotocin-diabetic mice. J Nat Prod 1998; 61: 397-400. http://dx.doi.org/10.1021/np970277l

[10] Lemus I, Garcia R, Delvillar E, *et al.* Hypoglycemic activity of four plants used in Chilean popular medicine. Phytother Res 1999; 13: 91-4.
http://dx.doi.org/10.1002/(SICI)1099-1573(199903)13:2<91::AID-PTR350>3.0.CO;2-8

[11] Litthilert P. Effect of mulberry (Morus alba Linn.) leaves extracts on plasma glucose level in streptozotocin-induced diabetic rats. Bangkok: Mahidol University; 2001.

[12] Sharaf A, Mansour MY. Pharmacological studies on the leaves of Morus alba, with special reference to its hypoglycemic activity. Planta Med 1964; 12: 71-6.
http://dx.doi.org/10.1055/s-0028-1100152

[13] Kimura T, Nakagawa K, Kubota H, *et al.* Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. J Agric Food Chem 2007; 55: 5869-74.
http://dx.doi.org/10.1021/jf062680g

[14] Kwon HJ, Chung JY, Kim JY, *et al.* Comparison of 1-deoxynojirimycin and aqueous mulberry leaf extract with emphasis on postprandial hypoglycemic effects: *In vivo* and *in vitro* studies. J Agric Food Chem 2011; 59: 3014-9.
http://dx.doi.org/10.1021/jf103463f

[15] Hansawasdi C, Kawabata J. Alpha-Glucosidase inhibitory effect of mulberry (Morus alba) leaves on Caco-2. Fitoterapia 2006; 77: 568-73.
http://dx.doi.org/10.1016/j.fitote.2006.09.003

[16] Mudra M, Ercan-Fang N, Zhong L, *et al.* Influence of mulberry leaf extract on the blood glucose and breath hydrogen response to ingestion of 75 g sucrose by type 2 diabetic and control subjects. Diabetes Care 2007; 30: 1272-4.
http://dx.doi.org/10.2337/dc06-2120

[17] Yatsunami K, Ichida M, Onodera S. The relationship between 1-deoxynojirimycin content and a-glucosidase inhibitory activity in leaves of 276 mulberry cultivars (Morus spp.). J Nat Med 2008; 62: 63-6.
http://dx.doi.org/10.1007/s11418-007-0185-0

[18] Zhong L, Furne JK, Levitt MD. An extract of black, green, and mulberry teas causes malabsorption of carbohydrate but not of triacylglycerol in healthy volunteers. Am J Clin Nutr 2006; 84: 551-5.

[19] Li Y-G, Ji D-F, Zhong S, *et al.* Hybrid of 1-deoxynojirimycin and polysaccharide from mulberry leaves treat diabetes mellitus by activating PDX-1/insulin-1 signaling pathway and regulating the expression of glucokinase, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in alloxan-induced diabetic mice. J Ethnopharmacol 2011; 134: 961-70.
http://dx.doi.org/10.1016/j.jep.2011.02.009

[20] Sinsatienporn S, Boonrood U, Chavalittumrong P, *et al.* Clinical study of Morus alba Linn. on glycemic control and blood lipids in patients with type 2 diabetes: A preliminary study. Siriraj Med J 2006; 58: 1039-41.

Received on 31-10-2015 Accepted on 02-01-2016 Published on 23-02-2016

DOI: http://dx.doi.org/10.6000/1927-5951.2016.06.01.6