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Effect of GABA Tea Containing Mulberry Leaf on Oxidative Stress in Healthy Subjects with Weight Control

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Abstract

Oxidative stress plays a vital role in human diseases. GABA tea and mulberry leaves show antioxidant effects and scavenge free radicals. The mulberry leaf-GABA tea (ML-GABA tea) product has the effect of reducing body fat. Therefore, this study evaluates the effect of ML-GABA tea on antioxidant activity and obese-related gene expression. We analyzed 27 subjects, including 18 female and 9 men subjects. The results showed that malondialdehyde (MDA) in plasma and erythrocyte catalase enzyme activity decreased significantly ($p < 0.05$) after the subjects consumed ML-GABA tea for 6 weeks. There was no significant difference ($p > 0.05$) in the enzyme activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). Additionally, we found no significant change ($p > 0.05$) of adiponectin, leptin, IL-6, and visfatin gene expressions in the obese group after consuming ML-GABA tea for 6 weeks. In summary, our findings indicate that ML-GABA tea inhibits MDA formation in the body, reducing the activity of catalytic enzymes. ML-GABA tea is a healthy food that inhibits the formation of body fat and has antioxidant effects.

Keywords: Mulberry; GABA; Obesity; Malondialdehyde; Antioxidative

Abbreviations: MDA: Malondialdehyde; Superoxide dismutase: SOD; Glutathione peroxidase: GPx; Glutathione reductase: GR; γ-amino butyric acid: GABA; Central nervous system: CNS

Introduction

From 1975 to 2016, the global prevalence of obesity almost tripled. In 2016, approximately 13% of the global adult population (11% for men and 15% for women) were obese [1]. According to data from the Nutrition and Health Survey in Taiwan (NAHSIT), the overweight and obesity rate among adult men over 19 years old in Taiwan from 2013 to 2016 was 52.1%, a figure 14.7% higher than that of women [2]. These rates increased by 18.7% (male) and 4.4% (female) between 1993 and 1996, with a much higher rate increase among men [3]. Between 2013 and 2016, the prevalence of people classified as overweight was 25.3% (male) and 19.4% (female), increasing 6.3% and 2.2% over the 2005-2008 statistics. The number of people moving from the overweight to obese categories increased [2, 3].

An analysis of the top ten causes of death in Taiwan found that more than half of the causes of death are related to obesity, including heart disease, cerebrovascular disease, and diabetes. Changes in lifestyle and habits, diet, and environmental factors are affecting people's health. Rich diets and insufficient exercise are common causes of the accumulation of body fat, which is the leading cause of obesity and a risk factor for chronic diseases like myocardial infarction, diabetes, hypertension, gallstones, and bone and joint degeneration [4]. It also contributes to increased cancer rates, accelerates Alzheimer's and gallbladder disease, and shortens life expectancy. There are clear reasons why reducing excessive body fat to avoid obesity should be a goal for most people.

In 2002, Taiwan spent approximately 16.2 billion on obesity and metabolic syndrome care, accounting for 2.9% of the total healthcare expenditure (National Health Expenditure, NHE) [5]. This amount does not include medical treatment due to illness, time lost to hospitalization, and the economic loss caused by reduced productivity. For comparison, the medical cost of obesity-related diseases in the United States in 1998 was US $78.5 billion. The increased to US$147 billion per year in 2008, an increase of 87% over the 10 years [6]. When BMI is higher than 30 kg/m^2, it is related to a 2% decrease in income, a 3% increase in social transfer payments, and a 4% increase in health care costs. A BMI higher than 30 points is also associated with increased comorbidities, which explains the increase in direct and indirect costs. As we touched on above, in addition to increasing health and medical expenses, obesity is also related to absenteeism and unemployment. The decline in productivity and income causes socio-economic shocks [7]. Therefore, obesity causes not only personal health problems but also leads to national economic loss. The factors affecting the increase in obesity rates in Taiwan in recent years are at the (1) family level (family socioeconomic status, family life quality, parent size, changes in eating habits, and growth in static activities); (2) school level (reduced exercise time and the pressure of entering school cause a reduction in sleep time); and (3) social level (Western-style fast food, sugary drinks, media advertising influence, and obesity tax) [8].

Oxidative stress is positively related to the occurrence of cardiovascular disease, type 2 diabetes (2TDM), high blood pressure, stroke, cancer, and osteoarthritis. Being overweight increases cardiovascular disease, type 2 diabetes, high blood pressure, stroke, cancer, and osteoarthritis [9]. A large-scale experiment conducted by the American Cancer Society found that men who are 40% overweight are more likely to get prostate, rectal, and colon cancer. Incidents of

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breast, ovarian, uterine, gallbladder, cervical, and endometrial cancers increase in obese women. There is a high correlation between oxidative stress and body weight. When the body produces too many oxidizing substances, the damage caused when the antioxidant system cannot remove these oxidizing substances promptly is called oxidative stress [10]. When we put our bodies through tension, stress, smoking, hemodialysis, and intense exercise, it releases oxidative stress factors in large quantities, harming human health [9]. Therefore, the problem of excessive oxidative stress caused by obesity is an important research topic.

Gamma-amino butyric acid (GABA) is a significant neurotransmitter, expressed from the embryonic stage until death. It is the primary inhibitory neurotransmitter in the adult brain [11]. Together with the excitatory neurotransmitter glutamate, GABA regulates the inhibitory-excitatory balance necessary for the normal brain function of the mature brain [12, 13]. Therefore, pure, natural foods rich in GABA are considered contemporary health foods and daily nutritional supplements for modern people. Foods rich in GABA include Jiayelong tea (GABA tea), red yeast rice, germinated rice, fermented soybeans, fermented dairy products, mulberry leaves, soft-shelled turtle, and Huangqi (a traditional Chinese medicine). Aside from containing the highest amounts of GABA, Jiayelong tea also helps lower blood pressure, reduces cancer and oxidization risks, and is antibacterial [14].

Mulberry leaf is the leaf of the mulberry tree [15]. Communities worldwide have used this raw material in sericulture for thousands of years. Traditional Chinese Medicine practitioners use mulberry leaf to nourish the blood, clear wind, dissipate heat, invigorate the liver, provide ventilation, lower blood pressure, and increase urine production (diuresis). This versatile botanical is rich in nutrients, including 17 amino acids, carbohydrates, dietary fiber, vitamins, and trace elements such as zinc, iron, and calcium [16]. Mulberry leaves also contain flavonoids, such as isoquercetin, achyranthes sterol, adenine, GABA, choline, alkaloids (GAL-DNJ and Fagomine); total polysaccharides; and other physiologically active ingredients [16, 17]. Recent pharmacological studies have found that consumption of mulberry leaves lowers blood sugar levels, blood pressure, and blood lipids [18, 19]; has anti-aging, anti-inflammatory, and anti-viral effects; and prevents cancer cell formation [20]. Researchers have extracted mulberry leaf components for use in human or mammalian cultured cells. For example, they use mulberry leaf extract to conduct safety and anti-mutation experiments using the chromosomal abnormality test and somatic mutation methods. These results confirm the safety of mulberry leaves [21].

Our past studies found that 150 mg of GABA per 100 g of GABA tea can regulate blood pressure, blood lipids, and blood sugar [22], and improve heart muscle cell apoptosis in rats [23]. GABA also has an excellent antioxidant stress effect. In addition, mulberry leaves lower blood pressure and blood lipids. By researching a combined mulberry leaf-GABA health food, we can understand the effect of the two products on the oxidative stress of weight control subjects. This research aims to learn more about the relationship between mulberry leaf GABA tea on oxidative stress factors and antioxidant enzymes. We will observe the changes in oxidative stress factors and antioxidant enzymes and the effect on the expression of obesity genes in subjects during weight control.

Materials and Methods

Participants

We selected 27 participants for this study. As shown in Table 2, the average age was 32.48±12.08 years old, and the average BMI value was 32.63±4.10 kg/m2 (obesity is ≥ 27 kg/m^2). The subjects, classified as obese, were selected from the outpatient and community screening activities of the Family Medicine Department of the Chung Kang Branch of Cheng Ching General Hospital. The entire participant completed and signed a human trial consent form (HP110014). They took a self-administered GABA tea formula containing mulberry leaves orally once a day (1.5 g / 3 capsules) in the morning and after dinner for 6 weeks. The subjects maintained their typical lifestyle, diet, and exercise habits for the duration of the trial period. We collected blood samples and testing data in the 0th and 7th weeks of the experiment.

The inclusion criteria were: (1) healthy adults over 18 years of age with BMI ≥ 27 kg/m^2; (2) no heart, liver, kidney, endocrine, or other organ diseases; (3) non-psychiatric patients; and (4) no not on any medication.

The exclusion criteria were: (1) Type 1 diabetes (or later-born diabetes caused by pancreatic damage) or secondary diabetes (such as Cushing's syndrome and acromegaly). Severe complications of acute diabetes have occurred in the past 6 months, such as ketoacidosis or high osmotic pressure non-ketoacidosis state (coma). (2) Significant signs of diabetic complications, including symptomatic autonomic neuropathy, retinopathy, or gastro paresis. (3) In the four weeks before the first visit, the patient has an acute infection that may affect blood sugar control and has other medical conditions that may hinder the interpretation of the efficacy and safety data during the trial period. (4) Any of the following conditions occurred in the past six months: myocardial infarction, unstable angina or stroke, coronary artery bypass surgery, or percutaneous coronary intervention. (5) Congestive heart failure requiring medical treatment. (6) Any of the following ECG abnormalities: Torsade de Pointes, persistent, clinically relevant ventricular tachycardia, sick sinus syndrome or atrial fibrillation (flutter), second-level atrioventricular conduction block, first Tertiary atrioventricular conduction block, or QTc prolongation (over 500ms). (7) Chronic respiratory diseases requiring oxygen supply or long-term medication. (8) Suffered from leukemia and lymphoma (excluding basal cell carcinoma) and other malignant tumors in the past five years. (9) Liver diseases, such as cirrhosis or chronic active hepatitis. (10) One unit (500ml) or more of blood donation, loss of at least one unit of blood in the past two weeks, or blood transfusion in the past eight weeks. (11) Long-term insulin therapy in the past six months (under the condition that no other diseases occur in the meantime or receiving treatment for more than four weeks). (12) Treatment with Ia, Ib, and Ic or class III antiarrhythmic drugs. (13) Treatment with any drugs (i.e., cytostatic) with known and frequent toxicity to vital organ systems in the past three months. (14) Significant laboratory abnormalities, including: ALT and AST values at first assessment were three times higher than the upper limit of the normal range; the direct bilirubin value at the first assessment was 1.3 times higher than the upper limit of the normal range; serum creatinine value ≥ 2.0 mg/dL; a laboratory abnormality confirmed to be clinically significant after repeated measurements in the first evaluation; or the oleic ester of fasting triadic was greater than 500 mg/dL in the first evaluation. (15) A history of substance abuse (including alcohol), mental illness, or untrustworthiness in the past two years. (16) Pregnancy. (17) Congenital metabolic diseases.

Samples

Both the mulberry leaf and the GABA tea come from the Alishan tea area in Chiayi, Taiwan. The raw materials are anaerobically fermented three times, dried, and crushed (60 mesh) before being encapsulated. There is a 1:50 ratio of mulberry leaf to GABA tea, and each capsule

weighs 0.5 g. Before the test began, all samples were stored at-80 [22].

Experimental measurements

The participants' body fat, height, weight (calculated body mass index, BMI), and waist and hip circumference (calculated waist-to-hip ratio, WHR) were measured in weeks 0 and 7 of the study. An Omron BP 742 monitor (Omron Healthcare, Inc., Bannockburn, and IL., USA) was used to measure systolic and diastolic blood pressure and pulse and calculate the mean arterial pressure (MAP) of all participants. A 24-hour diet recall record method was used to collect the participants' 3-day (2 days of the week and one holiday) food intake data. The daily dietary intake included total calories and calculated macronutrients (proteins, lipids, carbohydrates) [24].

20 mL of blood was collected from the participants and analyzed in the 0th and 7th weeks of the experiment. Blood sugar, blood lipid parameters (triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), liver function parameters (glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), kidney function parameters (blood urine nitrogen (BUN), creatinine, uric acid) were automatically analyzed using blood biochemistry instruments (Kit, Randox Laboratories Ltd, United Kingdom). Oxidative stress factor plasma malondialdehyde (MDA) was analyzed. Antioxidant enzyme analysis items included superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [25]. The enzyme immunoassay method was used to analyze the serum with a kit of reagents using an ELISA Reader to quantify the gene protein. The known specific antibody was used to bind the solid phase, and then after the test substance was added, the excess was washed away. The second enzyme-labeled antibody was added. After washing off the second antibody, the substrate was added, and a colorimeter was used to estimate the enzyme activity. This concentration was related to the concentration of the antigen in the test substance. We used the test reagents produced by R&D System to analyze the quantification of leptin and adiponectin. The minimum concentration of leptin was 0.15 ng/mL, and adiponectin was 1 ng/mL. The quantification of IL-6 [26] and visfatin, adiponectin [27, 28] was analyzed with a test reagent produced by Phoenix Pharmaceuticals. Blood cell information (Complete Blood Count, CBC) was analyzed using an automated blood cell counter (Cell-Dyne 3700, Abbott, USA) to analyze the number of red blood cells and white blood cells, hemoglobin, hematocrit ratio, average red blood cell volume, platelets, and other blood cell analysis factors in the all participants.

Statistical analysis

The data for all participants was analyzed using SPSS version 22.0.0 (IBM; Chicago, IL, U.S.A.) and are reported as mean ± standard deviation (SD) and the number of people (%). The differences between the two groups are analyzed using Student's t- test. Comparison of the data before and after the test in the same group (week 0 and week 7) was analyzed by paired t- test. If $p < 0.05$, it is statistically significant.

Results and discussion

The average age of the 27 participants in this study was 32.48±12.08 years, and there was no significant difference between men and women ($p = 0.80$). Table 2 shows the participants' average BMI value (≥ 27 kg/m^2), waist circumference (male ≥ 90 cm; female ≥ 80 cm), WHR value (male ≥ 0.9; female ≥ 0.85), and body fat percentage (male ≥ 25%; female ≥ 30%), all at higher than normal values. However, the average blood pressure (< 135/85 mmHg), pulse (60-100 beats per minute), blood lipids, liver function, kidney function, CBC, serum minerals, and urine test data of all participants was within the normal range. Therefore, the participants in this study are described as simple obese patients.

Obesity is a complex disease that involves excessive body fat. It is not only a cosmetic problem but also a medical problem. Obesity often causes heart disease, diabetes, cardiovascular disease, and high blood pressure, increases cancer incidence, accelerates Alzheimer's disease, gallbladder disease, and shortens life expectancy [29]. Furthermore, as the degree of obesity increases, the severity of diabetes, hypertension, hyperlipidemia, hyperuricemia, and pulmonary dysfunction also increases. Table 2 shows that after 6 weeks of ML-GABA intervention in this study, the average body weight, BMI, waist cycle, hip cycle, MAC, and TSF values of the simple obese patients reduced significantly ($p<0.05$). The body weight, BMI, BFR, waist and hip cycle, MAC, and TSF of the female participants and the waist and hip cycle of male participants also reduced significantly ($p<0.05$). The value decrease among the male participants (-3.83 cm, -4.41 cm) was higher than that of the female participants (-3.47 cm, -3.00 cm). On the whole, the WHR values of the female participants (body weight, waist cycle) before and after the experiment (week 7) were significantly lower than those of the male participants ($p <0.05$), and their body weight, BMI, BFR, MAC, and TSF values decreased more than those of the men. The male BMI value of the participants in this study was 2.06 kg/m2 higher than that of the women. While there was no significant difference ($p = 0.52$), the body fat rate of females was higher than that of the males (6.44% more, $p <0.001$). The results showed that ML-GABA had a better effect on improving body weight, BMI, BFR, MAC, and TSF of simple obese women than simple obese men.

According to the 1996-2007 death records of the Taiwan Health Insurance Database, body mass index (BMI) has a U-shaped relationship with the overall mortality rate. At the lowest mortality rate, the average BMI is 22-26 kg/m2 [30]. From 1989 to 1992, people over 40 years of age underwent medical examinations to analyze the overall mortality rate. The rate of overweight / first-degree obese / second-degree obese patients and cardiovascular disease mortality trended upward gradually [31]. The Six-Community Hypertension Intervention Project (SCHIP) conducted from 1982 to 1983 studied males and females aged 20-65. After 24 years of follow-up evaluation, researchers found that the mortality rate of obese people was higher than that of non-obese people. In addition, the odds ratio of diabetes, cardiovascular disease, cancer, and overall mortality gradually increased in the overweight and obesity groups [32]. ML-GABA improves the body weight, BMI, BFR, MAC, and TSF of women with simple obesity, reducing comorbidities caused by simple obesity.

Previous studies have shown that 100 g of GABA tea containing 150 mg of GABA can help regulate blood pressure, blood lipids, and blood sugar [22] and improve heart muscle cell apoptosis in rats [23]. The tea also contains theanine, EGCG, total polyphenols, caffeine, reducing sugars, and free amino acids similar to those found in black and green tea, as shown in Table 1. Table 2 shows that all participants' average blood pressure and pulse were within the normal range (< 135 / 85 mmHg). After 6 weeks of ML-GABA intervention, the average systolic blood pressure decreased by 2.96 mmHg: 1.78 mmHg (male) 3.56 mmHg (female). However, there was no significant difference. According to the results of Selmer et al., when reducing systolic blood pressure by 4% or lowering it by 2 mmHg, coronary artery death can be reduced. The mortality rate of sudden death can be reduced by 6%, and the death rate of all deaths can by 5% [33]. A 5 mmHg reduction in diastolic blood pressure can reduce coronary artery mortality by

Table 1: The contents of components in GABA tea/mulberry leave extract.

Samples	Contents
GABA (mg/100g)	171.95± 2.41
Theanine (mg/g)	40.25 ± 1.37
EGCG (mg/g)	28.07 ± 0.34
Total polyphenol (mg/100g)	2.71 ± 0.05
Caffeine (mg/g)	19.67 ± 3.63
Reduced sugar (mg/100g)	5.94 ± 0.38
Free amino acids (mg/100g)	11.78 ± 0.67
* Values are presented as mean ± SD.	

Table 2: The parameter values estimated for participants.

Variance		All subjects	P value	Man, N=9	P value	Females, N=18	P value	M-F P value
Age (year)		32.48±12.08		33.44±10.70		32.00±12.98		0.8
Body weight(kg)	W0	87.19±17.27		100.17±11.91		80.71±15.99		0.02
	W7	86.03±17.10	0	99.34±11.51	0.11	79.38±15.62	0.01	0.02
BMI (kg/m^2)	W0	32.63±4.10		34.00±3.04		31.94±4.46		0.52
	W7	32.20±4.06	0	33.73±3.07	0.12	31.43±4.35	0.01	0.48
BFR (%)	W0	36.46±4.97		32.17±4.82		38.61±3.50		0
	W7	35.74±5.31	0.21	31.62±5.89	0.74	37.80±3.66	0.05	0
WC (cm)	W0	100.81±10.48		109.39±8.71		96.53±8.59		0.01
	W7	97.22±10.11	0	105.56±9.04	0	93.06±7.92	0	0.02
HC (cm)	W0	113.76±9.08		118.94±7.18		111.17±8.97		0.09
	W7	110.29±8.23	0	114.53±6.56	0	108.17±8.31	0	0.17
WHR	W0	0.89±0.05		0.92±0.04		0.87±0.05		0.06
	W7	0.88±0.05	0.3	0.92±0.04	0.82	0.86±0.04	0.19	0.03
MAC(cm)	W0	34.19±3.14		35.58±2.04		33.50±3.41		0.45
	W7	33.25±2.96	0.01	35.03±1.71	0.09	32.36±3.08	0.02	0.15
TSF(mm)	W0	35.02±5.54		32.28±5.12		36.39±5.35		0.08
	W7	30.81±6.47	0.01	28.94±3.71	0.11	31.75±7.40	0	0.18
SBP(mmHg)	W0	126.85±11.38		132.00±11.49		124.28±10.71		0.21
	W7	123.89±10.78	0.16	130.22±13.47	0.51	120.72±7.78	0.23	0.06
DBP(mmHg)	W0	78.00±9.30		81.11±8.64		76.44±9.46		0.35
	W7	78.59±7.61	0.66	80.11±7.08	0.6	77.83±7.95	0.45	0.35
MAP (mmHg)	W0	93.47±10.19		98.33±8.43		91.04±10.33		0.16
	W7	95.89±10.80	0.3	99.96±12.76	0.56	93.85±9.42	0.39	0.22
Pulse	W0	79.07±11.91		78.22±11.01		79.50±12.63		0.48
	W7	78.41±9.62	0.8	75.22±8.01	0.39	80.00±10.17	0.89	0.26
FBS (mg/dL)	W0	98.78±28.18		117.44±43.49		89.44±6.91		0.01
	W7	96.92±16.97	0.78	107.13±25.81	0.57	91.81±6.88	0.33	0.03
TG (mg/dL)	W0	125.44±52.29		132.22±38.55		122.06±58.71		0.64
	W7	117.25±45.67	0.56	106.75±16.69	0.1	122.50±54.59	0.98	0.44
TC (mg/dL)	W0	189.26±29.39		193.00±29.00		187.39±30.24		0.65
	W7	188.58±30.28	0.94	190.13±19.27	0.82	187.81±35.08	0.97	0.86
LDL-C (mg/dL)	W0	115.50±27.53		122.22±26.91		112.14±27.97		0.38
	W7	113.47±22.64	0.78	120.53±13.72	0.87	109.94±25.66	0.81	0.29
HDL-C (mg/dL)	W0	48.67±9.08		44.33±6.48		50.83±9.56		0.08
	W7	51.67±10.77	0.29	48.25±5.75	0.21	53.38±12.38	0.5	0.28
SGOT (U/L)	W0	23.78±12.91		27.00±8.32		22.17±14.63		0.37
	W7	22.58±10.05	0.72	29.38±13.97	0.67	19.19±5.18	0.45	0.02
SGPT (U/L)	W0	26.19±13.71		33.78±15.30		22.39±11.46		0.04
	W7	26.13±14.63	0.99	34.88±16.47	0.89	21.75±11.85	0.87	0.04
T-4	W0	6.95±1.31		6.59±1.93		7.13±0.88		0.32
	W7	6.95±1.07	0.99	7.00±1.53	0.63	6.93±0.83	0.5	0.88
BUN (mg/dL)	W0	11.11±3.95		11.67±1.32		10.83±4.77		0.61
	W7	11.43±5.88	0.82	12.00±2.93	0.76	11.13±7.05	0.89	0.74
Creatinine (mg/dL)	W0	0.94±0.21		1.08±0.13		0.88±0.21		0.01
	W7	0.95±0.28	0.94	1.04±0.11	0.5	0.91±0.33	0.76	0.29
Uric acid (mg/dL)	W0	6.13±1.61		6.96±1.74		5.71±1.41		0.06
	W7	5.96±1.36	0.7	6.71±1.58	0.77	5.59±1.11	0.78	0.05

Hb (mg/dL)	W0	13.70±1.40		15.18±0.93		12.97±0.93		0
	W7	13.99±1.36	0.47	15.30±0.95	0.79	13.33±1.02	0.28	0
Hct (%)	W0	41.52±3.57		44.67±2.34		39.94±3.00		0
	W7	42.44±4.02	0.39	46.28±3.18	0.25	40.52±2.88	0.57	0
Platelets (K/Ul)	W0	290.46±99.98		210.04±87.02		330.67±81.17		0
	W7	297.58±75.11	0.78	244.50±42.83	0.33	324.13±74.44	0.81	0.01
PCT (%)	W0	0.22±0.07		0.17±0.07		0.24±0.05		0.01
	W7	0.22±0.06	0.65	0.18±0.08	0.84	0.25±0.04	0.6	0.02

between 9% and 14% and eliminate 7% of all stroke deaths in Americans aged 45-64 years. The 2002-2007 Taiwanese Survey on Hypertension, Hyperglycemia, and Hyperlipidemia (TwSHHH) showed that obesity and blood pressure prevalence positively correlated with hyperglycemia and hyperlipidemia [34]. The results of this study indicate that after more than 6 weeks of ML-BABA intervention, the blood pressure of the simple obese participants decreased by > 2 mmHg. ML-GABA improves the systolic blood pressure of people with simple obesity, possibly preventing death from coronary artery disease and stroke.

In addition, it is worth noting that the average blood pressure and plasma FBS, TG, TC, HDL-C values , and LDL-C values of all participants are within the normal range (Table 2). After 6 weeks of ML-GABA intervention, the participants' plasma FBS, TG, TC, and LDL-C values decreased while the HDL-C value increased.

Data are presented as mean ± SD. a Values are presented as mean ± SD. Non-parametric statistics. *P<0.05. **P<0.01. † Paired t-test result for two groups compared at weeks 0 and 7. ‡Independent test result for between-group difference comparison. BMR: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; BFR: body fat rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; FBS: fasting blood sugar; TG: Triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; BUN: blood urea nitrogen; Hb: hemoglobin; Hct: hematocrit; PCT: Platelet hematocrit.

Previous studies of GABA tea show that it can effectively inhibit streptozotocin (STZ)-induced cardiac fibrosis, bring high fasting blood glucose back to normal values, reduce TC, TG, LDL-C values, and increase HDL-C values. GABA tea inhibits the mechanism of cardiac cell fibrosis by reducing FBS and weakening the expression of TNF-α and/or Fas/Fas ligand (FasL) protein, and further reducing the path of cardiac cell apoptosis [35]. These studies prove that GABA tea has potential anti-diabetic properties. Another study pointed out those flavonoids extracted from mulberry leaves play an important role in reducing blood lipids and that GABA and dietary fiber have similar effects [21]. GABA can also induce fatty acid oxidation, promote lipolysis, and inhibit lipid production [36]. Mulberry root bark alcohol extract intervention may inhibit LDL-induced atherosclerosis modification and lipid peroxide formation in hypercholesterolemia rats [37]. Andallu confirmed that mulberry leaf reduces the levels of triglycerides, cholesterol, and free fatty acids in serum in diabetic rats with STZ-induced hyperglycemia and hyperlipidemia. Mulberry leaf also promotes the conversion of cholesterol to cholic acid, reduces LDL- C and VLDL-C, and increases the value of HDL-C [38]. These studies indicate that interventional ML-GABA could lower blood pressure, blood lipids, and blood sugar in adults with simple obesity.

The ML-GABA tea tested in this study was found to reduce the lipid peroxidation index in the body and increase the activity of serum antioxidant enzymes (Figure 1). The serum malondialdehyde

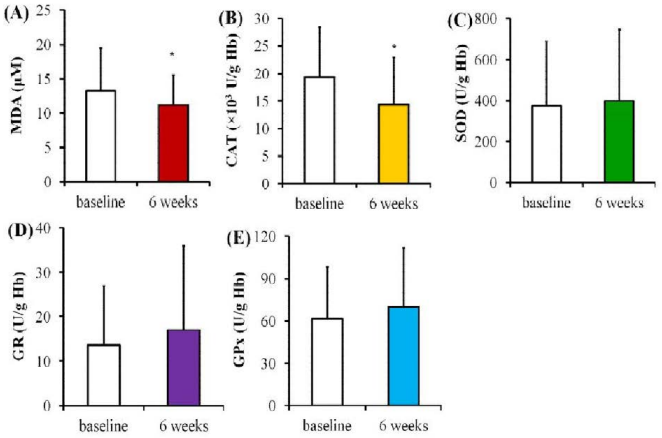

Figure 1: Measurements of MDA (A), CAT (B), SOD (C), GR (D) and GPx(E) before and after intervention. * P < 0.05, significantly different from baseline.

Table 3: Statistics of Clinical Trial of Mulberry Leaf GABA Tea-Diet (Obese Group).

	Week 0	Week 7	P Value
Protein (%)	16.33±4.20	14.95±2.82	0.08
Lipid (%)	20.28±8.85	21.44±7.47	0.48
Carbohydrate (%)	63.02±9.34	63.3255± 9.21	0.86
Total Calories (kcal)	1493.46±384.93	1528.67±415.37	0.11

(MDA) value of the 27 simple obese patients decreased significantly after 6 weeks of ML-GABA intervention ($p < 0.05$). A decrease in MDA content ($p > 0.05$) was observed in males and females, but there was a difference between the two groups. There were no significant differences between before and after the test. There was an increase in serum antioxidant enzyme activities, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) activities, but there was no significant difference ($p > 0.05$). Catalase in vivo catalase enzyme activity decreased significantly and has statistical significance ($p < 0.05$). MDA is a peroxide produced by lipids attacked by free radicals and can be used to indicate oxidative damage. An increase in oxidative damage or a decrease in antioxidant capacity will cause an increase in oxidative stress [25]. With its medicinal properties, GABA has important anti-hypertension, anti-diabetic, anti-cancer, anti-oxidation, anti-inflammatory, anti-microbial, and anti-allergic effects on human health. It also protects the liver, kidneys, and intestines [39]. GABA tea and mulberry leaf slow down metabolic diseases and inhibit inflammation [19, 23, 35, and 40]. Mulberry leaf extract is rich in flavonoids, and it has been found to scavenge blood lipid free radicals in diabetic rats [21]. In addition, studies have found that mulberry leaf extract has antioxidant properties [18-21]. Goralski and Sinal found a significant decrease in serum catalase in Wistar rats after administering mulberry leaves for 8 weeks [41]. Duarte et al. also demonstrated the effect of mulberry leaves on the activity of catalase enzymes in patients with hypercholesterolemia [42]. Catalase activity

is regulated by the signal-transmitting molecules of growth factors and cytokines. When oxidative stress increases, catalase enzyme activity will compensatively increase; with the decrease of oxidative stress, enzyme activity also decreases [43]. Gusti et al study suggests that related oxidative stress genetic determinants could significantly associate with obesity risk [44]. Different oxidized lipoproteins can induce catalase performance, so under appropriate oxidative stress, catalase in the body can be induced to produce anti-oxidative damage [45]. In one study, the intervention of EGCG-rich green tea extract for 6 weeks significantly increased blood antioxidant capacity and moderately reduced lipid peroxidation in men undergoing Cross Fit training [46]. If an obese person with metabolic syndrome is treated with green tea (4 cups per day), control (4 cups of water per day), or green tea extract (2 capsules and 4 cups of water per day) for 8 consecutive weeks, serum antioxidant enzymes (glutathione) Peroxidase (glutathione peroxidase), glutathione (glutathione), catalase (catalase), and plasma antioxidant capacity (plasma antioxidant capacity) significantly improve [47]. Although the serum antioxidant enzyme activity of the participants in this study increased, there was no significant difference. This may be because the participants were simple obese patients and the test period was only 6 weeks long. We speculate that if the ML-GABA were extended to 8 weeks or more, the effect of antioxidant damage would be more obvious.

After 6 weeks of ML-GABA intervention (Figure 2), the expression levels of serum leptin (pro-inflammatory) and visfatin in simple obese patients decreased. However, the expression levels of adiponectin (anti-inflammatory) and IL-6 gene protein (pro-inflammatory cytokines) did not change significantly, and there was no significant difference between the male and female groups ($p > 0.05$). According to a study by Duncan et al., a low adiponectin value (normal: 5-30 μg/mL) can predict the development of T2DM [26], while a high concentration of adiponectin can reduce the risk of T2DM in healthy people, decrease the Triglycerides of the liver and muscles, stimulate the oxidation of tissue fatty acids, and regulate inflammation. Adiponectin negatively correlates with body fat mass and BMI, so in the serum of patients with hyperinsulinemia and T2DM, the adiponectin concentration is lower than in normal people [Spranger et al. 2003]. Adiponectin acts as an anti-hyperglycemia, anti-atherogenic, and anti-inflammatory in human physiology. Serum leptin is a neuroendocrine hormone secreted by adipocytes. It is related to weight control and regulates overeating, insulin resistance, hyperlipidemia, and high blood pressure. If the concentration of leptin increases by one standard deviation (SD), the relative risk of cardiovascular disease will increase by 1.18 times [28]. When fat cells absorb too many calories, they release leptin in the serum and act on the hypothalamus, reducing appetite and increasing metabolic efficiency. Generally speaking, the serum leptin of obese people is higher than that of normal people, and its concentration is proportional to body fat. This means that most obese people are less susceptible to endogenous leptin, leading to significant leptin production. A pro-inflammatory factor is the visceral fat hormone (visfatin) secreted by adipocytes, macrophages, and inflamed endothelial tissue. Like leptin, it is also a fat hormone involved in obesity-related diseases. Visfatin induces vacuities in obese and type 2 diabetes patients and leads to instability of atherosclerotic plaques in patients. Atherosclerotic foam cells contain high visfatin levels, proving that visfatin is involved in the physiological mechanism of atherosclerosis and is also a serious risk factor for obesity and cardiovascular disease [27]. The participants in this study were simple obese without metabolic syndrome. After 6 weeks of ML-GABA intervention, leptin and visfatin levels dropped. We speculate that if the ML-GABA intervention period continued for 8 weeks or more, the benefit of lower leptin and visfatin levels might be more evident. This makes ML-GABA a health food candidate for people with simple obesity looking to prevent cardiovascular disease, diabetes, or other

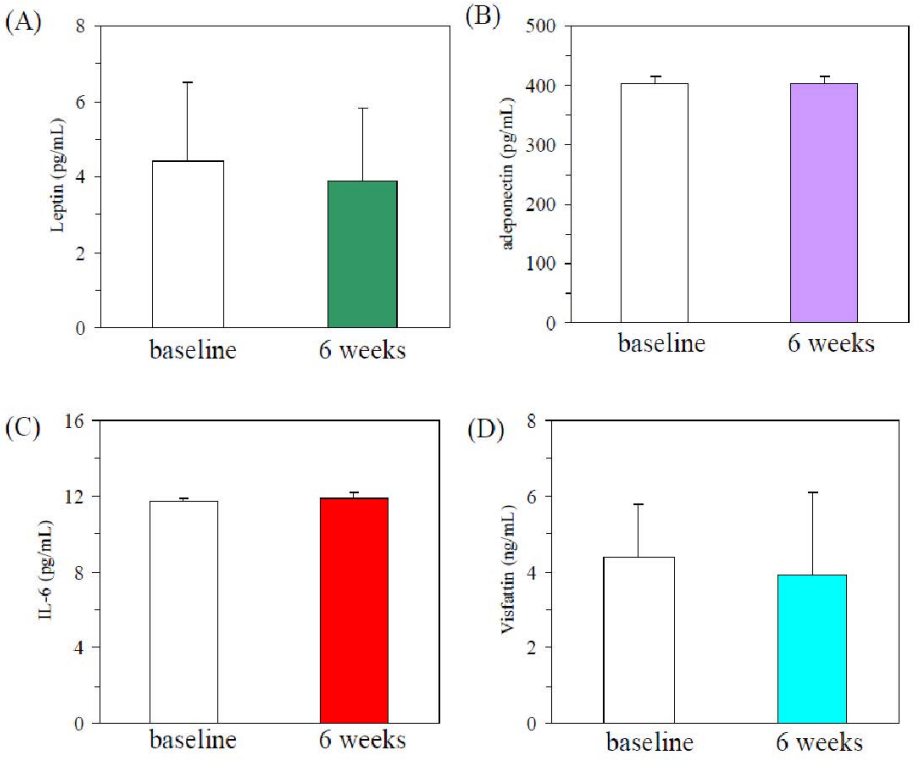

Figure 2: Effect of ML-GABA tea on the expression of leptin (A), adeno cetin (B), IL-6 (C) and visfatin (D) in obese group.

metabolic diseases.

Conclusion

This study found that ML-GABA tea is beneficial for simple obesity. After 6 weeks of ML-GABA intervention, obesity indicators (average weight, waist circumference, hip circumference, BMI, MAC, TSF values) and metabolic syndrome indicators (reduction of FBS, triglycerides, TC, and increased plasma HDL-C) improved in simple obese patients. In addition, oxidative damage in the body was reduced significantly, and participants maintained their antioxidant enzyme ability. Therefore, long-term use of ML-GABA may prevent increased metabolic syndrome indicators such as waist circumference, hypertension, hyperlipidemia, and lower HDL-C, thus preventing cardiovascular disease.

Disclosure statement

The authors have no conflicts of interest to declare.

Competing Interests

The authors have declared that no competing interest exists.

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Project contribution

Shu-E Lai: Collect data and analysis, study patient care, and achievement report writing. Chung-Huang Tsai: study patient care, human trial IRB project hosting, subject consent form explanations, and inspection report recommendations. Shur-Hueih Cherng: plan writing and funding application, antioxidant test technical guidance, and results report writing. Cheng-Chih Tsai: project content suggestions and funding application assistance. Hsueh-Fang Wang: plan writing and fund application, test progress monitoring, test specimen analysis and data sorting, achievement report writing and submission.

References

1. WHO (2021) Obesity and overweight Geneva, Switzerland: World Health Organization.
2. Lin SF, Fan YC, Chou CC, Pan WH, Bai CH (2020) Body composition patterns among normal glycemic, pre-diabetic, diabetic health Chinese adults in community: NAHSIT 2013-2016. PLoS One 15:e0241121.
3. Chang HC, Yang HC, Chang HY, Yeh CJ, Chen HH, et al. (2017) Morbid obesity in Taiwan: Prevalence, trends, associated social demographics, and lifestyle factors. PLoS One 12:e0169577.
4. Crain C, Shah P, Cook C, King H, Santanam N (2011) Obesity Related Markers in Catalase Transgenic Mice. Free Radic Biol Med 51:S65.
5. Fu T, Wen T, Yeh P, Chang H (2008) Costs of metabolic syndrome-related diseases induced by obesity in Taiwan. Obes Rev 9:68-73.
6. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W (2009) Annual Medical Spending Attributable To Obesity: Payer- And Service-Specific Estimates. Health Affairs 28.
7. Kjellberg J, Larson AT, Ibsen R, Hojaard B (2017) The socioeconomic burden of obesity. Obes Facts 10:493–502.
8. Tsai WL, Yang CY, Lin SF, Fang FM (2004) Impact of obesity on medical problems and quality of life in Taiwan. Am J Epidemiol 160:557-565.
9. Young IS, Woodside JV (2001) Antioxidants in health and disease. J Clin Pathol 54:176-186.
10. Sies H (1997) Oxidative stress:oxidants and antioxidants. Exp Physiol 82:291-295.
11. Gillis RA, Yamada KA, DiMicco JA, Williford DJ, Segal SA, et al. (1984) Central γ-aminobutyric acid involvement in blood pressure control. Fed Proc 43:32-38.
12. Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, et al. (2004) Interneurons of the Neocortical Inhibitory System. Nature reviews. Neuroscience 5:793-807.
13. Xu G, Broadbelt KG, Haynes RL, Folkerth .D, Borenstein NS, et al. (2011) Late Development of the GABAergic System in the Human Cerebral Cortex and White Matter. J Neuropathol Exp Neurol 70:841-858.
14. Tsushida T, Murai T (1987) Conversion of glutamine to γ-Aminobutyric acid in tea leaves under anaerobic conditions. Agric Biol Chem 51: 2865-2871.
15. Ercisli S, Orhan E (2007) Chemical composition of white (Morus alba), red (Morus rubra) and black (Morus nigra) mulberry fruits. Food Chem 103:1380-1394.
16. Nuengchamnong N, Ingkaninan K, Kaewruang W, Wongareonwanakij S, Hongthongdaeng B (2007) Quantitative determination of 1-deoxynojirimycin in mulberry leaves using liquid chromatography-tandem mass spectrometry. J Pharma Biomed Anal. 44:853-885.
17. Pawlowska A.M, Oleszek W, Braca A (2008) Quali-quantitative analyses of Flavonoids of Morus nigra L. and Morus alba L. (Moraceae) fruits. J Agric Food Chem 56:3377-3380.
18. Enkhmaa B, Shiwaku K, Katsube T, Kitajima K, Anuurad E, et al. (2005) Mulberry (Morus alba L.) leaves and their major flavonol quercetin 3-(6-malonylglucoside) attenuate atherosclerotic lesion development in LDL receptor-deficient mice. J Nutr 135:729-734.
19. Sugimoto M, Arai H, Taura Y, Murayama T, Khaengkhan P, et al. (2009) Mulberry leaf ameliorates the expression profile of adipocytokines by inhibiting oxidative stress in white adipose tissue in db/db mice. Atherosclerosis 204:388-394.
20. Colonna M, Danzon A, Delafosse P, Mitton N, Bara S, et al. (2008) Cancer prevalence in France: time trend, situation in 2002 and extrapolation to 2012. Eur J Cancer 44:115–122.
21. Chen J, Li X (2007) Hypolipidemic effect of flavonoids from mulberry leaves in triton WR-1339 induced hyperlipidemic mice. Asia Pac J Clin Nutr 16:290-294.
22. Wang HF, Tsai YS, Lin ML, Ou ASM (2006) Comparison of bioactive components in GABA tea and green tea produced in Taiwan. Food Chem. 96:648-653.
23. Wang H.F, Chuang S.M, Hsiao C.C, Cherng S.H (2011) A synergistic effect of GABA tea and copper(II) on DNA breakage in human peripheral lymphocytes. Food and Chemical Toxicology 49:955-62.
24. Wang HF, Lai SE, Tsai CC, Liao CC (2018) The Effect of Multi-Strain Probiotics Formula on Blood Pressure and Health Maintenance in Healthy Humans. J Nutr Diet 1:2.
25. Mimic-Oka J, Simic T, Djukanovic L, Reljic Z, Davicevic Z (1999) Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. Clin Nephrol 51:233-241.
26. Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, et al. (2004) Adiponectin and the development of type 2 diabetes. The Atherosclerosis Risk in Communities Study. Diabetes 53:2473-2478.
27. Lee WJ, Wu CS, Lin H, Lee IT, Wu CM, et al. (2009) Visfatin-induced expression of inflammatory mediators in human endothelial cells through the NF-kappaB pathway. Int J Obes(Lond). 33:465-472.
28. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, et al. (2001) Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study (WOSCOPS). Circulation 104:3052-3056.
29. Sarnali TT, PK MM (2011) Obesity and Disease Association: A Review. AKMMCJ 1:21-24.
30. Marek RJ, Ben-Porath YS, H-M-van-Dulmen M, Ashton K, Heinberg LJ (2017) Using the presurgical psychological evaluation to predict 5-year weight loss

outcomes in bariatric surgery patients. Surg Obes Relat Dis 13:514-521.

31. DeMaria E.J (2007) Bariatric surgery for morbid obesity. N Engl J Med 356:2176-83.
32. Pietras SM, Holick MF (2009) Refractory hypocalcemia following near-total thyroidectomy in a patient with a prior Roux-en-Y gastric bypass. Obes Surg19:524-526.
33. Selmer RM, Kristiansen IS, Haglerod A, Graff-Iversen S, Larsen HK (2000) Cost and health consequences of reducing the population intake of salt. J Epidemiol Community Health 54:697-702.
34. HPA (2015) 2002 Survey on the Prevalence of Hypertension, Hyperglycemia and Hyperlipidemia in Taiwan. Health Promotion Administration, Ministry of Health and Welfare.
35. Cherng SH, Huang CY, Kuo WW, Lai SE, Tseng CY, et al. (2014) GABA tea prevents cardiac fibrosis by attenuating TNF-alpha and Fas/FasL-mediated apoptosis in streptozotocin-induced diabetic rats. Food Chem Toxicol 65:90-96.
36. Kobayashi Y, Miyazawa M, Kamei A, Abe K, Kojima T (2010) Ameliorative effects of mulberry (Morus alba L.) leaves on hyperlipidemia in rats fed a high-fat diet: induction of fatty acid oxidation, inhibition of lipogenesis, and suppression of oxidative stress. Biosci Biotechnol Biochem 74:2385-2395.
37. El-Sayyad HIH, El-Sherbiny MA, Sobh MA, Abou-El-Naga AM, Ibrahim MAN, et al. (2011) Protective Effects of Morus alba Leaves Extract on Ocular Functions of Pups from Diabetic and Hypercholesterolemic Mother Rats. Int J Biol Sci 7:715-728.
38. Andallu B, Vinay Kumar AV, Varadacharyulu NC (2009) Lipid abnormalities in streptozotocin-diabetes: Amelioration by Morus indica L. cv Suguna leaves. Int J Diabetes Dev Ctries 29:123-128.
39. Ngo DH, Vo TS (2019) An updated review on pharmaceutical properties of gamma-aminobutyric acid. Molecules 24:2678.
40. Chuang S, Wang HF, Hsiao CC, Cherng S (2012) Zinc Ion Enhances GABA Tea-Mediated Oxidative DNA Damage. J Agric Food Chem 60:1586-1594.
41. Goralski KB, Sinal CJ (2007) Type 2 diabetes and cardiovascular disease: Getting to the fat of the matter. Can J Physiol Pharmacol 85:113-132.
42. Duarte MMMF, Moresco RN, Duarte T, Santi A, Bagatini MD, et al. (2010) Oxidative stress in hypercholesterolemia and its association with Ala16Val superoxide dismutase gene polymorphism. Clin Biochem 43:1118-1123.
43. Yano S, Yano N (2002) Regulation of catalase enzyme activity by cell signaling molecules. Mol Cell Biochem 240:119-130.
44. Gusti AMT, Qusti SY, Alshammari EM, Toraih EA, Fawzy MS (2021) Antioxidants-Related Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione-S-Transferase (GST), and Nitric Oxide Synthase (NOS) Gene Variants Analysis in an Obese Population: A Preliminary Case-Control Study. Antioxidants (Basel) 10:525.
45. Meilhac O, Zhou M, Santanam N, Parthasarathy S (2000) Lipid peroxides induce expression of catalase in cultured vascular cells. J Lipid Res 41:1205-1213.
46. Sadowska-Krępa E, Domaszewski P, Pokora I, Żebrowska A, Gdańska A, et al. (2019) Effects of medium-term green tea extract supplementation combined with CrossFit workout on blood antioxidant status and serum brain-derived neurotrophic factor in young men: a pilot study. J Int Soc Sports Nutr 16.
47. Basu A, Betts NM, Mulugeta A, Tong C, Newman E, et al. (2013) Green tea supplementation increases glutathione and plasma antioxidant capacity in adults with the metabolic syndrome. Nutrition Research 33:180-187.