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ORIGINAL RESEARCH

Serum Antioxidant Parameters are Significantly Increased in Patients with Type 2 Diabetes Mellitus after Consumption of Chinese Propolis: A Randomized Controlled Trial Based on Fasting Serum Glucose Level

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ABSTRACT

Introduction: Propolis is a natural product with many biological activities. The present study was designed to evaluate the effects of Chinese propolis on glucose metabolism, antioxidant function, and inflammatory cytokines in patients with type 2 diabetes mellitus (T2DM).
Methods: In the 18-week study, recruited T2DM patients were randomly divided into a Chinese propolis group (900 mg/day) ($n = 31$) and a control group ($n = 30$) according to fasting serum glucose levels at baseline.
Results: At the end of the study, no significant difference was found between the groups in serum glucose, glycosylated hemoglobin, insulin, aldose reductase, or adiponectin. However, serum GSH, flavonoids, and polyphenols were significantly increased, and serum lactate dehydrogenase activity was significantly reduced in the Chinese propolis group. Meanwhile, serum IL-6 was significantly increased in the Chinese propolis group.
Conclusion: Chinese propolis is effective at improving antioxidant function in T2DM patients, partly by increasing serum antioxidant parameters.

Keywords: Antioxidant function; Chinese propolis; Type 2 diabetes mellitus

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INTRODUCTION

Propolis is a resinous hive material produced by bees from exudates and buds of numerous plants in combination with secreted substances from bee metabolism, pollen, and beewaxes. Propolis is rich in active components such as polyphenols (including flavonoids), terpenoids, sugars, hydrocarbons, minerals, vitamins, amino acids, and other bioactive constituents [1–3]. Propolis has been used for many years in folk medicine because it possesses various biological properties, including antioxidant, antimicrobial, antiparasite, anticancer, and antiinflammatory activities [4–8].

Previously, propolis was demonstrated to improve glucose metabolism and insulin sensitivity, decrease the plasma insulin resistance index, ameliorate oxidative stress, and prevent

or delay the occurrence of diabetic complications in diabetic rats [2, 9, 10]. We showed that both Chinese and Brazilian green propolis decreased the serum glucose level and improved antioxidant function in rats with DM [11]. In a clinical trial, we found that Brazilian green propolis improved antioxidant function and modulated inflammatory cytokines in T2DM patients after an 18-week intervention. Based on a chemical analysis, Brazilian green and Chinese propolis were found to differ significantly in antioxidant component composition. The contents of flavonoids, zinc, and selenium were lower in Brazilian green propolis than in Chinese propolis, whereas the polyphenol content was higher [12]. We hypothesize that propolis from different geographical areas may act differently because they differ in chemical composition. Therefore, the study reported in the present paper aimed to investigate the effects of Chinese propolis on glucose metabolism, antioxidant function, and inflammatory cytokines in patients with T2DM.

METHODS

Study Subjects

From May to September 2013, 61 T2DM patients aged 35–78 years were recruited from the Department of Endocrinology, Pingjin Hospital, Tianjin, China. T2DM was diagnosed in accordance with the criteria of the American Diabetes Mellitus Association [13]. Patients with allergies, those using other functional foods or health products containing antioxidants, those taking medications (including hormonal contraceptives), substance abusers (e.g., alcohol intake >60 g/day for men and >40 g/day for women) [14], smokers (smoking index ≥ 400) [15], and those with serious endocrine, cardiovascular, renal, respiratory, gastrointestinal, hematological, or central nervous system diseases, acute inflammation or infection, active cancers, or psychiatric disorders were excluded from the study. Serious conditions or diseases were checked and confirmed by senior doctors based on international or national criteria. For example, T2DM patients with severe heart failure, chronic kidney disease, or severe Parkinsonism were excluded [16–18]. Pregnant or lactating women were also excluded.

The protocol was approved by the ethics committee of the Tianjin Institute of Health and Environmental Medicine (TIHE-TY-20130428). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all patients before they were included in the study. This trial was not registered because we signed a contract with By-Health Ltd. in which registration was not required.

Study Design

The design of the present study was not randomized completely. At baseline, we divided the enrolled patients into Chinese propolis and control groups based on fasting serum glucose levels (the fasting blood glucose criterion used in the current study was $\geq$7.0 mmol/L). Briefly, fasting serum glucose values were ranked from the lowest to the highest. The two patients with consecutive glucose values were grouped by lot. It was an open-label study. Subjects in the Chinese propolis group took Chinese propolis capsules provided by Feng-Language Co., Ltd. (Hangzhou, China) at a dose of 900 mg daily for 18 weeks. The dose was extrapolated from animal experiments reported previously [11, 19].

Body height, weight, and waist and hip circumferences were measured at baseline. Body mass index (BMI) was calculated as body weight (kg)/(body height (m))^2, and waist-to-hip ratio (WHR) as waist (cm)/hip (cm). Fasting blood samples were collected at baseline, and the fasting period was 8 h, from 23:00 pm to 7:00 am. Fasting blood samples from the antecubital vein were collected by vacuum tubes and sent to the clinical laboratory of Pingjin Hospital. Serum was separated by centrifugation within 4 h. Parameters such as glucose, glycosylated hemoglobin, and insulin were assayed within 48 h. Some of the blood samples were brought back to our laboratory at <4 °C. One

milliliter of whole blood was taken to prepare samples for hemolysis. The remaining blood samples were centrifuged for serum collection and stored at − 20 °C prior to the assessment of antioxidant parameters, activity of aldose reductase, adiponectin, and cytokines. Fasting blood samples were also collected at the end of the trial, and serum biochemical parameters were assayed again accordingly.

All treatments, exercise regimens, or diabetic diets were continued unchanged during the intervention period. Medications for type 2 diabetes, such as metformin, acarbose, glimepiride, repaglinide, and insulin, were consumed or injected daily as usual. In other words, the patients' usual medications were not altered during the intervention period. Some patients monitored their blood glucose at home; during the follow-up, those patients reported that their glucose levels were stable.

Dietary Survey

Experienced interviewers conducted a consecutive 5-day dietary survey using a 24-h recall method. The energy and nutrient intakes were calculated based on the food intake and Chinese food composition data compiled by the Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention [20].

Measurement of Blood Biochemical Parameters

At the beginning and the end of the intervention, fasting blood samples from the antecubital vein were collected by vacuum tubes. Serum glucose and insulin were assayed using commercial kits purchased from BioSino Biotechnology and Science Inc. (Beijing, China) and Huanri Inc. (Shandong, China), respectively. Serum glycosylated hemoglobin was determined using a glycosylated hemoglobin analyzer (Bio-Rad Laboratories, Hercules, CA, USA). Serum aldose reductase, adiponectin, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α) were measured using enzyme-linked immunosorbent assay (ELISA) kits obtained from BD Biosciences (Lake Franklin, NJ, USA).

Serum antioxidant capacity was assayed via the ferric-reducing antioxidant power (FRAP) described by Benzie and Strain [21]. Serum malondialdehyde (MDA) was analyzed spectrophotometrically by reaction with thiobarbituric acid [22]. The activities of serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and lactate dehydrogenase (LDH) as well as the content of reduced glutathione (GSH) and that of carbonyls were measured using commercial kits purchased from Jiancheng Bioengineering Institute (Nanjing, China). Total flavonoid content was measured with an aluminum chloride colorimetric assay [23]. Total polyphenol content in serum was determined spectrophotometrically using the Folin–Ciocalteu method [24].

Statistical Analysis

Statistical analysis was performed using the SPSS 10.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation. Data were checked for normality by performing a Kolmogorov–Smirnov test before carrying out any further analysis. When the data distribution was normal, Student's t test was used to analyze the difference between the two groups. The insulin, aldose reductase, and adiponectin data were transformed logarithmically because they were not normally distributed. $p < 0.05$ was considered to indicate statistical significance.

RESULTS

General Characteristics of the Patients at Baseline

Sixty-one patients were initially recruited to participate in this study, and 55 of those patients completed the intervention. Six patients from the Chinese propolis group withdrew from the study because of allergies to Chinese propolis (four subjects) or for personal reasons (two subjects). No subjects in the control group withdrew from the experiment. As

Table 1 General characteristics of the subjects at baseline

Parameter	Control	Chinese propolis
Subjects (M/F)	30 (14/16)	31 (11/20)
Age (year)	60.6 ± 8.4	57.7 ± 7.5
Body height (cm)	166.0 ± 8.5	164.6 ± 8.0
Body weight (kg)	74.5 ± 9.9	70.0 ± 10.4
WHR	0.9 ± 0.1	0.9 ± 0.1
BMI (kg/m^2)	26.6 ± 2.6	25.2 ± 2.3
Glucose (mmol/L)	8.3 ± 2.4	8.7 ± 2.8
Glycosylated hemoglobin (%)	7.8 ± 1.2	8.2 ± 1.7
Insulin (μIU/mL)	1.8 ± 0.3	1.1 ± 0.3
FRAP (mmol/L)	0.7 ± 0.2	0.7 ± 0.2
SOD (U/mL)	107.0 ± 25.8	111.7 ± 16.0
GSH-Px (U/L)	172.8 ± 96.6	161.9 ± 91.0
LDH (U/L)	233.5 ± 59.8	253.8 ± 110.8
GSH (g/L)	19.4 ± 6.8	20.3 ± 8.0
MDA (nmol/mL)	16.9 ± 8.2	18.7 ± 6.0

shown in Table 1, there was no significant difference between the two groups in body weight, BMI, and serum biomedical parameters related to glucose metabolism and antioxidant function. There was also no significant difference between the two groups at baseline in male and female (data were not shown).

Dietary Intakes of Energy and Nutrients

There was no significant difference in the dietary intake of either energy or nutrients between the two groups (Table 2).

Glucose Metabolism

There was no significant difference between the glucose metabolism data at baseline and the corresponding data at 18 weeks in the control and Chinese propolis groups (Table 3).

Table 2 Daily intakes of energy and nutrients in patients with T2DM

Parameter	Control ($n = 30$)	Chinese propolis ($n = 25$)
Energy (kcal)	1495.2 ± 346.5	1445.9 ± 448.4
Protein (g)	59.6 ± 15.9	56.3 ± 16.1
Lipids (g)	42.8 ± 17.1	45.8 ± 25.2
Cholesterol (mg)	471.9 ± 226.4	458.6 ± 163.8
Carbohydrates (g)	223.9 ± 52.5	207.9 ± 58.3
Fiber (g)	12.4 ± 4.7	12.5 ± 4.1
Retinol (μg RE)	470.5 ± 223.4	498.2 ± 313.4
Thiamin (mg)	0.8 ± 0.3	0.8 ± 0.4
Riboflavin (mg)	0.8 ± 0.3	0.9 ± 0.4
Niacin (mg)	11.0 ± 3.9	11.1 ± 4.3
Ascorbic acid (mg)	94.1 ± 38.9	100.1 ± 66.0
Tocopherol (mg)	13.2 ± 8.5	15.6 ± 6.3
Potassium (mg)	1809.4 ± 540.4	1769.8 ± 558.1
Sodium (mg)	831.9 ± 408.0	808.8 ± 375.9
Calcium (mg)	512.8 ± 195.2	521.1 ± 350.1
Iron (mg)	19.6 ± 9.9	19.2 ± 9.3
Zinc (mg)	8.6 ± 2.3	8.2 ± 2.5
Selenium (μg)	48.3 ± 16.6	46.1 ± 15.3

Antioxidant Function and Inflammatory Cytokines

After consuming Chinese propolis, serum GSH, flavonoids, and polyphenols were all significantly increased compared with the control group: by 236.4% ($p = 0.000$), 24.5% ($p = 0.004$), and 6.3% ($p = 0.019$), respectively. Interestingly, serum LDH was significantly decreased ($p = 0.004$), and IL-6 was increased ($p = 0.004$) in the Chinese propolis group compared to the control group. However, no significant difference in serum FRAP, SOD, GSH-Px, MDA, carbonyls, IL-1β, and TNF-α was observed between the two groups (Table 4).

Table 3 Comparison of the glucose metabolism data between baseline and the end of the trial in T2DM patients

Parameter	Control		Chinese propolis	
	Baseline	18 weeks	Baseline	18 weeks
Glucose (mmol/L)	8.3 ± 2.4	8.4 ± 2.4	8.7 ± 2.8	8.5 ± 2.0
Glycosylated hemoglobin (%)	7.8 ± 1.2	7.6 ± 1.2	8.2 ± 1.7	7.9 ± 1.4
Insulin (uIU/mL)	1.2 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.0 ± 0.2

Table 4 Antioxidant function and inflammatory cytokines in T2DM patients at the end of the trial

Parameter	Control ($n = 30$)	Chinese propolis ($n = 25$)
FRAP (mmol/L)	0.7 ± 0.1	0.7 ± 0.3
GSH (g/L)	2.2 ± 1.0	7.4 ± 1.4*
Total flavonoids (mg/L)	51.5 ± 13.1	64.1 ± 15.4*
Total polyphenols (mg/L)	199.9 ± 12.6	212.4 ± 24.2*
SOD (U/mL)	102.8 ± 3.1	104.3 ± 3.4
GSH-Px (U/L)	253.7 ± 74.9	262.2 ± 56.4
LDH (U/L)	1446.7 ± 202.1	1224.9 ± 318.5*
MDA (mmol/L)	3.9 ± 1.2	4.3 ± 0.9
Carbonyls (nmol/mg prot)	0.6 ± 0.1	0.6 ± 0.1
IL-6 (pg/mL)	3.1 ± 0.9	3.7 ± 1.2*
IL-1β (pg/mL)	18.7 ± 3.5	18.6 ± 3.8
TNF-α (pg/mL)	20.7 ± 3.7	20.6 ± 4.1

* $p < 0.05$ compared with control

Serum GSH was significantly increased at the end of the trial in both males and females in the Chinese propolis group. However, LDH activity was notably decreased in both genders in the Chinese propolis group at the end of the trial. Total polyphenols, total flavonoids, and IL-6 were all increased in both females and males in the Chinese propolis group at the end of the trial, although only the females showed statistically significant increases in these parameters (Table 5).

DISCUSSION

Diabetes mellitus (DM) is one of the most common metabolic disorders in humans, and is characterized by hyperglycemia due to a shortage of and/or insufficient action of insulin [25]. In type 2 diabetes mellitus (T2DM), the most common type of DM, insulin resistance decreases the response of the peripheral tissues to insulin [26]. Significant increases in reactive oxygen species (ROS) production and oxidative damage have been observed in patients with DM [27, 28], accompanied by lower serum levels of GSH, impaired antioxidant enzymatic activities, and higher serum levels of protein carbonyls and MDA [29–32]. Evidence from both experimental and clinical studies suggests that oxidative stress is involved in the development of insulin resistance and β-cell dysfunction, and plays a major role in the pathogenesis of T2DM [33–36]. Meanwhile, hyperglycemia also contributes to oxidative stress through several pathways: polyol, hexosamine, protein kinase C, glycolysis, and advanced glycation end-product production [37, 38]. Therefore, it is very important to improve antioxidant function in T2DM in order to protect against oxidative stress.

Several studies have revealed that polyphenols in natural products can help to considerably reduce oxidative stress in animals and patients with T2DM [39–42]. Propolis is one of the richest sources of plant polyphenols, including flavonoids, and presents strong antioxidant activity [43–46]. Previous studies have measured the total polyphenol content in Chinese propolis as 139.12–275.59 mg/g, and the total flavonoid content as 127.58–227.24 mg/g [12, 47]. In the present

Table 5 Effects of Chinese propolis on each gender by the end of the trial

Parameter	Male		Female	
	Control	Chinese propolis	Control	Chinese propolis
Glucose (mmol/L)	8.5 ± 2.5	8.6 ± 2.2	8.4 ± 2.3	9.4 ± 3.1
Glycosylated hemoglobin (%)	7.6 ± 0.9	7.8 ± 1.1	7.8 ± 1.4	7.9 ± 1.5
Insulin (μIU/mL)	1.2 ± 0.3	0.9 ± 0.2	1.2 ± 0.3	1.1 ± 0.2
Adiponectin (mg/L)	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Aldose reductase (μg/L)	0.7 ± 0.4	1.1 ± 0.5	1.4 ± 0.6	1.4 ± 0.4
Total polyphenols (mg/L)	204.4 ± 13.1	208.0 ± 13.3	196.2 ± 11.3	214.4 ± 25.1*
Total flavonoids (mg/L)	55.8 ± 13.4	61.2 ± 12.1	48.1 ± 12.2	65.8 ± 17.1*
GSH (g/L)	2.0 ± 0.8	7.2 ± 1.0*	2.0 ± 1.5	7.4 ± 1.6*
FRAP (mmol/L)	0.8 ± 0.1	0.7 ± 0.3	0.7 ± 0.1	0.7 ± 0.2
SOD (U)	101.7 ± 3.5	103.8 ± 5.1	103.6 ± 2.5	104.4 ± 2.5
GSH-Px (U)	281.1 ± 71.2	267.1 ± 56.9	231.4 ± 72.3	259.9 ± 57.7
LDH (U/L)	1466.4 ± 195.5	1242.0 ± 176.5*	1431.0 ± 212.6	1292.6 ± 208.0*
MDA (nmol/ml)	4.3 ± 1.3	4.23 ± 0.64	3.7 ± 1.1	4.3 ± 1.0
Carbonyls (nmol/mg prot)	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
IL-6 (pg/mL)	3.2 ± 0.9	3.7 ± 1.7	2.9 ± 0.9	3.7 ± 0.9*
IL-1β (pg/mL)	19.2 ± 3.0	18.3 ± 4.5	18.3 ± 4.0	18.7 ± 3.6
TNF-α (pg/mL)	21.3 ± 3.0	17.9 ± 4.6	20.1 ± 4.4	21.5 ± 3.7

* $p < 0.05$, compared with control

study, we demonstrated that consumption of Chinese propolis significantly elevated serum GSH, flavonoid, and polyphenol levels in patients with T2DM after 18 weeks of treatment, indicating that Chinese propolis was effective at increasing the availability of antioxidants. Mujica et al. also found that serum GSH content was significantly increased after propolis treatment in a human population in Chile, which is consistent with our results [48]. In comparison with Brazilian green propolis [49], we found that Chinese propolis increased serum total flavonoids to a greater degree. This appears to be because the total flavonoid content in Chinese propolis is higher than that in Brazilian green propolis (163.65 vs 98.46 mg/g) [12]. Interestingly, females seemed to be more sensitive to flavonoid and polyphenol consumption in the present study. This might be related to the gender-dependent hepatic activity of the cytochrome P450 family [50].

It was reported that propolis has a remarkable effect on glucose metabolism in vitro and in vivo. Ueda et al. demonstrated that propolis protects against hyperglycemia by promoting glucose uptake and utility [51]. Other researchers found that propolis modulated glucose metabolism by decreasing glucose levels, increasing plasma insulin levels, improving insulin sensitivity, and reducing serum glycated hemoglobin in rats with diabetes. Besides those effects, propolis also improved antioxidant function by inhibiting lipid peroxidation, enhancing the activities of antioxidant enzymes, and improving antioxidant function in rats with diabetes mellitus [9, 11, 19, 52–55].

Regrettably, we could not confirm the hypoglycemic effects of Chinese propolis in

T2DM patients in the present study. Similar results were recently reported by Zhao et al. [49] and Fukuda et al. [56], who found that Brazilian green propolis did not significantly improve glucose metabolism. We cannot currently explain this discrepancy between animal models and clinical trials. More studies are needed to validate the possible role of Chinese or Brazilian green propolis in improving glucose metabolism in patients with T2DM.

LDH, an enzyme involved in L-lactate metabolism, is an important marker in the pathophysiology and therapy of T2DM [57]. Under normal conditions, LDH release is limited. However, both cell damage and inflammation are involved in the pathogenesis of chronic diseases such as T2DM, and these are accompanied by enhanced serum LDH activity [57, 58]. In this study, the notable reduction in serum LDH activity suggests that the administration of Chinese propolis protects against cellular injury and inflammation in patients with T2DM, possibly by increasing the availability of antioxidants such as GSH, flavonoids, and polyphenols.

IL-6 is considered a proinflammatory cytokine [59]. It was reported that T2DM patients show higher levels of inflammatory cytokines such as IL-6, which may contribute to the chronic inflammation associated with T2DM [60]. However, studies have found that IL-6 suppresses inflammation response in several animal models [61, 62], and that it plays a crucial role in controlling the levels of proinflammatory cytokines [63, 64]. Therefore, Scheller et al. stated that IL-6 has both anti- and proinflammatory properties [65]. In this study, we found that serum IL-6 was significantly increased after Chinese propolis treatment, which may be due to the stimulatory action of propolis towards the secretion of IL-6. We believe that increasing IL-6 helps to improve the chronic low-grade inflammation associated with T2DM.

However, there were several limitations of this study. A placebo was not administered to the control group, and only a high dose of Chinese propolis was given to the treatment group. The trial was also not a double-blind trial. Moreover, the effective components in Chinese propolis have not been identified. In addition, a human trial indicated that the long-term use of the artificial antioxidant *RRR*-α-tocopheryl acetate may be harmful to patients with diabetes [66]. Propolis is rich in natural antioxidants such as polyphenols. Considering the biochemical effects of artificial antioxidants and natural antioxidants, further studies should be done to discern any possible harmful effects of Chinese propolis consumption.

CONCLUSION

In summary, this study demonstrates that Chinese propolis is effective at increasing serum antioxidant parameters such as GSH, flavonoid, and polyphenol levels in T2DM patients.

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Compliance with Ethics Guidelines. The protocol was approved by the Ethics Committee of Tianjin Institute of Health and Environmental Medicine (TIHE-TY-20130428). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all patients before they were included in the study.

Data Availability. The datasets obtained and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contribution Weina Gao and Lingling Pu conceived and designed the experiments. Weina Gao, Lingling Pu, Jingyu Wei, Zhanxin Yao, Yawen Wang, Tala Shi, and Liting Zhao performed the experiments. Weina Gao, Liting Zhao, and Changya Jiao analyzed the data. Weina Gao, Lingling Pu, and Changjiang Guo prepared the manuscript.

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