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Quality Analysis of Buckwheat, Corn Whiskers, Jerusalem Artichoke Formula Teas Glycolipid Metabolism Cell Model Study

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Abstract In this study, the content of water, water extract, total ash, fat, amino acids, proanthocyanidins, flavonoids and trace elements was determined in the formula tea composed of buckwheat, corn whisker and Jerusalem artichoke, and the effect of formula tea extract on glycolipid metabolism of *HepG2* cells was evaluated by cck-8 staining absorbance (OD value), oil red O staining, triglyceride (TG) value, glucose content and liver glycogen content by *HepG2* cell fat accumulation model. Reveal its effects on blood glucose value, blood lipids and related mechanisms. The nutrient test results show that the medicinal tea product meets the green tea bag tea standard GB/T24690-2018, and is rich in fat, amino acids, proanthocyanidins, flavonoids, total sugars, proteins and trace elements. The results of the glycolipid metabolism cell model showed that there was no significant difference in the survival rate of *HepG2* cells after the treatment of this formula tea compared with the control, indicating that there was no obvious killing effect on cells. In addition, the tea soup extract had obvious hypoglycemic and lipid-lowering effects on *HepG2* cell models, and could promote the conversion of glucose into hepatic glycogen, but had no significant effect on the content of triglycerides in cells. This formula tea can be used as a daily drink for diabetics, effectively regulating blood sugar and improving their quality of life.

Keywords: *formula tea, quality analysis, glycolipid metabolism, fat, total free amino acid*

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1. Introduction

Diabetes mellitus is a group of carbohydrate, protein, and fat metabolism disorders caused by absolute or relative insufficient or relative insulin secretion or utilization disorders, with hyperglycemia as the main marker [1]. According to the report of the International Diabetes Federation, the global prevalence of diabetes in people aged 20-79 years was 10.5% (536.6 million people) in 2021 and increased to 12.2% (783.2 million people) by 2045, with the highest prevalence in people aged 75-79 years old, and the prevalence of diabetes in China rose to 11.9%, of which the prevalence of diabetes in the elderly was as high as 30.2% [2,3,4]. Diabetes can cause vascular and nerve lesions, and various complications [5], and will also cause infections that are difficult to heal and wounds that are difficult to heal Treatment of multiple diseases is difficult, and high or low blood sugar during diabetes treatment can also lead to direct death. Among them, the core pathogenesis of Type 2 diabetes mellitus (T2DM) is insulin resistance (IR) and islet β cell dysfunction. Insulin resistance (IR) is a pathological condition that causes hyperinsulinemia and impaired glucose tolerance due to the inability of peripheral tissues (eg, skeletal muscle, liver and fat) to effectively respond to normal insulin cycle concentrations, and is the central link of glycolipid metabolism disorders [6], which in turn causes fat metabolism disorders. HepG2 cells are derived from human hepatic embryonic tumor cells, retain the basic biological characteristics of normal liver cells, and their surface expression of high-affinity insulin receptors regulate glucose uptake, glycogen syntheses activity, lipid production, etc., because of its relatively stable culture environment, less influencing factors, easy control of experimental conditions and short experimental period, etc., can ideally simulate the glucose metabolism environment in vivo, is an ideal cell model for studying insulin resistance [7].

It is one of the traditional treatments of traditional Chinese medicine and has a long history in China by

taking special drinks made from tea or traditional Chinese medicine with therapeutic effects. In this study, the quality analysis and in vitro cytology glycolipid metabolism experimental study of the formula tea products with hypoglycemic effect developed by the project team were carried out, and the nutrients, such as moisture, water extract, total ash, fat, amino acids, proanthocyanidins, flavonoids, trace elements, etc., were analyzed, and HepG2 cells were used as a research model to analyze the effects of formula tea soup extract on the content of triglycerides, glucose and liver glycogen in HepG2 cells, reveal the mechanism of their effects on blood glucose and blood lipids, and provide theoretical support for the development of medicinal tea products.

2. Materials and Methods

2.1. Test medicinal Tea

The formula tea for this study was developed by the medicinal tea research team of the Sorghum Research Institute of Shanxi Agricultural University using the unique natural resources of Shanxi Province and selecting buckwheat, corn whiskers, Jerusalem artichoke and other medicinal and edible Chinese medicinal materials based on the theory of traditional Chinese medicine food therapy.

2.2. Nutrient Content Analysis

The nutritional composition of tea powder and tea soup was determined separately, 2g of tea powder was taken, and the tea soup was steeped in a ratio of 1:50 (tea powder: boiling water) for 20min, and repeated 3 times per treatment. The national standards used for the determination of physical and chemical indexes (moisture, water extract, total ash) and the determination of fat, total free amino acids, trace elements, proanthocyanidins and other nutrients are shown in Table 1. The preparation and content determination of the standard curve of flavonoid refer to the method of [8,9], the method of determining the total sugar content refers to the method of Li Yu et al. [10], and the method of protein content determination in tea powder and tea soup is based on the method of Luo Famei and Zheng Qingmei respectively [11,12].

Table 1. Reference to national standards for nutritional composition testing

Detection index	Reference standard
Moisture	The People's Republic of China National standard GB 5009.3-2016
Water extract	The People's Republic of China National standard GB/T 8035-2013
Total ash content	The People's Republic of China National standard GB 5009.4-2016
Fat	The People's Republic of China National standard GB 5009.6-2016
Total free amino acid	The People's Republic of China National standard GB/T 8314-2013
Trace element	The People's Republic of China National standard GB/T30376-2013
Proanthocyanidin	The People's Republic of China National standard DB12/T 885-2019

2.3.1. In Vitro Cytology Glycolipid Metabolism Experiments

Taken 1 bag of tea (6g) and soaked these in 300ml of boiling water, take the extract solution after 20min, concentrated it by rotary evaporator, and add 500ul to the cell culture medium for HepG2 cell culture, using water as the control. The HepG2 cell fat accumulation model was purchased from the Concorde Cell Bank. CCK-8 and Oil Red O kits were purchased from So label Biotechnology Co., Ltd., and BCA protein quantitative kits were purchased from Thermo Fisher Scientific Co., Ltd.

2.3.2. *HepG2* Cell Culture

HepG2 cell cryopreservation tubes were placed in a 37°C water bath until melted, the cell suspension was transferred to a centrifuge tube containing fresh complete culture medium, cultured overnight at constant temperature, passages when the cells adhered to 80% ~ 90%, observed by inverted microscope, when the cell space increased, retracted nearly round and began to fall off, transfer the cell suspension in a centrifuge tube, centrifuge for 5min, and discard the supernatural. Then add fresh culture medium and blow into a cell suspension, passed these 1:3 to a flask containing fresh culture medium, and placed it in an incubator. Take logarithmic growth phase cells for subsequent experiments.

2.3.3. Detection Method

Cell viability determination (cck-8 method): discard HepG2 cell culture medium, washed 1~2 times with PBS solution, added 1mL of culture medium containing cck-8 (5 mg/mL MTT solution 100μL, 900μL culture medium), and set the blank zero group, continue the culture for 4h, and terminated the culture. The automatic microplate reader detects the OD value at a wavelength of 450nm and calculates the cell viability: cell viability (%) = (A1-A0)/(A2-A0), in the formula, the absorbance of the A0-zeroing group; A1—absorbance of the sample set; A2—The absorbance of the control group. Oil red O staining: suck up HepG2 cell culture medium and add 2mLPBS solution to wash the cells 1~2 times. Added 2 mL of 10% formalin solution and leave at room temperature for 10 min. Changed to a new 10% formalin solution, leave it at room temperature for 1 h, discarded formalin, and washed the cells twice with 2 mL of distilled water. Added 2 mL of 60% isopropanol and leave at room temperature for 5 min, aspirated isopropanol, and carefully blow stem cells at room temperature or with a hair dryer. Added 1mL of oil red O working solution to each well, placed these at room temperature for 10min, discard oil red O, wash the cells with 1mL of distilled water 4 times, and observed the staining with a microscope. After discarding the distilled water, let the cells dry. Added 1mL of 100% isopropanol per well, gently shaked for 10~15min to dissolve the dye completely, zero with 100% isopropanol, and used a spectrophotometer colorimetric at a wavelength of 500nm. Triglyceride (TG), glucose, liver glycogen content determination: discard HepG2 cell culture medium and added 2mLPBS solution to wash the cells 1-2 times. Add 0.25% trypsin-digested cells for 5~10min, suspend the cells, centrifuge at 1000r/min for 10min, discard the

supernatant, and then add 0.2mLPBS, ultrasonic broth under ice water bath conditions to prepare homogenate. The prepared homogenate was determined by TG kit, glucose content by glucose oxidase method, and protein content by BCA protein concentration determination kit. The cell homogenate was precipitated with absolute ethanol overnight, and then the glycogen content was determined by anthrone method to calculated the liver glycogen content.

2.3.4. Statistical Analysis

The above test data was tested by Prism7 software, and the test data were repeated three times. Excel 2010 was used to analyze all the experimental data Analyze of variance (ANOVA) was proceed using DPS7.05 software. SD method was used for multiple comparison (α=0.05).

3. Results

3.1. Effects of Nutrient

The nutritional components of tea powder and tea soup were tested separately, and the test results are shown in Table 2. The test results showed that the formula tea powder had the highest protein content, followed by fat, flavonoids, total sugars, and contained a small amount of amino acids and proanthocyanidins; The content of trace elements was potassium, calcium, sodium, magnesium, manganese and zinc from high to low; In tea soup, fat extract content is the largest, followed by protein, flavonoids, according to the general tea drinking habits, can supplement some daily nutritional elements of diabetics.

Table 2. Results of variance analysis of nutritional composition of hypoglycemic tea

Detection index		Tea powder (%)	Gruel of millet flour and sugar (/100ml)
Fat		8.15±0.22	5.54g±0.13
Amino acid		0.82±0.02	20.96mg±0.08
Proanthocyanidin		0.112±0.014	3.206mg±0.023
Flavone		3.70±0.04	63.0mg±0.80
Total sugar		3.48±0.01	7.04mg±0.03
Protein		25.45±0.33	856.8mg±5.96
Microelement	Potassium (mg/L)	1.59×10^4	326
	Sodium mg/L	2.48×10^3	98.7
	Calcium (mg/L)	3.38×10^3	59.6
	Magnesium (mg/L)	1.40×10^3	39.1
	Zinc (μg/L)	36.4	446
	Manganese (μg/L)	47.5	686
	Selenium (μg/L)	ND	ND

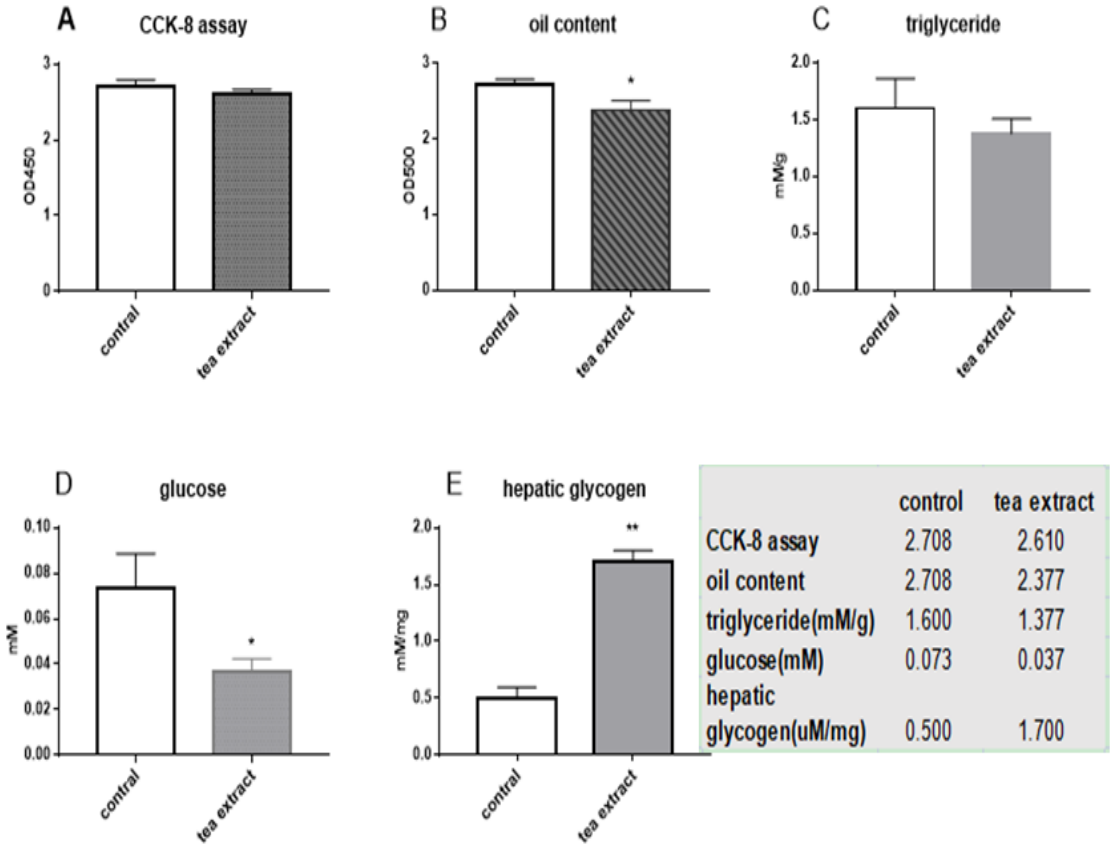

Figure 1. Effect of tea soup extract on glycolipid metabolism in *HepG2* cell model (Note: * indicates a significant difference (P<0.05), ** indicates a very significant difference (*P*<0.01).)

3.2. Analysis of in Vitro Cytology Glycolipid Metabolism Experiment

The results showed that the cell survival rate (OD450) of HepG2 cells treated with tea soup extract was 2.61, compared with 2.7 in the control group, and the OD500 with oil red O staining was 2.377 and 2.708 ($P<0.5$) in the control group. The contents of triglycerides were 1.377 and 1.6mM/g, respectively. The glucose content was 0.0366 and 0.0733mM, respectively, which were decreased by 50.07% ($P<0.5$) compared with the control group. The level of liver glycogen (1.7 uM/mg) were increased 2.4-fold compared with the control (0.5 uM/mg) and was very significantly elevated ($P<0.01$) details are mentioned in (Figure 1).

4. Discussions

Modern medical research shows that buckwheat as raw material, through the combination of traditional technology and modern technology made of drinks, rich in bioflavonoids, chlorophyll, crude protein, trace elements and other nutrients, often drink buckwheat tea can promote the repair of islet β cells, improve glucose tolerance, reduce blood sugar; It can also reduce body fat accumulation, soften blood vessels, promote blood circulation, etc., and also help lower blood lipids and blood pressure [13]. Corn water can improve edema, clear blood fever, and facilitate urine. Corn whisker water can also regulate the effect of glucose metabolism, and the corn whisker polysaccharide contained in it can relieve high blood sugar caused by elevated adrenaline, and can also promote liver sugar in the human body Protosynthesis, accelerate gluconeogenesis. The total flavonoids in corn whisker water can reduce serum low-density lipoprotein cholesterol levels, increase high-density lipoprotein cholesterol, apolipoprotein A content, regulate blood lipids, reduce blood pressure, in addition, corn whisker water has a protective effect on the liver [14]. Jerusalem artichoke has a two-way regulating effect on blood sugar, that is, on the one hand, it can reduce the blood sugar of diabetic patients, and on the other hand, it can increase the blood sugar of hypoglycemic patients. Studies have shown that Jerusalem artichoke contains a substance very similar to the endogenous insulin structure of the human pancreas, and when urine sugar appears in the urine, eating Jerusalem artichoke can control urine sugar, indicating that it has the effect of lowering blood sugar. When people have low blood sugar, eating Jerusalem artichoke can also relieve blood lipids and lower blood pressure [15,16]. Established 50 diabetic mouse models and divided them into high, medium and low dose groups of Jerusalem artichoke protein, and found that the blood glucose values of the high and medium dose groups were reduced, and the low dose group had no hypoglycemic effect. The formula tea used in this experiment is composed of tartary buckwheat, corn whisker, Jerusalem artichoke and other medicinal and food homologs, which has the effect of lowering blood sugar and lipid.

Nutritional composition analysis with a tea-to-soup ratio of 1:50 found that nutrients such as protein, fat, free amino acids, and proanthocyanidins could effectively supplement the nutritional needs of diabetic patients and improve body weight and blood sugar in diabetic patients [17]. In addition, tea soup contains a certain amount of trace elements, which as important oxidants or antioxidants of the body, play an important role in the occurrence and development of diabetes and related chronic complications.

Zinc plays an important role in the biosynthesis of insulin, in the presence of zinc ions, insulin and pre-insulin dimer can combine zinc ions to synthesize hexamer, hexamer can be considered to be the most basic form of pre-insulin to insulin, zinc can promote the synthesis and stability of hexamer, can make proinsulin effectively synthesize insulin; Magnesium is an important regulator of a variety of enzymes, and magnesium can affect the activity of insulin β subunit tyrosine kinase in peripheral tissues, thereby affecting glucose uptake in peripheral tissues, and plays an important role in the process of insulin resistance [18]. Diabetics take this formula tea as a daily reference tea, which can timely supplement the above trace elements, thereby regulating the body's blood sugar balance. As an important organ of glycolipid metabolism, the liver can sense and integrate nutrients, hormones and other stimuli, which plays a vital role in the balance of glycolipid metabolism in the body. In the fasted state, glucagon from pancreatic α cells and glucocorticoids from the adrenal glands can activate hepatic gluconeogenesis and fatty acid oxidation, providing energy supply; After eating, insulin is released from the pancreas β cells and reaches the liver cells, inhibiting gluconeogenesis and promoting glycogen synthesis and new fat production. However IR often leads to excessive activation of gluconeogenesis and lipogenesis, resulting in hyperglycemia, hepatic steatosis, and hyperlipidemia, which are typical symptoms of type-II diabetes. Therefore, in this experiment, the glycolipid metabolism effect of formula tea was evaluated by using in vitro *HepG2* cell fat accumulation model to test its efficacy. The test results showed that there was no significant difference in the survival rate of *HepG2* cells after treatment with this formula tea compared with the control (Figure 1A), indicating that there was no obvious killing effect on cells. In addition, the tea soup extract had obvious hypoglycemic and lipid-lowering effects on *HepG2* cell models (Figure 1B, Figure 1D), and could promote the conversion of glucose to hepatic glycogen (Figure 1E), but had no significant effect on the content of intracellular triglycerides (Figure 1C).

5. Conclusion

This formula tea can be used as a daily diet for diabetic patients, so as to effectively regulate blood sugar, smoothly and comfortably reduce postprandial blood sugar values, fundamentally improve the symptoms of diabetes, and improve the quality of life of diabetic patients.

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