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Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves

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ABSTRACT

Ethnopharmacological relevance: *Nelumbo nucifera* Gaertn. leaves have been used as medicinal herbs in the past 1300 years, specifically utilized to cure hyperlipidemia, hyperglycemia, and obesity. It has been recorded in the most famous medicinal book in China for more than 400 years. The present study aims to identify the potential therapeutic activities of the flavonoids isolated from *Nelumbo nucifera* leaves.
Materials and methods: *Nelumbo nucifera* leaf flavonoids (NLF) were tested for the inhibition of lipase, α-glucosidase, and α-amylase activities *in vitro*. A single dose of NLF was administered by oral gavage in mice for acute toxicity. Wistar rats with high-fat diet-induced hyperlipidemia and two other animal models were used to evaluate the hypolipidemic effects of NLF.
Results: Our *in vitro* biochemistry tests revealed that the NLF showed high inhibitory activity against porcine pancreatic lipase, α-amylase, and α-glucosidase with IC_{50} values of 0.38 ± 0.022, 2.20 ± 0.18, and 1.86 ± 0.018 mg/mL, respectively. Furthermore, the NLF significantly lowered the lipid components, such as the total cholesterol, triglycerides, low-density lipoprotein cholesterol, and malondialdehyde, in various established *in vivo* systems and raised the high-density lipoprotein cholesterol. Moreover, the NLF alleviated high-fat diet-induced lipid accumulation in the liver.
Conclusions: The results demonstrate that NLFs can effectively ameliorate hyperlipidemia and inhibit the key enzymes related to type 2 diabetes mellitus. Our findings may provide new pharmacological basis for the treatment of hyperlipidemia, hyperglycemia, and obesity using NLFs.

1. Introduction

Previous studies indicate that the excessive intake of calories can lead to many chronic diseases, such as type 2 diabetes mellitus (T2DM), obesity, hyperlipidemia, and cardiovascular diseases (Etoundi et al., 2010). Among them, obesity is the most common complication of hyperlipidemia and T2DM and they seem to share common causative factors, chemical abnormalities, and clinical complications (John, 1993; Shamseddeen et al., 2011). Hyperlipidemia significantly contributes to the manifestation and development of atherosclerosis (AS) and coronary heart diseases (CHDs), which are the most common causes of morbidity and mortality in the United States and worldwide (Yokozawa et al., 2003; Philippe and Daniel, 2004). Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia and disturbances in carbohydrate, fat, and protein metabolisms, resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include the long-term damage, dysfunction, and failure of various organs (World Health Organization, 1999). To date, about 5% of the global population is affected by this disease (World Health Organization, 2002). The prevalence of T2DM is increasing annually and is expected to rise above 300 million by 2025 (Bailey and Day, 2004).

Nelumbo nucifera Gaertn., commonly known as lotus, is an aquatic plant belonging to the family Nelumbonaceae and widely

Abbreviations: *A*, absorbance; AI, atherogenic index; ALT, glutamate pyruvate transaminase; AST, aspartate aminotransferase; BW, body weight; *C*, concentration; CHDs, coronary heart diseases; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; CVD, cardiovascular disease; AS, atherosclerosis; DNPB, 2,4-dinitrophenyl butyrate; HFD, high fat diet; HDL-C, high-density lipoprotein-cholesterol; IAEC, Institutional Animal Ethical Committee; LDL-C, low-density lipoprotein-cholesterol; KM, Kun Ming; MDA, malondialdehyde; NLF, *Nelumbo nucifera* leaf flavonoids; OECD, Organization for Economic Cooperation and Development; pNPG, p-nitrophenyl-α-D-glucopyranoside; PPL, porcine pancreatic lipase; PPB, potassium phosphate buffer; T2DM, type 2 diabetes mellitus; TG, triglyceride; TC, total cholesterol; ZBT, Zhi-bi-tuo.

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distributed in China, India, and Japan. Its roots, seeds, and young leaves are usually used as vegetables in Asia (Sridhar and Bhat, 2007). The *Nelumbo nucifera* was first recorded in the oldest book of Chinese Poetry(1100-600 BC): The She King (James, 1991), and its medicinal function, obtained through the leaves, was first recorded in the Tang Dynasty (618–907 AD) by Meng Shen (Xie, 1984). As a traditional Chinese medicine, the *Nelumbo nucifera* leaf has demonstrated many uses, such as for cleaning heat, resolving summer heat, and stopping bleeding (Bensky et al., 2004), and has also been used as an effective drug for the treatment of hematemesis, epistaxis, hemoptysis, hematuria, and metrorrhagia (Ou, 1989). The use of lotus leaf for the treatment of hyperlipidemia, hyperglycemia and obesity was first recorded during the Ming Dynasty (1368–1644 AD) by Dai Sigong (Dai, 1955). Furthermore, the Compendium of Materia Medica also declared that the lotus leaf exhibits "Qu Yu Xue Liu Hao Xue" (cleaning the deposit and dredging vessels), anti-obesity, hypolipidemic, and anti-AS activities (Tao et al., 2001). Hence, the porridge and beverages made from lotus leaf are becoming increasingly popular. The main functional compositions of lotus leaves are flavonoids and alkaloids, and many of them have been identified, including isoquercetin, hyperin, kaempferol, astragalin, and myricetin (Shoji et al., 1987; Elegami et al., 2003). Moreover, the antioxidant and hepatoprotective effects (Huang et al., 2010) and the alleviation of high-fat diet (HFD)-induced hepatic injuries and oxidative stress (Lin et al., 2009) exhibited by *Nelumbo nucifera* leaf extracts have been reported. However, to the best of our knowledge, the lotus leaf components that mainly control these effects have not been investigated. In the present study, the total flavonoid content was isolated from lotus leaves, their potential therapeutic activities of hyperlipidemia, hyperglycemia and obesity were tested, and their safety was confirmed.

2. Materials and methods

2.1. Chemicals

Porcine pancreatic lipase (PPL, type II ≥100 U/mg), yeast α-glucosidase (≥10 U/mg), α-amylase (≥250 U/mg), and orlistat (≥98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acarbose (BJ05882) was purchased from Bayer & Co., Inc. (Beijing, China). Simvastatin (09412) was purchased from Merck & Co., Inc. (Hangzhou, China). Rutin (99.8%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals used for the analyses were of AnalaR grade and obtained from the Chinese Medicine Group of the Shanghai Chemical Reagent Corporation (Shanghai, China). Cholesterol and cholic acid were obtained from the Beijing Chemical Reagent Corporation (Beijing, China).

Zhi-bi-tuo (1008012, ZBT), a generally accepted clinical drug for preventing hyperlipidemia and fatty liver disease in China, was adopted as the positive control in the HFD-induced hyperlipidemia experiment and was purchased from the Chengdu Di'ao Pharmaceutical Group Co., Ltd. (Chengdu, China). The effective components of ZBT are well-known traditional Chinese medicines, with a monascus content of over 90%.

2.2. Plant materials

Nelumbo nucifera Gaertn. (family Nelumbonaceae) leaves were collected at the full-leaf stage in August 2010 in Wuhan City and authenticated according to the Pharmacopoeia of the People's Republic of China (2010) by the corresponding author (Wuhan University, Wuhan, China) (Chinese Pharmacopoeia Commission, 2010). A voucher specimen (no. 809) was deposited at the Institute of Traditional Chinese Medicine and Natural Products, School of Pharmaceutical Sciences, Wuhan University, China.

2.3. Crude extract preparation

The total flavonoid content from air-dried, powdered (less than 0.25 mm) *Nelumbo nucifera* leaves (20 kg) were extracted using 70% ethanol (200 L) for 90 min at 80 °C. The solvent was evaporated under reduced pressure to 10 L. The extract was absorbed with a macro resin (D_{101}, 40 kg), which was then washed with water (50 L), 15% and 50% alcohol (200 L) in succession. The 50% alcohol eluent was evaporated under reduced pressure to 8 L, extracted using ethyl acetate (8 L, × 10), then pooled the ethyl acetate fraction together, and dried to obtain the *Nelumbo nucifera* leaf flavonoids (NLF) as a powder.

2.4. Total flavonoid contents determination

The total flavonoid content of the extract was determined by colorimetric assay (Moreno et al., 2000). Briefly, 0.1 g NLF was diluted with 80% aqueous ethanol to 1.0 mL. Subsequently, a 0.5 mL aliquot was added to test tubes containing 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M aqueous potassium acetate, and 4.3 mL of 80% ethanol. After incubation for 40 min at room temperature, the absorbance was determined at 415 nm using an ultraviolet (UV)–visible spectrophotometer (Shimadzu UV-1700, Japan). A calibration curve was generated using rutin as reference compound. The results were expressed as milligrams of rutin equivalents per gram of dry extract. The total flavonoid content of the tested sample was calculated using the following formula: $A = 35.23C - 0.004$, where A is the absorbance, C is the concentration (mg/mL), 35.23 is the slope, and −0.004 is the y-intercept.

2.5. Animals and diets

Adult male Wistar rats (180–200 g) and Kun Ming (KM) male mice (18–22 g) were purchased from the Hubei Research Center as laboratory animals. The animals were housed in a semibarrier system under controlled room temperature (23 ± 1 °C) and humidity (55 ± 5%) with 12 h light and dark cycles. Diet and water were provided *ad libitum*. Normal laboratory feed was prepared by mixing calculated amounts of 45% corn meal, 25% wheat flour, 10% milk powder, 5% cooking oil, 13% millet husk, 1% salt mixture, and 1% multivitamin tablets. The HFD consisted of well-mixed 1% cholesterol, 0.5% cholic acid, 10% cooking oil, 10% egg yolk, and 78.5% normal laboratory feed. The study received clearance from the Institutional Animal Ethical Committee of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Wuhan University, China.

2.6. PPL inhibition assay

The ability of the test extracts to inhibit PPL was determined using the method described previously (Zheng et al., 2010) with slight modifications. 2,4-Dinitrophenyl butyrate (DNPB) substrate was synthesized using the method described by Mosmuller et al. (1992). Briefly, 5 mg/mL of PPL stock solutions were prepared in 0.1 mM potassium phosphate buffer (PPB, pH 6.0) and stored at −20 °C. Extracts (0.5 mL) or orlistat with 0.5 mL enzyme in PPB (0.1 mM, pH 7.2, 1% Tween 80) were pre-incubated at 30 °C for 1 h. Subsequently, 7.5 mL of PBB and 0.5 mL of 1 mM DNPB were added. After incubation for 5 min at 30 °C, the supernatant was measured at 360 nm using a UV–visible spectrophotometer. The inhibitory activity was calculated as follows: Inhibition (%) = $[1-(A_{sample}/A_{control})] \times 100$. The IC_{50} values were calculated by logarithmic regression analysis.

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