香港浸會大學
HONG KONG BAPTIST UNIVERSITY

HKBU Scholars

DOCTORAL THESIS

Anti-obesity effects of flavonoids and saponins from adzuki bean

Liu, Rui

Date of Award:
2014

Link to publication

General rights
Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

Download date: 04 Feb, 2023

Abstract

Overweight and obesity are becoming a major public health issue. Pancreatic lipase is a key enzyme to catalyze the hydrolysis of 50-70% of dietary fat in the digestive system. Inhibition of pancreatic lipase activity can block fat absorption in gastrointestinal tract, further control obesity incidence. On the other hand, α-glucosidase is also a key enzyme to hydrolysis polysaccharides and disaccharides into glucose in small intestine. Inhibition of α-glucosidase activity can block digestion and absorption of carbohydrates, further control metabolism disorders such as diabetes and obesity. Food legumes are widely used in people's life with multiple pharmacological activities. The author extracted total phenolics and saponin components from 13 commonly consumed food legumes produced in China, did a systematic comparative study investigating their inhibitory effects against digestive enzymes (pancreatic lipase and α-glucosidase), and screened adzuki bean (*Vigna angularis* L.) as the further target bean. The results showed that the different concentrations of total phenolic extract from adzuki bean (0.25, 0.5, 0.75, 1 mg/mL) inhibited hydrolysis of triolein about 24.1, 24.0, 30.7, and 36.3%, respectively, while different concentrations of total saponin extracts from adzuki bean (0.25, 0.5, 0.75, 1 mg/mL) inhibited hydrolysis of triolein about 15.9, 23.5, 30.1, and 29.2%, respectively. On the other hand, phenolic extract of adzuki bean at the concentration from 0.25 to 1 mg/mL exhibited much more than 80% α-glucosidase inhibitory activity, while saponin extract of adzuki bean exhibited 56.4 to 68.7% α-glucosidase inhibitory activity. Based on the results of pancreatic

lipase inhibitory activity, at the concentration of 1 mg/mL, phenolics extract and saponin extract of adzuki bean (mean 32.5%) and pinto bean (mean 27%) had the stronger inhibitory effects. Moreover, phenolics extract and saponin extract of adzuki bean (mean 79.25%) and pinto bean (mean 72.85%) also had the stronger inhibitory effects on α-glucosidase activity. In addition, adzuki bean is widely used and has diverse application in foods and drugs. In a word, it indicated that adzuki bean is one of the best target beans to further study anti-obesity and anti-diabetes effects via cell and animal models.

Flavonoids and saponins in adzuki bean were obtained and characterized by high performance liquid chromatography with diode array detection and electro spray ionization-tandem multi-stage mass spectrometry in succession. Among 15 compounds identified, four flavonoids (catechin, vitexin-*4″-O*-glucoside, quercetin-*3-O*-glucoside, and quercetin-*3-O*-rutinoside) and six saponins (azukisaponin I, II, III, IV, V, and VI) in adzuki bean were further quantified by external calibration method using high performance liquid chromatography mass spectrometry with the program of time segment and extract ion chromatogram analysis.

Animal model is a good way to intuitively evaluate the anti-obesity effect of adzuki bean. So the anti-obesity effects of adzuki bean in mice fed with a high-fat diet was investigated. ICR female mice were fed with a high fat diet administrated orally with different doses of adzuki bean extracts for eight weeks. Total extract, flavonoids and saponins of adzuki bean enhanced lipolysis (166.1%, 175.6%, and

152.6%, respectively). Compared to the final body weight (33.6 g) of the high-fat diet group, oral administration (300 mg/kg per day) of total extract, flavonoids and saponins of adzuki bean significantly reduced the final body weight of mice, and significantly decreased the adipose tissue accumulation. The adzuki bean intervention also significantly reduced the levels of serum triglyceride, total cholesterol, low density lipoprotein-cholesterol, and liver lipid. Adzuki bean demonstrated the anti-obesity effects on mice fed with a high fat diet, such effects may mediated through the inhibitory effects of flavonoids and saponins from adzuki bean on α-glucosidase and pancreatic lipase activities, and lipolysis enhancement effect of active components from adzuki bean.

Obesity is characterized biologically at the cellular level by as an increase in the number of preadipocutes and an increase in the size of adipocytes differentiated from preadipocytes in adipose tissue. 3T3-L1 cell model was used to reveal the mechanism of anti-obesity effects of adzuki bean in the present study. The results showed that adzuki bean total extract, adzuki bean flavonoids, adzuki bean saponins, four mono flavonoides (catechin, vitexin-4″-*O*-glucoside, quercetin-*3*-*O*-glucoside, and quercetin-*3*-*O*-rutinoside) and six mono saponins (azukisaponin I, II, III, IV, V, and VI) exhibited inhibitory effect of proliferation of 3T3-L1 cells, and the inhibitory rate of proliferation of 3T3-L1 cells was about 20%. Mature adipocytes were stained by Oil Red O and the lipid accumulated exhibited red color, while the preadipocytes could not been stained. The data of the effects of adzuki bean samples on lipid accumulation during the differentiation

period of 3T3-L1 cells revealed that azukisaponin II had the highest inhibitory effect (49.72%) with a dose dependent manner from 50 to 500 μg/mL. It indicated that adzuki bean may block the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes which contribute to anti-obesity effects. Most of adzuki bean samples significantly blocked the triglyceride accumulation and enhanced lipolysis by increseaing the released of glycerol during differentiation of 3T3-L1 cell. Additionally, adzuki bean samples except catechin significantly inhibited glycerol-3-phosphase dehydrogenase activity in 3T3-L1 cells, which indicated that adzuki bean samples had the ability to inhibit triglyceride synthesis.

Real-time polymerase chain reaction and Western blot methods were used to investigate the intervention effects of adzuki bean total extract, adzuki bean flavonoids, adzuki bean saponins, quercetin-*3*-*O*-rutinoside, and azukisaponin II isolated from adzuki bean on the related gene expressions. These adzuki bean samples inhibited differentiation of 3T3-L1 cell by decreasing the expression of peroxisome proliferator-activated receptor γ and CCATT/enhancer-binding protein α (major adipocyte transcription factors) at messenger ribonucleic acid level and protein level in adipocytes. Additional, they inhibited adipogenesis by decreasing the expression of fatty acid synthase, promoting lipolysis by increasing the expression of hormone-sensitive lipase and adipose triglyceride lipase, made energy balance by regulating the expression of leptin, adenosine monophosphate -activated protein kinase, and tumor necrosis factor α, and induced cell apoptosis by increasing the expression of bax and decresing the expression of B-cell

lymphoma 2. These findings provide insight into the molecular mechanisums through regulation of the related gene expression in 3T3-L1 adipocytes. It also suggested that adzuki bean had a positive effect in prevention and treatment of adipogenesis-related obesity, and might be a good option of functional foods to control obesity.

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my supervisor Prof. Zongwei CAI, and my co-supervisor Dr. Baojun XU for their invaluable advice and support both in lab and life. They provide me a lot of opportunity to explore and develop my potentials in scientific inquiry. Their guidance, inspiration, and continued encouragement are greatly appreciated.

I also would like to thank professors in food science program at UIC for their sincere help to improve this work, which greatly enhanced my understanding on the subjects in theoretical view of experiments.

My sincere thanks must go to my group members for their continued support and helpful discussion.

I also would like to show my appreciation to Prof. Yinan ZHENG and Prof. Wencong LIU for their help in chemical experiments and animal studies.

At last, I also would like to thank my family for their love, support and encouragement.

Table of Contents