Gooseberry Anthocyanins Alleviates Insulin Resistance by Regulating Ceramide Metabolism in High Fat Diet Mice

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Research Article

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Abstract

In order to explore the effect and mechanism of Gooseberry anthocyanins (GA) on reducing the ceramide content to reduce Insulin Resistance (IR) in mice. Molecular docking, Real-Time PCR and Western Blotting were used to explore the molecular mechanism and the effects of different doses GA in high-fat diet (HFD) mice. The results showed that the binding energy of anthocyanins on CerS6 was in the range of -8.2 to 5.2 kcal/mol, with low energy parameters and good binding positions. GA could effectively inhibit visceral fat, liver index, TC, TG and LDL-C, and improved HDL-C, GSH-Px and SOD. GA decreased the level of Insulin sensitivity index from -5.15 to -4.54 and improved insulin sensitivity and insulin resistance in HFD mice. Real-Time PCR and Western blotting results showed that GA could reduce mRNA levels of inflammatory factors IL-6 and TNF-α, inhibit the expression of CerS6, PKCζ, PPARγ, CD36 and enhance the expression of SphK2, Akt, p-Akt/Akt, ISR. This study revealed the molecular mechanism of improving insulin resistance of GA in S1P/Cer signaling pathway, and provided theoretical support for preventive treatment of obesity related diseases by polyphenols.

Introduction

Fat leads to IR in liver, abnormal accumulation of fat in liver tissue can increase TC and TG; Obesity can lead to chronic inflammation, further induce IR[1].

Studies showed that IR can cause many cardiovascular diseases, especially atherosclerosis, coronary heart disease (CHD), etc, and cardiovascular disease is one of the most important complications of diabetes. Research showed that ceramide accumulation is linked to obesity, and the ceramide was proved to be related to insulin resistance[2]. IR is referring to insulin sensitivity decreased, and the body secretes more insulin in order to regulate blood glucose at normal levels[3]. Mainly manifested as: obesity; damage or sugar tolerance is reduced; serum triglyceride increases and high density lipoprotein cholesterol decreased; atherosclerosis Temperature and hyperuricemia, etc[4]. Insulin signal transduction plays an extremely important role in insulin to play a physiological effect, transduction can reduce the physiological effects of insulin, thereby inducing IR[5].

The gooseberry variety is Pixwell, is a Filial generation, the female parent is a European and American hybrid-Oregon, and the male parent is an American species (Ribes missouriense)[6]. The fruit is purple-red when fully mature. Its fruit contains rich nutrients, contains rich amino acids, vitamins, polyphenol ketones. The main monomer components of GA are: delphinidin 3-O-rutinoside and cyanidin 3-O-rutinoside. Studies showed that anthocyanins have a variety of biological activities, such as anti-tumor[7], anti-virus, anti-oxidation[8], anti-inflammation, improving metabolic disorders[9], inhibit the proliferation of hepatocellular carcinoma cells[10], inhibit colon cancer[11], age-related neurodegenerative diseases, lowering blood pressure[12], regulating blood lipids[13], improving insulin resistance, effectively delay the aging of mice[14] and so on. It has certain curative effect on cardiovascular disease, diabetes and other chronic diseases. In the investigation, we found that gooseberry has good ability of anti-oxidation, anti-inflammation and scavenging free radicals, preventing osteoporosis, lowering blood pressure and
protecting radiation and cadmium[15–17]. Gooseberry fruit powder and fruit juice have significant effects on the prevention of alcoholic fatty liver and non-alcoholic fatty liver[18]. Gooseberry showed good antioxidant and anti-inflammatory ability in the previous pre-experiment. It is considered that gooseberry has a good development potential and further explore the mechanism of gooseberry in reducing IR in HFD mice. Gooseberry is an excellent variety introduced by Liaoning Province, China. Its yield is very high in the cold weather. The development of gooseberry resources will promote the cultivation of berries and promote the common development of berry agriculture and food industry.

The purpose of this study is to identify and clarify the mechanism of GA alleviates IR by reducing ceramide in HFD mice. The mice with HFD induced by High-fat emulsion were used as models to study the effect of GA on HFD and its possible mechanism. This provides theoretical support for the treatment of obesity-related diseases in humans and animals.

Materials And Methods

2.1 Materials and reagents

Gooseberry fruit provided by Liaoning Academy of Forestry (Shenyang, China). The extraction process of anthocyanin refers to the method used in our laboratory and the anthocyanins were isolated as pure compounds (content > 95%): delphinidin 3-O-rutinoside and cyanidin 3-O-rutinoside. Chloral hydrate was purchased from Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China). All other chemicals and solvents used in the experiment were analytical grade.

2.2 Animals and treatments

Six-week-old healthy male Kunming (km) mice, weighing 33 ± 2g, were provided by Liaoning Changsheng Biotechnology Co., Ltd. (Beijing, China). [approval number: SCXK2019-0001]. 50 male Kunming mice were randomly divided into 5 groups: normal control group (CTL); high fat diet group (HFD), The formula of high-fat emulsion (100g) was shown in Table 1; high dose GA group (GA 40 mg/kg, HGA); low dose GA group (GA 20 mg/kg, LGA); and positive control group (EGCG 20 mg/kg). GA and EGCG were given to mice by intragastric administration. The entire modeling process lasted 2 months. During the eight-week experiments, the high-fat emulsion was given to mice by the gavage method, and the dose of intragastric administration was 0.2ml/10g. The body weight and blood glucose levels of the mice were recorded once a week, and observe their disease status every day. After the dry expectation, the mice fasted overnight, anesthetized by intraperitoneal injection of 0.1ml of 10% chloral hydrate, and then killed by spinal dislocation. The whole animal experiment process was completed in the Animal Biosafety Laboratory of College of Life Sciences, Liaoning University.
Table 1
Experimental mice high-fat emulsion formula.

<table>
<thead>
<tr>
<th>High-fat emulsion formula</th>
<th>content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard</td>
<td>25g</td>
</tr>
<tr>
<td>Tween-80</td>
<td>25ml</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10g</td>
</tr>
<tr>
<td>Propylthiouracil</td>
<td>1g</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>20ml</td>
</tr>
<tr>
<td>Sodium deoxycholate</td>
<td>2g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>30ml</td>
</tr>
</tbody>
</table>

2.3 Molecular Dynamic Simulations (MD) and Docking

The AutoDock vina1.1.2 software package was used for MD simulation. The crystal structure of Human Ceramide synthase 6 (CerS6) which used for this study was downloaded from the Uniprot (https://www.uniprot.org/). MGL Tools 1.5.6 software was used for molecular processing of pre-docking receptors and ligands. AutoDock vina1.1.2 was used to study the interaction between anthocyanins and CerS6. The three-dimensional structure of anthocyanins were downloaded from Pubchem-Open Chemistry Database (pubchem.ncbi.nlm.nih.gov/substance). AutoDockTools-1.5.6 software was used to prepare CerS6 and anthocyanin molecules before docking. The docking analysis of this research used Lamarckian genetic algorithm in the AutoDock docking method[19].

2.4 Serum lipid index

Serum insulin secretion were measured by mouse insulin kit; TC, TG, HDL-C and LDL-C were measured by TC, TG, HDL-C and LDL-C Kit, using the Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China). The experiment was repeated three times to avoid errors.

2.5 Oxidative stress related enzyme indicators

The activities of serum SOD by SOD kit and GSH-Px by GSH-Px kit (Dingguo Biotechnology Co., Ltd. (Beijing, China) were measured to reflect oxidative stress. The experiment was repeated three times to avoid errors.

2.6 Insulin sensitivity index and resistance index

Fasting insulin (FINS) level and fasting blood glucose concentration (FPG) were measured, cut off food for 8 hours ahead of time. Serum insulin levels were measured using the ELISA kit (Dingguo Biotechnology Co., Ltd. (Beijing, China) and blood glucose levels were measured by microglucometer. Insulin resistance index was expressed by Homeostasis model assessment-Insulin Resistance (HOMA-IR)
of steady-state model. Insulin sensitivity index (ISI) and HOMA-IR were calculated by the following
formula[20].

\[
\text{HOMA-IR} = \frac{\text{FINS(mIU/L)} \times \text{FPG(mmol/L)}}{22.5}
\]

\[
\text{ISI} = \ln\left[\frac{1}{\text{FPG(mmol/L)} \times \text{FINS(mIU/L)}}\right]
\]

2.7 Histopathological examination of Liver Tissues

Liver tissues fixed with 4% formaldehyde solution for 8 hours. Tissues were paraffin and sectioned, then
stained with hematoxylin and eosin (H&E)[21]. Tissue sections were observed and photographed under
microscope.

2.8 RNA extraction and Real-Time PCR

The primer design and Real-Time PCR were based on Primer 5.0 software and shown in Table 2. Total
RNAs were extracted with Trizol (Tiangen Biotech (Beijing) Co., Ltd.) reagent and purified. Refer to the
reaction instructions to synthesize cDNA[22, 23]. Then PCR amplification was performed.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Upstream primer</th>
<th>Downstream primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>5’TAGTCCTTCCCTACCACCACATTCC-3’</td>
<td>5’TTGGTCTTAAGCCACTCCTTC-3’</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5’-CCCTCACACTCAGATCTTCTTC-3’</td>
<td>5’-GCTACGACGTGGGCTACAG-3’</td>
</tr>
<tr>
<td>β-actin</td>
<td>5’-AGGCAAAACCGTGAAAAGATG-3’</td>
<td>5’-AGGCAAAACCGTGAAAAGATG-3’</td>
</tr>
</tbody>
</table>

2.9 Western blotting analysis

The protein samples were separated with 10% SDS-PAGE and transferred to nitrocellulose membrane[24].
Tris buffered saline solution containing Tween-20 (Beijing Solarbio Technology Co., Ltd, China) was
added to 5% skimmed milk to block the membrane, then added the primary antibody and incubated
overnight at 4°C (dilution factor and protein molecular weight was shown in Table 3), and washed the
membrane with 1×TBST for three times, 10 minutes each time. Added the secondary antibody (dilute the
secondary antibody with 5% BSA solution) and incubated for 1.5 hours at room temperature. Washed the
membrane 3 times with 1×TBST, 10 min each time. Expose under the exposure machine, used Image J
software to analyze the gray value.
Table 3
Dilution factor of protein.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution factor</th>
<th>protein molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti- CerS6 antibody</td>
<td>1: 500</td>
<td>44KDa</td>
</tr>
<tr>
<td>Anti- PKCζ antibody</td>
<td>1: 500</td>
<td>80KDa</td>
</tr>
<tr>
<td>Anti- Akt antibody</td>
<td>1: 1000</td>
<td>60KDa</td>
</tr>
<tr>
<td>Anti- p-Akt antibody</td>
<td>1: 1000</td>
<td>60KDa</td>
</tr>
<tr>
<td>Anti- SphK2 antibody</td>
<td>1: 300</td>
<td>69KDa</td>
</tr>
<tr>
<td>Anti- PPARγ antibody</td>
<td>1: 500</td>
<td>57KDa</td>
</tr>
<tr>
<td>Anti- CD36 antibody</td>
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</tr>
<tr>
<td>Anti- ISR antibody</td>
<td>1: 500</td>
<td>33KDa</td>
</tr>
<tr>
<td>Anti-β-actin antibody</td>
<td>1: 2000</td>
<td>42KDa</td>
</tr>
<tr>
<td>HRP GoatAnti-Mouse IgG</td>
<td>1: 5000</td>
<td>-</td>
</tr>
<tr>
<td>HRP GoatAnti-Rabbit IgG</td>
<td>1: 5000</td>
<td>-</td>
</tr>
</tbody>
</table>

2.10 Statistical Analysis

Statistical processing results were analyzed with statistical software (GraphPad Prism 8.3.0). Data were tested for differences between groups using T-test. When P > 0.05, there is no significant difference in the results; when P < 0.05, P < 0.01 or P < 0.001, the result was considered significant.

Results

3.1 Computational analysis of the binding between anthocyanins and CerS6

Docking simulations were used to study two possible anthocyanin binding sites on CerS6. The combination of the model during docking was shown in Fig. 1. Anthocyanins may bind to CerS6, and the binding region was shown in Fig. 1. From the results of molecular docking analysis, the amino acids composing the binding site of CerS6 and delphinidin 3-O-rutinoside are:(LYS-125, PRO-126, GLU-344, LYS-125, THR-128), binding energy of delphinidin 3-O-rutinoside is -5.2 kcal/mol; the binding site of CerS6 and cyanidin 3-O-rutinoside are: (GLN-73, GLN-75, GLN-119, ASN-72, GLY-73, PRO-74), binding energy of cyanidin 3-O-rutinoside is -8.2 kcal/mol.

3.2 Effects of GA on body weight in HFD mice

GA intervention was given to mice at the same time of intragastric administration of high-fat emulsion. The weight changes during the 8-week experimental period were shown in Table 4. From the initial body
weight before death, the average body weight of mice in the CTL group from 31.24 ± 1.05 g to 44.17 ± 1.87 g, HFD group from 30.99 ± 1.14 g to 53.73 ± 2.24 g. There are significant differences in body weight between CTL group, HFD group and GA group, It shows that HFD can effectively promote the weight gain of mice, and GA can alleviate the weight gain of mice. It could be seen in Fig. 2 that the weight growth rate of mice in HFD group was 1.76 times that of CTL group, the weight growth rate was significantly increased, the weight growth rate of mice in LGA and HGA groups was significantly lower than that in HFD group.

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Time(week)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31.24</td>
<td>±1.05</td>
<td>34.93</td>
<td>±0.97</td>
<td>36.17</td>
<td>±1.23</td>
<td>37.89</td>
<td>±1.42</td>
<td>40.13</td>
</tr>
<tr>
<td>HFD</td>
<td></td>
<td>30.99</td>
<td>±1.14</td>
<td>34.51</td>
<td>±1.02</td>
<td>38.84</td>
<td>±1.81</td>
<td>46.57</td>
<td>±0.97</td>
<td>48.24</td>
</tr>
<tr>
<td>LGA</td>
<td></td>
<td>30.62</td>
<td>±0.87</td>
<td>35.40</td>
<td>±1.13</td>
<td>39.03</td>
<td>±1.46</td>
<td>42.73</td>
<td>±1.67</td>
<td>45.56</td>
</tr>
<tr>
<td>HGA</td>
<td></td>
<td>31.37</td>
<td>±0.96</td>
<td>34.79</td>
<td>±0.79</td>
<td>37.92</td>
<td>±1.56</td>
<td>42.03</td>
<td>±1.47</td>
<td>44.77</td>
</tr>
<tr>
<td>EGCG</td>
<td></td>
<td>32.16</td>
<td>±0.62</td>
<td>35.41</td>
<td>±0.86</td>
<td>38.43</td>
<td>±1.46</td>
<td>41.24</td>
<td>±1.68</td>
<td>44.13</td>
</tr>
</tbody>
</table>

### 3.3 Effects of GA on visceral fat and liver index in HFD mice

As shown in Fig. 3(A), the visceral fat of HFD group was 1.81g, which was significantly higher than that of CTL group (0.74g). The visceral fat of high and low dose GA group decreased compared with HFD group, and the visceral fat value of HGA group decreased to 1.29g, indicating that high fat emulsion could promote the accumulation of adipose tissue in mice, and GA could slow down the accumulation of adipose tissue in HFD mice.

The liver index of CTL group was 3.57% as shown in Fig. 3(B). The liver index of the HFD group was 5.35%, significantly higher than CTL group. Compared with the HFD group, the intervention of EGCG group and HGA group significantly reduced the liver index by 4.75% and 4.69%, and the effect was better than that of the LGA group. It is suggested that high-fat emulsion could increase the liver index of mice, and GA could reduce the liver index of high-fat mice in a dose-dependent manner.

### 3.4 Effect of GA on serum biochemical indexes in HFD mice

As shown in Fig. 4, the contents of TC and TG in the serum of mice in HFD group were higher than those in CTL group. The contents of TC and TG in serum of HGA group and LGA group were decreased in varying degrees, which indicated that GA could effectively reduce the content of TC and TG in serum of
mice. The content of LDL-C in serum of mice in HFD group was higher than that in CTL group, and the content of HDL-C in serum in HFD group was lower than that in CTL group. The content of LDL-C in serum of HGA and LGA group showed a decreasing trend compared with that of HFD group. Compared with HFD group, the content of HDL-C in serum of HGA and LGA group showed an upward trend. The results showed that GA could reduce the content of LDL-C and increase the content of HDL-C in serum of HFD mice.

3.5 Effects of GA on oxidative stress related enzyme activity in HFD mice

As shown in Fig. 5, the levels of SOD and GSH-Px are related to oxidative stress. The decrease of SOD and GSH-Px levels indicates the decrease of antioxidant capacity, which will lead to the increase of ROS and oxidative stress. The levels of SOD and GSH-Px in CTL group were 106.69U/mL and 136.44U/mL. Oxidative damage occurred in the liver of mice in HFD group, and the levels of SOD and GSH-Px in HFD group mice decreased significantly to 69.49U/mL and 96.74 U/ML. HGA group returned to 105.86U/mL and 117.07U/mL, which were significantly higher than those in HFD group. The results showed that GA could significantly increase the levels of SOD and GSH-Px in hyperlipidemic mice, and the oxidative stress indexes of HGA group and EGCG group were close to those of CTL group. The results showed that GA could alleviate oxidative stress in mice in a dose-dependent manner.

3.6 Effects of GA on insulin sensitivity and insulin resistance index in HFD mice

As shown in Fig. 6, after 8 weeks of GA intervention, the level of ISI in HFD group was significantly lower than that in CTL group. With the increase of GA concentration, the level of ISI increased, and the level of ISI in HGA group increased to 4.54, which increased significantly. It is proved that the intervention of GA can effectively improve the insulin sensitivity of mice. At the same time, the HOMA-IR level of CTL mice was 2.47, and the HOMA-IR level of HFD mice was 6.57, which was significantly higher than that of CTL group, which proved that HFD mice produced IR successfully. With the increase of GA concentration, the IR index decreased significantly, and the HOMA-IR level of HGA group decreased to 5.75. The results showed that GA could alleviate IR in mice.

3.7 Effect of GA on liver morphology in HFD mice

As shown in Fig. 7, the hepatocytes in the CTL group arranged regularly, there were almost no fat vacuoles and steatosis in the visual field, the structure was normal, and there were no obvious pathological changes; in the HFD group, the hepatocytes were accompanied by a certain degree of steatosis, irregular structural arrangement, steatosis, enlarged hepatocytes, obvious intracellular fat infiltration and fat vacuoles of different sizes, resulting in hepatocyte degeneration. After intervention of GA, the liver cells of the intervention group were significantly improved compared with HFD group, and the degree of steatosis of liver cells was alleviated, which was close to the level of the CTL group. The results showed that GA could improve the fat accumulation in liver of HFD mice.
3.8 Effects of GA on the expression of inflammatory cytokines in HFD mice

It could be seen from Fig. 8, the mRNA expression of IL-6 and TNFα in liver tissue of HFD group was higher than that of CTL group. Compared with HFD group, the mRNA expression of IL-6 and TNFα in EGCG and GA groups showed a downward trend, indicating that GA can effectively down-regulate the mRNA expression of IL-6 and TNFα in liver tissue of HFD mice, reduce inflammation in HFD mice.

3.9 GA on S1P/Cer signaling pathway protein in liver tissue of HFD mice

S1P-SP-Cer metabolism influence insulin signaling via Akt phosphorylation and CD36 (Fig. 9). As shown in Fig. 10, compared with the CTL group, the expression of CerS6 protein and the downstream protein PKCζ in the liver of the HFD group were significantly increased, and the phosphorylation of the downstream protein Akt and p-Akt/Akt were significantly inhibited. Compared with the HFD group, GA intervention could significantly promote the phosphorylation of Akt and significantly increase the expression of p-Akt/Akt, and significantly inhibit the expression of CerS6 and PKCζ proteins.

It could be seen from Fig. 11, compared with the CTL group, the expression of SphK2 protein in the liver of the HFD group was significantly decreased, and the expression of PPARγ and its downstream proteins CD36 was significantly increased; the expression level of insulin receptor (ISR) was significantly down-regulated. Compared with the HFD group, GA intervention could significantly up-regulate the expression of SphK2 protein and significantly inhibit the expression levels of PPARγ and CD36 proteins, while GA intervention could significantly up-regulate the expression of ISR.

Conclusion

This study showed that GA could improve hepatic steatosis in HFD mice. GA could reduce the activity of TG, TC and LDL-C; increased the activity of HDL-C in HFD mice. GA could increase the GSH-Px and SOD activity of liver tissue and alleviate oxidative stress in mice. GA improved IR by inhibits the S1P/Cer signaling pathway, upregulated the expression of ISR, p-Akt, p-Akt/Akt and SphK2, and reduced the deposition of the expression of CerS6, PPARγ, CD36 and PKCζ. In conclusion, GA could significantly improve insulin resistance in liver tissue. Research showed that GA had protective effects against obesity and insulin resistance. GA could inhibit ceramide synthesis in HFD mice and reduce IR, which had a certain effect on the prevention and treatment of IR. This study provided a theoretical basis for GA to improve insulin resistance in obese mice.

Discussion

Insulin resistance often occurs in obesity, hypertension, high blood pressure and many cardiovascular diseases[25][26]. Anthocyanins can increase insulin sensitivity by increasing the expression of IR, p-IR and...
p-Akt, then promoting the body's glucose absorption, thereby achieving the effect of weight loss[27]−[28].

Studies have found that the level of ceramide in obese patients is significantly increased, and ceramide plays an important regulatory role in the occurrence of obesity, insulin resistance, atherosclerosis and other diseases[29]. Ceramide blocks the activation of Akt / PKB pathway in two ways[30]. The first is that ceramide mediates the up-regulation of protein phosphatase 2A (PP2A) activity, dephosphorylates Ser473 and Thr308 sites of Akt, leading to the damage of Akt signaling pathway; the second is that atypical PKCζ activated by ceramide reduces the phosphorylation of Akt at Thr34, thereby inhibiting Akt membrane translocation and subsequent activation[31]. S1P has a positive regulatory effect on cells, while ceramide and sphingosine (SP) have a negative regulatory inhibitory effect on cells. Sphingosine-1-phosphate (S1P) has the exact opposite effect of ceramide and SP, which can be converted into each other before. Sphingosine kinases (SphKs) are the main rate-limiting enzymes that catalyze SP into S1P. The enzyme has two subtypes: SphK1 and SphK2[32]. SphK2 is the key rate-limiting enzyme that catalyzes the conversion of SP to S1P[33]. Studies have shown that over-expression of SphK2 can improve liver endoplasmic reticulum stress and reduce liver lipid deposits[34]. Studies showed that change the level of intracellular S1P could alter the binding of S1P-PPARγ and the transcriptional activity of PPARγ. At the same time, S1P could activate anti-apoptotic pathways such as PI3K/Akt through S1P receptor and sphingosine kinase to improve IR[25][26].

This study show the therapeutic role of gooseberry anthocyanins in HFD mice. Compared with medicines, anthocyanins are considered safer and with less adverse effects. These results indicate that anthocyanins can improve glucose and lipid metabolism.

Declarations

Compliance with ethical standards

All animal experiments adhere to the ARRIVE guidelines.

Ethical approval

The article does not contain any studies with human particioants performed by any of the authors.

Competing Interests

The authors declare no conflict of interests.

Author Contribution Statement

Jie Wei analyzed the data and designed the research study. Xian Tang wrote the paper, Jinpeng Huang, Chenjuan Zhang, Hongwei Liu and Jun Gao performed the research.

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Data Availability Statements

All date generated or analysed during this study are included in this published article.

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Figures

Figure 1
The docking models of two anthocyanins and proteins were analyzed by Autodock software. Displays a panorama of binding modes between cyaniding 3-O-rutinoside(A), delphinidin 3-O-rutinoside(B), and Cers6. The most conformations of the binding energy are -8.2 and -5.2 kcal/mol.

Figure 2

Effect of GA on body weight growth rate of mice. Compared with the CTL group (*) p<0.05, (**) p<0.01, (***) p<0.001; compared with the HFD group (#) p<0.05, (##) p<0.01, (###) p<0.001.

A

B
Figure 3

Effect of GA on Visceral fat (A) and Liver index (B) in mice. Compared with the CTL group (*) p<0.05, (**) p<0.01, (***) p<0.001; compared with the HFD group (#) p<0.05, (##) p<0.01, (###) p<0.001.

Figure 4

Effect of GA on Serum biochemical indexes in mice: TC (A), TG (B), HDL-C (C), LDL-C (D). Compared with the CTL group (*) p<0.05, (**) p<0.01, (***) p<0.001; compared with the HFD group (#) p<0.05, (##) p<0.01, (###) p<0.001.
Figure 5

Effects of GA on serum related enzyme indexes in mice: SOD activity (A), GSH-Px activity (B) Compared with the CTL group(*) p<0.05,(**) p<0.01,(***) p<0.001; compared with the HFD group(#) p<0.05,## p<0.01,### p<0.001.

Figure 6

Effect of GA on ISI level (A) and HOMA-IR level (B) staining in mice. Compared with the CTL group(*) p<0.05,(**) p<0.01,(***) p<0.001; compared with the HFD group(#) p<0.05,## p<0.01,### p<0.001.
Figure 7

Effects of GA on liver pathology. Histological examination of liver samples from CTL (A), HFD (B), LGA (C), HGA (D) and EGCG (E) group mice. Scale bar: 100μm.
Figure 8

Effects of GA on the expression of inflammatory cytokines in mice. IL-6 mRNA expression in HFD mice (A); TNF-α mRNA expression in HFD mice (B). Compared with the CTL group (*) p<0.05, (**) p<0.01, (***) p<0.001; compared with the HFD group (#) p<0.05, (##) p<0.01, (###) p<0.001.
Figure 9

GA restored insulin signaling by suppressing ceramide synthesis: Effect of S1P-SP-Cer metabolism on insulin signal transduction
Figure 10

Regulating effect of GA on S1P/Cer signaling pathway in the liver tissue. (A) expression of CerS6 in HFD mice, (B) expression of p-Akt in HFD mice, (C) expression of p-Akt/Aktin HFD mice (D) expression of PKCζ in HFD mice. Compared with the CTL group(*) p<0.05,(**) p<0.01,(***) p<0.001; compared with the HFD group(##) p<0.05,(###) p<0.01,(####) p<0.001.
Figure 11

Regulating effect of GA on S1P/Cer signaling pathway in the liver tissue. (A) expression of SphK2 in HFD mice, (B) expression of PPARγ in HFD mice, (C) expression of CD36 in HFD mice, (D) expression of ISR in HFD mice. Compared with the CTL group (* p<0.05, ** p<0.01, *** p<0.001); compared with the HFD group († p<0.05, ‡ p<0.01, §§ p<0.001).