Anti-obesity effect of escin: a study on high-fat diet-induced obese mice

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Abstract. – **OBJECTIVE:** Obesity is characterized by excess fat accumulation and closely associated with insulin resistance and type 2 diabetes. We aimed at exploring the potential effect and mechanism of escin for the treatment of obesity using network pharmacology, and to verify the effect of escin on obese mice.

MATERIALS AND METHODS: Escin targets were predicted by DrugBank and SwissTarget database. Potential targets for the treatment of obesity were identified based on the DisGeNET database. Comparative analysis was used to investigate the overlapping genes between escin targets and obesity treatment-related targets. Using STRING database and Cytoscape to analyze interactions among overlapping genes, hub genes were identified. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were conducted in DAVID. High-fat diet (HFD) -induced obese mice were used to observe the anti-obesity effects of escin. The body weight, relevant biochemical markers and HE staining of fat and liver tissues were determined after escin was administered for 18 weeks.

RESULTS: We screened 53 overlapping genes for escin and obesity. The mechanism of intervention of escin in treating obesity may involve 10 hub targets (STAT3, MTOR, NR3C1, IKBKB, PTGS2, MMP9, PRKCA, PRKCD, AR, CYP3A4). The screening and enrichment analysis revealed that the treatment of obesity using escin primarily involved 10 GO enriched terms and 13 related pathways. *In vivo*, escin can reduce the body weight of obese mice induced by HFD and improve lipid metabolism through lowering triglycerides (TG), total cholesterol (TC), and density lipoprotein (LDL) levels and increasing high density lipoprotein (HDL) levels and decreasing leptin level and increasing adiponectin (ADPN) level. Escin can regulate glucose metabolism caused by obesity through decreasing fasting glucose, postprandial blood glucose and regulating the level of insulin. These obese mice induced by HFD displayed the increased insulin resistance that was associated with the increased inflammatory cytokines, including interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1). Escin may antagonize the increase of MCP-1 and partially antagonize the low-grade inflammation caused by obesity. From the morphological changes of fat and liver tissues stained by HE stain, escin could decrease the size of adipocytes and improve liver necrosis and fatty degeneration in obese mice fed by HFD.

CONCLUSIONS: The network pharmacology of escin in treating obesity may involve 10 hub targets (STAT3, MTOR, NR3C1, IKBKB, PTGS2, MMP9, PRKCA, PRKCD, AR, CYP3A4), 10 GO enriched terms and 13 related pathways. *In vivo*, escin can be potentially used to prevent or treat obesity through reducing the weight, improving glucose and lipid metabolism, partially antagonizing the low-grade inflammation, and improved insulin resistance.

Key Words:
  Escin, Obesity, Network pharmacology, Insulin resistance, Type 2 diabetes, Inflammatory cytokines.

Introduction

Obesity is a complex condition influenced by diet, developmental stage, age, physical activity, and genetic factors, and it is a major risk factor for a number of chronic diseases including diabetes, hypertension, atherosclerosis and cancer[1,2]. Some studies have shown that more than 80% of people with type 2 diabetes were obese[3]. Obesity indicates excessive fat accumulation and adipose tissue expansion, which is the result of increase in adipocyte size (hypertrophy) and adipocyte number (hyperplasia)[4]. Moreover, the hypertrophy of adipocytes is considered to be an important factor associated in reducing insulin sensitivity and secretes higher levels of

inflammatory factors, including leptin, interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and secretes lower level of anti-inflammatory factor-adiponectin (ADPN)[5]. Adipocyte hypertrophy and inflammatory responses are closely associated with the development of insulin resistance in obese individuals.

Escin is a triterpenoid saponin composed of four loops: A, B, C and D, and it is extracted from the seed of *Aesculus turbinata BLUME* that has been used as treating certain conditions, including varicose veins, hematoma, and venous congestion[6,7]. Previous studies have shown that escin had obvious anti-inflammatory, anti-edema, hypoglycemic effects and inhibiting alcohol absorption effects. In recent years, it has been reported that escin had the potential to inhibit the increase of blood glucose and to inhibit the lipase activity[8,9]. So, we speculated that escin might improve obesity-related diseases. In this study, we used the network pharmacology to reveal the pharmacological mechanism of escin in the treatment of obesity from potential targets and pathways. *In vivo*, the body weight, insulin, blood glucose, blood lipid and inflammatory factors were selected to study the effects of escin on obesity, inflammation and insulin resistance.

Materials and Methods

Screening of Target Proteins for Escin

The DrugBank (https://go.drugbank.com/) and SwissTarget database (http://www.swisstargetprediction.ch/) were employed to obtain the target proteins for escin.

The Acquisition of Gene Targets for Obesity

The target genes for obesity in this study were collected from DisGeNet databases (http://www.disgenet.org/) by entering the key words of "obesity".

Intersection of Drug Genes and Disease Genes

Further, we matched escin targets with obesity targets to obtain overlapping targets. The Venn Diagram Platform (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to draw a Venn diagram.

PPI Network Construction

PPI data were attained from the STRING database (https://stringdb.org/), which is a database that can predict known protein interactions[10]. Firstly, the above-mentioned overlapping therapeutic targets were subjected to PPI analysis using the STRING database. In the platform, "Multiple proteins" were selected, and species were set as "Homo sapiens". The target interaction information was obtained according to the confidence score of 0.4. The results were imported into the Cytoscape 3.8.2 software where the interaction networks were drawn and analyzed.

Identification of Hub Genes

After establishing the PPI network, the networks were constructed using Cytoscape 3.8.2. The Cytoscape plug-in cytoHubba was used to filter out hub genes. Important hub genes could be selected through calculation and analysis of the network structure and the weighted reconnection between nodes. We calculated it by "Degree".

Gene Ontology and KEGG Pathway Enrichment Analysis

GO and KEGG enrichment analysis were obtained by DAVID (https://david.ncifcrf.gov/). Three aspects are contained in GO enrichment analysis: biological process (BP), molecular function (MF), and cellular component (CC)[11]. The enrichment results of GO ($p<0.01$, count >10) and KEGG ($p<0.01$, count >5) were analyzed with p and count as evaluation criteria.

Experimental Study of Escin In Vivo

Drugs and Materials

The escin was in the form of sodium aescinate and purchased from Shandong Green Leaf Pharmaceutical Co.Ltd. (20170408, Shanghai, China). Rosiglitazone tablets were purchased from Chengdu Hengrui Pharmaceutical Co., Ltd. (170403, Chengdu, China). Biochemical kits for insulin, TC, TG, HDL, LDL, MCP-1, TNF-α, IL-6, Leptin and ADPN were purchased from Shanghai Enzyme-linked Biotechnology Co.Ltd. (Shanghai, China). Other experimental reagents and materials were obtained by the relevant biological reagent companies.

Animal Experiment

Male 6-week-old C57BL/6J mice were purchased from Beijing Huafukang Biotechnology Co.Ltd (SCXK: Beijing, China; 2014-0004),
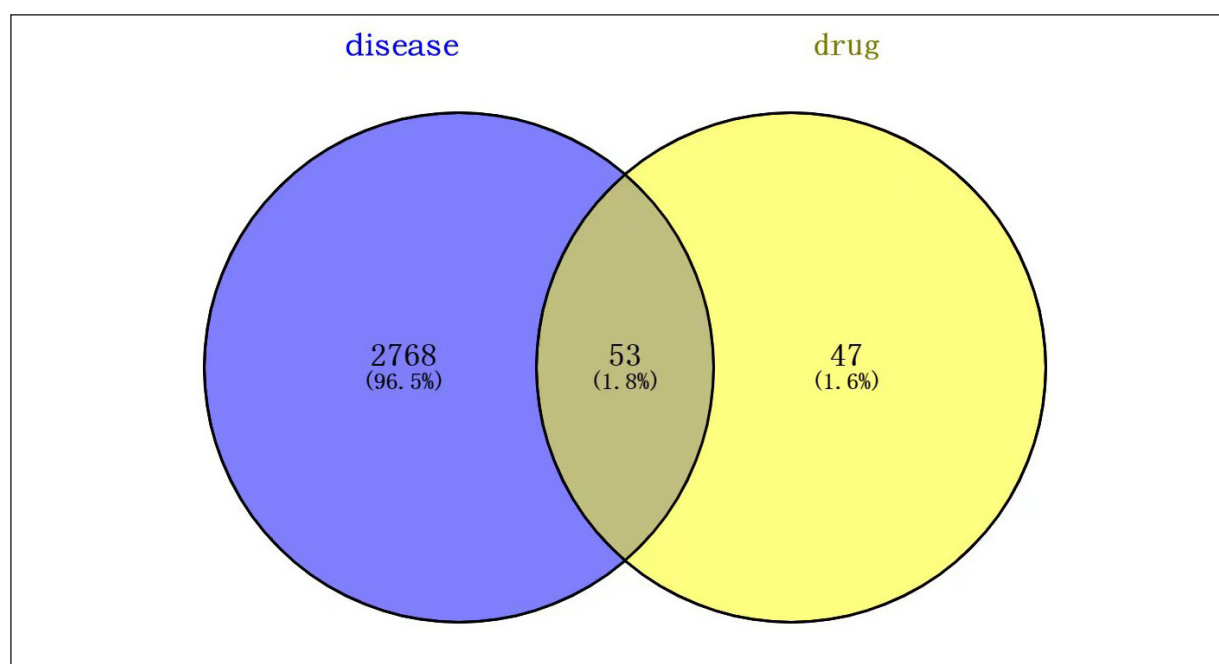
Figure 1. The Venn diagram of escin and obesity-related targets. The 53 overlapping genes between the disease and drug.

and housed in an air-conditioned animal room with a 12-h light/12-h dark cycle at a temperature of $21 \pm 2°C$ and humidity of $50 \pm 5\%$. They were fed with a commercial diet for 1 week at the SPF Animal Experiment Center of Shenyang Pharmacy University. This study was approved by the Ethics Committee for the Animal Experiment Center of Shenyang Pharmacy University (Number: SYPU-IACUC-S2017-09.13-101). Then, the mice were randomly divided into 5 groups according to the body weight (15 mice per group): control group (standard diet), high-fat diet (HFD) group, LDE group (HFD + 7 mg/kg escin), HDE group (HFD + 40 mg/kg escin), and RSG group (HFD + 40 mg/kg rosiglitazone). Except for the control mice, the other mice were fed with 45% high fat diet and taken orally at different doses for 18 weeks. Escin and rosiglitazone were dissolved in distilled water and sonicated for 10 min for gavage administration. Mouse body weight and blood glucose were determined weekly, and glucose tolerance test (GTT) was measured at week 15. After week 18, all mice were cut off food and water at night. The next morning, the blood was taken, and the serum was separated and preserved at -80°C. Adipose tissue and liver tissue were taken from the mice, and half of the tissues was fixed with polymethanol for the preparation of paraffin samples, and the other half was washed with saline, dried, and stored at -80°C.

Glucose Tolerance Test

At week 15, all mice were fasted for 12 h overnight, and were given 0.1 ml/10 g glucose (15%) in the next morning, and blood glucose was measured 2 h later.

Serum Biochemistry

Serum insulin, TC, TG, LDL and HDL were detected according to the kit instructions. Serum leptin, ADPN, TNF-α, MCP-1 and IL-6 were determined by ELISA method.

Histopathology

White adipose tissues (epididymal fat, renal fat, and mesenteric fat) and liver tissues were dehydrated and embedded in paraffin, sectioned (4 μm) on a polycut microtome, and stained with hematoxylin and eosin (H&E). Pathological observation of these tissues was made under light microscopy (CX41RF, Olympus Optical Co., Tokyo, Japan).

Statistical Analysis

Statistical analysis was performed by using SPSS 22.0 software (SPSS Inc., Armonk, NY, USA). All data were presented as mean ± S.E.M.
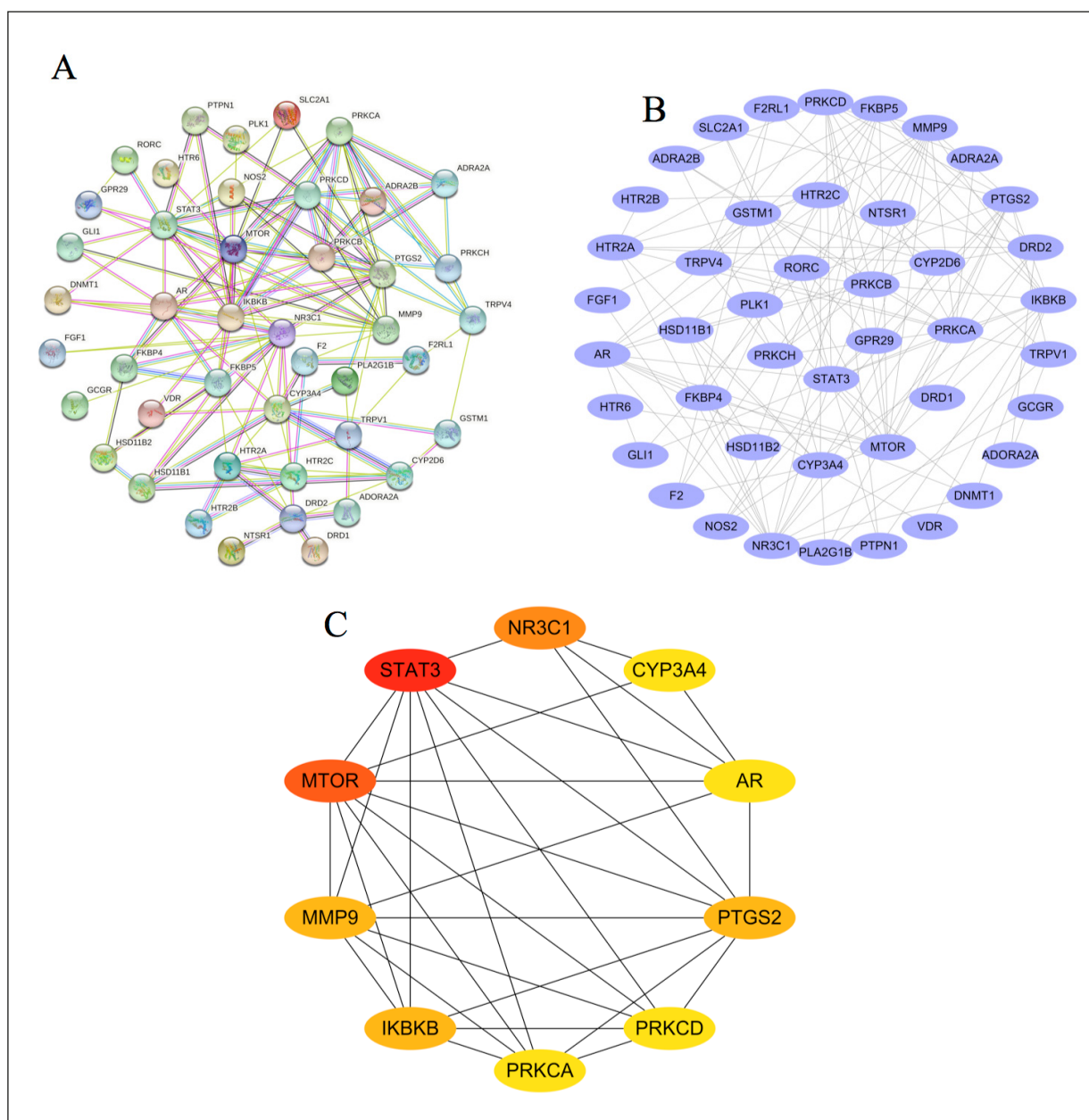
Figure 2. The protein-protein interaction (PPI) network (**A**, STRING database; **B**, Cytoscape) of 53 overlapping genes of escin and obesity-related targets. **C**, Top 10 hub genes in the PPI network. STAT3: signal transducer and activator of transcription 3, MTOR: mechanistic target of rapamycin kinase, NR3C1: nuclear receptor subfamily 3 group C member 1, IKBKB: inhibitor of nuclear factor kappa B kinase subunit beta, PTGS2: prostaglandin-endoperoxide synthase 2, MMP9: matrix metallopeptidase 9, PRKCA: protein kinase C alpha, PRKCD: protein kinase C delta, AR: androgen receptor, CYP3A4: cytochrome P450 family 3 subfamily A member 4.

One-way ANOVA was used to count for significant differences between multiple groups, Fisher's least significant difference (LSD) test was used to determine the differences between each group. The significant differences among groups were set at $p<0.05$.

Results

Target Prediction and Analysis of Escin on Network Pharmacology

A total of 100 escin potential targets was obtained from swisstarget database. Meanwhile,
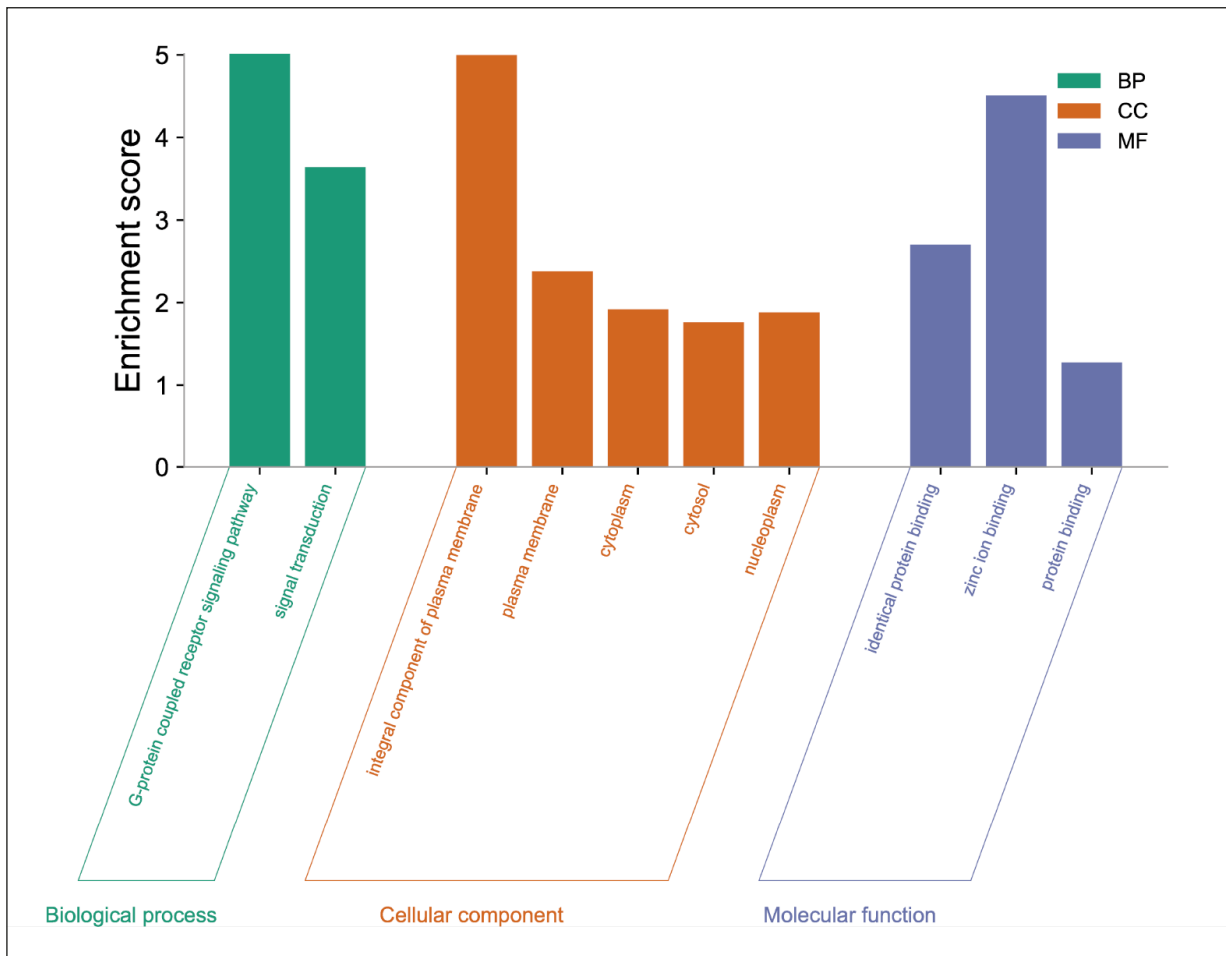
Figure 3. Gene Ontology enrichment analysis of 53 overlapping genes. The top Gene Ontology enriched terms with $p < 0.01$ and count >10 were screened.

2821 obesity-related genes were obtained from the DisGeNET database. By taking the intersection of drug and disease targets, we got 53 genes (Figure 1), which meant that these genes might play a major role in escin treatment of obesity.

Construction and Analysis of Target Protein PPI Network

For the purpose of figuring out how overlapping gene interact, we uploaded the information of them to the STRING database and then structured a PPI network. It contained 44 nodes and 126 edges according to the high confidence (0.4) on protein level (hide disconnected nodes in the network) (Figure 2A). The results showed that the protein interactions involved 44 nodes and 126 edges by Cytoscape (Figure 2B). The top ten target proteins (STAT3, MTOR, NR3C1, IKBKB, PTGS2, MMP9, PRKCA, PRKCD, AR, CY-P3A4) were identified to be visualized by Cytoscape according to the degree (Figure 2C). These genes are considered as hub genes that play an important role in treating of obesity.

Gene Ontology Enrichment Analysis

GO enrichment analysis included 3 different levels: BP, MF, and CC. We selected the top 10 according to the p and count value as shown in Figure 3. The results indicated that escin treated obesity through various BP, including G-protein coupled receptor signaling pathway and signal transduction. GO terms under CC class were integral component of plasma membrane, plasma membrane, cytoplasm, cytosol, and nucleoplasm. GO terms in the MF classification were zinc ion binding, identical protein binding, and protein binding.
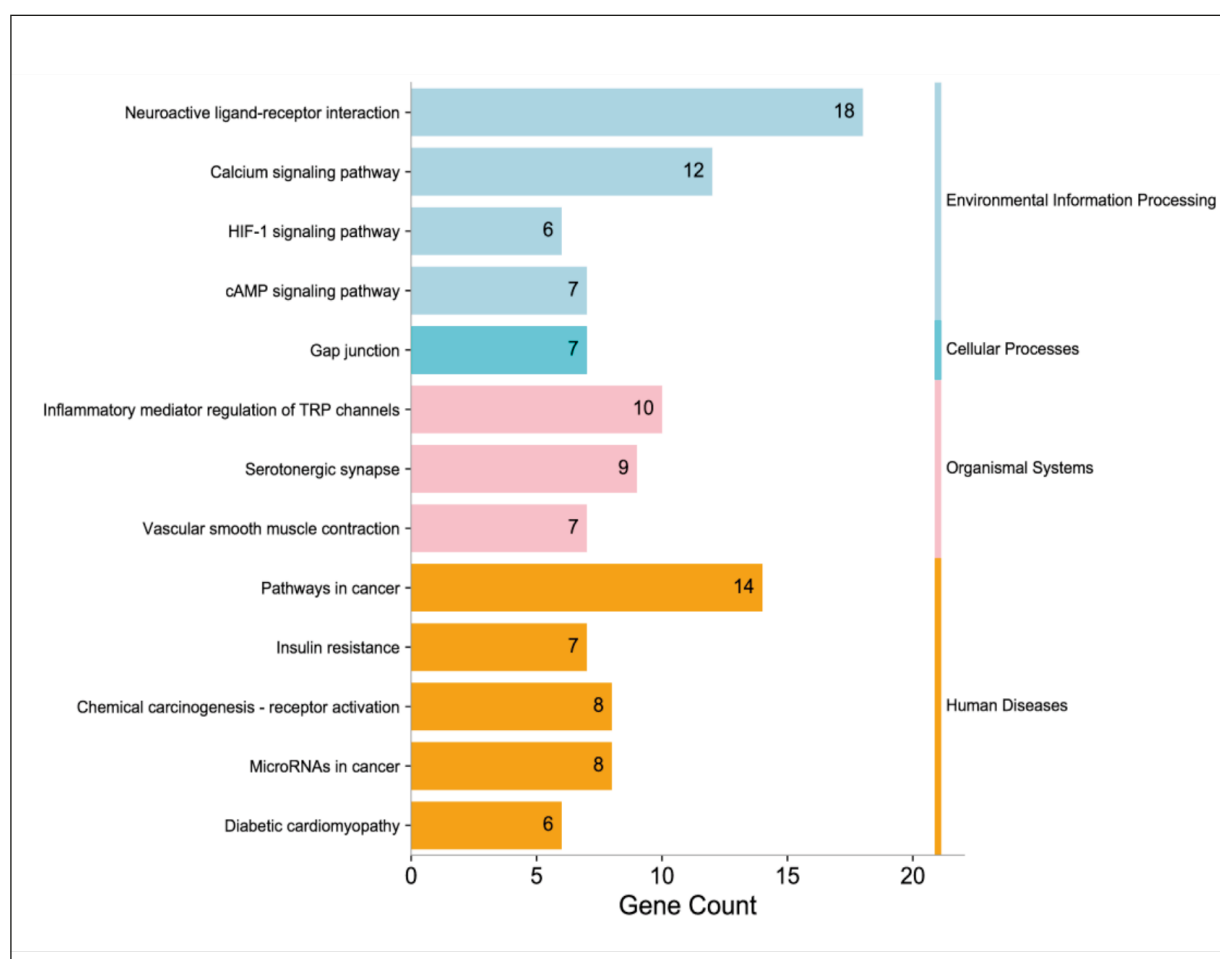
Figure 4. KEGG pathway enrichment analysis of 53 overlapping genes. *p* < 0.01 and count <5.

KEGG Enrichment Analysis

To further explore how escin affected obesity through these target genes, we uploaded these 53 genes to the DAVID database and performed KEGG enrichment analysis. A total of 13 significant treatment pathways (Figure 4) were screened ($p<0.01$, count >5), including Neuroactive ligand-receptor interaction, inflammatory mediator regulation of TRP channels, calcium signaling pathway, serotonergic synapse, pathways in cancer, gap junction, insulin resistance, vascular smooth muscle contraction, chemical carcinogenesis - receptor activation, HIF-1 signaling pathway, cAMP signaling pathway, MicroRNAs in cancer, and diabetic cardiomyopathy. The top pathway was based on statistical significance and then ranked in descending order (Table I). We find that a pathway includes multiple proteins, and a protein is also involved in multiple pathways.

Results of the Experimental Study of Escin In Vivo

Effect of Escin on Body Weight of Mice Induced by HFD

The obese C57BL/6J mice induced by HFD were used to investigate the effect of escin on obesity. Mouse body weight in control group, HFD group, LDE group, HDE group and RSG group fed for 18 weeks were shown in Figure 5A. There was no statistical difference in initial body weight (Figure 5B). From 11 to 18 weeks, mice fed with high fat weighed significantly higher than the control group, whereas mice given with escin at the dose of 40 mg/kg and rosiglitazone weighed significantly less than mice in HFD group. The result could be also seen in the bar graph of mouse body weight at week 18 (Figure 5C). These results demonstrated that the HFD-induced obesity model was successful and escin had the effect of reducing weight.

Table I. KEGG pathway analysis based on target-pathway network.

Pathway ID	Pathway Name	Count	*p*-value	Gene Name
hsa04080	Neuroactive ligand-receptor interaction	18	1.30E-11	GCGR, HTR2B, HTR1B, HTR2C, TRPV1, HTR2A, F2, NR3C1, ADRA1A, ADRA2B, ADRA2A, HTR6, ADORA2A, ADORA3, F2RL1, DRD1, DRD2, NTSR1
hsa04750	Inflammatory mediator regulation of TRP channels	10	6.29E-09	PRKCH, PRKCB, TRPV4, PRKCD, HTR2B, F2RL1, HTR2C, PRKCA, TRPV1, HTR2A
hsa04020	Calcium signaling pathway	12	1.63E-07	HTR6, ADORA2A, NOS2, PRKCB, HTR2B, HTR2C, PRKCA, DRD1, HTR2A, FGF1, ADRA1A, NTSR1
hsa04726	Serotonergic synapse	9	4.18E-07	HTR6, PRKCB, CYP2D6, HTR2B, HTR1B, HTR2C, PRKCA, HTR2A, PTGS2
hsa05200	Pathways in cancer	14	1.31E-05	GSTM1, NOS2, PRKCB, STAT3, SLC2A1, PRKCA, F2, FGF1, GLI1, PTGS2, MMP9, MTOR, IKBKB, AR
hsa04540	Gap junction	7	1.49E-05	PRKCB, HTR2B, HTR2C, PRKCA, DRD1, HTR2A, DRD2
hsa04931:	Insulin resistance	7	4.78E-05	IKBKB, PTPN1, PRKCB, STAT3, PRKCD, SLC2A1, MTOR
hsa04270	Vascular smooth muscle contraction	7	1.59E-04	PRKCH, ADORA2A, PLA2G1B, PRKCB, PRKCD, PRKCA, ADRA1A
hsa05207	Chemical carcinogenesis - receptor activation	8	2.90E-04	AR, GSTM1, PRKCB, VDR, STAT3, PRKCA, CYP3A4, MTOR
hsa04066	HIF-1 signaling pathway	6	5.23E-04	NOS2, PRKCB, STAT3, SLC2A1, PRKCA, MTOR
hsa04024	cAMP signaling pathway	7	2.23E-03	HTR6, ADORA2A, PDE4D, HTR1B, DRD1, DRD2, GLI1
hsa05206	MicroRNAs in cancer	8	2.70E-03	IKBKB, DNMT1, PRKCB, STAT3, PRKCA, PTGS2, MMP9, MTOR
hsa05415	Diabetic cardiomyopathy	6	7.94E-03	PRKCB, PRKCD, SLC2A1, PRKCA, MMP9, MTOR

Effects of Escin on Glucose Tolerance and Insulin in the Mice Induced by HFD

The serum blood glucose at 2 h after the meal and insulin level after the 8-week administration were determined and displayed in Figure 6. Two hours postprandial glucose and insulin level in HFD group were significantly higher than those in the control group, which indicated that the HFD-fed mice have successfully resulted in impaired glucose metabolism and reduced glucose tolerance. Both postprandial blood glucose and insulin level were significantly lower in HDE and RSG group than HFD group, indicating that escin had significant effects on reducing blood glucose and improving glucose metabolism.

Effects of Escin on Lipid Related Parameters in the Mice Induced by HFD

The concentrations of plasma lipids (TG, TC, LDL and HDL), leptin and ADPN are shown in Figure 7. Compared with the control group, the level of TG, TC and LDL significantly increased, the level of HDL significantly decreased in the HFD group. Compared with the HFD group, the TG, TC and LDL level significantly decreased, the HDL level significantly increased in both HDE and RSG group. The leptin level was significantly higher in HFD group than in control group. Compared with the HFD group, the leptin levels were significantly lower in HDE group and RSG group. The ADPN level was lower in the HFD group than in the control group, and the level of ADPN in HDE group and RSG group was significantly higher than that in the HFD group. These results suggested that escin could regulate blood lipids and improve lipid metabolism.

Effects of Escin on Inflammation Factors in Obese Mice Induced by HFD

The effects of escin on MCP-1, TNF-α and IL-6 were shown in Figure 8. It could be seen from the Fig-
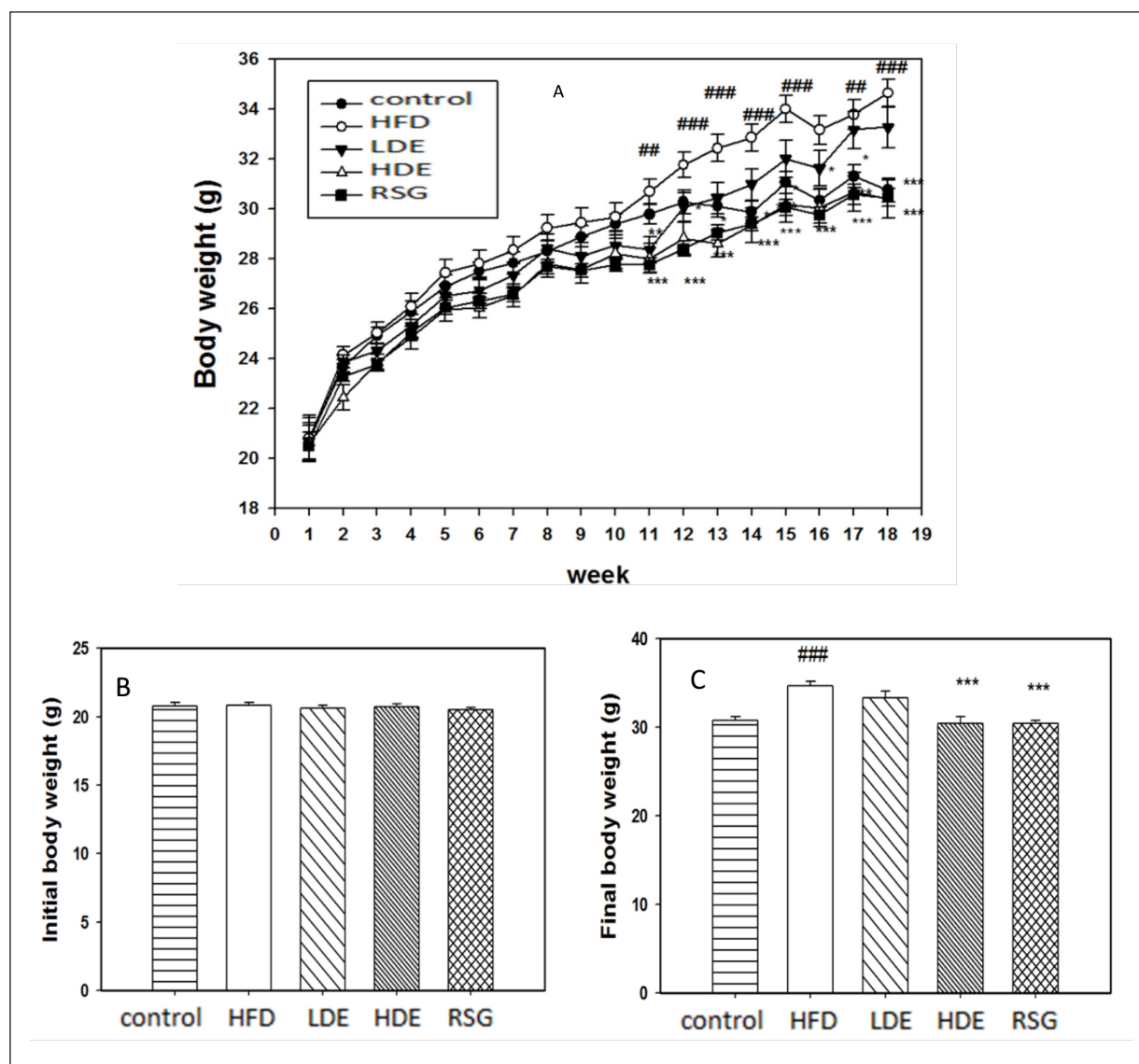
Figure 5. Effect of 18-week escin treatment on body weight in mice induced by high-fat diet. **A**, 18-week body weight; **B**, Initial body weight; **C**, Final body weight. Control group: standard chow diet; HFD group: HFD only; LDE: HFD + 7 mg/kg escin; HDE group: HFD + 40 mg/kg escin; RSG: Positive group, HFD + 40 mg/kg rosiglitazone. Results are expressed as the mean ± standard error of the mean (SEM) (n=15). ## $p < 0.01$, ### $p < 0.001$ compared with the control group; *$p<0.05$,** $p<0.01$,***$p <0.001$ compared with the HFD group.

ure 8 that the content of inflammatory factors such as MCP-1, TNF- α and IL-6 increased in obese mice fed by HFD, but escin only reduced the content of MCP-1, indicating that escin only partially antagonized the low-grade inflammatory caused by obesity.

Effects of Escin on Adipose Tissue and Liver Morphology in Obese Mice Induced by HFD

Histological observation showed a strong lipid accumulation in adipose tissues and liv-er stained by HE stain in HFD mice compared to the control mice (Figure 9: 200×, Figure 10: 400×). Hypertrophy of the adipocytes was observed in the epididymal, renal and mesenteric fats in the HFD mice. The average diameter of epididymal, renal and mesenteric adipocytes in the HFD group was significantly larger than that in the control group, and the diameter of adipocytes in HDE and RSG group was lower than that in the HFD group. However, the change of LDE group was not obvious. The results showed

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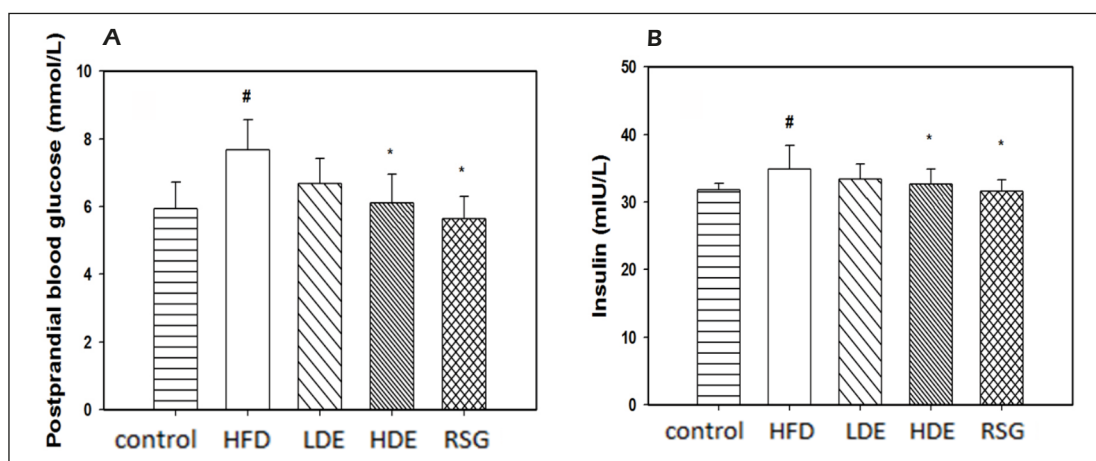
Figure 6. Effect of escin on postprandial blood glucose and insulin in mice induced by high-fat diet. (**A**) postprandial glucose, (**B**) insulin. Control group: standard chow diet; HFD group: HFD only; LDE: HFD + 7 mg/kg escin; HDE group: HFD + 40 mg/kg escin; RSG: Positive group, HFD + 40 mg/kg rosiglitazone. Results are expressed as the mean ± SD (n=9). #$p < 0.05$ compared with the control group; *$p < 0.05$ compared with the HFD group.

that escin could decrease the size of adipocytes in obese mice fed by HFD.

In the control group, the structure of hepatic lobule was complete and clear, the hepatic cell cord was arranged radially with the central vein as the center, the hepatic sinusoid was clear, the liver cell morphology was normal, and the cytoplasm was abundant. The hepatocytes in the HFD group possessed serious somatic swelling and fatty degeneration, indicating that the mice had developed a high degree of hepatic steatosis induced by HFD. In HDE and RSG group, the somatic swelling and fatty degeneration of hepatocytes is alleviated compared to those of HFD group, which proved that escin could improve liver necrosis in the mice induced by high-fat diet.

Discussion

Due to the harmful effect of obesity on human health, it is urgent to develop new types of anti-obesity drugs. Natural products are promising alternative due to their effective biological activities and with potentially less side effects, and escin is such the natural bioactive substance.

In the current work, we predicted the possible molecular mechanisms of escin on obesity using network pharmacology. According to PPI network and degree, the rankings of main targets (STAT3, MTOR, NR3C1, IKBKB, PTGS2, MMP9, PRKCA, PRKCD, AR, CYP3A4) are the hub genes involved in escin's anti-obesity. Many studies in the literature suggest that these hub genes are also associated with obesity[12-20]. For instance, in the study of Marek et al[21] on young subjects with uncomplicated obesity, obese subjects had lower insulin sensitivity, lower adipose tissue JAK1, and JAK2 expression and higher adipose tissue expression of LEP, STAT3, MIF, CCL2, MMP9, and IL18. In addition, these genes are also strongly linked to inflammation[22-26]. Some studies have shown that anti-obesity drugs and in-depth mechanistic studies of anti-obesity were closely associated with anti-inflammation[16,27]. Therefore, we had reason to believe that the genes screened in this paper can be used as key genes for obesity and escin. In addition, we found that PRKCA, PRKCD and CYP3A4 have been relatively poorly studied on obesity.

The GO enrichment analysis showed that GO terms in the CC classification was more than BP or MF, suggesting that CC may be the main mechanism. Pathway enrichment analysis showed that the related diseases were diabetic cardiomyopathy, insulin resistance and so on. For organismal systems, pathway enrichment included inflammatory mediator regulation of TRP channels, etc. Based on bioinformatic analysis, we initially performed routine blood glucose and blood lipid tests to verify the effect of escin on obesity, and in
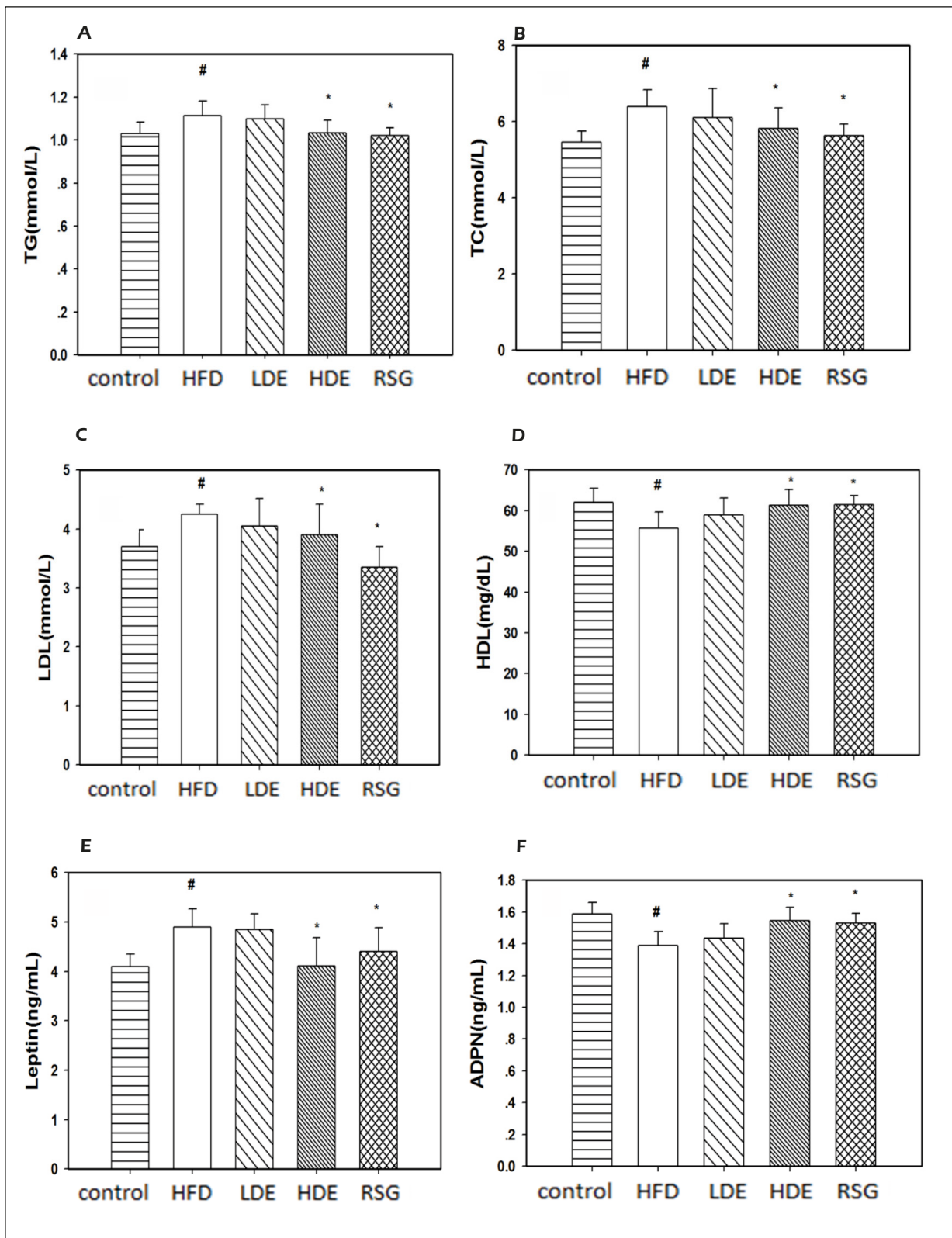
Figure 7. Effect of escin on TG, TC, LDL, HDL, Leptin and ADPN in mice induced by high-fat diet. (**A**) TG; (**B**) TC; (**C**) LDL; (**D**) HDL; (**E**) Leptin; (**F**) ADPN. Control group: standard chow diet; HFD group: HFD only; LDE: HFD + 7 mg/kg escin; HDE group: HFD + 40 mg/kg escin; RSG: Positive group, HFD + 40 mg/kg rosiglitazone. Results are expressed as the mean ± SD (n=9). # $p < 0.05$, compared with the control group; * $p < 0.05$ compared with the HFD group.
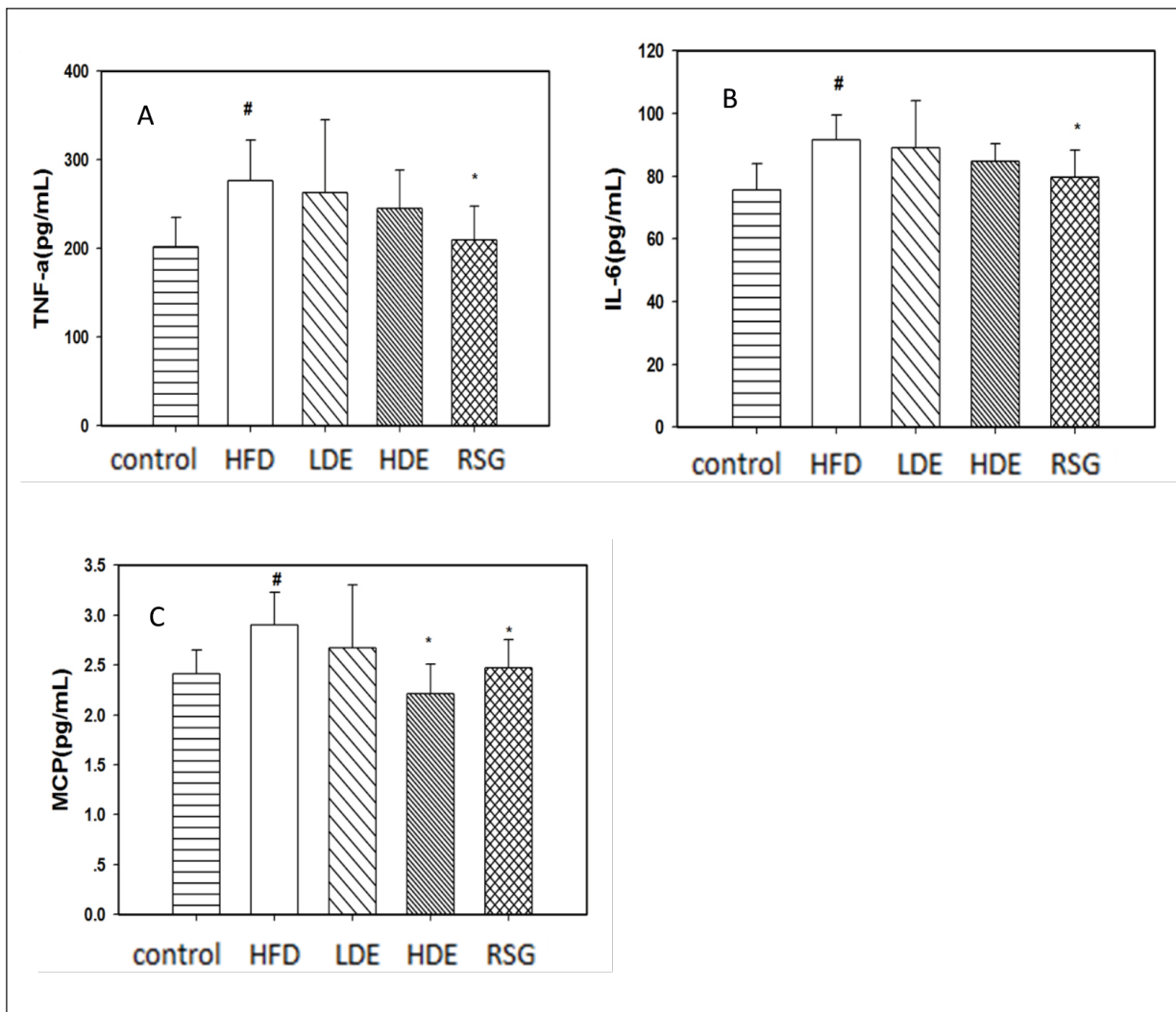
Figure 8. Effect of escin on TNF-α, IL-6 and MCP in mice induced by high-fat diet, as assessed by enzyme-linked immunosorbent assay. **A**, TNF-α; **B**, IL-6; **C**, MCP-1. Control group: standard chow diet; HFD group: HFD only; LDE: HFD + 7 mg/kg escin; HDE group: HFD + 40 mg/kg escin; RSG: Positive group, HFD + 40 mg/kg rosiglitazone. Results are expressed as the mean ± SD (n =9). # $p < 0.05$, compared with the control group; * $p < 0.05$ compared with the HFD group.

pathogenesis, we mainly discussed the effects of escin on inflammatory cytokines.

For obesity studies, diet-induced obesity in rodents has been used as an animal model to investigate environmental effects[28]. In our present study, the HFD-induced obesity in C57BL/6 mice was clearly confirmed by many factors including body weight gain, fat accumulation, dyslipidemia, hyperglycemia, and hyperinsulinemia.

To verify the effects of escin on fat metabolism, glucose metabolism and insulin resistance in HFD-induced C57BL/6 mice, this paper showed oral administration of escin, at a dose of 40 mg/kg, dramatically improved HFD-induced obesity in many parameters. During the 18-week feeding

and administration cycle, mice at the dose of 40 mg/kg escin weighed significantly lower than the model mice from 11 to 18 week. The result is consistent with Hu et al[8] findings on escin inhibiting fat absorption in the intestine by inhibiting pancreatic lipase and reducing weight gain.

Escin can improve lipid aggregation and abnormal lipid metabolism (Figure 7, Figure 9 and Figure 10). The adipocyte size of epididymal fat, renal fat and mesenteric fat was significantly increased in HFD groups, and escin significantly reduced adipocyte size in epididymal fat, renal fat and mesenteric fat. Serum TC, TG and LDL were significantly increased, and HDL was significantly decreased in obese mice induced by HFD. Escin
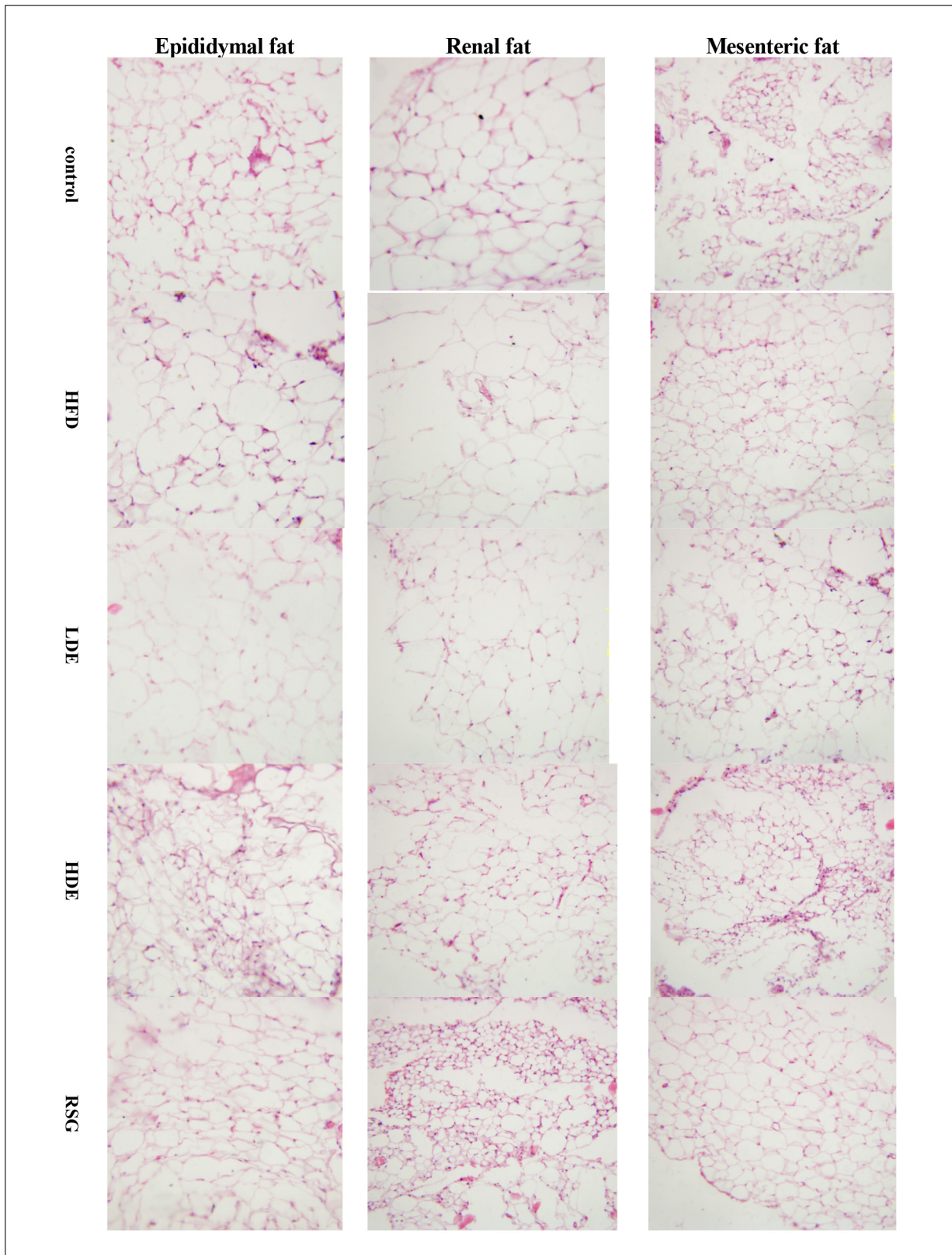
Figure 9. Effect of escin on the morphological effect of epididymal fat, renal fat and mesenteric fat in mice induced by high-fat diet (200×). Control group: standard chow diet; HFD group: HFD only; LDE: HFD + 7 mg/kg escin; HDE group: HFD + 40 mg/kg escin; RSG: Positive group, HFD + 40 mg/kg rosiglitazone.
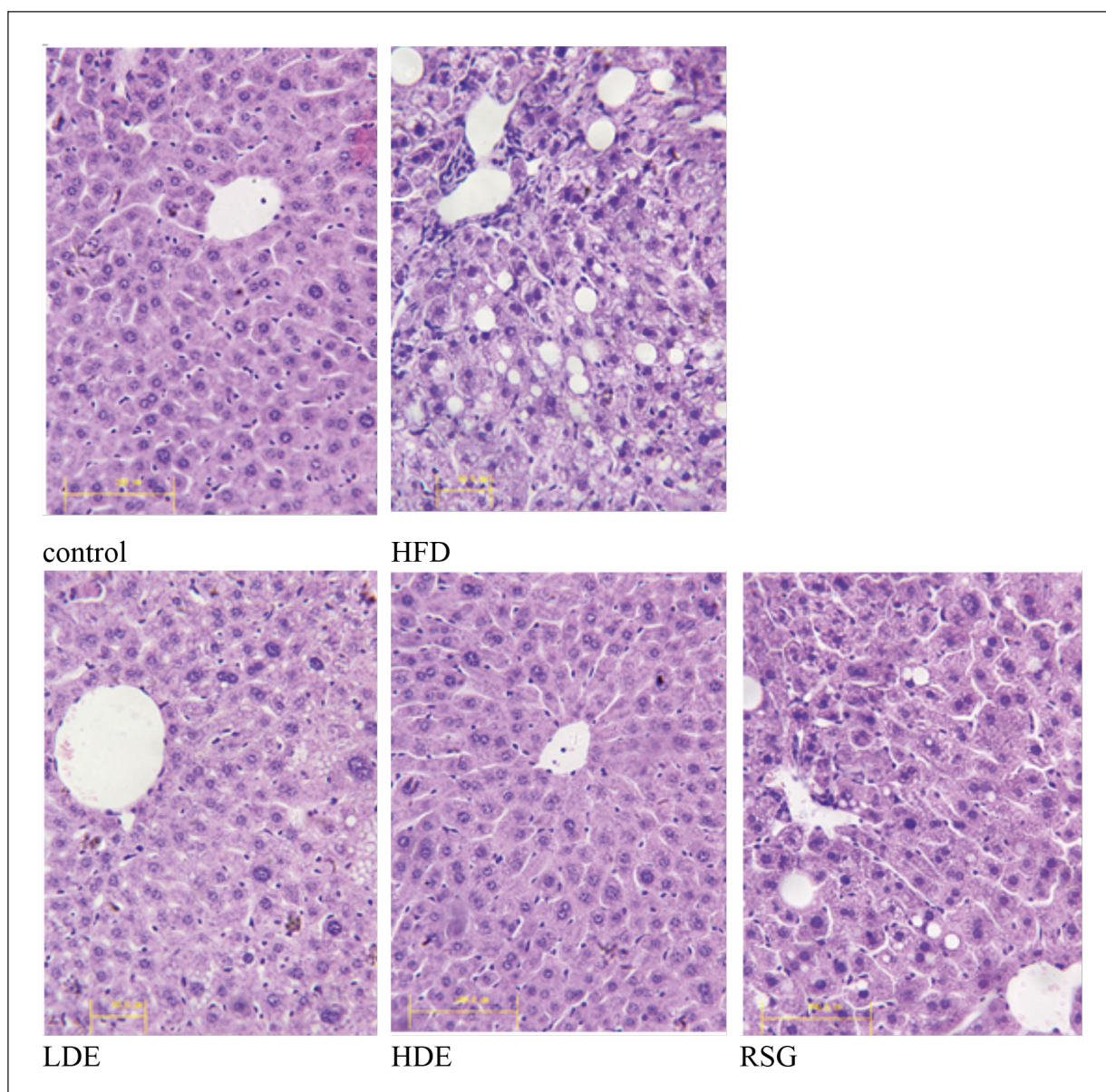
Figure 10. Effect of escin on the morphological effect of the liver in mice induced by high-fat diet (400×). Control group: standard chow diet; HFD group: HFD only; LDE: HFD + 7 mg/kg escin; HDE group: HFD + 40 mg/kg escin; RSG: Positive group, HFD + 40 mg/kg rosiglitazone.

can significantly reverse serum high TC, TG, and LDL, as well as low HDL and improved abnormal lipid metabolism induced by HFD. The excessive lipid accumulation in the liver caused by obesity can led to the hepatic steatosis (fatty liver)[29,30]. Our results showed that escin can reverse HFD-induced somatic swelling and fatty degeneration, indicating that escin can improve hepatic steatosis mainly by reducing fat accumulation.

Obesity contributes to the occurrence and development of type 2 diabetes. Fasting blood glu-

cose, postprandial blood glucose and insulin are important parameters to measure the contribution of obesity to type 2 diabetes[31]. Two hours postprandial blood glucose is a commonly method to detect the degree of insulin resistance. It reflects the ability of postprandial insulin to rapidly reduce blood glucose level and maintain the stable blood glucose levels[32]. It can be recognized that the individual has insulin resistance if the 2-h postprandial blood glucose was higher than 7.8 mmol/L, and the individual can be regarded as a diabetic

if the 2-h postprandial blood glucose was more than 11.1 mmol/L. In our study, the initial fasting blood glucose in mice induced by high-fat diet did not directly reflect the differences between HFD group and HDE group (data not displayed), but escin could ameliorate the increase of postprandial blood glucose and insulin in obese mice induced by HFD, which meant it could improve the insulin resistance of obese mice.

Both leptin and adiponectin are the hormones mainly produced by adipocytes and involved in obesity-related energy homeostasis and neuroendocrine regulation of appetite and satiety[33,34]. Obesity is associated with leptin and insulin resistance leading to hyperinsulinemia and hyperleptinemia. Adiponectin modulates glucose and lipid metabolism by stimulating fatty acid oxidation in almost all its major target tissues and negatively correlated with obesity[35]. In the present study, the leptin levels in HFD model group were significantly higher than those in the control group, while the adiponectin levels in the model group were significantly lower than those in the control group. Escin could reverse the high leptin and low adiponectin levels caused by obesity and improve the glucose and fat metabolism. The result of leptin is consistent with the literature[36]. The effect of escin on adiponectin has not been reported so far.

Obesity is associated with low-grade inflammation, which may exacerbate insulin resistance[37,38]. In our study, TNF-α, IL-6 and MCP-1 were significantly increased in obese mice induced by high fat diet, and escin could only antagonize the increase of MCP-1, suggesting that escin only partially antagonized the low-grade inflammation caused by obesity. This is different from what has been shown in other experimental models[6,39-41], and we found that the reason may be due to different experimental models.

Conclusions

In summary, we screened 53 overlapping genes for escin and obesity. The mechanism of intervention of escin in treating obesity may involve 10 hub targets (STAT3, MTOR, NR3C1, IKBKB, PTGS2, MMP9, PRKCA, PRKCD, AR, CYP3A4). The screening and enrichment analysis revealed that the treatment of obesity using escin primarily involved 10 GO enriched terms (G-protein coupled receptor signaling pathway, signal transduction, integral component of plasma membrane, plasma membrane, cytoplasm, cytosol,

nucleoplasm, zinc ion binding, identical protein binding, and protein binding in various BP, CC class and MF classification) and 13 related pathways (neuroactive ligand-receptor interaction, inflammatory mediator regulation of TRP channels, calcium signaling pathway, serotonergic synapse, pathways in cancer, gap junction, insulin resistance, vascular smooth muscle contraction, chemical carcinogenesis – receptor activation, HIF-1 signaling pathway, cAMP signaling pathway, MicroRNAs in cancer, and diabetic cardiomyopathy). *In vivo*, escin improved high-fat diet-induced obesity, reduced obese mouse weight, improved glucose and lipid metabolism, partially antagonized the low-grade inflammation state caused by obesity, and improved insulin resistance. All these effects suggested that escin can be used as a natural product for the prevention and treatment of obesity. The effects of escin on 10 hub targets, 10 GO enriched terms and 13 related pathways against obesity need to be further confirmed.

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