Study on the Mechanism of Lupenone for Treating type 2 Diabetes by Integrating Pharmacological Evaluation and Network Pharmacology

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Research

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Abstract

Background: Lupenone (LUP) is the active ingredient of *Rhizoma Musae*, which has good anti-diabetes effects, but the underlying mechanism is unclear. In this study, animal experiments combined with network pharmacology were used to explore the mechanism of LUP for treating diabetes.

Methods: Type 2 diabetic rats with insulin resistance (IR) were induced by a high-fat diet and streptozotocin. the fasting blood glucose (FBG), index of oxidative stress, blood lipids, and IR-related targets in skeletal muscle and adipose were detected. a network pharmacology-based strategy was also adopted to clarify the mechanism of LUP for treating diabetes by improving IR.

Results: LUP decreased the FBG levels and synthesis of glycogen, improved oxidative stress and lipid metabolism disorders, and increased the gene and protein expression of insulin receptor, insulin receptor substrate (IRS)-1, IRS-2, glucose transporter type 4 (GLUT-4) in skeletal muscle and peroxisome proliferator-activated receptor γ , IRS-1, IRS-2, GLUT-4 in adipose. Network pharmacology analysis revealed that LUP improves IR by multiple targets (like INS, TP53, TNF, SRC and ESR1) and signal pathways.

Conclusion: These results suggested that the mechanism of LUP for treating diabetes is closely related to improving IR. LUP has the potential to develop as a new drug for the treatment of type 2 diabetes.

Background

Diabetes mellitus is a chronic disease and it is characterized by hyperglycemia [1]. There are three main types of diabetes including type 1 diabetes, type 2 diabetes (T2DM), and gestational diabetes. Among them, T2DM is the most common. The T2DM put a huge burden on the global health-care systems [2]. Drugs currently used clinically for the treatment of T2DM have many limitations and adverse reactions, such as gastrointestinal reactions, hypoglycemia, edema, osteoporosis, and lactic acidosis [3]. Therefore, it is necessary to find an ideal drug for the safe and effective prevention and treatment of T2DM. Insulin resistance (IR) is one of the main factors leading to T2DM and its complications [4].

IR refers to a decrease in the response of biological target tissues (mainly including liver, muscle, and adipose) to normal physiological concentrations of insulin. Modern research finds that improving IR is an effective way to treat T2DM. The traditional Chinese medicine could treat T2DM through improving the IR, especially in increasing the sensitivity of muscle, adipose tissue, and liver to insulin [5–7]. Our research team has confirmed that *Rhizoma Musae* (the dried rhizome of *Musa basjoo* Sieb. et Zucc.), a traditional Chinese medicine, has a good anti-diabetic effect, and the lupenone (LUP) is isolated from the *Rhizoma Musae*, has good inhibitory effect on protein tyrosine phosphatase 1B (PTP1B) and α -glucosidase *in vitro* [8–10]. Furthermore, LUP could reduce the fasting blood glucose (FBG) in diabetic mice and improve the oral glucose tolerance and insulin tolerance in T2DM rats [11–13]. These results indicate that LUP has definite anti-diabetic effects. However, the mechanism of LUP in the treatment of T2DM has not been elucidated. Here, the aim of this study is to elucidate the mechanism of LUP to improve IR using animal experiments and network pharmacology methods.

Materials And Methods

Reagents

LUP was isolated from *Rhizoma Musae* by chromatographic separation on a silica gel column, and its structure was elucidated by comparison of its spectral data (mp, MS, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$) with reference standards [9]. The purity of LUP was $\geq 98\%$. Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in the study were of analytical grade.

Animals

All experiments were performed on 6-week-old male Sprague-Dawley rats (200 ± 20 g) obtained from Chongqing Tengxin Biological Technology Co., Ltd. (Chongqing, PR China). The colony was maintained under controlled conditions of temperature at $23 \pm 2^\circ\text{C}$, $50 \pm 5\%$ humidity, 12 h light/dark cycle. All the animals in the study were cared for and treated humanely according to the national legislations of China, as well as local guidelines. The animal experiments were approved by Guizhou University of Traditional Chinese Medicine and the Ethics Committee for Animal Experiments of West China Hospital of Sichuan University (under registration: 2013029A).

Induction Of Type 2 Diabetic Rats And Treatment

After acclimatization for one week, the rats were randomly divided into normal group and high-fat diet (HFD) group. The rats in normal group were fed with standard diet *ad libitum*, and the HFD group was fed with high-fat diet (15% cholesterol, 15% coconut oil, 15% granulated sugar, and 55% standard diet *ad libitum*). After feeding for 4 weeks, all rats were fasted for 12 h, then the HFD group animals were injected twice with low-dose streptozocin (STZ; first with 30 mg/kg, i.p.; second with 28 mg/kg, i.p. 24 h later) to induce partial insulin deficiency, and the normal group were intraperitoneal injected with sodium citrate buffer. After injection 72 h, the FBG levels of the rats were measured, and the rats with FBG of ≥ 11.1 mmol/L were regarded as T2DM rats. T2DM rats were further randomly divided into T2DM group, positive group, and three LUP treatment groups. The normal group and T2DM group were received the vehicle treatment (0.03% Tween 80; Twice daily), while the positive group were administered the rosiglitazone (1.00 mg/kg; Twice daily), and the LUP groups were administered with 2.0, 4.0, and 8.0 mg/kg LUP (Twice daily), respectively. After 2 weeks of treatment, 6 rats in each group were sacrificed by cervical dislocation, and the skeletal muscle and adipose tissues were preserved at -80°C until use for quantitative real-time PCR test; After 4 weeks of treatment, the rest rats were fasted overnight, and then blood samples were collected under anesthesia. The blood samples were centrifuged at 3500 rpm for 10 min, and then the serum was collected for biochemical analyses. The rats were sacrificed by cervical dislocation, the pancreas was fixed with 10% neutral buffered formalin for the histological analysis, and the liver tissues were fixed with Carnoy fixative solution used to perform periodic acid-Schiff staining, and

the skeletal muscle and adipose tissues were preserved at -80°C until use for testing protein expression. No death was recorded and no rats were dropped out of the analysis.

Determination Of Body Weight And Fbg

After the animal starts to dose, body weight of rats in all groups was measured every three days. All rats were fasted for 12 h every weekend to measure the FBG levels using the One-touch Ultra Blood Glucose Monitoring System (Johnson & Johnson Medical (China) Ltd., Shanghai, PR China).

Biochemical Parameter Analysis

The levels of serum leptin (LEP), hemoglobin A1c (HbA1c), superoxide dismutase (SOD), Malondialdehyde (MDA), Glutathione peroxidase (GSH-PX), Adiponectin (ADPN), and free fatty acid (FFA) in serum was analyzed by commercial ELISA kit according to the manufacturer's instructions (Abcom, Cambridge, UK). The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were detected by automatic biochemical analyzer (Olympus AU640, Japan).

Periodic acid-schiff (PAS) staining

PAS staining for glycogen and examined with light microscopy at 100× magnification were performed. Briefly, liver was subsequently routinely processed, embedded in paraffin. Sections of 5 µm thickness were cut. The sections of the liver tissue were deparaffinized and hydrated, and then immersed in 0.5% periodic acid solution for 10 min and rinsed in distilled water for another 5 min, twice. Then the sections were placed in Schiff reagent for 10 min and wash in water for 5 min before counterstained in hematoxylin for 2 min. Stained reddish dots or threadlike structures in the liver cells were considered to be a positive result.

Histological Analysis

The pancreas were fixed in 10% neutral buffered formalin for 48 h. Samples were subsequently routinely processed, embedded in paraffin. Sections of 5 µm thickness were cut, and stained with hematoxylin and eosin (HE) for general histopathological examination under a light microscope.

Quantitative Real-time Pcr

The total RNA of skeletal muscle and adipose tissue was isolated using trizol reagent (Invitrogen, Carlsbad, CA), and total RNA was used to synthesize cDNA using the PrimeScript RT reagent kit

according to the manufacture’s protocol (TaKaRa Bio, Dalian, China), then the amplification reactions were carried out in 96 well reaction plates (Bio-Rad, Hercules, CA, USA) with 20 µL reaction volume. GAPDH was used as endogenous control, and the fold change in the level of target mRNA between control and treatment groups were normalized by the level of GAPDH. The change in gene expression was calculated by $2^{-\Delta\Delta C_t}$ [14]. Primer sequences for analysis of insulin receptor (InsR), insulin receptor substrate (IRS)-1, IRS-2, and glucose transporter type 4 (GLUT-4) mRNA in skeletal muscle and proliferator-activated receptor (PPAR)-γ, IRS-1, IRS-2, and GLUT-4 mRNA in adipose tissue were described in Table 1.

Table 1
PCR primers used in all quantitative PCR assays

Primer: gene	Forward	Reverse
<i>Gapdh</i>	5'-CCA AGG TCA TCC ATG ACA AC-3'	5'-TGT CAT ACC AGG AAA TGA GC-3'
<i>Insr</i>	5'-GCT GGT GTT TTC CTC ATA GAT TG-3'	5'-CTG TAG GTC AGG TTT GGT TTT TG-3'
<i>Irs1</i>	5'-CCT GAC ATT GGA GGT GGG TCT T-3'	5'-GAA TCT TCG GCA GTT GCG GTA T-3'
<i>Irs2</i>	5'-TGC GAA CAG CCG TCG GTG AC-3'	5'-GAC CGG TGA CGG CTG AAC GG-3'
<i>Glut4</i>	5'-AGC CAG CCT ACG CCA CCA TA-3'	5'-GGA CCC ATA GCA TCC GCA AC-3'
<i>Ppary</i>	5'-CTG GCC TCC CTG ATG AAT AA-3'	5'-GGC GGT CTC CAC TGA GAA TA-3'

Western Blotting

Proteins were extracted from skeletal muscle and adipose tissue by using lysis buffer. The proteins were then separated by SDS-PAGE (10% or 15%) and electrophoretically transferred onto polyvinylidene fluoride membranes. The membranes were probed with anti-INSR (1:20000; Abcam, Cambridge, UK), anti-IRS-1 (1:500; Abcam, Cambridge, UK), anti-IRS-2 (1:5000; Abcam, Cambridge, UK), anti-GLUT-4 (1:2000; Abcam, Cambridge, UK), and anti-PPAR-γ (1:5000; Abcam, Cambridge, UK) overnight at 4°C, and then incubated with a HRP-coupled secondary antibody. Detection was performed using a ChemiDoc XRS⁺ (BioRad, Hercules, CA, USA) image analysis system.

Compound And Ir Related Targets

The targets of LUP were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, [http://tcmspw.com/tcmsp.- php](http://tcmspw.com/tcmsp.-php)), Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>), and BATMAN-TCM (<http://bionet.ncpsb.org/batman->

tcm/index.php). The genes targets associated with IR were screened by comprehensive database of human genes and gene phenotypes (OMIM, <http://www.omim.org/>), DurgBank database (<https://www.drugbank.ca/>), and GeneCards (<https://www.genecards.org/>).

Construction Of "component-target-disease" Network

The LUP-related targets and IR-related targets were linked into a "component-target-disease" network, and the interaction between proteins was realized by STRING database (<http://string-db.org/>). The above network was visualized using Cytoscape ver. 3.7.1 software (<http://www.cytoscape.org/>).

Biological Process Analysis And Pathway Analysis

Gene Ontology (GO) functional annotation and KEGG pathway analysis were performed to explore related biological processes and signaling pathways.

Ppi Network Analysis And Screening Of The Core Targets

Protein protein interaction (PPI) network analysis was performed by STRING database. In "component-target-disease" network, there are three key topology parameters in the network including betweenness centrality, closeness centrality, and degree, which can be used to screen the more important nodes [15]. Thus, the nodes with degree and betweenness centrality value two times higher than the median value of all nodes, and simultaneously satisfy the closeness centrality value greater than median value of nodes were chosen as the core targets.

Molecular Docking

The *mol2 format of LUP was downloaded at the TCMSP database, and the 3D structure (*PDB format) format of the targets were obtained from the PDB protein database (<https://www.rcsb.org/>). PyMol software was used to perform dehydration and hydrogenation of the proteins, and the docking was performed using AutoDock Vina software, and the docking results were analyzed and visualized.

Results

LUP improves body weight loss and decreases the FBG in T2DM rats

LUP inhibited the body weight loss induced by STZ. The results were shown in Fig. 1a. After 2 weeks of treatment, the LUP and rosiglitazone significantly decreased the FBG level ($P < 0.05$). After 3 weeks of treatment, LUP (2.0 and 8.0 mg/kg) decreased the FBG level ($P < 0.05$). After 4 weeks of treatment, LUP

(4.0 and 8.0 mg/kg) could significantly decrease the FBG level of rats ($P < 0.05$). The results showed that LUP has a stable hypoglycemic effects in T2DM rats (Fig. 1b).

LUP decreases the levels of LEP, HbA1c, MDA and increases the ADPN levels and GSH-PX activity in T2DM rats

After treated with LUP for 4 weeks, LUP (8.0 mg/kg) and rosiglitazone significantly reduced the levels of LEP in serum (Fig. 2A). In addition, rosiglitazone and LUP (4.0 and 8.0 mg/kg) significantly reduced the serum HbA1c levels in T2DM rats (Fig. 2C). Rosiglitazone and LUP (8.0 mg/kg) also significantly reduce serum MDA levels and increase the serum GSH-PX activity (Fig. 2D and Fig. 2F). Furthermore, rosiglitazone and LUP (2.0 and 8.0 mg/kg) increased the serum ADPN levels (Fig. 2B). However, LUP had only a slight effect on serum SOD activity (Fig. 2E).

Lup Improves The Lipid Metabolism Disorder In T2dm Rats

Compared to the normal group, the levels of FFA, TG, and TC of the DM group were significantly increased, suggesting that the lipid metabolism disorder occurred in the T2DM rats. LUP (8.0 mg/kg) significantly reduced the levels of FFA, TG, and TC in serum ($P < 0.05$). In addition, LUP (2.0 and 4.0 mg/kg) significantly decreased the TC levels and LUP (4.0 mg/kg) reduced the levels of TG in serum (Fig. 3a-3c). Furthermore, rosiglitazone and LUP (2.0 mg/kg) significantly reduced the ratio of TC/HDL-C (Fig. 3f). However, LUP had no significant effect on LDL-C and HDL-C levels in serum (Fig. 3d and Fig. 3e).

LUP increases the synthesis of hepatic glycogen in T2DM rats

The liver cell morphology of T2DM group was complete, but the interstitial was hyperplasia, the cell gaps of hepatocytes were large, cells were irregularly arranged, and the intercellular and intracellular PAS staining was low and relative homogeneity was poor. While, in the normal, rosiglitazone, and the LUP (8.0 mg/kg) group, the structure of hepatocytes was tight and neat, with no cell gaps. Furthermore, the intercellular and the intracellular PAS staining were diffusely colored and had good homogeneity. Compared to the T2DM group, in the LUP (2.0 and 4.0 mg/kg) group, the interstitial hyperplasia was less, cells gap was smaller, the cells were arranged regularly, and PAS-positive cells were significantly increased. The rosiglitazone and LUP could increase the synthesis of hepatic glycogen in T2DM rats (Fig. 4A).

LUP improves the pathological changes of the pancreas in T2DM rats

The structure of the exocrine part of pancreatic tissue in rosiglitazone and LUP (2.0, 4.0, and 8.0 mg/kg) group was normal, the endocrine part of pancreatic tissue had a small amount of fatty degeneration and no obvious inflammatory cell infiltration was observed (Fig. 4B).

LUP increases the mRNA and protein expression of InsR, IRS-1, IRS-2, GLUT-4 in skeletal muscle and PPAR- γ , IRS-1, IRS-2, GLUT-4 in adipose of T2DM rats

The mRNA expression of InsR, IRS-1, IRS-2, and GLUT-4 in skeletal muscle and PPAR- γ , IRS-1, IRS-2, and GLUT-4 in adipose of T2DM rats was decreased (Fig. 5a-5h). LUP (4.0 and 8.0 mg/kg) and rosiglitazone significantly increased the expression of InsR, IRS-1, IRS-2, and GLUT-4 mRNA in skeletal muscle ($P < 0.05$). In addition, LUP (2.0 mg/kg) significantly increased the expression of IRS-1 mRNA in skeletal muscle ($P < 0.05$). Furthermore, LUP (8.0 mg/kg) and rosiglitazone significantly increased the expression of PPAR- γ , IRS-1, IRS-2, and GLUT-4 mRNA in adipose tissue ($P < 0.05$). In addition, LUP (2.0 and 4.0 mg/kg) significantly increased the expression of IRS-1 and GLUT-4 mRNA in adipose, and LUP (4.0 mg/kg) could significantly increase the expression of PPAR- γ mRNA in adipose tissue ($P < 0.05$).

The protein expression of InsR, IRS-1, IRS-2, and GLUT-4 in skeletal muscle and PPAR- γ , IRS-1, IRS-2, and GLUT-4 in adipose of diabetic rats was significantly decreased (Fig. 6A-6H). Rosiglitazone significantly increased the protein expression of IRS-1, IRS-2, and GLUT-4 in skeletal muscle and PPAR- γ , IRS-2, and GLUT-4 in adipose tissue ($P < 0.05$). LUP (8.0 mg/kg) significantly increased the protein expression of InsR, IRS-1, IRS-2, and GLUT-4 in skeletal muscle and PPAR- γ , IRS-1, IRS-2, and GLUT-4 in adipose tissue ($P < 0.05$). In addition, LUP (4.0 mg/kg) increased the protein expression of PPAR- γ in adipose tissue ($P < 0.05$).

Network Analysis

An interactive network of LUP to improve IR was constructed, visualized using different color and shape patterns (Fig. 7). Among them, the red triangle is the chemical component, the pink diamond is disease, and the green circle is the common target genes of the component and the disease.

Go Functional Annotation And Kegg Pathway Analysis

GO functional annotation was performed. 139 potential targets were used to perform the biological functional enrichment using the R packages ggplot2 and cluster Profiler. A total of 2724 biological processes with a total P value of ≤ 0.01 were enriched. Biological process was most strongly correlated with response to steroid hormone, protein kinase B signaling, gland development, etc. (Fig. 8A); In cellular component, there was 110 biological processes with P value ≤ 0.01 , including neuronal cell body, dendritic spine, neuron spine, Golgi lumen, etc. (Fig. 8B); In molecular function, there were 150 biological processes with P value ≤ 0.01 , among which G protein-coupled receptor binding, histone deacetylase binding, 1-phosphatidylinositol-3-kinase activity were the stronger (Fig. 8C).

A total of 164 pathways were screened using the KEGG enrichment analysis ($P \leq 0.01$), and some of the signal pathways are shown in Fig. 9. The LUP might improve IR by acting on Prostate cancer, Endocrine

resistance, AGE-RAGE signaling pathway in diabetic complications, Proteoglycans in cancer, Kaposi sarcoma-associated herpesvirus infection, etc. The targets of LUP's direct effect on IR is shown in Fig. 10.

Ppi Network Analysis And The Core Targets Results

The targets were introduced into the STRING database, and the specie was “homo sapiens”, then the interaction network of the protein targets was obtained, as shown in Fig. 11. There was a total of 139 nodes and 1357 edges in the network. In addition, the results of topological analysis in the network showed that the median value of Betweenness Centrality, Closeness Centrality, and Degree were 0.000577, 0.534351, and 18, respectively. According to the screening criteria, 24 hub targets of LUP were obtained. The results are shown in Table 2.

Table 2
Topological analysis of core targets in "lupenone-target-disease" network

Gene name	BetweennessCentrality	ClosenessCentrality	Degree
INS	0.068121	0.756757	95
TP53	0.02875	0.679612	74
TNF	0.023754	0.669856	71
SRC	0.022054	0.660377	68
ESR1	0.013141	0.633484	59
JUN	0.009126	0.625	56
PTGS2	0.008946	0.611354	51
HSP90AA1	0.01071	0.608696	50
CTNNB1	0.009412	0.606061	49
MTOR	0.008296	0.606061	49
AR	0.007686	0.598291	46
MAPK14	0.005711	0.595745	45
IL10	0.006232	0.59322	44
SIRT1	0.005683	0.59322	44
PIK3CA	0.005147	0.588235	42
IL4	0.005161	0.585774	41
KIT	0.004878	0.585774	41
RELA	0.003582	0.585774	41
HIF1A	0.004726	0.583333	40
EDN1	0.004736	0.578512	38
PIK3R1	0.004544	0.578512	38
MDM2	0.004322	0.578512	38
AGT	0.006154	0.576132	37
CRP	0.005963	0.576132	37

Molecular Docking Results

The conformation with the highest score (lowest affinity value) was selected, and the docking conformation was visualized using PyMol software. The results showed that the LUP had good affinity with the proteins corresponding to the genes including INS, TP53, TNF, SRC, ESR1, JUN (Fig. 12). These six genes were the most relevant genes for LUP to improve IR according to the network analysis.

Discussion

In this study, we found that LUP could inhibit the body weight loss and decreased the FBG levels. Furthermore, LUP improved the lipid metabolic disorders and reduced the excessive oxidative stress in T2DM rats. Moreover, LUP promoted the synthesis of hepatic glycogen and improved the pathological changes of pancreas. LUP could improve the IR via regulation of gene and protein expression in T2DM rats. In addition, network pharmacology analysis revealed that LUP improve IR by multiple targets and signal pathways. These results indicate that LUP has the potential to be developed as a new drug for the treatment of T2DM.

T2DM rats induced by HFD/STZ are often accompanied by weight loss [16, 17], but there are conflicting reports on the changes in body weight of T2DM rats after drug treatment [18]. In this study, LUP could inhibit the body weight loss of T2DM rats. In addition, LUP decreased the LEP level, which is closely related to the appetite, body weight, neuroendocrine functions and glycaemia [19]. The above results suggested that LUP has a good effect on weight maintenance of T2DM rats. As we know, HbA1c was formally included in the diagnostic index of T2DM by the American Diabetes Association (ADA) in 2010. LUP could reduce the FBG and HbA1c levels in T2DM rats, and the result is consistent with our previous work [11]. Furthermore, the IR is closely related to the hepatic glycogen metabolism disorders, LUP could increase the synthesis of hepatic glycogen, so LUP might improve the IR by regulating gluconeogenesis in T2DM rats.

Lipid metabolism disorders are closely related to the cardiovascular diseases and T2DM [20], and TC/HDL-C ratio is a reasonable blood lipid index in predicting the cardiovascular risk of T2DM patients. Our results showed that LUP significantly decrease the levels of TC, TG, and TC/HDL-C ratio, which indicates that LUP can improve the lipid metabolism disorders and may reduce cardiovascular risk in T2DM.

The oxidative stress caused by obesity, hyperglycemia, FFA, and inflammation could lead to the islet β cell damage [21]. As report, long-term exposure to high concentrations of FFA will damage islet β cell function and reduce the secretion of insulin, and then the gluconeogenesis is increased, eventually IR is observed [22]. Moreover, ADPN can increase the muscle and liver sensitivity to insulin by increasing FFA oxidation [23], and ADPN level is an important marker for improvement of IR [24]. LUP could decrease FFA levels and increase ADPN secretion in T2DM rats, indicating that LUP could improve the IR of T2DM rats in this work. Furthermore, levels of SOD, MDA, and GSH-PX are closely related to the islet β cells damage [25, 26]. LUP increased the GSH-PX activity and reduced MDA levels in T2DM rats. Interestingly, LUP also

inhibited the mild inflammatory response in pancreas tissue. Taken together, LUP might protect the T2DM via inhibiting the inflammation, and the result is consistent with our previous work [27].

InsR/PI3K/GLUT-4 signaling pathway plays an important role in improving IR in skeletal muscle and adipose tissue [28]. This study found that LUP could increase the gene and protein expression of InsR in skeletal muscle and GLUT-4 in skeletal muscle and adipose tissue. Network pharmacology analysis results showed that LUP can improve IR by acting on PI3K (Fig. 10). These results indicate that LUP may increase the sensitivity of skeletal muscle and adipose to insulin, at least partly, by activating the InsR/PI3K/GLUT-4 signaling pathway. According to the report, the expression of IRS-1 in skeletal muscle and adipose tissue of T2DM patients is significantly decreased [29, 30]. Both IRS-1 and IRS-2 function in peripheral carbohydrate metabolism, and IRS-2 has the major role in β -cell development and compensation for peripheral IR [31]. In present work, LUP up-regulated the gene and protein expression of IRS-1 and IRS-2 in skeletal muscle and adipose tissue, suggesting that LUP may improve the IR via acting on the family of insulin substrates in T2DM. In addition, PPAR- γ was known to play a major role in the improvement of insulin sensitivity through stimulating differentiation of small adipocytes from preadipocyte [32]. Here, LUP might improve the IR by up-regulating the expression of PPAR- γ . All in all, the above results showed that LUP improves the skeletal muscle and adipose tissue sensitivity to insulin through increasing the gene and protein transcription related to IR in T2DM.

Combining the drug-target network with the biological system network can provide new approaches and strategies for new drug development [33]. Network pharmacological studies have found that LUP improve IR through multiple targets and multiple pathways. The biological process is mainly related to the regulation of cell proliferation, signal transduction, metabolism, cell differentiation, etc. the result suggested that LUP could improve IR by regulating multiple biological processes. KEGG pathway analysis also showed that LUP improve IR involving multiple signaling pathways, like AGE-RAGE signaling pathway in diabetic complications, IR, Type II diabetes mellitus. Advanced glycation end products (AGEs) play a significant role in the pathogenesis of multiple diabetic complications [34]. These results indicated that LUP might be used for the prevention and treatment of T2DM and diabetic complications. Of course, many signaling pathways related to cancer, tumor, atherosclerosis, and infection were predicted, indicating that LUP may have various pharmacological activities, which deserves further research.

Conclusion

LUP could improve the lipid metabolism disorder and alleviate the excessive oxidative stress in T2DM. Furthermore, as expected, LUP improved the IR via increasing the sensitivity of skeletal muscle and adipose tissue to insulin by regulating related gene and protein expression in T2DM. Our preliminary results indicate that LUP has the potential to be developed as a new drug for the treatment of T2DM.

Abbreviations

ADPN: Adiponectin; FBG: Fasting blood glucose; FFA: Free fatty acid; GLUT-4: Glucose transporter type 4; GO: Gene Ontology; GSH-PX: Glutathione peroxidase; HbA1c: Hemoglobin A1c; HDL-C: High-density lipoprotein-cholesterol; HE: Hematoxylin and eosin; HFD: High-fat diet; InsR: Insulin receptor; IRS: Insulin receptor substrate; LDL-C: Low-density lipoprotein-cholesterol; LEP: Leptin; LUP: Lupenone; MDA: Malondialdehyde; OMIM: Comprehensive database of human genes and gene phenotypes; PPAR: Proliferator-activated receptor; PPI: Protein protein interaction; PTP1B: Protein tyrosine phosphatase 1B; SOD: Superoxide dismutase; STZ: Streptozotocin; TC: Total cholesterol; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; TG: Triglyceride; T2DM: Type 2 diabetes

Declarations

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Authors' contributions

Xiangpei Wang, Feng Xu, and Hongmei Wu contributed to the study conception and design, acquisition, analysis and interpretation of data, and writing of the article. Yuanmin Wang and Ye Yang contributed to the acquisition and interpretation of data and also to the final critical revision of the article. All authors read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

All experiments in the present study were approved by the were approved by Guizhou University of Traditional Chinese Medicine and the Ethics Committee for Animal Experiments of West China Hospital

of Sichuan University (under registration: 2013029A).

Consent for publication

The manuscript is approved by all authors for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al (2017) IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 128: 40-50.
2. Peiró C, Lorenzo Ó, Carraro R, Sanchez-Ferrer CF (2017) IL-1 β Inhibition in Cardiovascular Complications Associated to Diabetes Mellitus. *Fron Pharmacol* 8: 363.
- Radziuk J, Pye S (2001) Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis. *Diabetes-metab Res* 4: 250-272.
3. Donath MY (2014) Targeting inflammation in the treatment of type 2 diabetes: time to start. *Nat Rev Drug discov* 6: 465-476.
4. Guo S, Ouyang H, Du W, Li J, Liu M, Yang S, et al (2021) Exploring the protective effect of *Gynura procumbens* against type 2 diabetes mellitus by network pharmacology and validation in C57BL/KsJ db/db mice. *Food Funct* 4: 1732-1744.
5. Tang JJ, Li JG, Qi W, Qiu WW, Li PS, Li BL, et al (2011) Inhibition of SREBP by a Small Molecule, Betulin, Improves Hyperlipidemia and Insulin Resistance and Reduces Atherosclerotic Plaques. *Cell Metab* 1: 44-56.
6. Sheng JQ, Tu XY, Wei XX, Li Y, Xu GL (2014) Effects of puerarin on insulin resistance in HepG2 cell line. *Lishizhen Med Mater Med Res* 8: 1831-1833.
7. Li GS, Liu YH, Li XY, Gao ZN, Huang L, Liu YL (2016) Regulations of berberine on gene expression of BMP4 transcriptional pathways to improve visceral white adipose tissues insulin resistance in type 2 diabetic hamsters. *Chin J Chin Mater Med* 3: 514-520.
8. Na M, Kim BY, Osada H, Ahn JS (2009) Inhibition of protein tyrosine phosphatase 1B by lupeol and lupenone isolated from *Sorbus commixta*. *J Enzyme Inhib Med Chem* 4: 1056-1059.
9. Wang XP, Hao JJ, Xu SN (2012) The Chemical Constituents in Ethyl Acetate Extraction from the *Rhizoma Musae*. *Lishizhen Med Mater Med Res* 23: 515-516.

10. Seong SH, Roy A, Jung HA, Jung HJ, Choi JS (2016) Protein tyrosine phosphatase 1B and α -glucosidase inhibitory activities of *Pueraria lobata* root and its constituents. *J Ethnopharmacol* 194: 706-716.
11. Xu F, Wu H, Wang X, Yang Y, Wang YM, Qian H, et al (2014) RP-HPLC characterization of lupenone and β -sitosterol in *Rhizoma Musae* and evaluation of the anti-diabetic activity of lupenone in diabetic Sprague-Dawley rats. *Molecules* 9: 14114-14127.
12. Wu HM, Xu F, Hao J, Yang Y, Wang XP (2015) Antihyperglycemic activity of banana (*Musa nana* Lour.) peel and its active ingredients in alloxan-induced diabetic mice. *3rd International Conference on Material, Mechanical and Manufacturing Engineering*: 231-238.
13. Wu HM, Xu F, Wang YM, Qian HB, Wang XP (2017) Effects of lupenone on the general condition, impaired glucose tolerance and insulin tolerance in Type 2 Diabetes Mellitus with insulin resistance. *Lishizhen Med Mater Med Res* 5: 1035-1037.
14. Xu F, Wang F, Wen T, Sang W, Zeng N (2017) Inhibition of NLRP3 inflammasome: a new protective mechanism of cinnamaldehyde in endotoxin poisoning of mice. *Immunopharmacol Immunotoxicol* 5: 296-304.
15. Huang X, Zhang M, Wu H, Wang X, Xu F (2021) Study on the active ingredients and potential targets of rice bran petroleum ether extracts for treating diabetes based on network pharmacology. *Comb Chem High T Scr* 6: 790-802.
16. Sharma AK, Bharti S, Ojha S, Bhatia J, Kumar N, Ray R, et al (2011) Up-regulation of PPAR γ , heat shock protein-27 and -72 by naringin attenuates insulin resistance, β -cell dysfunction, hepatic steatosis and kidney damage in a rat model of type 2 diabetes. *Br J Nutr* 11: 1713-1723.
17. Wei Y, Gao J, Qin L, Xu Y, Wang D, Shi H, et al (2017) Tanshinone I alleviates insulin resistance in type 2 diabetes mellitus rats through IRS-1 pathway. *Biomed Pharmacother* 93: 352-358.
18. Gheibi S, Kashfi K, Ghasemi A (2017) A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. *Biomed Pharmacother* 95: 605-613.
19. Coppari R, Bjørbaek C (2012) Leptin revisited: its mechanism of action and potential for treating diabetes. *Nat Rev Drug Discov* 9: 692-708.
20. Loza-Rodríguez H, Estrada-Soto S, Alarcón-Aguilar FJ, Huang F, Aquino-Jarquín G, Fortis-Barrera Á, et al (2020) Oleanolic acid induces a dual agonist action on PPAR γ / α and GLUT4 translocation: A pentacyclic triterpene for dyslipidemia and type 2 diabetes. *Eur J Pharmacol* 883: 173252.
21. Halim M, Halim A (2019) The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes). *Diabetes Metab Syndr* 2: 1165-1172.

22. Zhande R, Mitchell JJ, Wu J, Sun XJ (2002) Molecular mechanism of insulin induced degradation of insulin receptor substrate-1. *Mol Cell Biol* 4: 1016-1026.
23. Wang XJ, Jiang ZS, Huang JK (2009) The study of relationship between adiponectin and insulin resistance in type 2 diabetic patients with obesity. *Chin J Arterioscler* 7: 535-538.
24. Yukihiro Y, Hiroshi H, Ikuo S, Kanako N, Takao S (2013) Adiponectin, an Adipocyte-Derived Protein, Predicts Future Insulin Resistance: Two-Year Follow-Up Study in Japanese Population. *J Clin Endocrinol Metab* 1: 87-90.
25. Coskun O, Kanter M, Korkmaz A, Oter S (2005) Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β -cell damage in rat pancreas. *Pharmacol Res* 2: 117-123.
26. Sun W, Zheng XZ, Nian H, Xu QL (2007) Effects of puerarin on MDA content, SOD and ATPase activities in mitochondria of pancreas in diabetic rats. *Chin J Gerontol* 12: 1141-1143.
27. Xu F, Yang L, Huang X, Liang Y, Wang X, Wu, H (2020) Lupenone is a good anti-inflammatory compound based on the network pharmacology. *Mol Divers* 1: 21-30.
28. Liu X, Jin QG, Luo Q (2012) Effects of aerobic exercise and dietary control on InsR/PI3K/GLUT4 signaling pathway in skeletal muscle of rats with type 2 diabetes. *Chin J Sports Med* 11: 988-993.
29. Ducluzeau PH, Perretti N, Laville M, Andreelli F, Vega N, Riou JP, et al (2001) Regulation by insulin of gene expression in human skeletal muscle and adipose tissue: evidence for specific defects in type 2 diabetes. *Diabetes* 5: 1134-1142.
30. Hammarstedt A, Jansson PA, Wesslau C, Yang X, Smith U (2003) Reduced expression of PGC-1 and insulin-signaling molecules in adipose tissue is associated with insulin resistance. *Biochem Biophys Res Commun* 2: 578-582.
31. Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF (1999) Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signaling. *Nat Gene* 1: 32-40.
32. Kadowaki T, Hara K, Yamauchi T, Terauchi Y, Tobe K, Nagai R (2003) Molecular mechanism of insulin resistance and obesity. *Exp Biol Med* 10: 1111-1117.
33. Hopkins AL (2007) Network pharmacology. *Nat Biotechnol* 10: 1110-1111.
34. Sergi D, Boulestin H, Campbell FM, Williams LM (2021) The Role of Dietary Advanced Glycation End Products in Metabolic Dysfunction. *Mol Nutr Food Res* 1: e1900934.

Figures

Figure 1

Lupenone prevents the body weight loss (a), decreases the fasting blood glucose (b) in diabetic rats. Data presented as mean $\pm$ SD. $n=9-10$ for the body weight change and fasting blood glucose. * $P<0.05$ versus the DM group, # $P<0.05$ versus the normal group. SD: standard deviation.

Figure 2

Lupenone decreased the levels of LEP (A) and HbA_{1c} (C), increased the ADPN (B) levels, and reduced the excessive oxidative stress in the serum of diabetic rats. Lupenone decreases the levels of MDA (D) and

reduced GSH-PX (F) activity, but has no significantly effect on the SOD (E) activity. Data are means $\pm$ SD. There are nine rats in normal group, and ten rats in the other groups. *P<0.05 versus the DM group, #P<0.05 versus the normal group. SD: standard deviation.

Figure 3

Lupenone improves the lipid metabolic disorder in diabetic rats. Lupenone decreases the FFA (a), TC (b), and TG (c) levels. Lupenone has no significantly effect on the levels of LDL-C (d) and HDL-C (e), but the 2.0 mg/kg lupenone could reduce the TC/HDL-C (f) ratio. Data are means $\pm$ SD. There are nine rats in normal group, and ten rats in the other groups. *P<0.05 versus the DM group, #P<0.05 versus the normal group. SD: standard deviation.

Figure 4

Lupenone increases the synthesis of hepatic glycogen (A; 100× magnification) and improves the pathological changes of the pancreas (B; 400× magnification), six rats per group. Stained reddish dots or threadlike structures in the liver cells were considered to be as positive result, rosiglitazone and lupenone increased the synthesis of hepatic glycogen in diabetic rats (A). Acinar cell steatosis (short arrows) and small irregular-shaped islet (long arrows) was observed in pancreas, rosiglitazone and lupenone protected the structure of the exocrine part of pancreatic tissues and decreased the fatty degeneration (B).

Figure 5

Lupenone increases the mRNA expression in skeletal muscle and adipose tissue related to insulin resistance of diabetic rats. Lupenone increases the mRNA expression of InsR (a), IRS-1(b), IRS-2 (c), and GLUT-4 (d) in skeletal muscle and PPAR-γ (e), IRS-1 (f), IRS-2 (g), and GLUT-4 (h) in adipose of diabetic rats. Data are means ±SD from six rats per group. *P<0.05 versus the DM group, #P<0.05 versus the normal group. SD: standard deviation.

Figure 6

Lupenone increases the protein expression in skeletal muscle and adipose tissue related to insulin resistance of diabetic rats. Lupenone increases the protien expression of InsR (A), IRS-1(B), IRS-2 (C), and GLUT-4 (D) in skeletal muscle and PPAR-γ (E), IRS-1 (F), IRS-2 (G), and GLUT-4 (H) in adipose of diabetic rats. Data are means ±SD from six rats per group. *P<0.05 versus the DM group, #P<0.05 versus the normal group. SD: standard deviation. K = Normal, M = DM, Y = Rosiglitazone, D = 2.0 mg/kg Lupenone, Z = 4.0 mg/kg Lupenone, G = 8.0 mg/kg Lupenone.

Figure 7

"lupenone-target-disease" interaction network. The red triangle is the chemical component, the pink diamond is disease, and the green circle is the common target genes of the component and the disease, and line represents each side, indicating the interaction between the components and the targets.

A

B

C

Figure 8

GO enrichment analysis of target proteins. GO enrichment analysis include biological process (A), cellular component (B), and molecular function (C).

Figure 9

KEGG pathway analysis of the targets.

Figure 11

Interaction network of targets, PPI enrichment P-value: $P < 1.0e-16$.

INS (1ZNI Affinity=-6.3)

TP53 (3ZME Affinity=-6.4)

TNF (5MU8 Affinity=-5.3)

SRC (6PDJ Affinity=-5.3)

ESR1 (3OSA Affinity=-5.2)

JUN (1JNM Affinity=-5.9)

Figure 12

Molecular docking results of lupenone and the corresponding protein of the gene targets.