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ORIGINAL ARTICLE

# Predicting the Targets of Mangiferin in the Treatment of Diabetes Mellitus on Network Pharmacology and Analysis of its Metabolites *in vivo*

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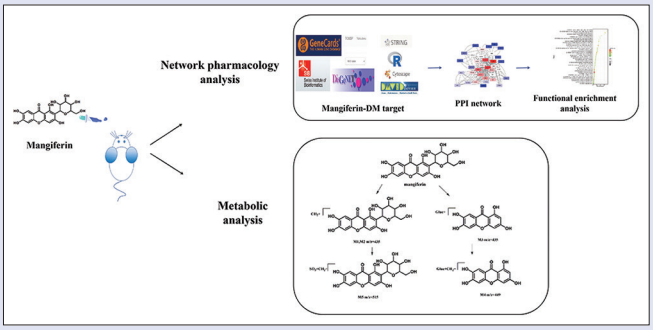
## ABSTRACT

**Background:** The number of diabetic patients worldwide continues to increase. Diabetes mellitus (DM) has become one of the three chronic diseases threatening human health. Mangiferin is a kind of xanthone with multiple biological activities. Studies have confirmed that it has good activity in treating DM. **Objectives:** The purpose of our study was to investigate the targets and mechanism of mangiferin in the treatment of DM and to analyze its metabolites *in vivo*. **Materials and Methods:** We predict the targets and mechanism of mangiferin in DM in view of network pharmacology (NP). Mangiferin's targets were completed using Gene Cards, Swiss Target Prediction, and TCMSP database. DisGeNET database retrieved DM-related targets. The common targets were put into STRING platform to construct a protein–protein interaction (PPI) network model. DAVID platform and Cytoscape were used to achieve GO analysis and KEGG signal pathway enrichment analysis. Then, in metabolites study, LC-MS/MS was used to analyze the metabolites of mangiferin in plasma collected from SD rats. **Results:** A total of 37 targets for the coaction of mangiferin and DM were screened. Tumor necrosis factor (TNF), epidermal growth factor (EGF), prostaglandin-endoperoxide synthase 2 (PTGS2), estrogen receptor 1 (ESR1), hypoxia-inducible factor-1 alpha (HIF1A), nuclear factor-κB p65 (RELA), protein kinase C alpha (PRKCA), and Interleukin-2 (IL2) were selected as the most important targets. The biological functions related to DM were mainly enriched in the signaling pathway in phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt), mitogen-activated protein kinase (MAPK), nuclear factor-kappa B (NF-kappa B), hypoxia inducible factor-1 (HiF-1), and mammalian target of rapamycin (mTOR). With reference to the obtained accurate relative molecular mass, chromatographic retention behavior, and characteristic fragment ions, a total of six metabolites including the original drug were analyzed and identified. **Conclusion:** This study provides key data for the mechanism of action and targets research on mangiferin, and it provides a basis for its further development into hypoglycemic drugs.

**Key words:** Diabetes mellitus, mangiferin, mechanism of action, metabolites *in vivo*, network pharmacology

## SUMMARY

- A total of 37 targets for the coaction of mangiferin and DM were screened. The biological functions related to DM were mainly enriched in signaling pathway in PI3K-Akt, MAPK, NF-kappa B, HiF-1, and mTOR.
- A total of six metabolites including the original drug were analyzed and identified.
- It provides a reference for the research of mangiferin in the treatment of DM.




**Abbreviations used:** DM: Diabetes mellitus; NP: Network pharmacology; PPI: Protein–protein interaction; TNF: Tumor necrosis factor; EGF: Epidermal growth factor; PTGS2: Prostaglandin-endoperoxide synthase 2; ESR1: Eestrogen receptor 1; RELA: Nuclear factor-κB p65; PRKCA: Protein kinase C alpha; IL2: Interleukin-2; PI3K: Phosphoinositide 3-kinase; Akt: protein kinase B; MAPK: Mitogen-activated protein kinase; NF-kappa B: Nuclear factor-kappa B; HIF1: Hypoxia inducible factor-1; mTOR: Mammalian target of rapamycin; T2DM: Type 2 diabetes; BP: Biological process; CC: Cellular component; IR: Insulin resistance.

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## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease linked to various long-term complications. DM and its complications have become a chronic non-communicable disease that seriously affects human health and quality of life. It is estimated that the number of DM patients will rise to 40 million by 2025. DM, especially type 2 diabetes (T2DM), has seriously threatened human health.[1,2] At present, the drugs used in clinical practice have been difficult to obtain the ideal therapeutic effect due to their drug resistance and other adverse reactions. Therefore, new drug research and development for DM is becoming imminent. Mangiferin (2-β-D-glucopyranoside-1,3,6,7-tetrahydroxy-9H-xanthone), a natural product extracted from traditional Chinese medicine, is able to reduce blood sugar and treat diabetic complications. Mangiferin is a carbon ketone of tetrahydroxypyrone



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glycosides belonging to flavonoids. It is mainly distributed in Gentianaceae, Iridaceae, Garciniaceae, and Leguminosae. In recent years, several studies have reported a variety of pharmacological activities of mangiferin, including hypoglycemic, antilipidemic, anti-hyperuricemic, antitumor, antioxidative, anti-inflammatory, and immunoregulatory.[3,4]

Network Pharmacology (NP) is a new discipline that applies biology and multidirectional theory to analyze biological system networks and selects specific signal nodes to design multitarget drug molecules.[5,6] NP can construct a "drug-target-disease" interaction network by inferring the correlation between small molecules and genes, proteins, metabolites and other targets and networks. It can comprehensively and systematically explore the mechanism of a drug linked to a disease. Mangiferin has a good hypoglycemic effect, but its mechanisms of action are not fully understood. Based on the NP, this study constructed a complex network of drugs and biomolecules that interact with mangiferin and DM to explore drug targets and the relationship between drugs, endogenous proteins, and biological pathways. After the drug is absorbed by the body, there will be a series of chemical reactions resulting in the transformation of the drug structure: metabolism, also known as biotransformation. The metabolic reaction mainly occurs in the liver but can also occur in other organs or tissues such as kidney, lung, stomach, intestine, and blood. Metabolic reactions are usually divided into two types. For type I, functional groups should be introduced into the drug structure mainly through oxidation, reduction, hydrolysis, isomerization, and other reactions to increase its polarity and become an excretable form that is easier to excrete. Type II reactions refer to the combination of drugs and their metabolites with endogenous glucuronic acid, sulfuric acid, glutathione, or amino acids, to increase their water solubility and polarity, and make their excretion from the body easier. The metabolism of drugs in the body is closely related to their pharmacological effects. Due to metabolism in the body, the activity of the drug can be enhanced, decreased, or lost. Additionally, drugs can also be metabolized to produce toxic metabolites. Therefore, in order to more comprehensively evaluate the mode of action of mangiferin,

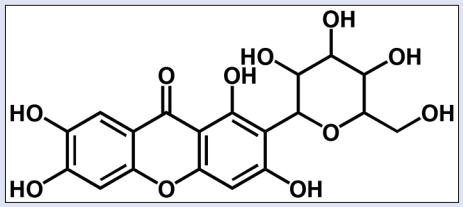


**Figure 1:** The structure of mangiferin (molecular formula: $C_{19}H_{18}O_{11}$)

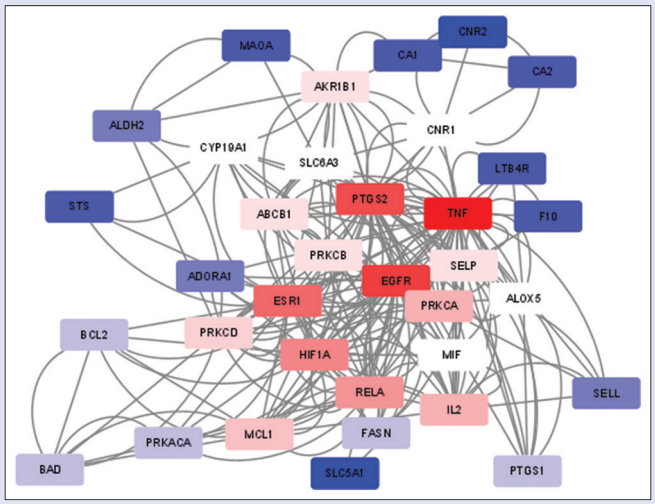


**Figure 3:** PPI network diagram of mangiferin-DM co-targets

**Table 1:** High-performance liquid gradient conditions in identification

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.0 | 98 | 2 |
| 0.2 | 98 | 2 |
| 6.0 | 50 | 50 |
| 7.0 | 2 | 98 |
| 8.0 | 2 | 98 |
| 8.1 | 98 | 2 |
| 9.0 | 98 | 2 |

**Table 2:** Mass spectrometry conditions

| Parameter | Electrospray positive ionization mode | |
|---|---|---|
| | Selective ion scan (MIM) | Enhanced ion scan (EPI) |
| Ion spray voltage (kV) | −4500 | −4500 |
| Curtain gas | 40 | 40 |
| Temperature (oC) | 500 | 500 |
| Ion source gas 1 | 50 | 50 |
| Ion source gas 2 | 50 | 50 |
| Interface heater | ON | ON |
| Declustering potential | −80 | −80 |
| Entrance potential | −10 | −10 |
| Collision energy | 5 | −40 |

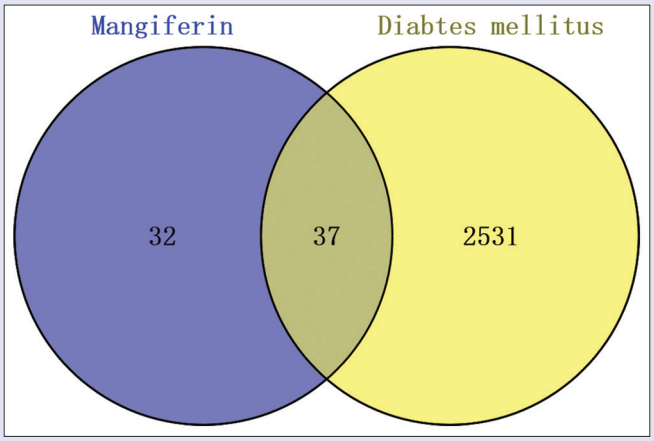


**Figure 2:** Venn diagram of mangiferin-DM co-targets

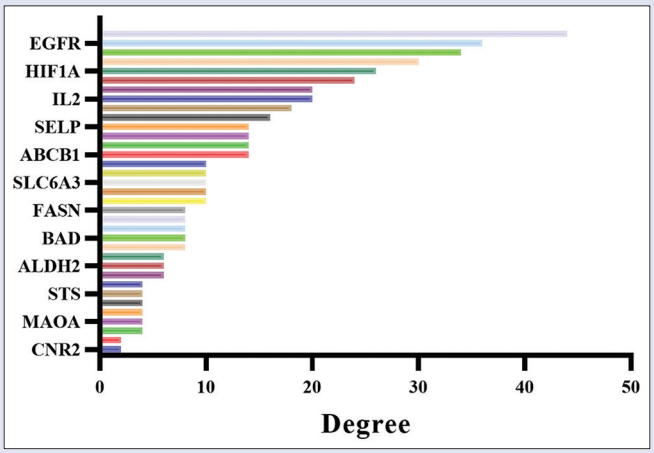


**Figure 4:** Degree value of mangiferin-DM co-targets

the metabolism of mangiferin was studied in SD rats' plasma, using LC-MS/MS to identify and analyze the structure of the tested compound mangiferin and its metabolites. This research aims to understand the action mechanism of mangiferin and its metabolites.

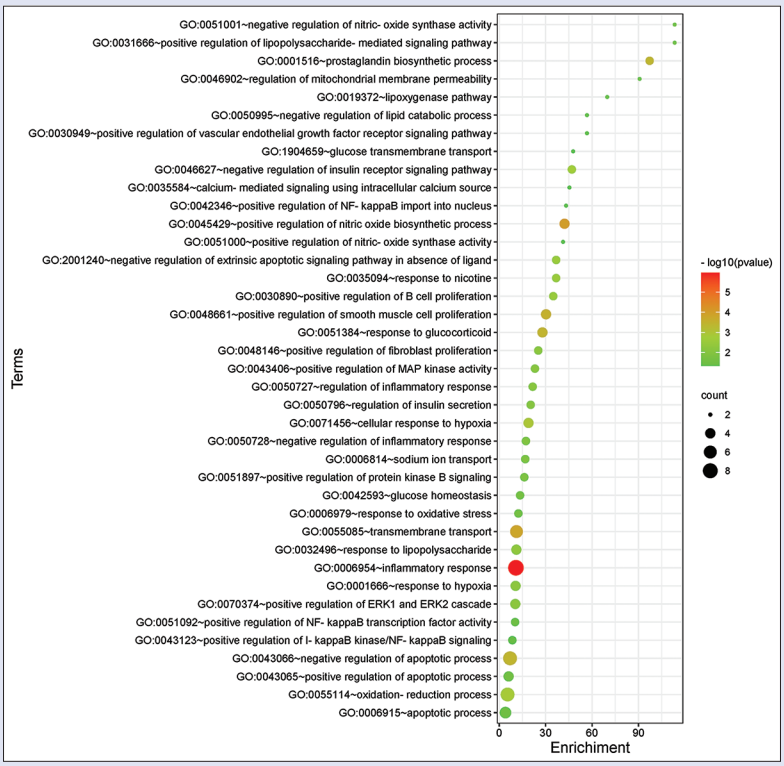


**Figure 5:** GO BP functional enrichment analysis

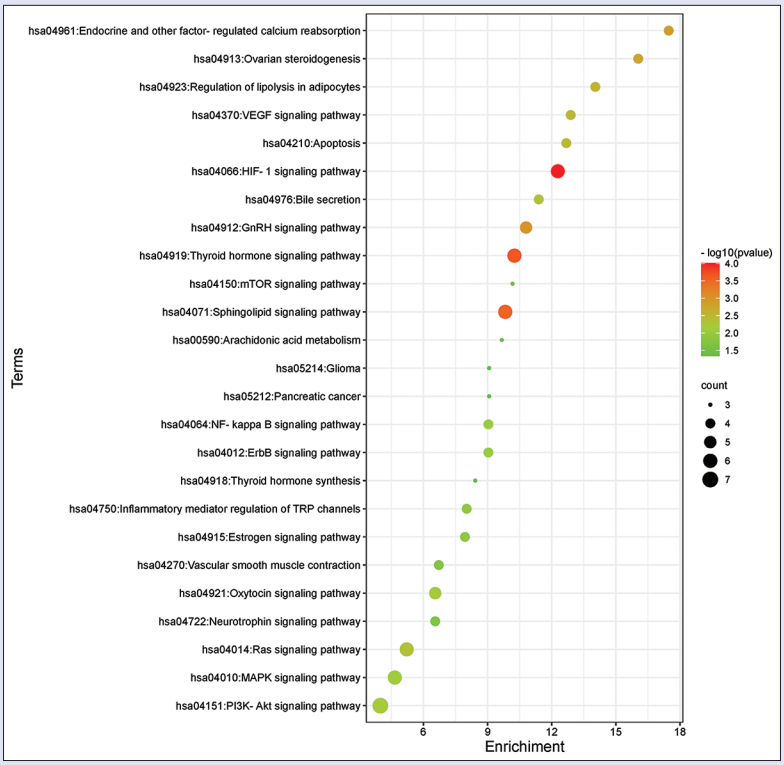


**Figure 6:** KEGG signal pathway analysis

## MATERIALS AND METHODS

### Mangiferin targets screening

Mangiferin's chemical structure was obtained from the small organic molecule biological activity database Pubchem (https://pubchem.ncbi.nlm.nih.gov/). The keyword "mangiferin" was searched and saved as Smiles format. The structure was uploaded into Swiss Target Prediction (http://www. swisstargetprediction.ch/) database, and the search was limited to *Homo sapiens*. Gene Cards Database (https://www.genecards.org/), TCMSP database (https://www. tcmsp-e.com/) were also used to search for the keyword "mangiferin" to obtain its related targets. Finally, the targets obtained from the three databases were combined to delete duplicates.

### DM targets screening

DisGeNET (https://www.disgenet.org/) was used to search for the disease keyword as "Diabetes mellitus". False positives were removed.

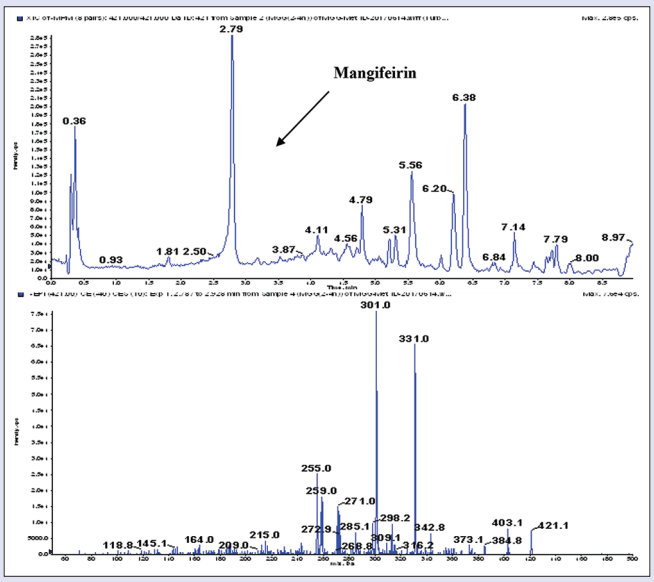


**Figure 7:** MIM ion chromatogram and MS/MS spectrum of mangiferin (*m/z* 421) in SD rats' plasma

### Mangiferin-DM co-targets interaction network

In order to clarify the interaction between mangiferin potential targets and DM-related targets, the selected mangiferin targets and DM targets were intersected. Venny 2.1.0 software (https://bioinfogp.cnb.csic.es/tools/venny/index. html) was used to draw a Venn diagram. A protein–protein interaction (PPI) network model was built on the STRING platform (https://www.string-db.org/) with the screened co-targets. Select the organism as "Homo sapiens", all the other parameters remain unchanged.

### Mangiferin-DM co-targets biofunctional interpretation and KEGG pathway analysis

KEGG signaling pathway was used to further understand the biological functions of the common target genes and their role. Coacting target genes were inserted into the DAVID platform (https://david.ncifcrf.gov/), and the biological function enrichment of the biological process (BP) was selected and analyzed using KEGG signal pathway, with a set significance of $P < 0.05$. Cytoscape software was used to draw the GO-BP biological function and KEGG signaling pathway network of mangiferin-DM co-targets.

### Test compound and animals

Mangiferin (batch number HM044244198) was purchased from Baoji Herbest Bio-Tech Co., Ltd., and the control quality scores were ≥98%. The male SD rats used in the experiment weighed 174 g–192 g and were purchased from Shanghai Xipuer-Bikai Experimental Animal Co., Ltd., production license number SCXK (Shanghai) 2013-0016, and laboratory animal certificate number: 2008001671544. All animal experiments are approved by the Experimental Animal Ethics Committee of Inner Mongolia Medical University.

### Identification of metabolites in SD rats' plasma

Plasma samples of rats with oral administration of mangiferin for 2 hours and 4 hours were mixed and analyzed. 600 μL acetonitrile was added to 200 μL plasma sample to precipitate proteins, and centrifugation was done at 13000 rpm for 10 min. The supernatant was collected, dried with nitrogen, and reconstituted with 200 μL of 30% acetonitrile. LC-MS/MS analysis was performed on a Shimadzu UPLC equipped with Shimadzu 30AD binary pump and Shimadzu 30ACMP sampler and on a Qtrap 5500 LC/MS instrument from Applied Biosystems, equipped with ESI ion source. The instrument control and data were made via Analyst

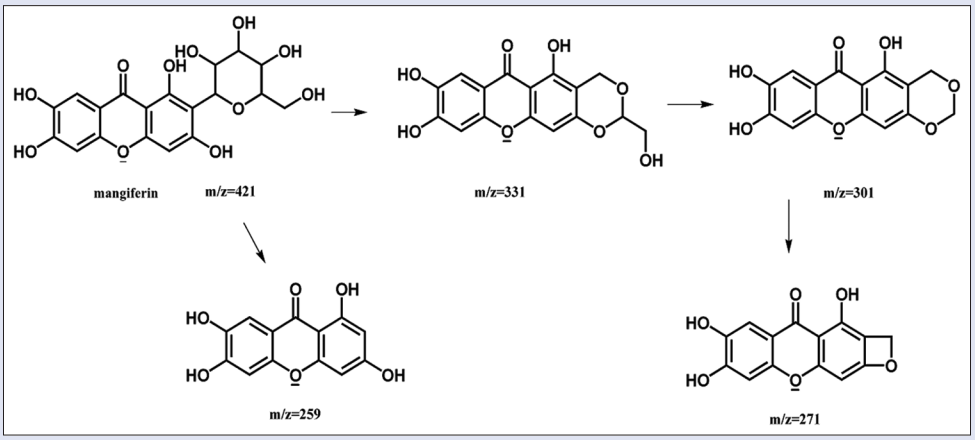


**Figure 8:** The cleavage pattern of mangiferin by mass spectrometry: Analysis of mangiferin metabolites

version 1.6.1 data analysis software. Chromatography conditions were as follows: chromatographic column was Waters CORTECS C18+ (2.7 μm, 50 mm × 2.1 mm) and column was set to room temperature; flow rate was 0.4 mL/min; injection volume was 2 μL; mobile phase was formed by 0.1% formic acid water (A) and 0.1% formic acid acetonitrile (B) in a gradient elution [Table 1]. Mass spectrometry conditions were as follows: scanning mode was set to negative ion MIM mode scanning; scanning range was *m/z* 200 ~ 700; MIM mode was based on the MRM scanning principle, the parent ion and fragment ion were set to the same value, CE value was set to the minimum value of 5. Specific mass spectrometry conditions are provided in Table 2.

# RESULTS

## Co-targets of mangiferin and DM

The structural formula of mangiferin was obtained using Pubchem database and saved in Smiles format [Figure 1]. The target information related to mangiferin is obtained by uploading the Smiles format of mangiferin to the Swiss Target Prediction. Gene Cards database and TCMSP database were used to search for the keyword "mangiferin" and obtain mangiferin-related targets. Results from three databases were combined and duplicates were deleted. A total of 69 targets for mangiferin were screened out. The DisGeNET database was used to search and screen DM disease targets. 2568 DM-related targets were obtained. The 69 mangiferin targets and 2568 DM targets were input into Venny 2.1 software to draw the Venn diagram. After the intersection of those two, 37 mangiferin-DM co-targets were obtained [Figure 2].

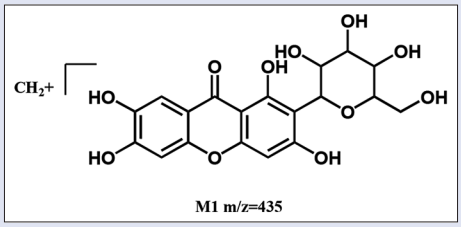


**Figure 9:** The chemical structure of M1

## Mangiferin-DM PPI network construction

The 37 mangiferin-DM co-targets were imported into the STRING database to obtain the PPI interaction relationship. Among them, there were 35 nodes and 231 edges. Each node represented a protein, and each edge represented the relationship between two proteins. More lines are related to a greater degree of association, that is, a greater degree of value [Figures 3 and 4]. It was found that among the co-targets of mangiferin-DM, tumor necrosis factor (TNF), epidermal growth factor (EGF), prostaglandin-endoperoxide synthase 2 (PTGS2), estrogen receptor 1 (ESR1), hypoxia-inducible factor-1 alpha (HIF1A), nuclear factor-κB p65 (RELA), protein kinase C alpha (PRKCA), and Interleukin-2 (IL2) were relatively important.

## GO function enrichment analysis

Using the DAVID database, GO function enrichment analysis of 37 mangiferin-DM co-targets was performed to obtain 137 GO entries ($P < 0.05$), of which 93 BP entries, 17 cellular component (CC), and 27 molecular functions. Multiple biological

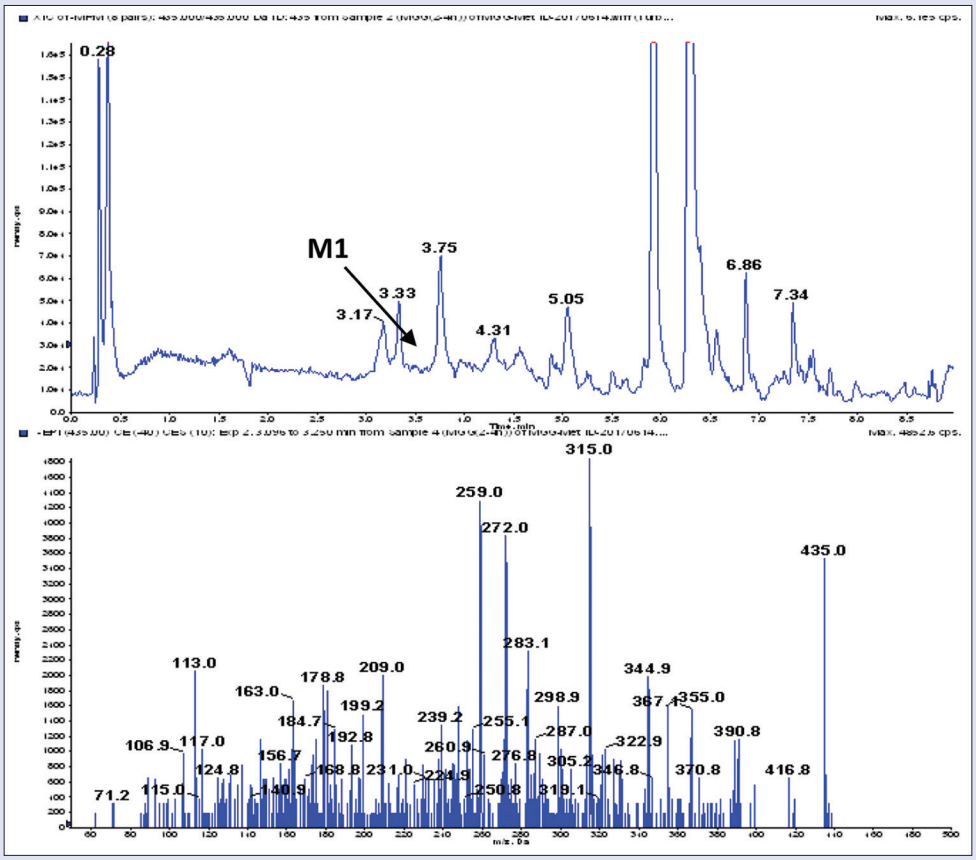


**Figure 10:** MIM ion chromatogram and MS/MS spectrum of M1 (*m/z* 435) in SD rats' plasma

functions were closely related to the occurrence and development of atrial fibrillation. Among them, BP related to DM is mainly concentrated in the inflammatory response, positive regulation of nitric oxide biosynthetic process, cellular response to hypoxia, oxidation-reduction process, and negative regulation of insulin receptor signaling pathway [Figure 5].

## KEGG signal pathway analysis

The 37 mangiferin-DM co-targets were imported into the DAVID platform for KEGG pathway enrichment analysis, and 52 signal pathways were screened ($P < 0.05$). Among them, many were directly related to DM, such as phosphoinositide 3-kinase-protein kinase B ((PI3K-Akt) signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway, nuclear factor-kappa B (NF-kappa B) signaling pathway, hypoxia inducible factor-1 (HiF-1) signaling pathway, and mammalian target of rapamycin (mTOR) signaling pathway [Figure 6].

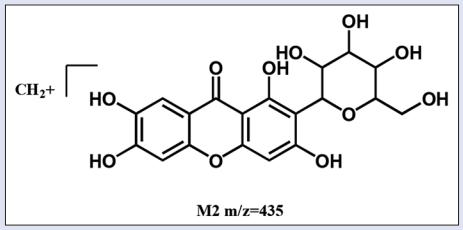


**Figure 11:** The chemical structure of M2

## LC-MS/MS behavior investigation of mangiferin

The retention time of mangiferin was 2.79 min, and its molecular ion peak was *m/z* 421. The EPI channel shows that its secondary fragments were *m/z* 403 ([MH-18]$^-$), 331 ([MH-90]$^-$), 301 ([MH-120]$^-$), 271 ([MH-150] $^-$), and 259 ([MH-162] $^-$). The MIM ion current diagram and the secondary mass spectrum are shown in Figure 7. The glucose structure in the mangiferin structure appeared to be prone to dehydration and deglycosylation reactions. The specific cleavage pathway is shown in Figure 8.

In this experiment, five mangiferin metabolites were identified in SD rats' plasma. The detailed substance information is as follows.

### *M1 (m/z 435)*

The retention time of M1 was 3.17 min, and the quasi-molecular ion peak was *m/z* 435 [M-H]$^-$detected by mass spectrometry. Compared with the substrate, it has increased by 14 amu. Its secondary mass spectrum showed that it produced fragments of *m/z* 417, 345, 315 compared with the fragment ions *m/z* 403, 331, and 301 of mangiferin, which increased by 14 amu, suggesting that M1 may undergo monomethylation reaction based on mangiferin (+14). The possible structural formula of M1 is shown in Figure 9. The MIM ion current diagram and the secondary mass spectrum are shown in Figure 10.

### *M2 (m/z 435)*

The retention time of M2 was 3.33 min, and the mass spectrometer detected its quasi-molecular ion peak at *m/z* 435, which corresponded to an increase of 14 amu, compared to that of the substrate. Its secondary

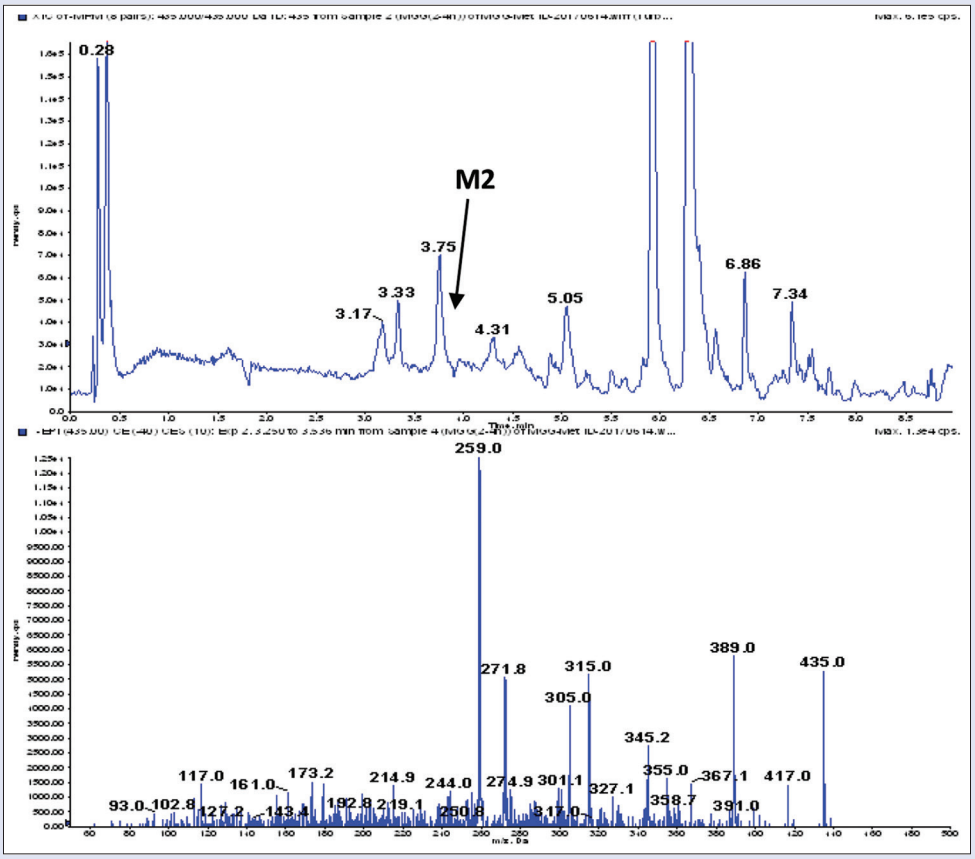


**Figure 12:** MIM ion chromatogram and MS/MS spectrum of M1 (*m/z* 435) in SD rats' plasma

mass spectrum was similar to that of M1, indicating that it was also a single methylation reaction. The possible structural formula of M2 is shown in Figure 11, and the MIM ion current diagram and secondary mass spectrum are shown in Figure 12.

### *M3 (m/z 435)*

The retention time of M3 was 3.75 min, and the mass spectrometry detected its quasi-molecular ion peak at *m/z* 435, which corresponded to an increase of 14 amu, compared with that of its substrate. The secondary mass spectrum showed that the main fragment ion produced was *m/z* 259 (−176), and no fragment ions of *m/z* 417, 345, 315, with an increase of 14 amu were found, suggesting that a monomethylation reaction occurred in M3, but not M1 and M2. According to the neutral loss of 176, it was suggested that M3 may be the product of glucuronic acid after the deglycosylation reaction on the basis of mangiferin. The possible structural formula of M3 is shown in Figure 13. The MIM ion current diagram and secondary mass spectrum are shown in Figure 14.

### *M4 (m/z 449)*

The retention time of M4 was 3.97 min, and the mass spectrometer detected its quasi-molecular ion peak at *m/z* 449, which corresponded to an increase of 28 amu compared with that of its substrate. Its secondary mass spectrum showed that the main fragment ions produced were *m/z* 273 (−176) and 258. Thus, M4 participated in a glucuronic acid binding reaction like M3. In addition, 14 amu was added to M4 (*m/z* 449) and M3 (*m/z* 435), indicating that M4 has undergone a methylation reaction from M3. The possible structural formula of M4 is shown in Figure 15. The MIM ion current diagram and the secondary mass spectrum are shown in Figure 16.

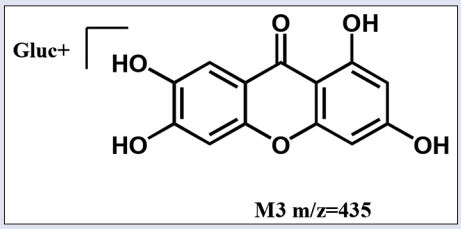


**Figure 13:** The chemical structure of M3

### *M5 (m/z 515)*

The retention time of M5 was 4.28 min, and its quasi-molecular ion peak was *m/z* 515 detected by mass spectrometry. The secondary mass spectrum showed that the main fragment ion produced was *m/z* 435 (−80). Sulfation reaction might have occurred on the basis of *m/z* 435. In addition, based on the remaining fragment ions *m/z* 345, and 315, the methylation reaction of M5 occurred. The possible structural formula of M5 is shown in Figure 17. The MIM ion current diagram and the secondary mass spectrum are shown in Figure 18.

## DISCUSSION

According to the analysis results, targets such as TNF, EGF, HIF1A, RELA, PRKCA, and IL2 are most likely to be the active targets of mangiferin against DM. Among them, TNF and IL2 are closely related to inflammation. PRKCA can effectively stimulate the abnormal increase of NF-κB levels, a large number of inflammatory mediators.[7,8] Insulin resistance (IR) is the main cause of T2DM, which has been recognized as a chronic low-grade inflammation state.[7] An important way to improve IR is to improve this state of chronic low-grade inflammation. Both TNF and IL2 are important indicators of an inflammatory response, which can directly or indirectly lead to IR.[9]

EGF, HIF1A, and RELA are currently key molecules in DM research. T2DM is often accompanied by obesity.[10] Obese patients may be

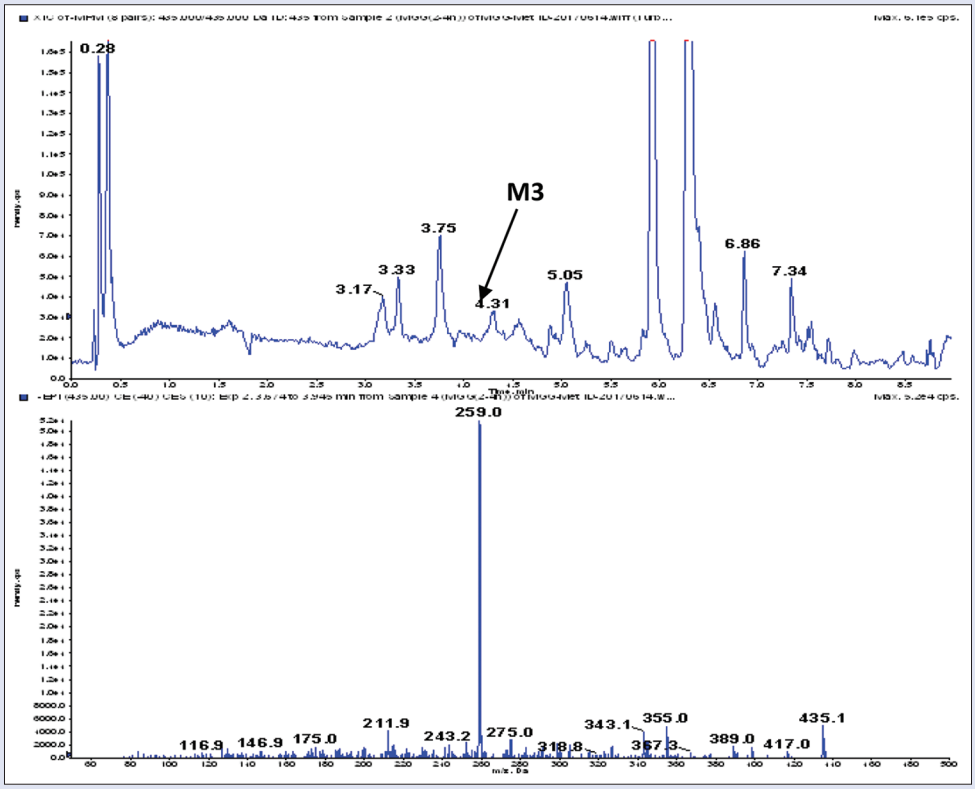


**Figure 14:** MIM ion chromatogram and MS/MS spectrum of M3 (*m/z* 435) in SD rats' plasma

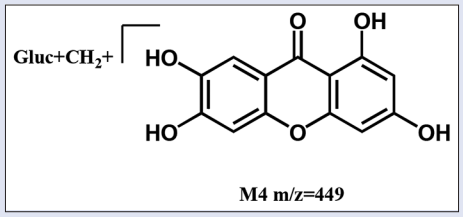


**Figure 15:** The chemical structure of M4

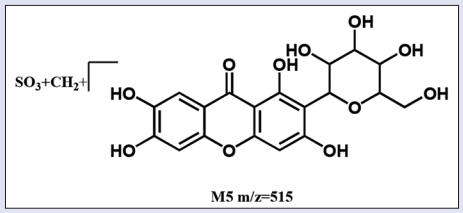


**Figure 17:** The chemical structure of M5

in a state of hypoxia due to the increased concentration of fatty cells, which leads to intensified inflammation and IR. HIF1A is a hypoxia-inducing factor, and its content also affects the occurrence and development of IR.[11] EGF can stimulate the proliferation and differentiation of vascular endothelial cells and promote the formation of blood vessels. This is of great significance in T2DM as EFG can help during the process of tissue. Diabetic wound healing and diabetic angiopathy are closely related to it.[12] This means that mangiferin may have the effect of regulating EGF to prevent or treat diabetic vascular disease.

The pathways obtained in GO BP analysis confirmed that it may regulate inflammation, oxidative stress, glucolipid metabolism, endocrine hormone regulation, hypoxia and other processes related to DM, such as inflammatory response, cellular response to hypoxia, oxidation-reduction process, negative regulation of insulin receptor signaling pathway, positive regulation of ERK1 and ERK2 cascade, positive regulation of MAPK activity, regulation of insulin secretion, positive regulation of Akt signaling, glucose homeostasis, response to oxidative stress, lipoxygenase pathway, glucose transmembrane transport, and regulation of I-Kappab kinase/NF-kappa B signaling, PI3K-Akt signaling pathway, MAPK signaling pathway, NF-kappa B signaling pathway, HiF-1 signaling pathway, and mTOR signaling pathway. These are all classic pathways that have been studied for IR. Therefore, mangiferin may have the potential to be developed into a hypoglycemic drug via improving chronic low-grade inflammatory response and tackling oxidative stress and hypoxia in obesity.

Then, LC-MS/MS was used to analyze the metabolism of the test compound mangiferin in SD rats' plasma. Five metabolites of mangiferin in SD rats' plasma were identified [Table 3]. The main metabolic pathways include the combination reaction between deglycosylation, methylation, sulfation, and glucuronidation or glucosidation [Figure 19]. Drugs currently used clinically for the treatment of DM have side effects such as strong drug resistance. As an active compound of traditional Chinese medicine, mangiferin has the potential to develop into a new hypoglycemic drug. The results obtained in this study will serve as a good reference for further research on mangiferin in the future.

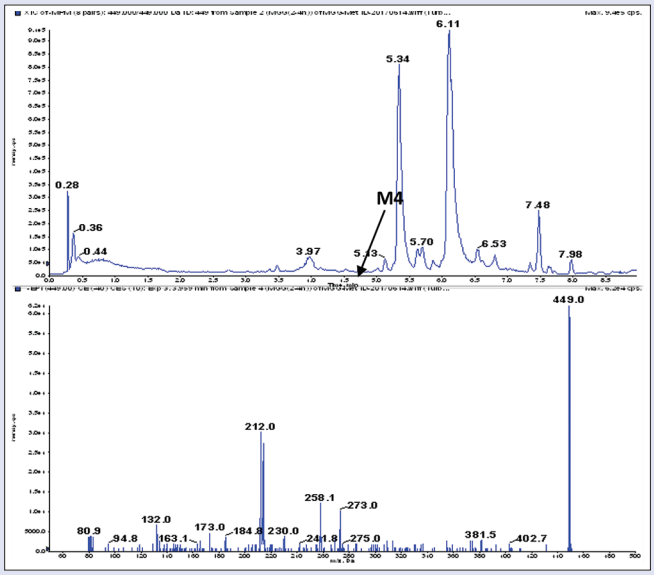


**Figure 16:** MIM ion chromatogram and MS/MS spectrum of M4 (*m/z* 435) in SD rats' plasma

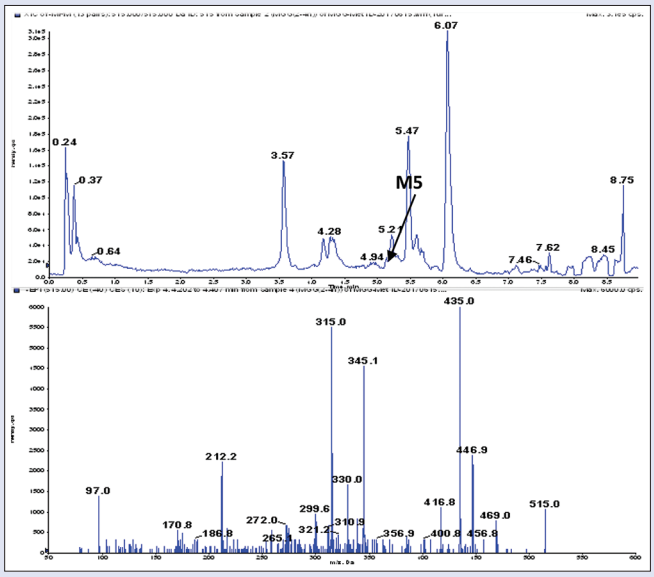


**Figure 18:** MIM ion chromatogram and MS/MS spectrum of M5 (*m/z* 435) in SD rats' plasma

**Table 3:** Metabolites of mangiferin observed in SD rats' plasma

| Metabolite ID | RT (min) | *m/z* | Path |
|---|---|---|---|
| Mangiferin | 2.79 | 421 | Parent |
| M1 | 3.17 | 435 | Methylation |
| M2 | 3.33 | 435 | Methylation |
| M3 | 3.75 | 435 | Deglycosylation, glucuronic acid binding |
| M4 | 3.97 | 449 | Deglycosylation, glucuronic acid-binding, methylation |
| M5 | 4.28 | 515 | Methylation, Sulfation |

Note: M=Metabolite

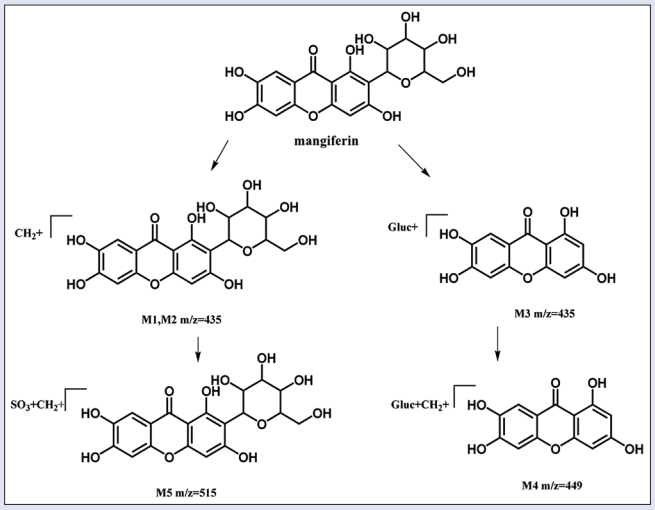


**Figure 19:** Possible metabolic pathways of mangiferin

## CONCLUSION

In summary, mangiferin mainly regulates 37 mangiferin-DM co-targets such as TNF, EGF, PTGS2, ESR1, HIF1A, RELA, PRKCA, and IL2, and affects PI3K-Akt, MAPK, NF-kappa B, HiF-1, and mTOR signaling pathways to treat DM. At the same time, a total of six metabolites including the original drug were analyzed and identified. This study laid the foundation for the further development of mangiferin.

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## Conflicts of interest

There are no conflicts of interest.

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