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NETWORK PHARMACOLOGY STUDY ON THE MECHANISM OF DIABETIC NEUROPATHY TREATMENT USING MANGIFERIN

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Summary

Network Pharmacology Study on the Mechanism of Diabetic Neuropathy Treatment Using Mangiferin

Mangiferin was the natural C-glycosyl xanthone compound with the various pharmacological activities, however, the absence of evidence for potential actions in diabetic neuropathy. Structure-based (molecular docking) and ligand-based (pharmacophore) methodologies, and network pharmacology could investigate the therapeutic potential of mangiferin for diabetic neuropathy due to simple and a system information of mechanism. Which structure-based methodologies, the diabetic neuropathy genes were determined by String db and converted to protein name with Uniprot. After mangiferin docking by Smina, the gene which coded the protein with the lower than -6 kcal/mol binding energy would be selected to the structure-based dataset. Also, the ligand-based dataset was composed of the genetics targets of mangiferin thought TargetNet and SwissTarget server. All gene belongs two datasets were the forces targets which further analyzed. Genemania and DAVID6.8 tools were used to obtain the GO and KEGG pathways. In results, 16 focus genes corresponding with 796 protein targets were revealed the mechanism of mangiferin in the diabetic neuropathy therapeutics. Furthermore, 91 biological processes and 29 pathways were disclosed according to the topological parameters of network pharmacology, specially, VEGF signaling pathway, HIF-1 signaling pathway, insulin resistance, and Alzheimer's disease. Mangiferin was suggested the good candidates for the diabetic uremic neuropathy and peripheral neuropathy onset and progression.

Keywords: *Diabetic neuropathy, Mangiferin, Molecular docking, Network pharmacology, Pharmacophore.*

1. Introduction

Diabetic neuropathy (DN) was a type of nerve damage that could be occur as least 50% of diabetic individuals. Depending on the affected nerves, diabetic neuropathy symptoms could be range from pain and numbness in legs and feet to problems with digestive system, urinary tract, blood vessels and heart. The difference symptoms were between patients for instance some patients have mild symptoms, for others with quite painful and disabling [1],[2]. In treatment, the glucose control therapy could be effectively halts the progression of diabetic neuropathy in patients with type I diabetes mellitus, however, the more modest effects with type II. In recent, several therapies have been designed to target the pathogenesis of diabetic neuropathy. Although, the unequivocal evidence of those was remain lacking [1].

Mangiferin was the C-glycosyl xanthone type with various pharmacological activities which isolated from natural sources as *Mangifera indica*, *Anemarrhena asphodeloides* or *Cyclopia sp.* Its capability on treatment diabetes mellitus and neuroprotection was confirmed by Aswal S *et al.* [3]. However, the evidence for potential actions in diabetic neuropathy was still unclear.

Network pharmacology (NP) was first identified by Hopkins in 2007. It could be established a "compound-protein/gene disease" network and revealed the regulation principles of small molecules in a high-throughput manner [4],[5]. Also, the discovery new targets of existing drug molecules would be proposed the difference application according to an unbiased investigation of potential target spaces [6].

Therefore, this study aimed at predicting the target and signaling pathways for mangiferin against diabetic neuropathy from a network pharmacology. The ADMElab server was used to evaluate the molecular and pharmacokinetic properties of mangiferin. The focus genetics targets were determined by structure-based (docking) and ligand-based (TargetNet and SwissTarget) methodologies. Furthermore, these targets were used for GO enrichment analysis, KEGG enrichment analysis. Finally, we systematically explained the targets and mechanism of mangiferin by constructing the pharmacological relationship network. Fig. 1 was the workflow of gene prediction and analysis process.

2. Materials and methods

2.1. Pharmacokinetic evaluation

To be effective as a drug, a potent molecule must be satisfied with many parameters to reach its target in the body in sufficient concentration

and stay there in a bioactive form long enough for the expected biologic events to occur. In drug research and development, assessment of absorption, distribution, metabolism, and excretion (ADME) is important [7]. Here, ADMElab servers were used to evaluate pharmacokinetic properties of mangiferin. The ADMElab server (https://admet.scbdd.com/home/index/) is a network tool that provides a set of mature and efficient predictive models to users for calculating molecular and pharmacokinetic properties.

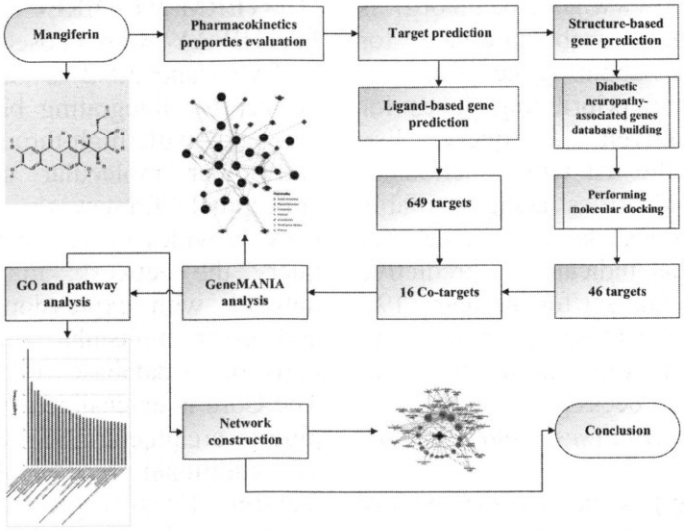

Fig. 1. The workflow for the network-pharmacology approach used in our study

2.2. Prediction and identification of diabetic neuropathy-associated targets

Collection of target protein for related diabetic neuropathy

The keyword "diabetic neuropathy" was selected to search for related genes in Cytoscape 3.8.2 using plugins String db. The confidence (score) for cutoff were set to the default value (0.4) and the number of proteins was limited to 100. The received gene name converted to protein name through the UniProt database (https://www.UniProt.org/). After removing repetitive protein targets, we acquired all protein targets for diabetic neuropathy.

Molecular docking

Molecular docking was performed with Smina (a fork of Autodock Vina). Smina added better control of scoring terms as well as a range of convenience functions for easy use from the command line (https://sourceforge.net/ projects/smina). With ligand preparation, ligand-mangiferin for molecular docking could be prepared to create 3-dimensional geometries in RDKit library assigned to addition hydrogen, generate conformations, and determination the minimization conformer in *MMFF94* force-field (kcal/mol). With protein preparation, the crystal structure of proteins - encoding diabetic neuropathy genes were saved as pdb format from RCSB Protein Data Bank (RCSB PDB, http://www.pdb.org/). The protein-ligand complex (pdb files) was split into protein (.pdb) and ligand (.sdf) files and assign ligand bond orders by SMILES strings from Ligand Export. Only the bioactive ligand file was utilized to define a binding site of molecular docking, the co-crystallized ligand files including the stabilizer agent, ion, metal (PEG, NO3, SO4, SCN, Cu, Fe…), and water would be unattended. The autobox_ligand function of docking Smina was applied and the binding pattern with the lower than -6 kcal/mol binding energy was selected for further analysis.

Focus genetics target of mangiferin

Related target of mangiferin also were obtained from TargetNet (http://targetnet.scbdd.com/ home/index/) and SwissTarget (http://www.swisstargetprediction.ch/, 2021 version). TargetNet is a server which can be used to provide target prediction results of small molecule through many QSAR models built on proven valence data to predict based on proven chemogenomic data for prediction. SwissTarget is a server can be used to evaluate the most probable macromolecular targets of a small molecule. This prediction is based on the 2D and 3D similarity alignment in the established active substance library [8]. The canonical smiles of

manfigerin were uploaded to TargetNet and SwissTarget with AUC ≥ 0.7 and "*Homo sapiens*", respectively. All the received targets were transferred to UniProtKB (https://www.uniprot.org/) to avoid mix-up across the databases and platforms.

2.3. Analysis genes using GeneMANIA

GeneMANIA (http://www.genemania.org) is a flexible, user-friendly web interface for generating hypotheses about gene function, analyzing gene lists and prioritizing genes for functional assays. Given a query list, GeneMANIA extends the list with functionally similar genes that it identifies using available genomics and proteomics data. GeneMANIA also reports weights that indicate the predictive value of each selected data set for the query [9]. The list of potential gene targets with selecting *Homo Sapiens* organism were queried to reveal results of the prediction process [10].

2.4. GO, Pathway Analyses, and Network Construction

GO (Gene Ontology) is an ontology widely used in bioinformatics, covering three aspects of biology: molecular function, biological process, and cellular component [11]. The cellular component refers to each part of the cell and the extracellular environment. Molecular functions can be described as molecular levels of activity, such as catalytic or binding activity. Biological process is a process that results from the orderly combination of one or more molecule functions [11]. DAVID is a web-accessible program that integrates functional genomic annotations with intuitive graphical summaries. Lists of gene or protein identifiers are rapidly annotated and summarized according to shared categorical data for Gene Ontology, protein domain, and biochemical pathway membership. DAVID assists in the interpretation of genome-scale datasets by facilitating the transition from data collection to biological meaning. In this study, DAVID 6.8 (https://david.ncifcrf.gov/home.jsp) were used to obtain GO and KEGG Pathway Analysis results, Functional Annotation Tool had been selected and four parameters "GOTERM-BP-DIRECT", "GOTERM-CC-DIRECT", "GOTERM-MF-DIRECT" "KEGG-PATHWAY" were chosen.

Cytoscape 3.8.2 is an open-source software project for integrating biomolecular interaction networks with high-throughput expression data and other molecular states into a unified conceptual framework. Cytoscape's software Core provides basic functionality to layout and query the network; to visually integrate the network with expression profiles, phenotypes, and other molecular states; and to link the network to databases of functional annotations. The Core is extensible through a straightforward plug-in architecture, allowing rapid development of additional computational analyses and features. Drug-Target Pathway Network were constructed by Cytoscape 3.8.2 to visualize and gain insight into the interactions of mangiferin with potential targets and protect mechanisms in diabetic neuropathy.

3. Results and Discussion

3.1. Molecular and pharmacokinetic properties of Mangiferin

The molecular and pharmacokinetic properties of mangiferin (PubChem CID: 5281647) were evaluated *in silico* with ADMElab server. The obtained data were shown in 1 and 2. Among five common rules of druglikeness including Lipinski, Ghose, Oprea, Veber, and Varma, the property value of mangiferin in the Ghose filter were the highest with 75% (Table 1). Additionally, the vital ADME properties were displayed in Table 2.

Table 1. Molecular properties of mangiferin by ADMElab server

	Lipinski	**Ghose**	**Oprea**	**Veber**	**Varma**
The number of ring			4		
The number of rigid bonds			31		
The number of rotatable bonds			2	2	2
Molecular weight	422.342	422.342			422.342
Hydrogen bond donor	8			8	8
Hydrogen bond acceptor	11			11	11
LogP	-0.716	-0.716			
Log D					-0.112
Molar refractivity		99.369			
Total number of atoms		48			
TPSA				21.28	
Matches (%)	50	75	66.67	33.33	60

Table 2. Pharmacological properties of mangiferin by ADMElab server

	Predicted values	Suggestions
LogS (Solubility)	-2.998 log mol/L (424.291 μg/mL)	> 10 μg/ml
LogD7.4 (Distribution Coefficient D)	-0.112	1~5
LogP (Distribution Coefficient P)	-0.716	0~3
Caco-2 Permeability	-6.512 cm/s	> -5.15 cm/s
PPB (Plasma Protein Binding)	67.433%	90%
BBB (Blood-Brain Barrier)	-	These features tend to improve BBB permeation: H-bonds (total) < 8-10; MW < 400-500; No acids.
T 1/2 (Half Life Time)	1.262 h	> 0.5 h

3.2. Prediction and identification of Diabetic neuropathy - associated targets

Molecular docking

From String database, the retrieve mapping in Uniprot, and RCSB Protein Data Bank, the final dataset of the docking target included 2627 the crystal structure of proteins in relationship with the diabetic neuropathy genes. The mangiferin docking results showed that the predicted binding affinity of 1070 protein targets corresponding to 46 gens which was less or equal than -6 kcal/mol.

The focus genetics target of mangiferin in relating the diabetic neuropathy

To identify gene targets of mangiferin, TargetNet and SwissTarget were used to reveal the bioactive target. Using the aforementioned method, 623 and 70 gene targets were receipted from TargetNet and SwissTarget, respectively. After removal of duplicates, 646 gene targets of mangiferin were determined. Of those, 16 gene targets were shared with the obtain gene targets (796 protein targets) from molecular docking. This was the focus genetics targets of mangiferin respecting the diabetic neuropathy therapy (*Supporting information Table S1*).

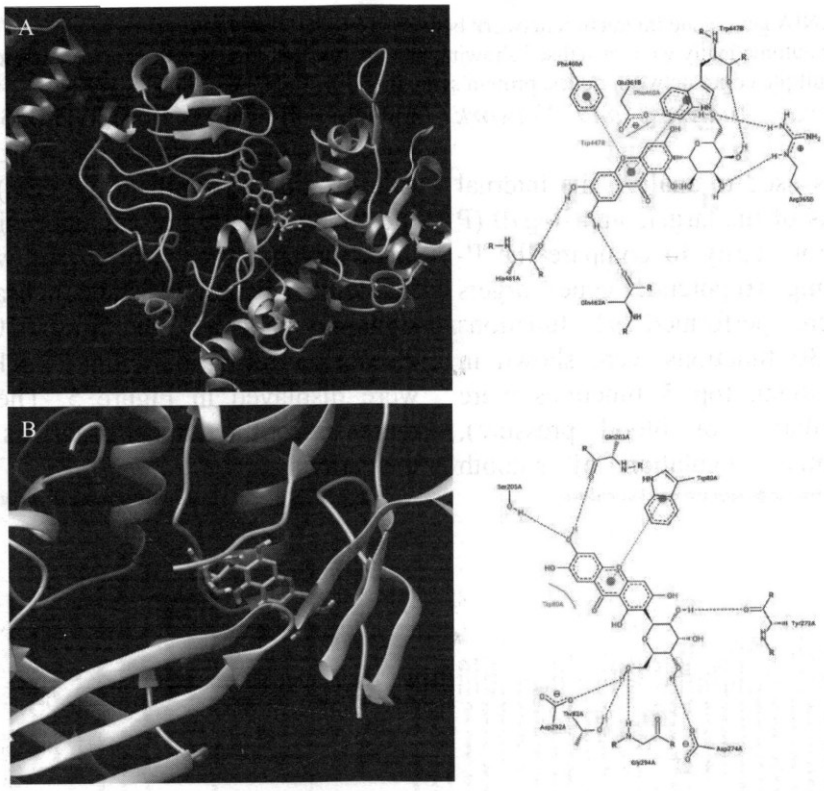

Fig. 2. Part of molecular docking results including mangiferin-5UOC (**A**) and mangiferin-5KCV (**B**). The compound structure was presented as a cyan stick, the protein structure was presented as a yellow ribbon in 3D chemical structure. The 2D chemical structure with the pi interaction and hydrogen bonding were shown as a green and black dotted lines respectively.

3.3. Analysis genes using GeneMANIA

Give the query above 16 genes list, GeneMANIA was shown their functionally similar genes at the gene level to illuminate the associations among colocalization, shared protein domains, co-expression, prediction, and pathways in the diabetic neuropathy of mangiferin (Figure 3). Among them, the weight of portions which had physical interactions and genetic interactions were 22.57 and 38.68%, respectively. The portions that had co-localization were 14.07%. The weight of these portions adds up to 75.32%. Furthermore, the portions that displayed similar co-expression characteristics occupy 10.86% of the weight. The portions that shared the same protein domains occupy 5.91% of the weight and genes that were predicted to interact with each other occupy 10.66% of the weight. These results indicated that the potential genes targets may comprehensively interact with each other and could function through alliance mechanisms.

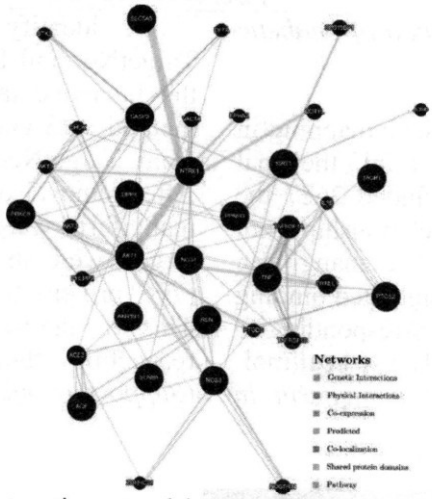

Fig. 3. A GeneMANIA gene-gene interaction network between 16 focus genetics target of mangiferin in the diabetic neuropathy therapeutic automatically were visualised showing interaction strength (edge thickness), interaction type (color), multiple edges between nodes, protein score (node size) defined using a stylesheet.

3.4. GO, Pathway Analyses, and Network Construction

DAVID 6.8 was used to analyze the internal interaction networks of the targets and -log10 (P value) were used for clarity to compare the P-value. After analyzing, 16 potential genes targets of mangiferin were performed 91 functions ($P \leq 0.05$) and top 30 functions were shown in Figure 4. Among them, top 5 functions were GO:0008217 (regulation of blood pressure), GO:0048661 (positive regulation of smooth muscle cell proliferation), GO:0051926 (negative regulation of calcium ion transport), GO:0006809 (nitric oxide biosynthetic process), GO:0045766 (positive regulation of angiogenesis). In addition, these targets enriched in 29 pathways ($P < 0.05$), including (Insulin resistance), hsa04066 (HIF-1 signaling pathway) and hsa05010 (Alzheimer's disease). Information about 29 KEGG pathways were displayed in Figure 5. The count values represent for the number of targets participated in the pathway.

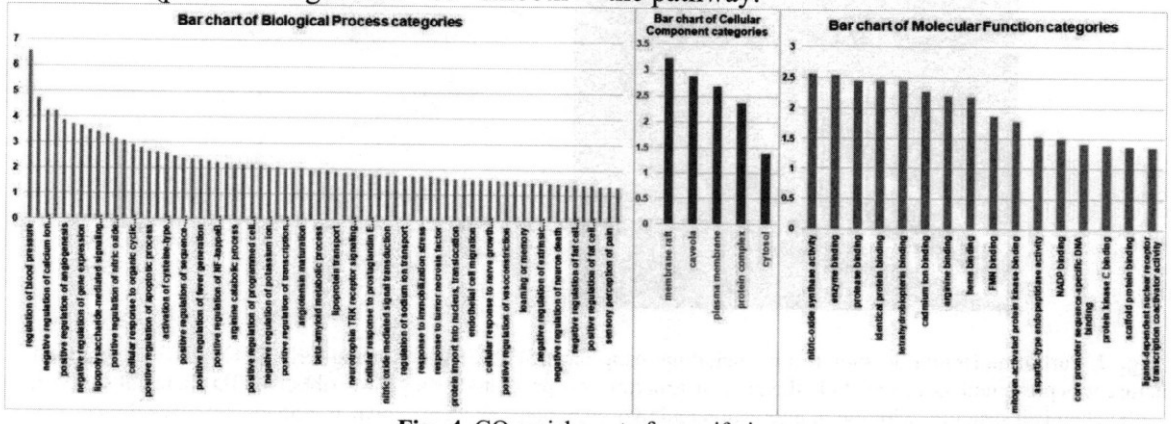

Fig. 4. GO enrichment of mangiferin.

Based on target identification and pathway analysis, the mangiferin- the focus targets-Pathway networks were constructed using Cytoscape 3.8.2. The network of potential targets using molecular docking and the network of predicted target collected from TargetNet and SwissTarget were shown in Figure 5.

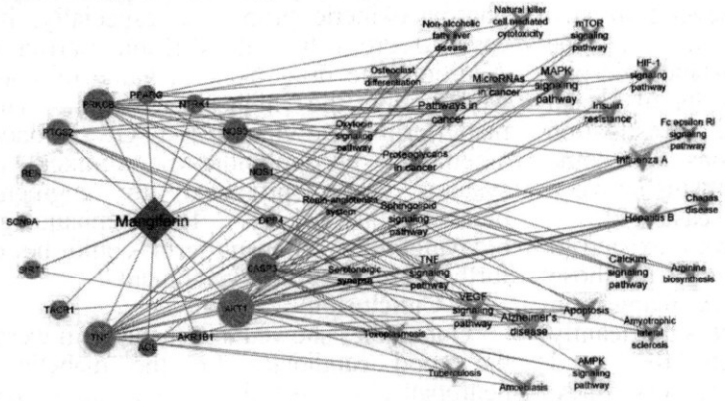

Fig. 5. Mangiferin-16 the focus genetics targets-29 KEGG Pathway network of magiferin in the diabetic neuropathy therapeutic.

3.5. Discussion

In this study, the pharmacological mechanism of mangiferin effecting the diabetic neuropathy were investigated using a bioinformatics methodologies including structure-based (molecular docking), ligand-based (TargetNet and SwissTarget), and network pharmacology. The structure-based and ligand-based results indicated that mangiferin had the strong binding force with 796 protein targets corresponding to the 16 genes association with the diabetic neuropathy. Basic on analysis GeneMANIA results, these genes could be interacted amongst themselves and functioned through alliance mechanisms.

In clinical, diabetic neuropathies were divided three groups inclusive of uremic neuropathy, diabetic peripheral neuropathy, and cardiac autonomic neuropathy (CAN). Diabetic neuropathies have complex disease etiologies for instance chronic hyperglycemia, hypertension, dyslipidemia, insulin resistance, and uremia could be the key factors that contribute to their pathogenesis. They caused changes in gene expression, molecular transport, inflammation, and oxidative stress. Genetic sensitivity and environmental factors were also recognized to relate and contribute to disease onset and progression [12]. Ini-Isabée Witzel *et al* suggested that polymorphism within ACE, AKR1B1, APOE, MTHFR, NOS3, and VEGF could act as the genetic risk factors for the diabetic peripheral neuropathy as well as uremic neuropathy that a statistically significant association has been published. Coincidentally, three gene as ACE, AKR1B1, and NOS3 also were appeared in catalogue of 16 focus genes target of mangiferin. Additionally, Vieira *et al* found that Mangiferin increases NOS3 expression in cultured tracheal rings by Western blot [13]. Mangiferin showed to inhibit the expression of TNF-alpha and Caspase-3 proteins *in vivo* and *in vitro* models [14]. This evidence suggested mangiferin could be usefully in therapies for the uremic and peripheral neuropathy diabetic.

Furthermore, 29 pathways and 91 biological processes were disclosed according to the topological parameters of network pharmacology. Go analysis showed that targets were mainly act in 5 functions: GO:0008217 (regulation of blood pressure), GO:0048661 (positive regulation of smooth muscle cell proliferation), GO:0051926 (negative regulation of calcium ion transport), GO:0006809 (nitric oxide biosynthetic process), GO:0045766 (positive regulation of angiogenesis). As impaired angiogenesis and endothelial dysfunction were hallmarks of diabetes, positive regulation of angiogenesis and positive regulation of smooth muscle cell proliferation functions could help promoting angiogenesis and reduce serious complications of impaired angiogenesis and endothelium consequences. Regulation of blood pressure function could also be used to protect blood vessels for patients with diabetes, helping to reduce serious complications such as stroke and brain hemorrhage [15]. Several studies *in vitro* and *in vivo* suggested that nitric oxide (NO) involve in the development of glomerular hyperfiltration in diabetes mellitus and enrich in nitric oxide biosynthetic process may improve the condition of diabetic patients [16]. Consistent with our expectations, these GO corresponding to the predicted targets of mangiferin is mainly related to diabetic neuropathy through angiogenesis and vascular protection processes.

Pathway analysis in diabetic neuropathy suggested that mangiferin mainly regulates (hsa04370) VEGF signaling pathway, HIF-1 signaling pathway, (hsa04931) insulin resistance, and Alzheimer's disease. Vascular endothelial growth factor (VEGF) which is a critical pro-angiogenic growth factors may use to stimulate angiogenesis for protecting blood vessels under high blood pressure in diabetes mellitus patients. Hypoxia-inducible factors (HIFs) are the key regulators of oxygen homeostasis in response to hypoxia. In diabetes, multiple tissues are hypoxic

but adaptive responses to hypoxia are impaired due to insufficient activation of HIF signaling. Pharmacological induction of HIF-1 could promote wound healing in experimental diabetes models have been confirmed in several *in vivo* study [17],[18]. Diabetic neuropathy could be affected by previous insulin resistance despite regular glycemic control. The results of recent epidemiological and basic science investigation have suggested possible associations and some common pathophysiological mechanisms between diabetes mellitus and Alzheimer's disease [19].

Several experimental evidence were existed to confirm the affection of mangiferin in those pathways. *In vitro* experimentation, mangiferin was proved that capable treatment significantly downregulated HG/hypoxia-induced HIF-α and VEGF expression levels in RRCECs cell lines. The ability for decreasing insulin resistance *in vivo* model were insisted in literature. And finally, mangiferin reduced acetylcholine and tumor necrosis factor (TNF)-alpha levels induced by scopolamine in mice brain as well as inhibited nuclear factor (NF)-kappaB activation in scopolamine or TNF-alpha-stimulated BV-2 microglial cells [20].

The disadvantage of mangiferin was the poor oral bioavailability basic on the prediction value of pharmacokinetic properties, especially, it is not easy to cross the Blood-Brain Barrier (BBB). Addition, the drug likeness value of mangiferin were only the highest as 75%. However, mangiferin using vitamin E-TPGS co-loaded self-assembled phosholipidic nano-mixed micellar systems were promised to augment oral bioavailability [21]. The enhancing oral bioavailability of mangiferin could be conduct further research and development.

4. Conclusion

Our studies showed that mangiferin were one of the good candidates for the diabetic uremic neuropathy and peripheral neuropathy onset and progression. At the same time, we analyzed the possible mechanism of action of mangiferin, which can be used to further develop safe and effective drugs. However, its shortcomings such as the poor oral bioavailability and the low BBB permeability also bring development barriers.

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