Diabetes, Metabolic Syndrome and Obesity

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Luteolin Alleviates Diabetic Nephropathy Fibrosis Involving AMPK/NLRP3/TGF-β Pathway

Rong Huang[1,*], Jun Zeng[1,*], Xiaoze Yu[1], Yunke Shi[2], Na Song[1], Jie Zhang[1], Peng Wang[1], Min Luo[1], Yiming Ma[2], Chuang Xiao[1], Lueli Wang[1], Guanhua Du[3], Hongyan Cai[4], Weimin Yang[1]

[1]School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming, Yunnan, People's Republic of China; [2]The First Department of Cardiology, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, People's Republic of China; [3]Key Laboratory of Drug Target Research and Drug Screen, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, People's Republic of China; [4]The Second Department of Cardiology, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Weimin Yang, School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming, Yunnan, 650500, People's Republic of China, Email ywmbessie@yeah.net; Hongyan Cai, The Second Department of Cardiology, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, 650032, People's Republic of China, Email caihy@ydyy.cn

Purpose: Luteolin is a promising candidate for diabetic nephropathy due to its potential anti-inflammatory and anti-fibrotic properties. This study explored the molecular mechanisms through which luteolin combats fibrosis in DN.

Methods: Potential targets affected by luteolin and genes associated with DN were collected from databases. Overlapping targets between luteolin and diabetic nephropathy were identified through Venn analysis. A protein-protein interaction network was constructed using these common targets, and critical pathways and targets were elucidated through GO and KEGG analysis. These pathways and targets were confirmed using a streptozotocin-induced mouse model. Luteolin was administered at 45 mg/kg and 90 mg/kg. Various parameters were evaluated, including body weight, blood glucose levels, and histopathological examinations. Protein levels related to energy metabolism, inflammation, and fibrosis were quantified.

Results: Fifty-three targets associated with luteolin and 36 genes related to diabetic nephropathy were extracted. The AGE-RAGE signaling pathway was the key pathway impacted by luteolin in diabetic nephropathy. Key molecular targets include TGF-β, IL-1β, and PPARG. Luteolin reduced body weight and blood glucose levels, lowered the left kidney index, and improved insulin and glucose tolerance. Furthermore, luteolin mitigated inflammatory cell infiltration, basement membrane thickening, and collagen deposition in the kidney. Luteolin up-regulated the protein expression of p-AMPKα (Th172) while simultaneously down-regulated the protein expression of p-NF-κB (p65), NLRP3, TGF-β1, α-SMA, and Collagen I.

Conclusion: Luteolin mitigated renal fibrosis by alleviating energy metabolism disruptions and inflammation by modulating the AMPK/NLRP3/TGF-β signaling pathway.

Keywords: diabetic nephropathy, network pharmacology, inflammation, TGF-β, fibrosis

Introduction

The global prevalence of diabetes is projected to exceed 10%, with a 46% increase in the absolute number of diabetic patients by 2045.[1] Diabetic nephropathy (DN) is one of the common microvascular complications caused by chronic hyperglycemia. Younger people are increasingly diagnosed with diabetes and are more likely than older people to develop diabetic complications due to poor blood glucose control.[2] In the early stages of DN, observable phenomena include thickening of the glomerular basement membrane and proliferation of mesangial cells. In contrast, the late stages of DN manifest tubulointerstitial fibrosis and mesenchymal lysis.[3] Clinical outcome studies have suggested that the use of glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose cotransporter protein-2 (SGLT2) inhibitors, in conjunction with other medications outlined in the Standards of Medical Care in Diabetes,[4,5] can mitigate the progression of DN and reduce cardiovascular risk.

Received: 31 January 2024
Accepted: 23 July 2024
Published: 30 July 2024

Hyperglycemia-induced mitochondrial dysfunction and subsequent excess production of reactive oxygen species (ROS) can lead to irreversible damage due to the initiation of epigenetic reprogramming, known as metabolic memory. Consequently, diabetic complications may persist even if glycemic control is within the normal range.[6] The pathogenesis of DN involves multiple cytokines and growth factors, with ROS and inflammatory cytokines notably accelerating the progression of renal fibrosis. Therefore, therapeutic approaches with antioxidant and anti-inflammatory effects prove to be effective strategies for managing DN.

More than 90% of patients with early-stage DN often face delayed diagnosis, underscoring the benefits of pre-treatments with safety considerations.[7] Luteolin, extracted from *Erigeron breviscapus* (Vant). Hand.-Mazz. in Yunnan, China, is classified as a flavonoid compound (Figure 1A).[8] Luteolin is naturally present in various traditional Chinese herbs and commonly consumed foods. Research has shown that luteolin can mitigate liver fibrosis by inhibiting tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1), sourced from *Lonicera japonica Thunb* and *Forsythiae Fructus*.[9] Additionally, luteolin has been found to reduce the production of advanced glycation end (AGE) products and ROS, mainly sourced from *Vernonia amygdalina*.[10]

Previous studies also indicate that luteolin can upregulate the expression of adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) and PKC isoforms, which helps mitigate H_2O_2-induced oxidative stress in huvec cells.[11] Furthermore, luteolin has been shown to inhibit the activation of the NOD-like receptor protein 3 (NLRP3) inflammasome, thus attenuating endoplasmic reticulum stress and inflammation in endothelial cells.[12,13] Luteolin has multiple therapeutic targets and may hold promise as a potential therapeutic strategy to mitigate kidney injury.[14] In

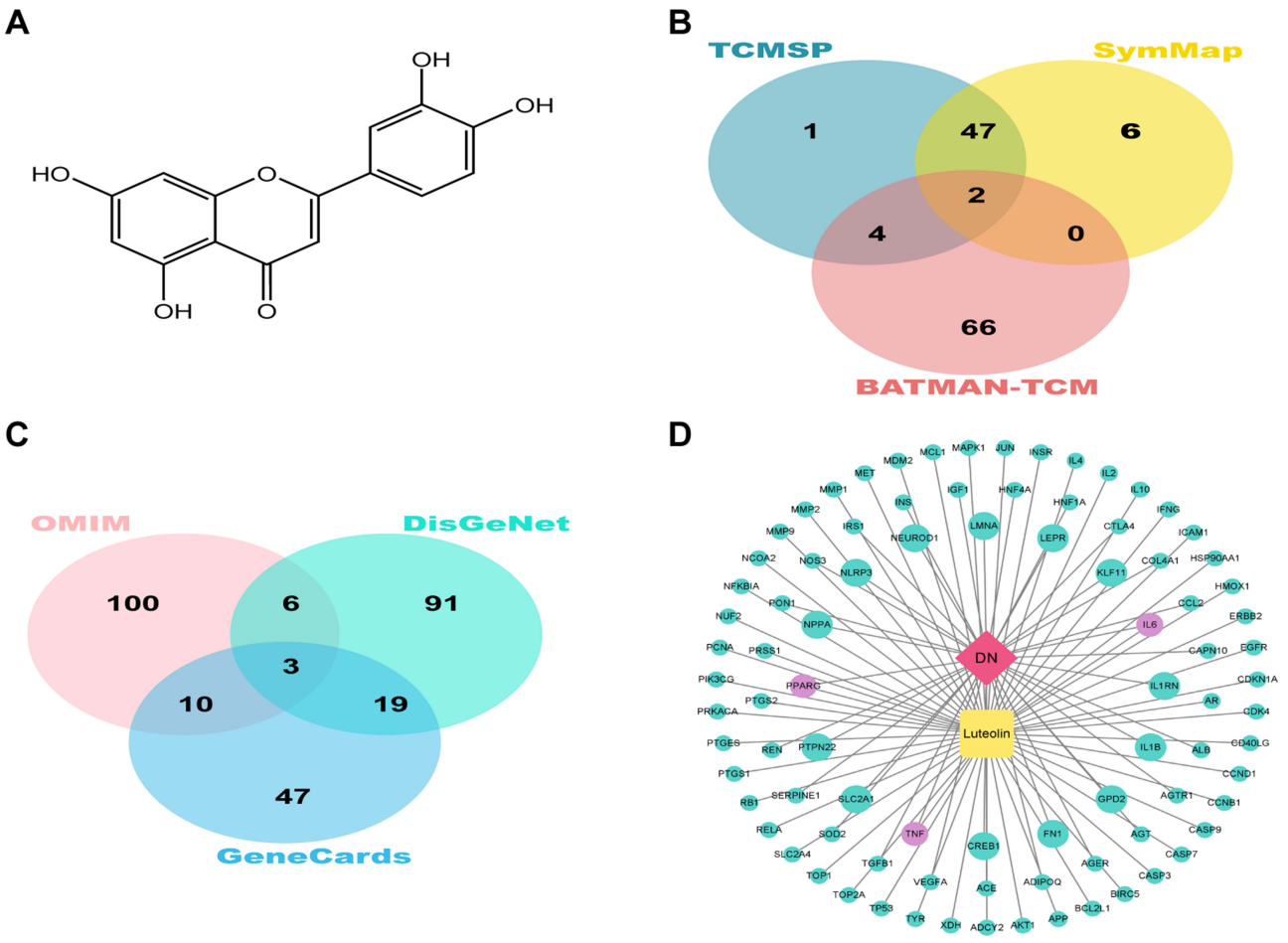

Figure 1 Luteolin targets and diabetic nephropathy disease genes. (**A**) Structural formula of luteolin. (**B**) Veen analysis of luteolin targets. (**C**) Venn analysis of diabetic nephropathy disease genes. (**D**) The network of luteolin-diabetic nephropathy.

particular, the long-standing history of the safety profile of luteolin in various studies makes it a candidate for consideration as a long-term drug option to slow the progression of DN in clinical practice.

Network pharmacology is an emerging discipline grounded in systems biology theory, offering a novel approach to elucidating the mechanisms by which Chinese medicine treats various diseases.[15] We can selectively identify key molecular and systemic targets and pathways by creating virtual screening and analyzing the "drug-component-target-pathway" network. This approach accelerates the development of new drugs while mitigating the costs associated with the process.

Inflammation and fibrosis represent significant risk factors for DN.[16] Following our network pharmacology analysis, we hypothesized that luteolin could improve DN by modulating the AMPK/NLRP3/TGF-β pathway. This research established a diabetic mouse model through streptozotocin (STZ) induction and administered luteolin. Our findings strongly suggest that luteolin effectively mitigates renal fibrosis by increasing phosphorylation and simultaneously suppressing the NLRP3 inflammasome and TGF-β expression. These results present a promising strategy for treating early-stage DN.

Methods

Network Pharmacology Analysis

Data Preparation

Luteolin targets were obtained from the TCM Systems Pharmacology Database (TCMSP, http://tcmspw.com/tcmsp.php), the SymMap database (SymMap, www.symmap.org), and the Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org.cn/batman-tcm/index.php/Home/Index/index) by using the keyword of luteolin, then Luteoin targets from TCMSP database, the SymMap database and BATMAN-TCM were all included for subsequent analysis and were not additional screened. Disease-related genes associated with DN were retrieved from the Online Mendelian Inheritance in Man database (OMIM, https://omim.org/), the Human GeneDatabase (GeneCards, https://www.genecards.org/) and the DisGeNET Database (DisGeNET, https://www.disgenet.org/home/) by using the keyword diabetic nephropathy and diabetic kidney disease, then disease genes with a Pheno map key of 3 were screened in the OMIM database, disease genes with a Relevance score >30 were screened in the Human GeneDatabase, and disease genes with a Score_gda >0.2 were screened in the DisGeNET database. A Venn analysis was employed to synthesize these datasets to identify potential targets for further analysis, and the overlapping targets were considered as a set of potential targets for subsequent investigations.

Network Construction

We imported luteolin targets and DN disease genes into STRING (https://string-db.org/) to construct the protein-protein interaction (PPI) network, and the above results were imported into Cytoscape 3.8.2 software for visualization. Important PPI network nodes were screened using the Cytoscape MCODE plug-in through MCC, MNC, DMNC algorithm, and key targets were obtained using Venn analysis. The analysis of Gene ontology (GO) functions that include biological processes (BP), cell components (CC), and molecular functions (MF) was performed using the David database (https://david.ncifcrf.gov/). $P < 0.05$ indicates significant differences. Gene mapping of common targets was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG).

In vivo Experiments

The Protocol of Animal Studies

Thirty male C57BL/6 mice, aged 6–8 weeks, were procured from the Kunming Medical University Laboratory Animal Center (Kunming, China). All mice were housed in a standard environment, provided ad libitum access to food and water, and subjected to a controlled environment (temperature: 20–24°C, humidity: 40%-70%, 12-hour light-dark cycle). The Animal Ethics Committee of Kunming Medical University approved all animal experiments (Kmmu20220042, approval date February 21, 2022). After one week of acclimatization, twenty-six mice were injected intraperitoneally with STZ dissolved in 10 mmol/L citrate buffer (pH 4.5) at 50 mg/kg/day for 5 days, and mice were respectively fasted for 12 h before intraperitoneal injection of STZ and 3 h after injection. The fasting blood glucose(FBG) of mice were

detected by collecting blood from the tail of mice using Sinocare blood glucose meter(GA-3, Sinocare). The diabetic mice model was established when the FBG ≥ 11.1 mM, Twenty-five mice were included diabetic model, and one mouse died during feeding.

After 10 weeks of continued feeding, mice were divided into normal group (N), STZ model group (STZ), STZ plus metformin (250 mg/kg) positive control group (Met), STZ plus low-dose luteolin (45 mg/kg) group (Lut-L), and STZ plus high-dose luteolin (90 mg/kg) group (Lut-H). Mice were administered luteolin by gavage daily for 8 consecutive weeks. Double-distilled water was administered to the N and STZ groups. The mice's body weight (BW) and blood glucose were monitored weekly.

Glucose Tolerance Test and Insulin Tolerance Test

Before glucose tolerance and insulin tolerance tests, mice were fasted for 6 h with free access to water. Mice were injected intraperitoneally with glucose at 1 g/kg of BW or insulin at 1 unit/kg of BW. Blood glucose was measured by collecting blood from the tail of mice using Sinocare blood glucose meter at 0, 15, 30, 60, and 120 min, respectively. The area under the curve (AUC) is calculated as follows: AUC= (FBG0+FBG15) × 15/2 + (FBG15+FBG30) × 15/2 + (FBG30+FBG60) × 30/2 + (FBG60+FBG120) × 60/2.

Pathological Observation

Following the euthanasia of the mice using 0.3% pentobarbital sodium, their kidneys were surgically removed, weighed, and then fixed in 4% paraformaldehyde for 48 h. Subsequently, 8 μm sections were cut from paraffin-embedded kidney tissues. These sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and Masson's trichrome (Masson) techniques. For histopathological analysis, kidney morphology was observed using a light microscope (Nikon microscope). HE staining was used to assess the inflammatory infiltrate. PAS staining was used to evaluate the extracellular matrix (ECM) and thickness of the basement membrane, while Masson staining was performed to assess kidney fibrosis. Semi-quantitative scoring of glomerular injury on a scale of 0 to 4 based on mesangial expansion and glomerulosclerosis, as follows: Mild mesangial expansion < 25% (0), Mild mesangial expansion > 25 (1), Severe mesangial expansion > 25% (2), At least one convincing Kimmelstiel-Wilson lesion (3), Global glomerular sclerosis > 50% (4). Semi-quantitative scoring of glomerular injury on a scale of 0 to 3 based on fibrosis in the cortex, as follows: No fibrosis (0), fibrosis< 25% (1), fibrosis between 25% and 50% (2), fibrosis > 50% (3).[17]

Western Blot Analysis

Tissue samples were homogenized in ice-cold RIPA lysis buffer (P0013B, Beyotime), supplemented with 1% protease inhibitor (11836153001, Roche) and phosphatase inhibitor (04906837001, Roche). After homogenization, the lysates were centrifuged at 17,000 rpm for 20 min at 4°C and quantified using the Enhanced BCA Protein Assay Kit (P0010S, Beyotime). Equal amounts of protein from tissue lysates were separated by 10% SDS-PAGE and subsequently transferred to a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was blocked and then incubated with primary antibodies overnight at 4°C with gentle agitation. The primary antibodies used included CST: anti-ASC (67824S), anti-NFκB p65 (8242S), anti-p-NFκB p65 (3033S), anti-β-actin (4970T); Abcam: anti-NLRP3 (ab263899), anti-IL-1β (ab254360), and anti-TGF-β1 (ab215715). Following incubation with primary antibodies, the membrane was washed six times with Tris-buffered saline solution/Tween (TBST) and subsequently incubated with secondary antibodies for 1.5 h at room temperature. β-actin was chosen as an internal control for data normalization in all samples in Western blot analysis. The results were captured using the Amersham Imager 600 imaging system, and the grayscale values were quantified using ScanImage software.

Data Analysis

Data are presented as mean ± standard error of the mean (SEM) derived from six independent experiments. The distribution and variance chi-square of data were first tested, and then multiple-group comparisons of normally distributed data was performed using one-way analysis of variance (ANOVA), followed by the minimum significant

difference test, with the statistical significance set at $P < 0.05$. Data analysis was performed using Sigmastat, and graphs were generated using GraphPad 9.

Results

Identification of Luteolin and DN-Related Targets

We collected luteolin targets from TCMSP, SymMap, and BATMAN-TCM, yielding 54, 55, and 72 targets, respectively. Through Venn analysis, we identified 53 common targets for the three data sets (Figure 1B and Supplementary Table 1). Similarly, we gathered DN disease genes from OMIM, DisGeNet, and GeneCards, which yielded 119, 119, and 79 genes, respectively. Through Venn analysis, we identified 36 DN disease genes shared between the three databases (Figure 1C and Supplementary Table 2). The "luteolin-DN target network" was constructed with Cytoscape to illustrate the connections between luteolin and DN based on their shared targets (Figure 1D).

PPI Network Analysis

The PPI network comprising 86 common luteolin-DN targets was analyzed using the STRING database (Figure 2A and Supplementary Table 3). The results of this STRING analysis were imported into Cytoscape for further examination. Node connectivity was assessed using a plug-in, in which higher-degree nodes indicate greater importance and functionality. The top 30 targets were selected using the CytoHubba plug-in, and among these, key targets were identified, including TGF-β, IL-1β, and peroxisome proliferator-activated receptor gamma (PPARG) (Figure 2B and Supplementary Table 4).

GO and KEGG Enrichment Analysis

The GO analysis yielded 176 BPs, 415 CCs, and 24 MFs from the David database (Figure 3A and Supplementary Table 5). Among these, the BPs were ranked by significance, covering a range of functions such as cell cycle regulation, drug response, positive regulation of NF-κB transcription factor activity, negative regulation of apoptotic processes, collagen catabolic processes, positive regulation of fibroblast proliferation, positive regulation of MAP kinase activity, and vascular endothelial growth factor production processes.

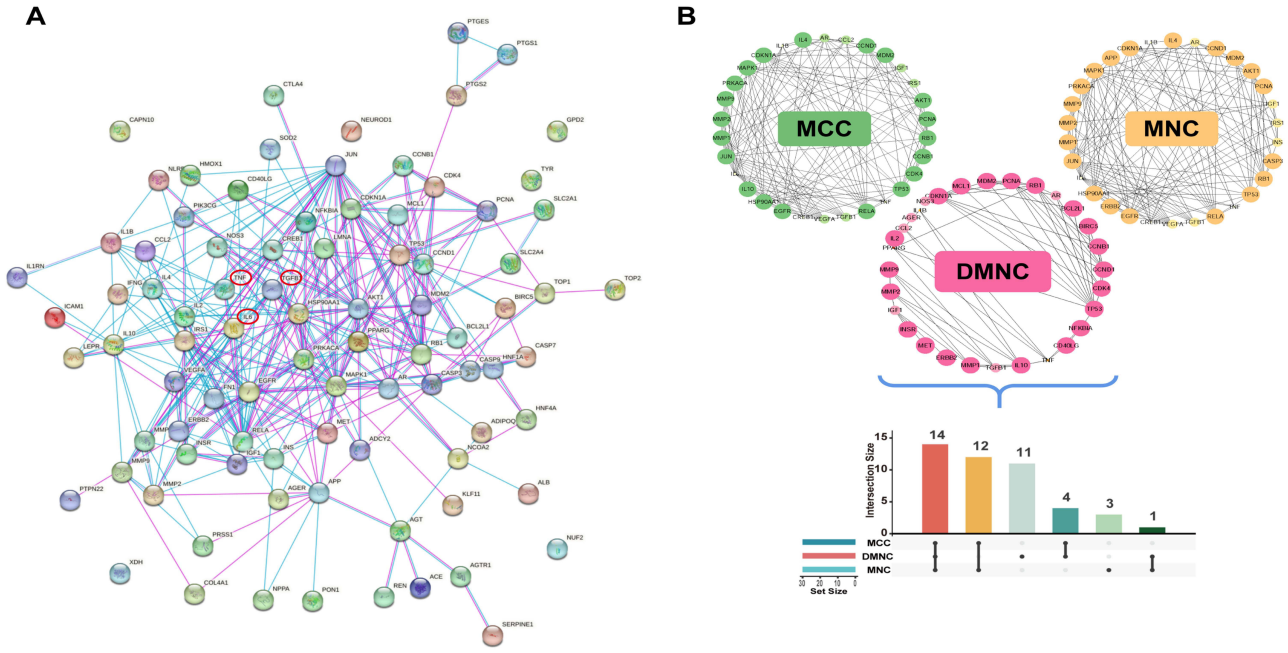

Figure 2 Luteolin-diabetic nephropathy interaction network of targets. (**A**) PPI network of luteolin-diabetic nephropathy common targets. (**B**) Upset plot of top 30 targets in luteolin-diabetic nephropathy common targets by MCC, MNC, DMNC algorithm.

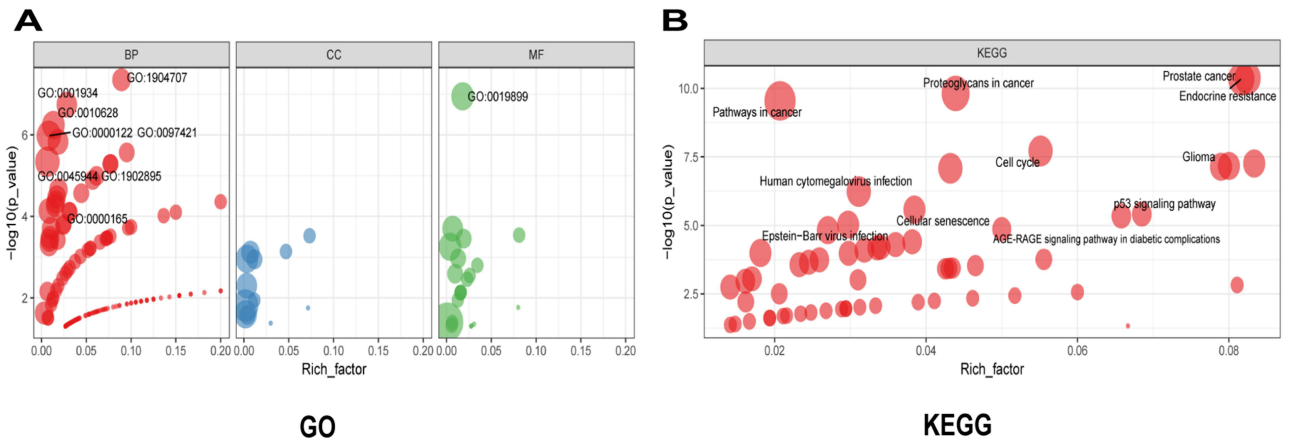

Figure 3 GO and KEGG enrichment analysis of target proteins. (**A**) GO enrichment analysis of the top gene in luteolin-diabetic nephropathy common targets. (**B**) KEGG enrichment analysis of the top gene in luteolin-diabetic nephropathy common targets.

In the KEGG analysis, enriched pathways were identified, including p53, AGE-RAGE, FoxO, IL-17, MAPK, PI3K-Akt, TNF, and JAK-STAT (Figure 3B and Supplementary Table 6). Furthermore, we observed the involvement of other pathways, such as cellular senescence, fluid shear stress, atherosclerosis, and the relaxin signaling pathway. These results suggest that luteolin may alleviate DN through anti-inflammation, antioxidation, and anti-fibrosis mechanisms.

Body Weight and Blood Glucose

Animal experiments were conducted according to the workflow described (Figure 4A). Compared to the N group, the STZ group exhibited a notable reduction in body weight and a significant increase in blood glucose levels and the left kidney index (calculated as the weight of the left kidney divided by body weight). However, the Met, Lut-L, and Lut-H treatment groups counteracted the trends associated with diabetic-induced weight loss and hyperglycemia, although these differences did not reach statistical significance (Figure 4B and C). The Met and Lut-H groups exhibited a statistically significant decrease in left kidney index compared to the STZ group (Figure 4D).

Intraperitoneal Insulin Tolerance Test (IPITT) and Intraperitoneal Glucose Tolerance Test (IPGTT)

Insulin and glucose tolerance were significantly higher in the STZ group than in the N group. The Met group significantly decreased insulin and glucose tolerance compared to the STZ group. In contrast, the Lut-L and Lut-H groups significantly decreased insulin tolerance but did not affect glucose tolerance (Figures 4E–H).

Pathological Observation

Compared to the N group, HE staining shows that STZ induction increased inflammatory cell infiltration. In particular, the Met, Lut-L, and Lut-H groups significantly reduced the inflammatory response of the kidney. Histologic analysis of PAS staining showed that the glomerular mesangium of the STZ group was significantly thicker than that of the N group, and the renal damage score was significantly increased. The thickening of the glomerular mesangial was reduced significantly in the Met, Lut-L, and Lut-H groups compared to the STZ group.

Histological analysis Masson staining shows that there were no apparent blue-stained collagen fibers in the glomerulus of the N group, while there were more blue-stained collagen fibers in the glomerulus of the STZ group, and the renal damage score was significantly increased. There was a decrease in blue-stained collagen fibers in the glomerulus of the Met, Lut-L, and Lut-H groups, which slowed the process of renal fibrosis in DN (Figure 5A–C).

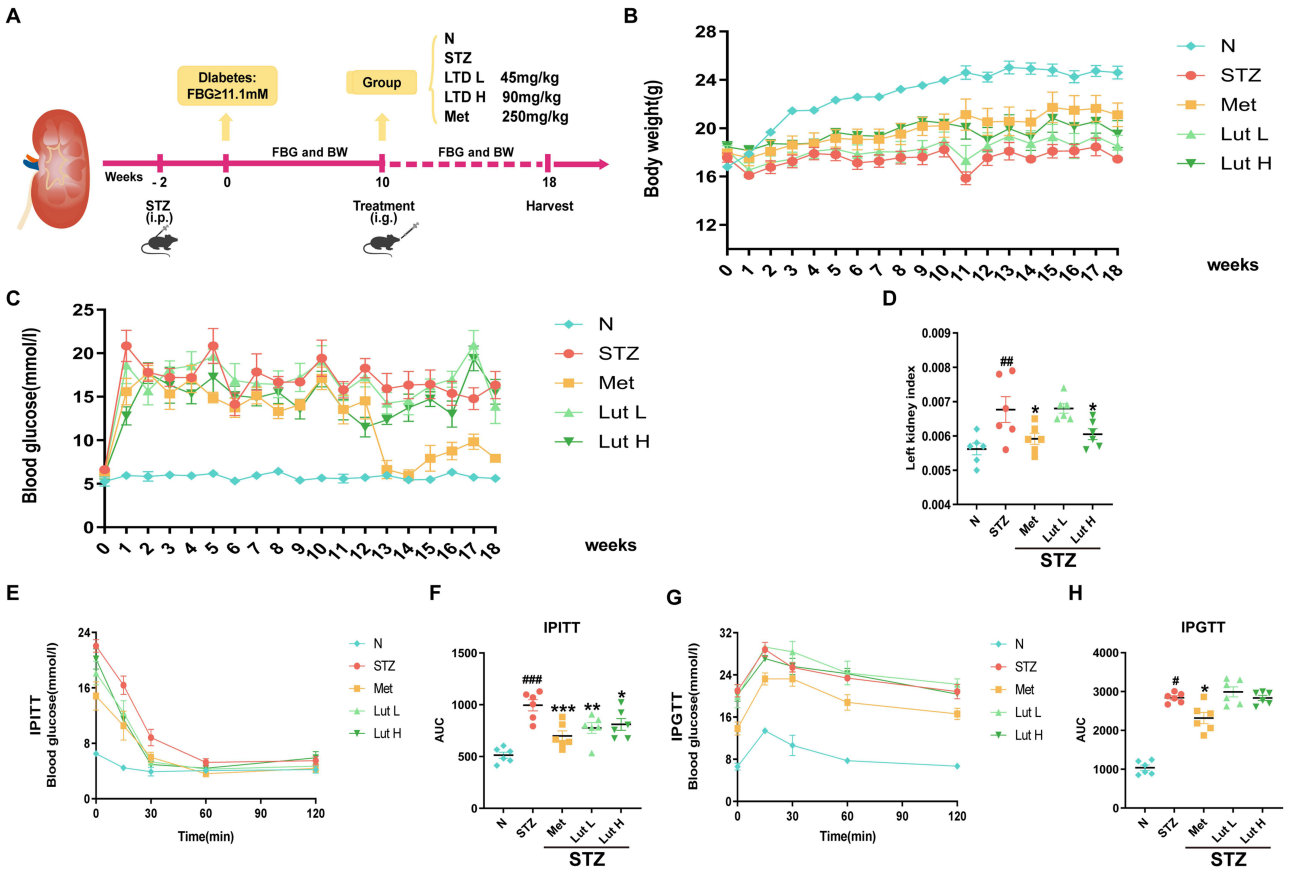

Figure 4 Luteolin attenuated diabetic nephropathy injury in diabetic nephropathy mice. (**A**) The process of animal experiment. (**B**) Body weight in diabetic nephropathy mice(n=6). (**C**) Fasting blood glucose in diabetic nephropathy mice(n=6). (**D**) Left kidney index in diabetic nephropathy mice(n=6). (**E**) The curve of IPITT in diabetic nephropathy mice(n=6). (**F**) IPITT AUC in diabetic nephropathy mice(n=6). (**G**) The curve of IPGTT in diabetic nephropathy mice(n=6). (**H**) IPGTT AUC in diabetic nephropathy mice(n=6). $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 shows compared with N group, *P<0.05, **P<0.01, ***P<0.001 shows compared with STZ group.

Luteolin Alleviates Energy Metabolism Disorders in DN Mice

The protein expression of phosphorylated AMPKα in the kidney of the STZ group was significantly down-regulated than in the N group. Protein expression of phosphorylated AMPKα was dramatically upregulated in the Met, Lut-L, and Lut-H groups, and there was no significant difference between the Lut-H and Lut-L groups (Figures 5D–G).

Luteolin Attenuates Inflammation in DN Mice

Protein expression of phosphorylated NF-κB was up-regulated in the kidney of the STZ group, subsequently initiating the inflammatory process. After STZ stimulation in mice (STZ group), protein expressions were significantly up-regulated compared to the N group, including the nod-like receptor protein containing pyrin 3 (NLRP3), the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), cleaved caspase-1, and cleaved IL-1β. However, the participation of Met, Lut-L, and Lut-H could regulate the expressions of these proteins. The Lut-H group had a stronger effect than the Lut-L group (Figures 6A–I).

Luteolin Attenuates Fibrosis in DN Mice

The protein expression of TGF-β1 was up-regulated in the STZ group, which led to a significant up-regulation of the expression of the smooth muscle alpha-actin (α-SMA) and collagen I protein compared to the N group. However, Met, Lut-L, and Lut-H could down-regulate the expressions of these proteins and improve renal fibrosis in DN mice. The Lut-H group had a stronger effect than the Lut-L group (Figures 6J–M).

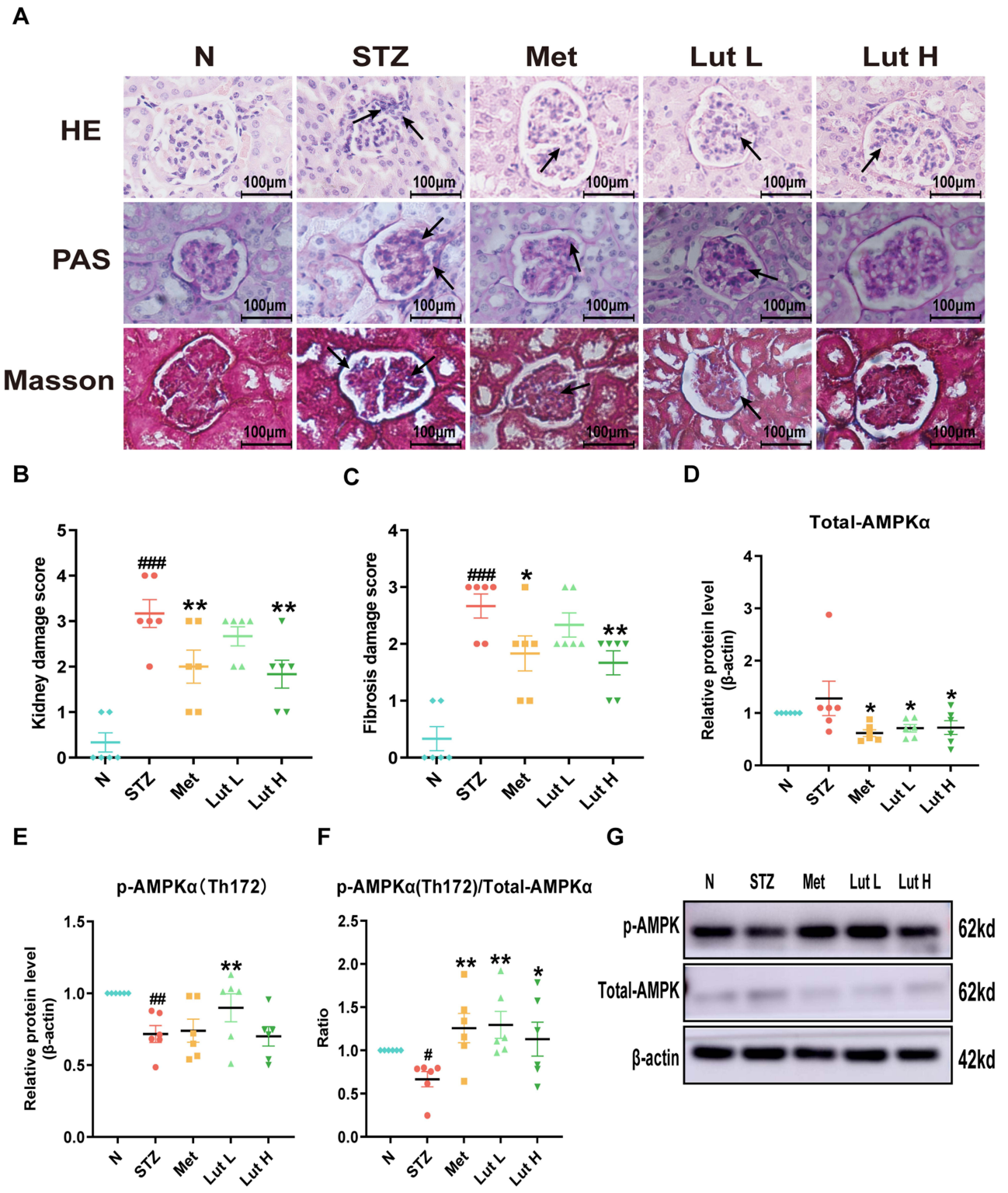

Figure 5 Luteolin relieved renal fibrosis in diabetic nephropathy mice. (**A**) Staining of HE, PAS and Masson of kidney in diabetic nephropathy mice(arrows indicate structural derangements of glomerulus). (**B**) Kidney damage score(n=6). (**C**) Fibrosis damage score(n=6). (**D**–**F**) The protein expression levels of AMPK and p-AMPK(Th172) of kidney in diabetic nephropathy mice, and were expressed as Mean ± SEM (n=6). (**G**) Representative protein blotting results of kidney in diabetic nephropathy mice. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 shows compared with N group, *P<0.05, **P<0.01 shows compared with STZ group.

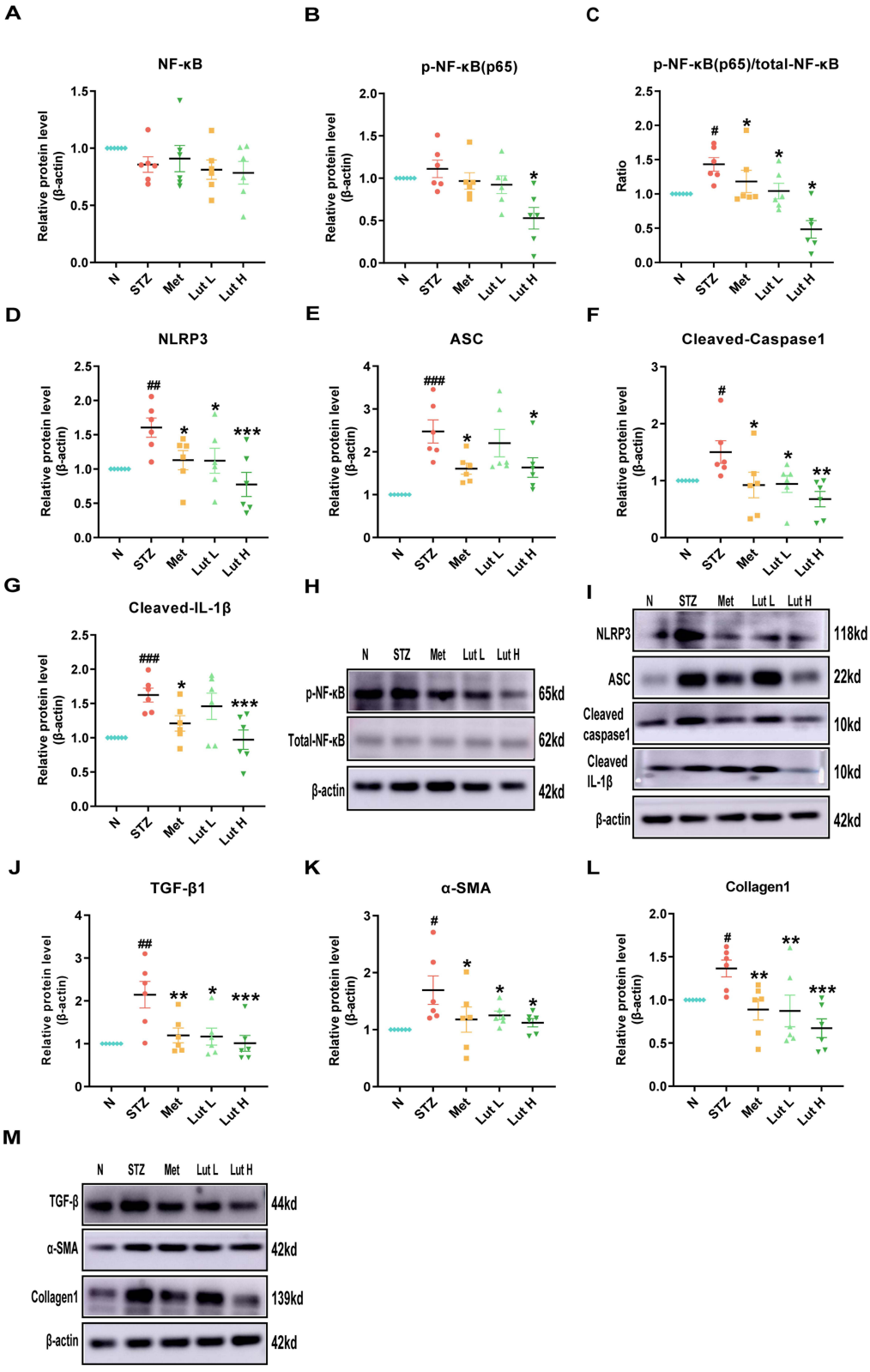

Figure 6 Luteolin alleviated renal fibrosis by down-regulating the protein expression of NF-κB and TGF-β1 in mice with diabetic nephropathy. (**A**–**G**)The protein expression levels of NF-κB, p-NF-κB(p65), NLRP3, ASC, Cleaved caspase1 and Cleaved IL-1β of kidney in diabetic nephropathy mice, and were expressed as Mean ± SEM(n=6). (**H** and **I**)Representative protein blotting results of kidney in diabetic nephropathy mice. (**J**–**L**)The protein expression levels of TGF-β1, α-SMA and Collagen1 of kidney in diabetic nephropathy mice, and were expressed as Mean ± SEM(n=6). (**M**) Representative protein blotting results of kidney in diabetic nephropathy mice. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 shows compared with N group, *P<0.05, **P<0.01, ***P<0.001 shows compared with STZ group.

Discussion

Diabetes mellitus is a significant risk factor for the development of end-stage renal disease (ESRD), making the early diagnosis and treatment of kidney injury a global research focus.[18] Chronic hyperglycemia exacerbates ROS production, immune cell infiltration, and the secretion of various cytokines.[19] Currently, only a limited number of drugs have been used to mitigate DN progression and improve renal function, including angiotensin-converting enzyme inhibitors (ACEIs), GLP-1 receptor agonists, and SGLT2 inhibitors.[20,21] Previous research has shed light on the mechanisms by which certain compounds work to ameliorate kidney complications in diabetes. For example, studies have shown that salidroside alleviates endothelial inflammation and oxidative stress induced by AGEs, partly through the AMPK/NF-κB/NLRP3 pathway.[22] Metformin, as an AMPK agonist, exerts its effects by upregulating mitophagy through the p-AMPK-Pink1-Parkin pathway, thus improving renal tubular interstitial fibrosis in high-fat diet/streptozotocin-induced diabetic mice.[23]

Luteolin has shown its potential to downregulate Nrf2-mediated oxidative stress and NF-κB-mediated inflammatory responses to reduce cardiac injury in STZ-induced diabetic mice.[24] Our study results align with these previous findings, showing that luteolin upregulates the expression of p-AMPKα and mitigates renal oxidative stress in STZ-induced diabetic mice. Furthermore, luteolin addresses energy metabolism dysfunction in the kidneys, corroborating previous research findings.

Renal biopsy tissues from diabetic patients have consistently revealed the presence of macrophage infiltration and activation of the NLRP3 inflammasome.[25,26] Numerous investigations have explored therapeutic strategies to modulate inflammatory pathways, targeting immune cells, pro-inflammatory cytokines, adhesion molecules, chemokines, and JAK-STAT and NF-κB signaling.[27] We observed that luteolin exhibited a significant down-regulation in the expression levels of proteins associated with inflammation in STZ-induced diabetic mice. This effect contributed to the attenuation of DN progression. In particular, activation of the NLRP3 inflammasome occurred in the early stages of DN, and luteolin effectively alleviated early DN symptoms by inhibiting activation of the NLRP3 inflammasome.

Furthermore, luteolin and luteolin-7-O-glucoside reduced the mRNA expression levels of multiple inflammatory cytokines and their receptors by inhibiting the STAT3 pathway.[28] The intricate relationship between immune responses, inflammation, and abnormal metabolism plays a crucial role in the pathogenesis of DN. In this context, luteolin is a promising agent for treating DN because of its demonstrated benefits in modulating these interconnected processes.

In DN, accumulation of ECM in the glomeruli is a prominent feature that progresses to glomerulosclerosis, ultimately leading to decreased renal function. This accumulation of ECM is closely associated with oxidative stress and autophagy. Recent studies have indicated that luteolin can ameliorate renal fibrosis induced by aristolochic acid in mice through the sirtuin-1/forkhead box class O3 (SIRT1/FOXO3) pathway.[29] In our study, we identified 89 potential target sites through data collection, with TGF-β, IL-1β, and PPARG emerging as key targets for treating diabetic kidney fibrosis. Our findings demonstrate that luteolin effectively inhibits ECM accumulation, thus attenuating renal fibrosis.

Luteolin significantly down-regulated the protein expression of TGF-β1, α-SMA, and Collagen I. TGF-β1, a pleiotropic cytokine, has potential as a strategy for treating DN.[30] The binding of TGF-β to its receptor can initiate the epithelial-mesenchymal transition (EMT) in the kidney, and this EMT process is influenced by factors such as inflammatory cytokines, AGEs, oxidative stress, hypoxia, and TGF-β1.[31] TGF-β regulates gene transcription related to the myofibroblast phenotype, associated with the differentiation of renal tubular epithelial cells into fibroblasts.[32] Our study screened to the key target of luteolin for diabetic nephropathy through network pharmacology, which can target the action of luteolin more accurately and validate it experimentally, and provide a line of thought for the pharmacological study of natural products and diseases. Luteolin improves DN through multi-target synergistic effects, and effectively mitigates renal fibrosis in mice with STZ-induced DN by targeting the AMPK/NLRP3/TGF-β signaling pathway (Figure 7), and this pathway were completely and systematically validated. This holds promise for the future, offering new strategies to prevent and treat renal fibrosis.

However, the specific effects of luteolin on different cell types in the kidney, including epithelial cells, podocytes, mesangial cells, and endothelial cells, were not explored in-depth in this study. Future investigations should investigate the molecular mechanisms of luteolin treatment for DN in various types of kidney cells.

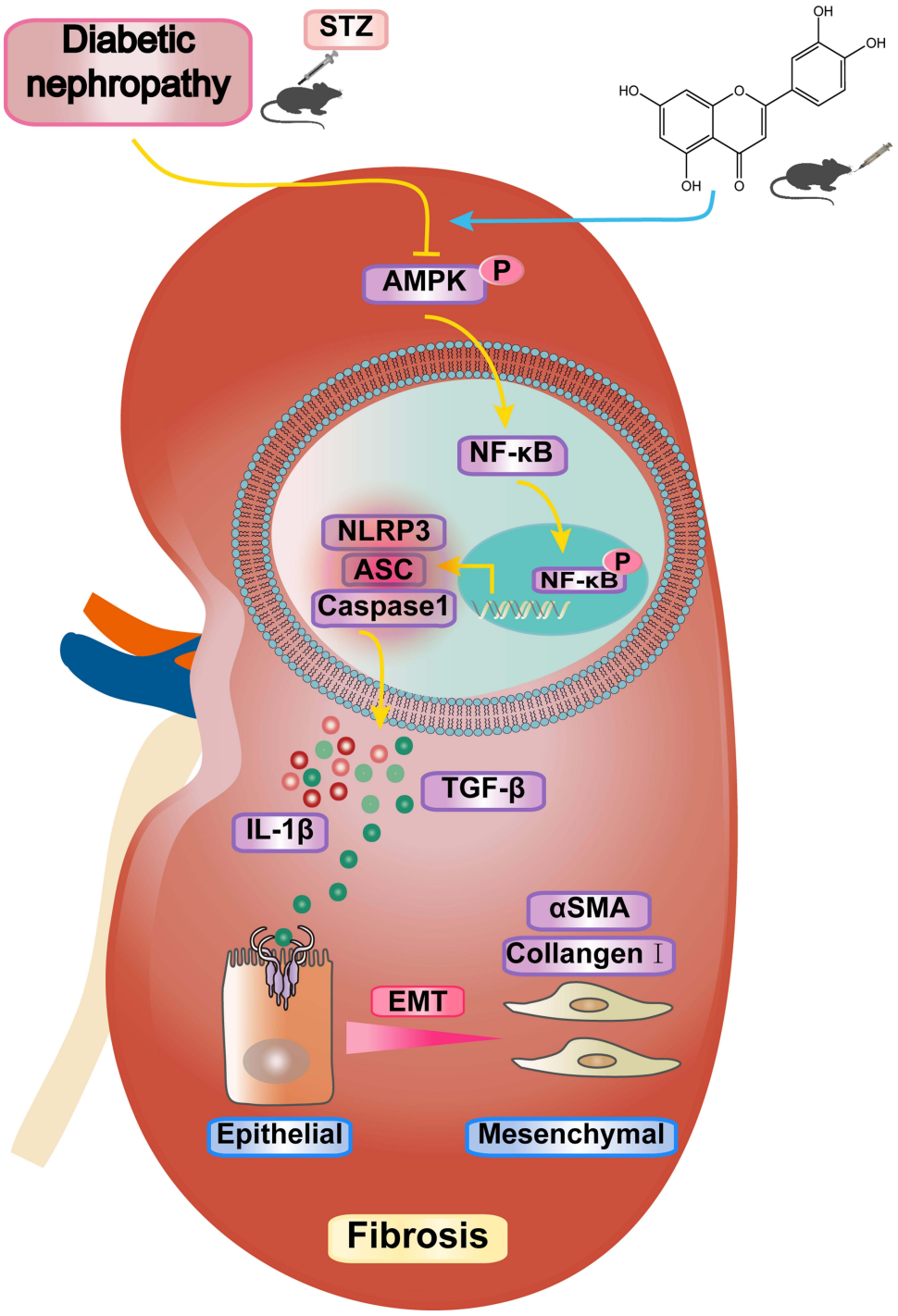

Figure 7 Luteolin alleviated renal fibrosis in STZ-induced diabetic nephropathy mice involving AMPK/NLRP3/TGF-β signaling pathway.

Conclusion

This study demonstrated that luteolin alleviated renal fibrosis in DN mice, which involved the regulation of the AMPK/NLRP3/TGF-β signaling pathway, and the multi-targets of luteolin are beneficial for treating renal fibrosis in DN.

Abbreviations

Diabetic nephropathy, DN; Epithelial-mesenchymal transition, EMT. Transforming growth factor-β1, TGF-β1.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author Weimin Yang upon reasonable request.

Ethics Approval

This study was approved by the Committee on Ethics of Kunming Medical University (protocol No. Kmmu20220042 dated 21-Feb-2022). All animal experiments meet the requirements of National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No.8023).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by [the National Natural Science Foundation of China #1] under Grant [number 82270372, 82260087, 81870037]; [Science and Technology Program of Yunnan Province #2] under Grant [number 202105AF150015, 202302AA310026, 202102AA310030]; [Kunming Development and Reform Commission] under Grant [number 202202], and [Special Foundation Projects of Joint Applied Basic Research of Yunnan Provincial Department of Science and Technology with Kunming Medical University#3]under Grant [number 202301AY070001-119].

Disclosure

The authors report no potential conflicts of interest in this work.

References

1. Sun H, Saeedi P, Karuranga S, et al. Idf diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabet Res Clin Pract*. 2022;183:109119. doi:10.1016/j.diabres.2021.109119
2. Pettus JH, Zhou FL, Shepherd L, et al. Incidences of severe hypoglycemia and diabetic ketoacidosis and prevalence of microvascular complications stratified by age and glycemic control in u.s. Adult patients with type 1 diabetes: a real-world study. *Diabetes Care*. 2019;42(12):2220–2227. doi:10.2337/dc19-0830
3. Azushima K, Gurley SB, Coffman TM. Modelling diabetic nephropathy in mice. *Nat Rev Nephrol*. 2018;14(1):48–56. doi:10.1038/nrneph.2017.142
4. Palmer SC, Tendal B, Mustafa RA, et al. Sodium-glucose cotransporter protein-2 (sglt-2) inhibitors and glucagon-like peptide-1 (glp-1) receptor agonists for type 2 diabetes: systematic review and network meta-analysis of randomised controlled trials. *BMJ*. 2021;372:m4573. doi:10.1136/bmj.m4573
5. Alicic RZ, Cox EJ, Neumiller JJ, Tuttle KR. Incretin drugs in diabetic kidney disease: biological mechanisms and clinical evidence. *Nat Rev Nephrol*. 2021;17(4):227–244. doi:10.1038/s41581-020-00367-2
6. Kato M, Natarajan R. Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. *Nat Rev Nephrol*. 2019;15(6):327–345. doi:10.1038/s41581-019-0135-6
7. Tuttle KR, Jones CR, Daratha KB, et al. Incidence of chronic kidney disease among adults with diabetes, 2015-2020. *N Engl J Med*. 2022;387(15):1430–1431. doi:10.1056/NEJMc2207018
8. Vargas F, Romecín P, García-Guillén AI, et al. Flavonoids in kidney health and disease. *Front Physiol*. 2018;9:394. doi:10.3389/fphys.2018.00394
9. Zhang J, Jin H, Feng X, et al. The combination of lonicerae japonicae flos and forsythiae fructus herb-pair alleviated inflammation in liver fibrosis. *Front Pharmacol*. 2022;13:984611. doi:10.3389/fphar.2022.984611
10. Djeujo FM, Stablum V, Pangrazzi E, Ragazzi E, Froldi G. Luteolin and vernodalol as bioactive compounds of leaf and root vernonia amygdalina extracts: effects on α-glucosidase, glycation, ros, cell viability, and in silico admet parameters. *Pharmaceutics*. 2023;15(5):1541. doi:10.3390/pharmaceutics15051541
11. Ou H, Pandey S, Hung M, et al. Luteolin: a natural flavonoid enhances the survival of huvecs against oxidative stress by modulating ampk/pkc pathway. *Am J Chin Med*. 2019;47(03):541–557. doi:10.1142/S0192415X19500289
12. Wu J, Xu X, Li Y, et al. Quercetin, luteolin and epigallocatechin gallate alleviate txnip and nlrp3-mediated inflammation and apoptosis with regulation of ampk in endothelial cells. *Eur J Pharmacol*. 2014;745:59–68. doi:10.1016/j.ejphar.2014.09.046
13. Yu Q, Zhang M, Qian L, Wen D, Wu G. Luteolin attenuates high glucose-induced podocyte injury via suppressing nlrp3 inflammasome pathway. *Life Sci*. 2019;225:1–7. doi:10.1016/j.lfs.2019.03.073

14. Tang G, Li S, Zhang C, Chen H, Wang N, Feng Y. Clinical efficacies, underlying mechanisms and molecular targets of Chinese medicines for diabetic nephropathy treatment and management. *Acta Pharm Sin B*. 2021;11(9):2749–2767. doi:10.1016/j.apsb.2020.12.020
15. Nogales C, Mamdouh ZM, List M, Kiel C, Casas AI, Schmidt HH. Network pharmacology curing causal mechanism. *Trends Pharmacol Sci*. 2022;43(2):136–150. doi:10.1016/j.tips.2021.11.004
16. Palella E, Cimino R, Pullano SA, et al. Laboratory parameters of hemostasis, adhesion molecules, and inflammation in type 2 diabetes mellitus: correlation with glycemic control. *Int J Environ Res Public Health*. 2020;17(1):300. doi:10.3390/ijerph17010300
17. Tervaert TWC, Mooyaart AL, Amann K, et al. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol*. 2010;21(4):556–563. doi:10.1681/ASN.2010010010
18. Lei Q, Xu F, Liang S, et al. Clinical acute kidney injury and histologic acute tubular-interstitial injury and their prognosis in diabetic nephropathy. *Nephron Clin Pract*. 2022;146(4):351–359. doi:10.1159/000520944
19. Zhang P, Li T, Wu X, Nice EC, Huang C, Zhang Y. Oxidative stress and diabetes: antioxidative strategies. *Front Med*. 2020;14(5):583–600. doi:10.1007/s11684-019-0729-1
20. Ni L, Yuan C, Chen G, Zhang C, Wu X. Sglt2i: beyond the glucose-lowering effect. *Cardiovasc Diabetol*. 2020;19(1):98. doi:10.1186/s12933-020-01071-y
21. Vergara A, Llorens-Cebrià C, Martos N, et al. The membrane-associated protein 17 (map17) is up-regulated in response to empagliflozin on top of ras blockade in experimental diabetic nephropathy. *Clin Sci*. 2023;137(1):87–104. doi:10.1042/CS20220447
22. Hu R, Wang M, Ni S, et al. Salidroside ameliorates endothelial inflammation and oxidative stress by regulating the ampk/nf-κb/nlrp3 signaling pathway in ages-induced huvecs. *Eur J Pharmacol*. 2020;867:172797. doi:10.1016/j.ejphar.2019.172797
23. Han YC, Tang SQ, Liu YT, et al. Ampk agonist alleviate renal tubulointerstitial fibrosis via activating mitophagy in high fat and streptozotocin induced diabetic mice. *Cell Death Dis*. 2021;12(10):925. doi:10.1038/s41419-021-04184-8
24. Li L, Luo W, Qian Y, et al. Luteolin protects against diabetic cardiomyopathy by inhibiting nf-κb-mediated inflammation and activating the nrf2-mediated antioxidant responses. *Phytomedicine*. 2019;59:152774. doi:10.1016/j.phymed.2018.11.034
25. Chen K, Zhang J, Zhang W, et al. Atp-p2x4 signaling mediates nlrp3 inflammasome activation: a novel pathway of diabetic nephropathy. *Int J Biochem Cell Biol*. 2013;45(5):932–943. doi:10.1016/j.biocel.2013.02.009
26. Qi W, Keenan HA, Li Q, et al. Pyruvate kinase m2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction. *Nat Med*. 2017;23(6):753–762. doi:10.1038/nm.4328
27. Chen J, Liu Q, He J, Li Y. Immune responses in diabetic nephropathy: pathogenic mechanisms and therapeutic target. *Front Immunol*. 2022;13:958790. doi:10.3389/fimmu.2022.958790
28. Zhang M, He L, Liu J, Zhou L. Luteolin attenuates diabetic nephropathy through suppressing inflammatory response and oxidative stress by inhibiting stat3 pathway. *Exp Clin Endocrinol Diabetes*. 2021;129(10):729. doi:10.1055/a-0998-7985
29. Li F, Wei R, Huang M, et al. Luteolin can ameliorate renal interstitial fibrosis-induced renal anaemia through the sirt1/foxo3 pathway. *Food Funct*. 2022;13(22):11896–11914. doi:10.1039/D2FO02477B
30. Wang L, Wang H, Liu T, Lan H. Tgf-beta as a master regulator of diabetic nephropathy. *Int J Mol Sci*. 2021;22(15):7881. doi:10.3390/ijms22157881
31. Avila-Carrasco L, Majano P, Sánchez-Toméro JA, et al. Natural plants compounds as modulators of epithelial-to-mesenchymal transition. *Front Pharmacol*. 2019;10:715. doi:10.3389/fphar.2019.00715
32. Ina K, Kitamura H, Tatsukawa S, Takayama T, Fujikura Y, Shimada T. Transformation of interstitial fibroblasts and tubulointerstitial fibrosis in diabetic nephropathy. *Med Electron Microsc*. 2002;35(2):87–95. doi:10.1007/s007950200011