Journal of Biochemical and Molecular Toxicology

WILEY

RESEARCH ARTICLE

Taxifolin Attenuates Cisplatin-Induced Acute Kidney Injury by Promoting Fatty Acid Oxidation

Xuejin Jin[1,2] | Lingkun Wang[3] | Miao Yuan[1,2] | Huanyan Tao[1,2] | Huiyan Zha[3] | Zheng Xu[4] | Guang Liang[1,2] | Xiangwei Xu[1,2] | Qian Zhou[1,2]

[1]Affiliated Yongkang First People's Hospital, Hangzhou Medical College, Yongkang, China | [2]Zhejiang TCM Key Laboratory of Pharmacology and Translational Research of Natural Products, School of Pharmaceutical Sciences, Hangzhou Medical College, Hangzhou, Zhejiang, China | [3]Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, Center for Drug Safety Evaluation and Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China | [4]Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, China

Correspondence: Xiangwei Xu (xuxiangwei@hmc.edu.cn) | Qian Zhou (qianz1220@hmc.edu.cn)

Received: 7 April 2025 | **Revised:** 12 September 2025 | **Accepted:** 29 September 2025

Funding: The study was supported by the General Scientific Research Project of Department of Education of Zhejiang Province (Y202456747 to Qian Zhou).

Keywords: acute kidney injury | cisplatin | Fabp4 | fatty acid oxidation | taxifolin

ABSTRACT

Cisplatin-induced nephrotoxicity frequently manifests as acute kidney injury (AKI), creating a major limitation for its widespread clinical application. Taxifolin, a phytochemical flavonoid, possesses potent free radical-scavenging activity and the ability to modulate inflammatory responses. Our study focused on assessing the renoprotective capacity of taxifolin in the context of cisplatin-induced kidney damage. Cell culture studies showed that taxifolin reduced damage to human tubular epithelial cells caused by cisplatin. Furthermore, we evaluated the protective effect of taxifolin on mice with cisplatin-induced nephrotoxicity. Oral administration of taxifolin effectively mitigated both functional impairment and structural injury in renal tubules, both when administered 2 days before and 2 h following cisplatin injection. Transcriptomic analysis of renal tissues via RNA-seq revealed that taxifolin's nephroprotective effects against cisplatin toxicity may involve Fabp4-regulated lipid metabolism pathways. In both cisplatin-induced AKI mouse models and renal tubular cells, taxifolin enhanced lipid metabolism, restored cellular energy (ATP) production, and upregulated PGC-1α/PPARα expression by reversing Fabp4-impaired suppression of fatty acid beta-oxidation. Collectively, these results indicate that taxifolin exerts nephroprotective effects against cisplatin-induced AKI by modulating Fabp4-dependent fatty acid oxidation pathways, positioning it as a potential therapeutic candidate for cisplatin-associated nephrotoxicity.

1 | Introduction

Acute kidney injury (AKI) is a critical clinical syndrome that poses significant threats to patient survival, characterized by high rates of morbidity and mortality. It affects approximately 10%–15% of all hospitalized patients and has been reported in more than 50% of patients admitted to intensive care units [1, 2]. Nephrotoxicity is one of the leading causes of AKI and serves as a major dose-limiting factor for the chemotherapeutic agent cisplatin (Cis) [3]. As a platinum-based drug with well-documented antineoplastic efficacy, cisplatin remains a cornerstone in the treatment of various solid tumors, including ovarian, osteosarcoma, and prostate carcinomas [4–6]. Nevertheless, its clinical utility is substantially limited by its nephrotoxic effects [7]. Approximately 25%–30% of adult patients receiving cisplatin develop AKI, and among long-term survivors (≥ 5 years), a

Xuejin Jin and Lingkun Wang contributed equally to this study.

significant proportion experience a persistent reduction in glomerular filtration rate [8, 9]. Unfortunately, there are currently no established effective treatment for cisplatin-induced AKI in clinical practice, highlighting a pressing and unmet medical need. Thus, the development of strategies to mitigate cisplatin-induced nephrotoxicity is urgently required and deserves immediate translational attention.

Cisplatin-induced nephrotoxicity occurs primarily in proximal tubular epithelial cells (PTECs), which constitute the major site of injury leading to AKI [10]. Damage to PTECs disrupts key cellular processes, including bioenergetic networks, intercellular communication, and proliferative homeostasis. Renal proximal tubular cells are highly metabolically active and rely substantially on fatty acid oxidation (FAO) to meet their considerable energy demands [11]. Impairment of FAO is strongly associated with AKI progression, as its suppression during renal injury leads to energy deficiency and lipotoxicity. These effects are characterized by reduced renal ATP levels, intracellular lipid accumulation, inflammatory activation, tubular cell damage, and cell death [12]. Fatty acid binding protein 4 (Fabp4) plays a crucial role in regulating FAO. Studies indicate that Fabp4 overexpression downregulates key oxidative respiratory factors in adipocytes, such as PGC-1α and PPARα—master transcriptional regulators of numerous genes involved in FAO [13, 14]. Such disruption in FAO directly compromises the survival of renal tubular epithelial cells, positioning metabolic dysfunction as a central mechanism in kidney injury [15]. During AKI process, diminished FAO results in intracellular lipid accumulation and a metabolic shift toward glycolysis for energy production [16]. This adaptive change in substrate utilization may further exacerbate renal damage. Additionally, impaired FAO can induce mitochondrial dysfunction and apoptosis, contributing to the progression of kidney injury [17]. Therefore, restoring FAO to normal levels represents a promising therapeutic strategy for mitigating cisplatin-induced AKI.

Flavonoids, a major subclass of plant-derived polyphenols, exhibit potent antioxidant and anti-inflammatory properties [18]. Growing evidence indicates that various flavonoids confer both therapeutic and preventive effects against cisplatin-induced AKI, primarily through modulation of reactive oxygen species (ROS) and inflammatory pathways [16, 19, 20]. Taxifolin, a naturally occurring flavonoid present in numerous plants, also demonstrates notable pharmacological activities, including strong antioxidant and anti-inflammatory effects [21, 22]. This bioflavonoid has been applied in the treatment of inflammatory bowel disease [23]. In models of spinal cord injury, taxifolin has been shown to mitigate neuroinflammation and microglial pyroptosis via specific inhibition of the PI3K/Akt signaling pathway [24]. Additionally, taxifolin activates the Nrf2 pathway, thereby reducing oxidative stress and apoptotic activity, suggesting its potential as a treatment for glucocorticoid-induced femoral head osteonecrosis [25]. In a mouse model of nonalcoholic steatohepatitis (NASH), taxifolin treatment effectively attenuated hepatic steatosis, chronic inflammation, and extracellular matrix remodeling—effects mediated in part through direct actions on hepatocytes to suppress lipid accumulation [26]. However, the renal protective potential of taxifolin against cisplatin-induced nephrotoxicity has not yet been systematically evaluated.

In the present study, we demonstrated the protective role of taxifolin against cisplatin-induced nephrotoxicity, both in vitro and in vivo. Mechanistically, this protection was mediated by enhancing FAO through the inhibition of Fabp4 expression. Our findings uncover a novel pharmacological mechanism of taxifolin, suggesting its potential as a therapeutic candidate for cisplatin-induced nephroprotection.

2 | Materials and Methods

2.1 | Reagents

Taxifolin (T1738, 98.9% purity) and Cisplatin (T1564, 99.63% purity) were obtained from TargetMol.

2.2 | Cell Culture

The human renal proximal tubular cell line (HK-2) was obtained from ATCC and was cultured in RPMI-1640 medium (BC-M-017; Biochannel), added with 10% FBS (BS-1102; OPCEL) and 1% penicillin-streptomycin (BC-CE-007; Biochannel). HK-2 cells were maintained under a humidified incubator with 5% CO_2 at 37°C.

2.3 | Cytotoxicity Assay

The SRB cytotoxicity assay was utilized to assess taxifolin sensitivity in HK-2 cells under cisplatin challenge. HK-2 cells were plated in 96-well plates at 3000 cells/well and treated with 5 μM cisplatin plus taxifolin at escalating concentrations (0, 2.5, 5, 10, 20 μM) for 24 h.

The cells were immobilized by adding 10% trichloroacetic acid at 4°C for 4 h. Subsequently, the cells were treated with SRB stain for 30 min, followed by rinsing with 1% acetic acid and air-drying in an inverted orientation at 60°C. To ensure complete dye solubilization, 10 mM Tris-base solution was dispensed into each well until the dye achieved complete dissolution. The absorbances at 540 nm of each well were measured using a microplate reader (Multiskan FC; Thermo Fisher Scientific). The cell survival fraction was calculated by dividing the optical density of the treated cells by the optical density of the untreated cells.

2.4 | Murine Model of Cisplatin-Induced Nephrotoxicity

Six- to eight-week-old male C57BL/6 mice were obtained from the certified Laboratory Animal Center of Hangzhou Medical College. All experimental procedures were conducted in strict compliance with China's Animal Welfare Guidelines, as sanctioned by the Institutional Animal Care and Use Committee (IACUC) of Hangzhou Medical College (Registration Number: 2023-111, ethical approval date: 3/15/2023). Housing parameters for the mice included SPF-certified accommodation with a programmed 12-h diurnal rhythm, maintained at (24 ± 2)°C

and 55% ± 5% relative humidity. During the experimental period, subjects received a nutritionally complete diet with continuous access to provisions.

To investigate the renal preventive efficacy of taxifolin against cisplatin-induced AKI, mice were randomly allocated into four distinct experimental cohorts: control group (CON, $n = 6$); cisplatin group (Cis, $n = 6$); cisplatin-induced AKI mice treated with 50 mg/kg taxifolin group (Cis + TAX 50, $n = 6$); and cisplatin-induced AKI mice treated with 100 mg/kg taxifolin group (Cis + TAX 100, $n = 6$). Taxifolin (50 and 100 mg/kg) was administered by gavage for 5 consecutive days. On the third day, cisplatin 20 mg/kg was intraperitoneally injected once a day.

To explore the therapeutic efficacy of taxifolin in mitigating cisplatin-induced AKI, mice were randomly allocated into four distinct experimental cohorts: control group (CON, $n = 6$); cisplatin group (Cis, $n = 6$); cisplatin-induced AKI mice treated with 50 mg/kg taxifolin group (Cis + TAX 50, $n = 6$); and cisplatin-induced AKI mice treated with 100 mg/kg taxifolin group (Cis + TAX 100, $n = 6$). Two hours after intraperitoneal injection of cisplatin (20 mg/kg), taxifolin (50 and 100 mg/kg) was administered by gavage for 2 consecutive days.

The experimental endpoint was marked by the humane sacrifice of animals under anesthesia, achieved through intraperitoneal administration of 0.3% pentobarbital sodium.

Whole blood was harvested via cardiac puncture and processed into serum through centrifugation at 2000 rpm at 4°C for 15 min for subsequent biochemical analysis. Renal tissues were cryopreserved in liquid nitrogen to preserve molecular integrity for downstream gene and protein expression analysis or fixed in 4% paraformaldehyde for histological examination.

2.5 | Serum Biochemical Measurements

Serum biochemical markers, specifically blood urea nitrogen (BUN) and creatinine (Cre), were assessed using an automated analyzer (Cobas c311; Roche) following standardized blood collection protocols.

2.6 | Renal Histopathology

Tissue samples were formalin-fixed, paraffin-embedded, and sectioned into 5 μm slices for H&E staining to enable histological examination and renal injury scoring. Pathological evaluation revealed renal tubular dilatation, necrotic changes, and cast presence in the examined tissue sections. The renal tubular injury was assessed using the afflicted tubule proportion: 0 (no injury), 1 (< 25%), 2 (25%–50%), 3 (50%–75%), and 4 (> 75%).

2.7 | RNA Extraction, RT-qPCR, and RNA-Seq

Total RNA was isolated from biological samples using RNAex reagent (AG21101; AG) and an RNA extraction kit (AG21024; AG). Using the Evo M-MLV reverse transcription system, the RNA template was reverse-transcribed into cDNA under optimized reaction conditions (AG11728; AG).

Real-time quantitative PCR was carried out in a 10 μL reaction volume using the SYBR Green Pro Taq HS premixed Kit (AG11733; AG) on the CFX Connect Detection System (Bio-Rad). Gene expression was measured via the $2^{-\Delta\Delta C_T}$ method, with all data normalized to *Gapdh*. A portion of the kidney tissue was utilized for RNA-seq.

2.8 | Western Blot Analysis

Protein extraction was performed from renal cortical samples employing RIPA cracking buffer (containing 1% protease and phosphatase inhibitors). The protein extract was separated by 10% SDS-PAGE followed by electrophoretic transfer to a 0.45 μM PVDF membrane (C3117; Millipore). Membrane blocking was performed using 5% nonfat milk powder for 1 h at room temperature, followed by three washing cycles and overnight incubation with primary antibodies at 4°C. HPR-conjugated secondary antibodies were applied for 1 h at 25°C. Subsequently, the membranes were washed again and visualized using a chemiluminescence imaging system (FUSION FX; Vilber GmbH). The relative differences in expression levels were normalized to GAPDH levels.

2.9 | Bodipy Staining

Both HK-2 cell culture and frozen renal sections were subjected to fixation using 4% PFA for 15 min, followed by incubation with Bodipy (C2053S; Beyotime) for 20 min in the dark at 24°C. Following nuclear staining with DAPI (10 μg/mL) for 5 min, fluorescence images were captured using an inverted microscope (EVOS M7000; Invitrogen).

2.10 | Oil Red Staining

For Oil Red O staining, the kidney tissues were fixed in 4% paraformaldehyde overnight, subsequently dehydrated using a sucrose gradient, and then embedded in Tissue-Tek OCT compound (Sakura). Next, 10 μm sections were stained with Oil Red O (Cat No. C0157S; Beyotime) for 20 min. Following successive rinsing steps with distilled water, the samples were processed for counterstaining using hematoxylin to enhance nuclear visualization. Histological staining images were obtained using a microscope (EVOS M7000; Invitrogen).

2.11 | Statistical Analysis

Data were analyzed using GraphPad Prism software (v 9.5.1) and ImageJ (v1.8.0) and expressed as means ± SD. One-way ANOVA followed by Tukey's *post-hoc* test was applied to identify statistical significance. $p < 0.05$ indicated statistical significance. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus Ctrl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus Cis group.

3 | Results

3.1 | Taxifolin Alleviates Cisplatin-Induced Injury in HK-2 Cells

Renal tubule epithelial cells are highly vulnerable to AKI, making them a relevant model for in vitro studies. To evaluate the renal protective effects of taxifolin, we utilized the human proximal tubule cell line HK-2. Cell viability was assessed under cisplatin challenge with increasing concentrations of taxifolin (0–20 μM). Using SRB assays, we identified a concentration-dependent protective effect of taxifolin, with significant cytoprotection observed within this range (Figure 1A). Consequently, 20 μM taxifolin was selected for further experiments. Bright-field imaging confirmed that taxifolin markedly attenuated cisplatin-induced morphological damage in HK-2 cells (Figure 1B). To elucidate the molecular mechanisms underlying taxifolin's protective effects, we analyzed the transcription of two well-established renal injury biomarkers: kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL). Quantitative RT-qPCR showed that taxifolin treatment significantly reduced the mRNA levels of both *KIM-1* and *NGAL* in cisplatin-treated HK-2 cells (Figure 1C,D). Furthermore, taxifolin reduced apoptosis in HK-2 cells, as evidenced by decreased levels of cleaved PARP (C-PARP) (Figure 1E,F). Together, these results demonstrate that taxifolin attenuates cisplatin-induced nephrotoxicity in HK-2 cells by improving viability, reducing injury biomarkers, and inhibiting apoptosis, suggesting its potential as a therapeutic agent for cisplatin-associated AKI.

3.2 | Taxifolin Pretreatment Prevents Cisplatin-Induced AKI *In Vivo*

To evaluate the protective effect of taxifolin against cisplatin-induced AKI in vivo, mice were pretreated with oral taxifolin (50 or 100 mg/kg) for 5 days. On Day 3, a single intraperitoneal injection of cisplatin (20 mg/kg) was administered (Figure 2A). After 72 h, cisplatin-treated mice developed significant AKI, as indicated by marked elevated levels of BUN and serum creatinine (Cre), consistent with impaired renal function. Importantly, taxifolin pretreatment significantly mitigated this renal dysfunction (Figure 2B,C). Histopathological examination further revealed that taxifolin markedly reduced cisplatin-induced kidney tissue damage (Figure 2D,E). At the molecular level, taxifolin also suppressed the cisplatin-mediated upregulation of injury biomarkers *KIM-1* and *NGAL* at the mRNA level (Figure 2F,G). These findings indicate that taxifolin pretreatment may preserve renal function by suppressing cisplatin-induced nephrotoxic pathways.

3.3 | Taxifolin Posttreatment Ameliorates Cisplatin-Provoked AKI *In Vivo*

To further evaluate the therapeutic potential of taxifolin, we investigated whether postexposure administration could ameliorate cisplatin-induced AKI. Taxifolin (50 or 100 mg/kg) was administered intraperitoneally at 2, 24, and 48 h after cisplatin challenge (Figure 3A). Taxifolin administration resulted in marked attenuation of serum BUN and Cre elevation in

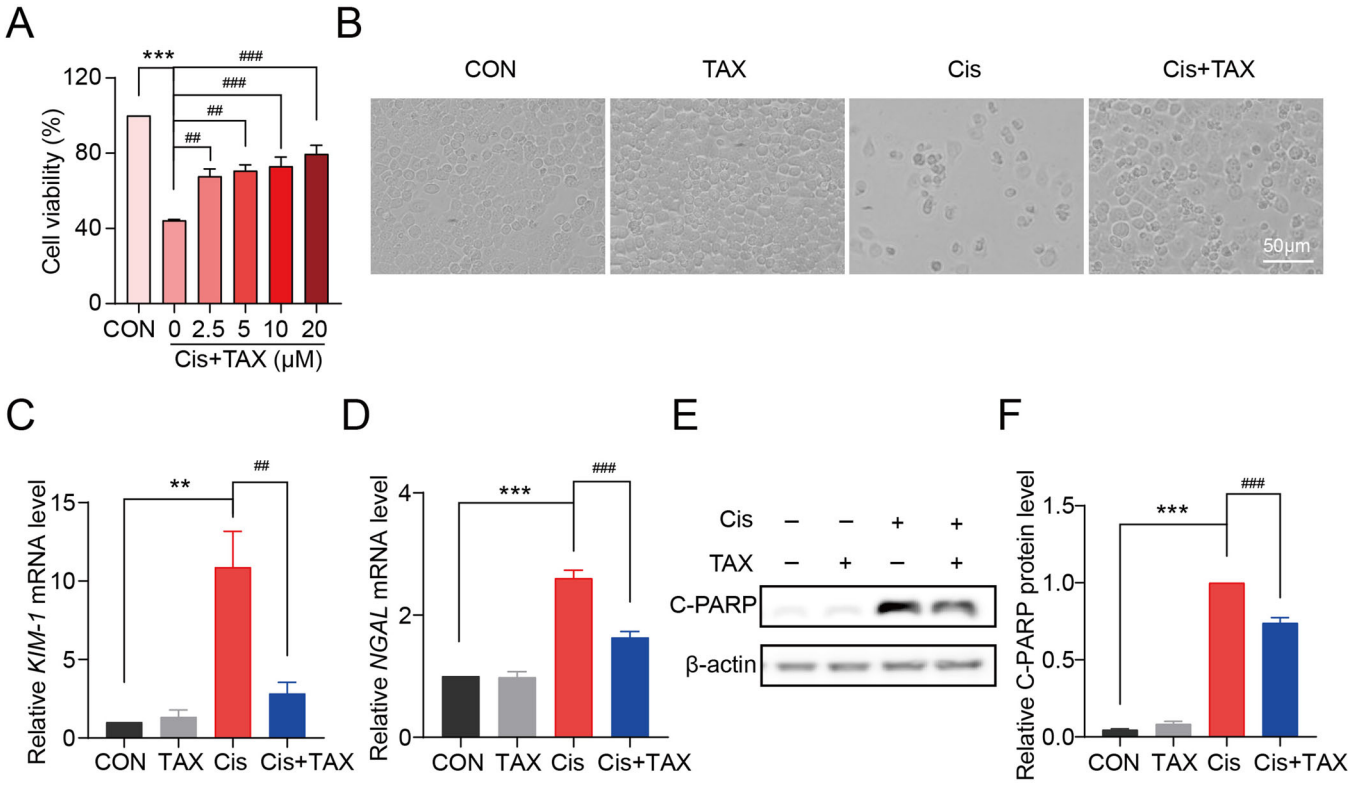

FIGURE 1 | Taxifolin reduced cisplatin-induced kidney injury in HK-2 cells. (A) Effect of taxifolin (0–20 μM) on HK-2 cells survival under cisplatin (5 μM) challenge. (B) HK-2 cell morphology was visualized using bright-field microscopy (scale bar = 50 μm). (C,D) RT-qPCR was employed to quantify *KIM-1* and *NGAL* mRNA expression, normalized to *Gapdh*. (E) Western blot analysis was performed to assess C-PARP protein levels, with β-actin serving as the loading control. (F) Quantification analysis of protein levels of C-PARP.

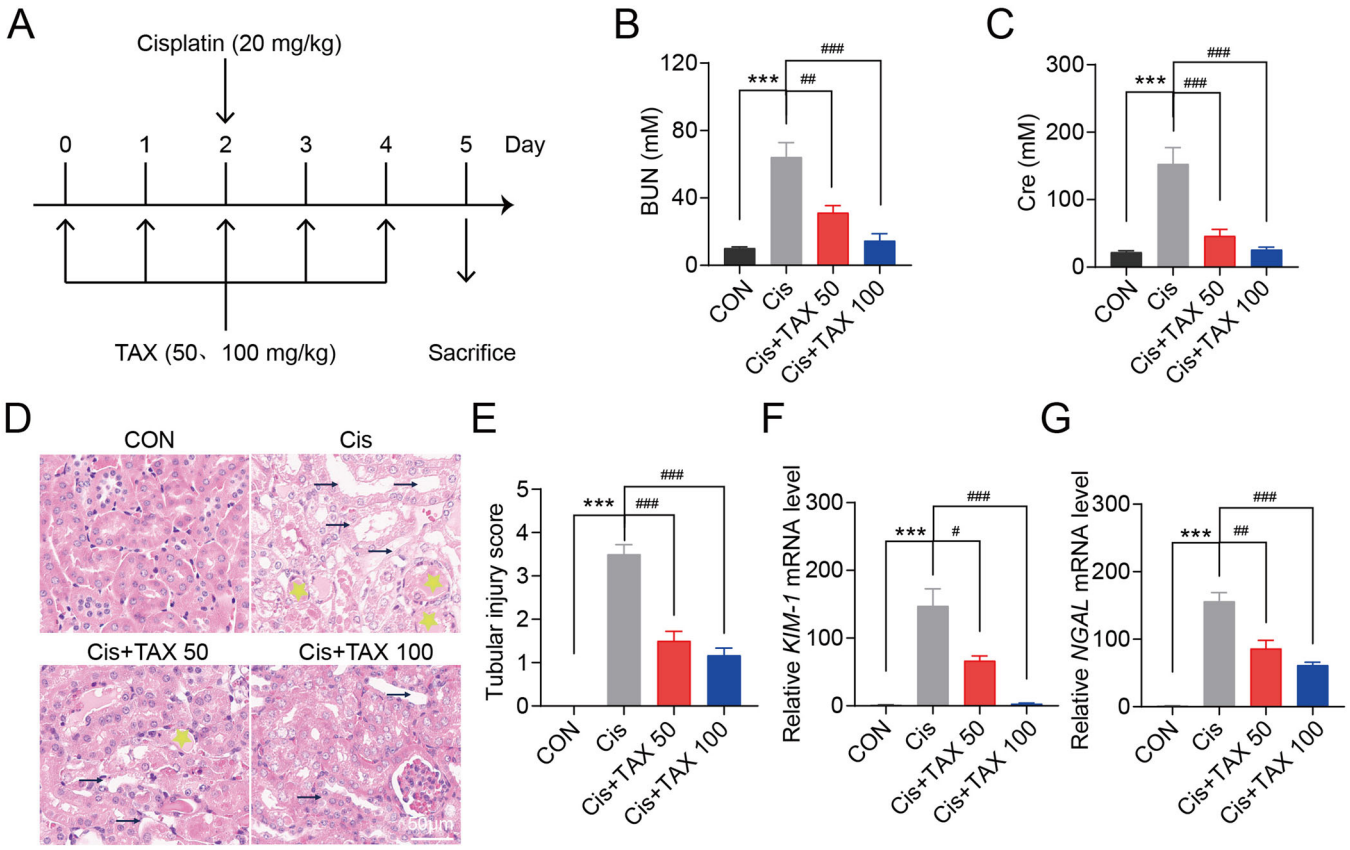

FIGURE 2 | Taxifolin prevented cisplatin-induced nephrotoxicity in vivo. (A) A diagrammatic depiction of administering taxifolin to prevent cisplatin-induced AKI. (B,C) Serum BUN and Cre levels were detected after 72 h cisplatin injection. (D) Representative images stained with hematoxylin and eosin. Arrows indicated tubular dilatation and necrosis, and asterisks indicated cast formation (scale bar = 50 μm). (E) Tubular injury score analysis. (F,G) RT-qPCR was used to detect *KIM-1* and *NGAL* mRNA expression in mouse renal samples, normalized to *Gapdh*. $n = 6$ animals per group.

cisplatin-treated mice (Figure 3B,C). H&E staining revealed that cisplatin exposure resulted in distinct tubular pathology, specifically tubular epithelial necrosis, luminal dilatation, and protein cast deposition (Figure 3D). Notably, taxifolin intervention significantly ameliorated renal histopathology (Figure 3E). The results of RT-qPCR suggested reduced *KIM-1* and *NGAL* gene expression posttreatment (Figure 3F,G). Thus, these data support the translational potential of taxifolin as a nephroprotective agent in cisplatin-induced AKI.

3.4 | Taxifolin Treatment Reduces Apoptosis and Inflammatory Response in Mouse Kidneys

Cisplatin-induced nephrotoxicity was closely associated with inflammatory activation and apoptotic cell death. To investigate whether taxifolin attenuates these processes in a mouse model of cisplatin-induced AKI, we evaluated the protein expression of the injury marker KIM-1 and the apoptotic marker C-PARP by Western blot. Cisplatin treatment significantly elevated both KIM-1 and C-PARP levels, which were markedly reduced by taxifolin administration (Figure 4A–C). We further examined the effect of taxifolin on the transcription of key inflammatory cytokines—*TNF-α*, *Il1b*, and *Il6*—using RT-qPCR. Taxifolin treatment significantly suppressed the mRNA expression of these pro-inflammatory factors in the kidneys of cisplatin-exposed mice (Figure 4D–F). Together, these results demonstrate that taxifolin effectively mitigates cisplatin-induced renal injury by attenuating both apoptosis and inflammation, highlighting its dual protective role in AKI.

3.5 | Transcriptomic Analysis of Taxifolin Function in Cisplatin-Provoked AKI Kidneys

To elucidate the molecular mechanisms through which taxifolin exerts its renoprotective effects in cisplatin-induced AKI, we performed RNA-seq analysis on renal tissues from taxifolin-treated and untreated mice. Gene Ontology (GO) enrichment analysis of the differentially expressed genes highlighted a pronounced enrichment in biological processes associated with lipid homeostasis, including fatty acid metabolism and lipid catabolism (Figure 5A). Thermal profiling of fatty acid metabolic genes identified Fabp4 as a significantly downregulated target following taxifolin treatment, implicating this adipogenic regulator in the compound's nephroprotective mechanism (Figure 5B). Cross-validation using RT-qPCR and Western blot analysis confirmed the heatmap findings, demonstrating reduced Fabp4 expression at both transcriptional and translational levels (Figure 5C–E). These findings suggest that taxifolin

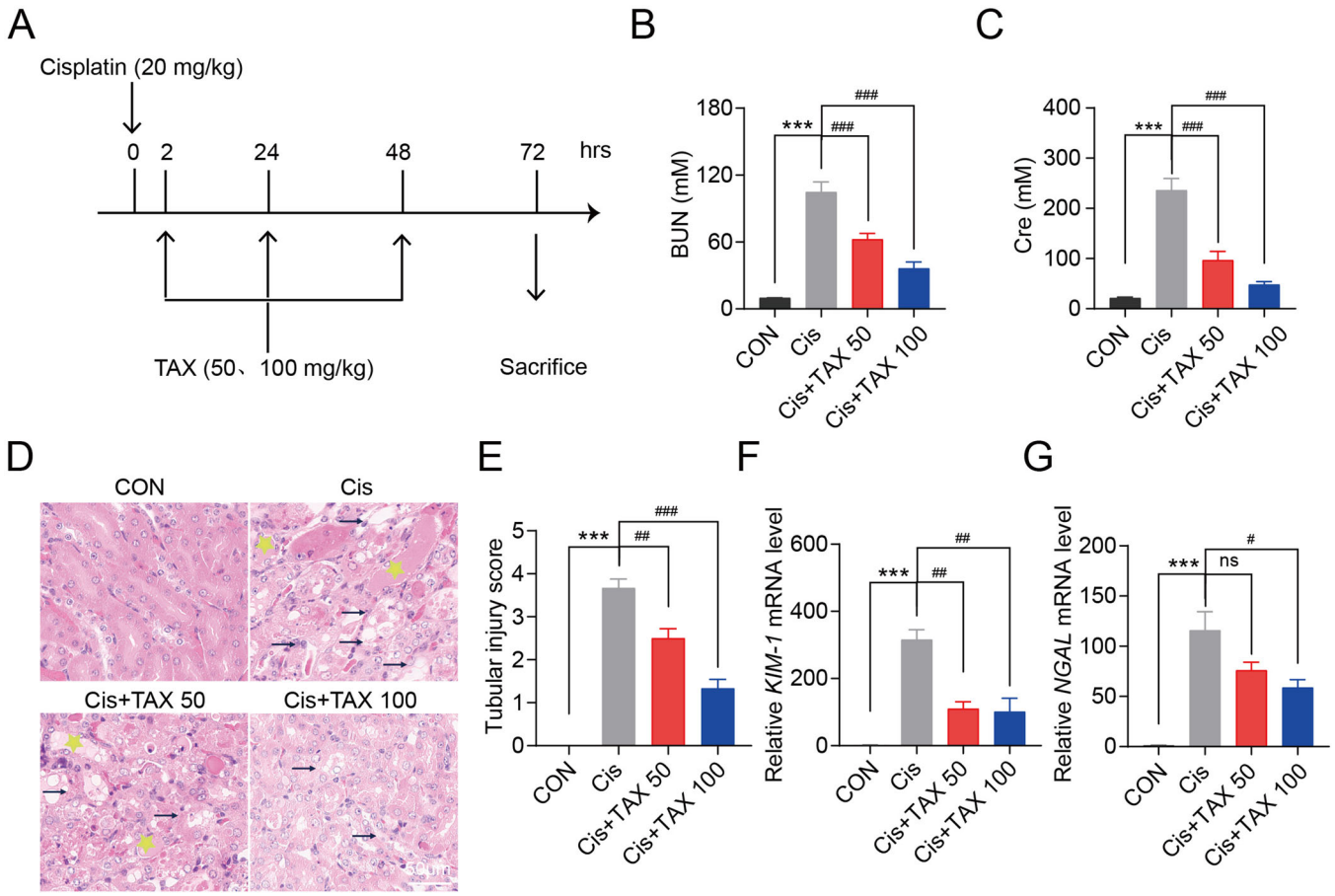

FIGURE 3 | Taxifolin attenuated cisplatin-induced AKI in mice. (A) A diagrammatic depiction of administering taxifolin to attenuate cisplatin-induced AKI. (B,C) BUN and Cre levels in serum were detected 72 h after cisplatin injection. (D) Representative images stained with H&E. Arrows indicated tubular dilatation and necrosis, and asterisks indicated cast formation (scale bar = 50 μm). (E) Tubular injury score analysis. (F,G) RT-qPCR was used to detect KIM-1 and NGAL mRNA expression in mouse renal samples, normalized to *Gapdh*. $n = 6$ animals per group.

may mitigate cisplatin-induced renal injury, at least in part, through modulation of lipid metabolic pathways, with Fabp4 serving as a critical regulatory node.

3.6 | Taxifolin Attenuates Cisplatin-Induced Impairment of FAO and Energy Deficiency

Previous studies have established that impaired FAO contributes to the pathogenesis of AKI, promoting aberrant lipid accumulation and subsequent tubular cell apoptosis. To investigate whether taxifolin modulates FAO in cisplatin-induced AKI, we quantitatively assessed intracellular lipid deposition. Oil red O staining revealed a substantial increase in neutral lipids following cisplatin exposure, which was significantly attenuated by taxifolin treatment (Figure 6A). Complementary lipid visualization using both Oil red O and Bodipy fluorescent dye confirmed pronounced lipid accumulation after cisplatin injury and consistently demonstrated that taxifolin effectively reduced lipid deposition across both detection methods (Figure 6B). We further explored the molecular mechanisms underlying this effect by examining key transcriptional regulators of lipid metabolism. PGC-1α and PPARα are known to synergistically promote FAO and cellular energy production. Western blot analysis showed that cisplatin downregulated both PGC-1α and PPARα protein expression, whereas taxifolin treatment markedly restored their levels (Figure 6C). Consistent with these in vivo findings, taxifolin also counteracted cisplatin-induced lipid metabolic dysregulation in renal tubular epithelial (HK-2) cells. It suppressed Fabp4 upregulation and restored ATP production (Figure 6D–F). Additionally, cisplatin treatment led to diminished PGC-1α and PPARα protein levels in HK-2 cells, whereas taxifolin administration resulted in their marked upregulation (Figure 6G). Together, these results indicate that taxifolin mitigates cisplatin-induced impairment of FAO by restoring the expression of central metabolic regulators and reducing lipid accumulation, thereby supporting renal tubular cell energy homeostasis.

4 | Discussion

Patients receiving cisplatin-based chemotherapy frequently develop AKI, a serious side effect for which effective clinical interventions remain limited despite ongoing research efforts [7]. In this study, we demonstrated through both in vitro and in vivo models that taxifolin confers significant protection against cisplatin-induced nephrotoxicity. RNA sequencing

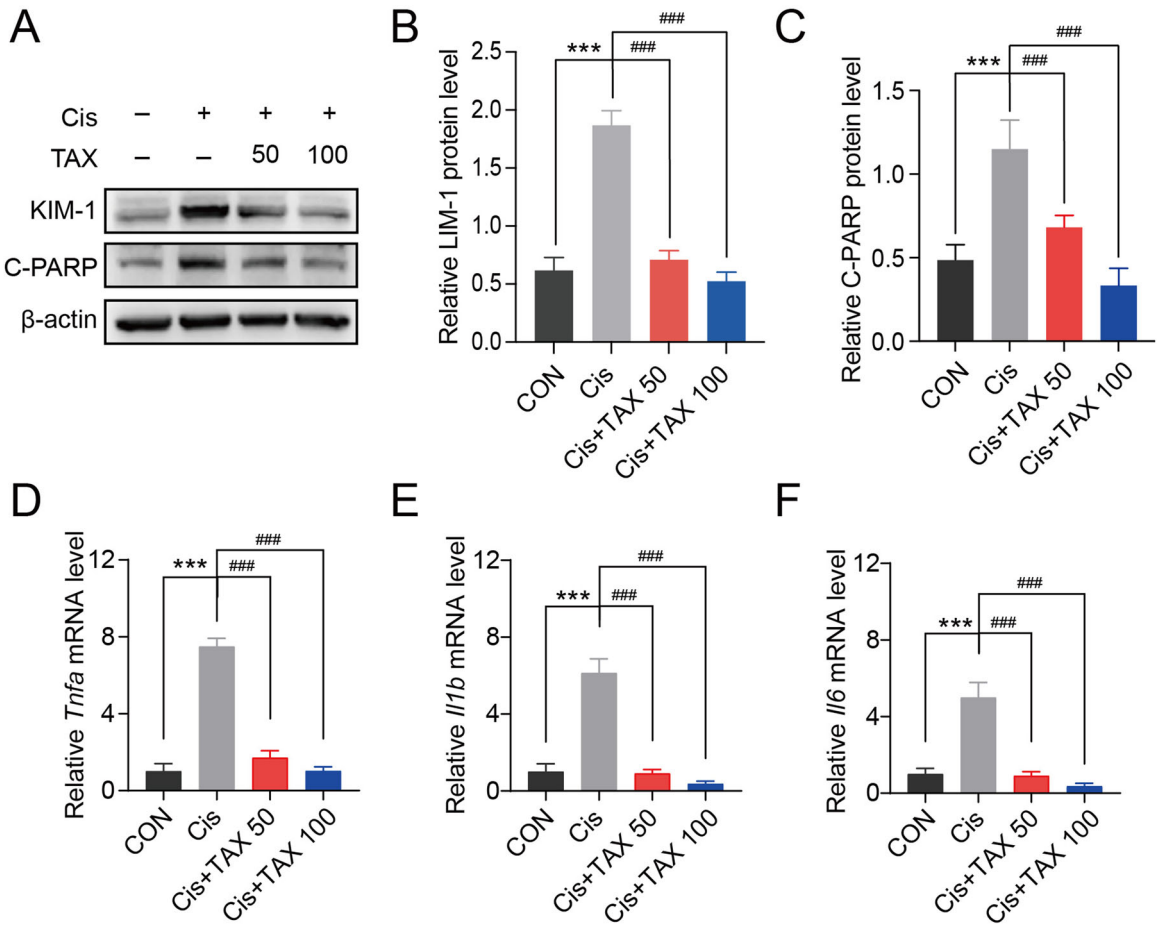

FIGURE 4 | Taxifolin inhibited apoptotic pathway and inflammation in the kidney tissues of mice administrated with cisplatin. (A) Representative Western blot analysis of KIM-1 and C-PARP protein levels in mouse kidney tissues. β-actin was used as the loading control. (B,C) Quantification analysis of protein levels of KIM-1 and C-PARP. (D–F) RT-qPCR was employed to detect mRNA levels of TNF-α, IL-1β, and IL-6, normalized to *Gapdh*. $n = 6$ animals per group.

analysis revealed that this protective mechanism likely involves the restoration of disrupted FAO. Specifically, we observed a marked upregulation of Fabp4—a key negative regulator of FAO—in cisplatin-treated mice and HK-2 cells. Taxifolin treatment effectively suppressed Fabp4 expression, resulting in enhanced FAO and concomitant reduction in renal injury in both experimental systems. These results underscore the potential of taxifolin as a promising therapeutic agent for the prevention or treatment of cisplatin-induced AKI.

Emerging evidence highlights the therapeutic promise of phytochemicals in managing cisplatin-induced nephrotoxicity, owing to their multitarget bioactive profiles and low propensity for eliciting drug resistance [19]. Taxifolin, a natural flavonoid with demonstrated anti-inflammatory and antioxidant activities, has been shown to protect against various forms of tissue injury [27–29]. However, its efficacy in alleviating cisplatin-induced AKI remained unclear. In this study, taxifolin significantly suppressed cisplatin-induced cytotoxicity, loss of membrane integrity, apoptotic signaling, and inflammatory activation in HK-2 renal tubular cells. Unlike conventional clinical agents that are typically administered after the onset of kidney injury, taxifolin demonstrated protective effects in both preventive and therapeutic settings. Pretreatment with taxifolin markedly improved renal function and reduced histopathological damage in cisplatin-challenged mice. Moreover, even when administered after cisplatin exposure, taxifolin significantly attenuated AKI-related alterations. These findings strongly support the clinical potential of taxifolin as a complementary agent for preventing and treating cisplatin-associated renal injury.

Dysregulation of fatty acid metabolism has been increasingly recognized as a key contributor to the pathogenesis of various kidney diseases [30–32]. Renal PTECs—the primary site of injury in cisplatin-induced AKI—depend heavily on FAO to meet their high energy demands [10]. Cisplatin impairs FAO, resulting in cellular energy deficit and lipotoxicity, which in turn drive a cascade of pathological events including reduced ATP production, aberrant triglyceride accumulation, inflammatory activation, and tubular cell damage [10, 16, 33]. Pharmacological enhancement of FAO has previously been shown to confer renal protection in both AKI and chronic kidney disease (CKD) models [11, 12], suggesting that restoring FAO homeostasis could represent a promising therapeutic strategy for cisplatin-induced AKI. In line with this, GO analysis indicated that the differentially expressed genes in our data set were predominantly enriched in processes such as fatty acid

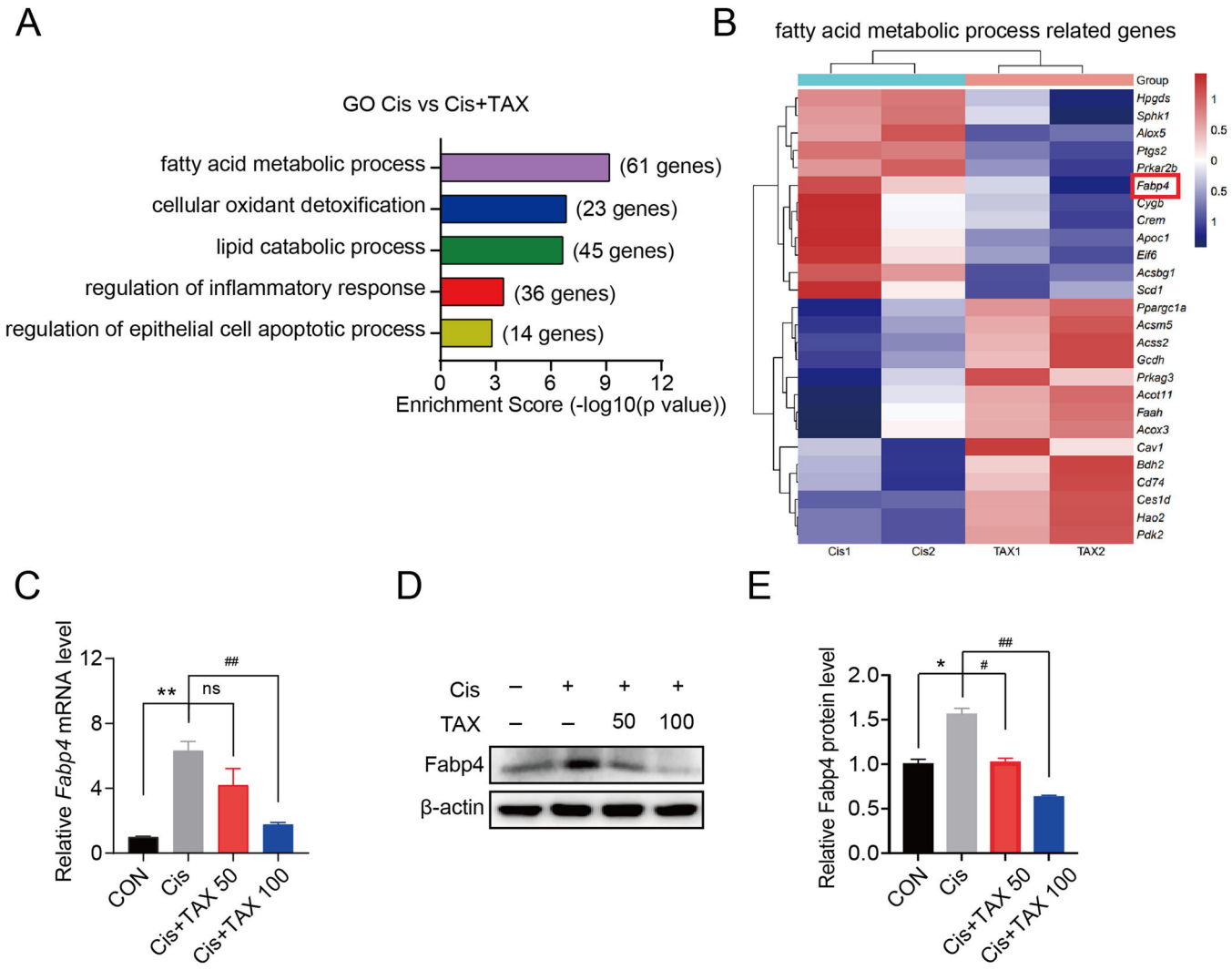

FIGURE 5 | RNA-seq analysis of the underlying regulatory networks mediating taxifolin's renoprotective effects in cisplatin nephropathy. (A) GO enrichment analysis. (B) Heatmap of genes enriched in lipid metabolic networks. (C) The mRNA expression of Fabp4 in mouse renal tissues were detected by RT-qPCR, normalized to *Gapdh*. (D,E) The protein levels of Fabp4 were determined by Western blot analysis, with subsequent quantification via ImageJ analysis. β-actin served as the normalization standard. $n = 6$ animals per group.

metabolism, cellular oxidant detoxification, and lipid catabolism, underscoring the central role of lipid metabolic pathways in taxifolin-mediated protection. Furthermore, we demonstrated that taxifolin treatment effectively attenuated cisplatin-induced lipid deposition, ATP depletion, and apoptotic cell death. These results suggest that taxifolin mitigates cisplatin nephrotoxicity at least in part through the restoration of FAO.

Fabp4 is well-established as a key mediator in the pathogenesis of metabolic diseases such as atherosclerosis and diabetes [34–36]. More recently, it has also been implicated in inflammatory responses and cell death across a range of pathological conditions [37, 38]. Growing evidence suggests that Fabp4 contributes to multiple mechanisms of renal injury. For example, Fabp4 expression is markedly upregulated in doxorubicin-induced nephrotoxicity, where it is associated with disrupted lipid metabolism and activation of apoptotic pathways [39]. Fabp4 levels in the kidney are closely linked to lipid homeostasis [14], and its elevation during kidney injury may exacerbate lipid metabolic disorders, thereby amplifying renal damage [40]. Additionally, Fabp4 appears to promote renal cell apoptosis, as studies have shown a positive correlation between Fabp4 expression and apoptosis rates in kidney injury models [41]. In this study, RNA-sequencing analysis suggested that Fabp4 may be involved in taxifolin-mediated regulation of FAO. We further confirmed that taxifolin significantly downregulated Fabp4 protein expression, while upregulating key transcriptional regulators of fatty acid metabolism, PGC-1α and PPARα, which are known to enhance FAO and cellular energy production.

5 | Conclusions

In summary, our data demonstrated that taxifolin effectively reduces cisplatin-provoked AKI via decreasing the levels of Fabp4, thereby correcting the abnormal FAO. These findings underscore the promising efficacy of taxifolin as a natural therapeutic intervention for managing cisplatin-related kidney injury in clinical settings.

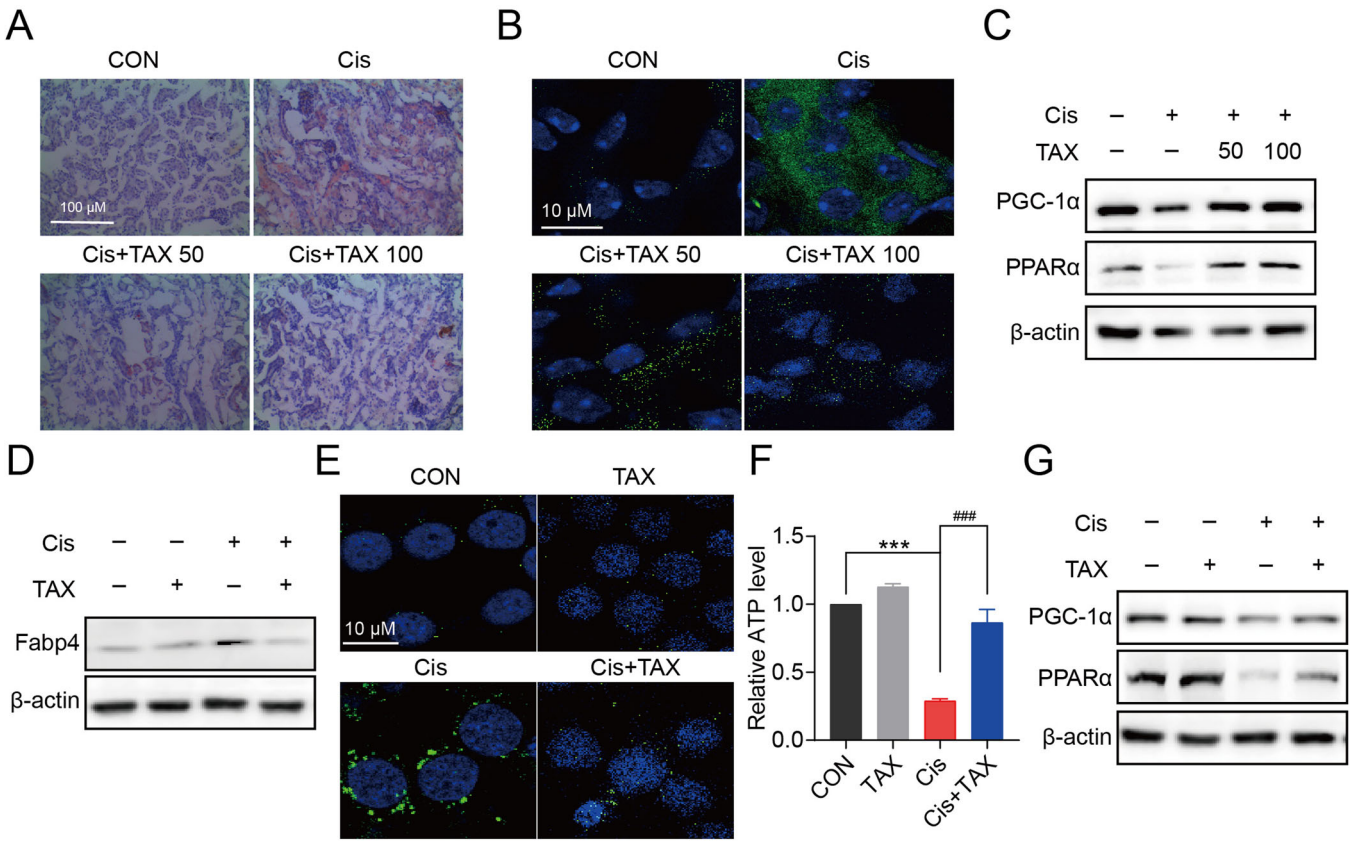

FIGURE 6 | Taxifolin counteracts cisplatin-induced FAO dysregulation and ATP depletion (A) Intrarenal lipid deposition was evaluated using Oil red O staining. (scale bar = 100 μm). (B) Bodipy staining in mouse kidneys (scale bar = 10 μm). (C) Protein expression of PGC-1α and PPARα in renal tissues. β-actin served as the normalization standard. (D) Western blot analysis of Fabp4 protein levels in HK-2 cells. (E) Bodipy analysis in HK-2 cells (scale bar = 10 μm). (F) Using the ATP colorimetric assay to determine the content of intracellular adenosine triphosphate. (G) Protein expression of PGC-1α and PPARα in HK-2 cells. β-actin served as the normalization standard.

Acknowledgments

The study was supported by the General Scientific Research Project of Department of Education of Zhejiang Province (Y202456747 to Qian Zhou).

Data Availability Statement

The data of this study are available from the corresponding author upon reasonable request.

References

1. R. Matsuura, K. Doi, and H. Rabb, "Acute Kidney Injury and Distant Organ Dysfunction–Network System Analysis," *Kidney International* 103 (2023): 1041–1055.

2. J. A. Kellum, P. Romagnani, G. Ashuntantang, C. Ronco, A. Zarbock, and H. J. Anders, "Acute Kidney Injury," *Nature Reviews Disease Primers* 7 (2021): 52.

3. D. H. Kim, H. I. Choi, J. S. Park, et al., "Farnesoid X Receptor Protects Against Cisplatin-Induced Acute Kidney Injury by Regulating the Transcription of Ferroptosis-Related Genes," *Redox Biology* 54 (2022): 102382.

4. L. M. Chambers, E. L. Esakov Rhoades, R. Bharti, et al., "Disruption of the Gut Microbiota Confers Cisplatin Resistance in Epithelial Ovarian Cancer," *Cancer Research* 82 (2022): 4654–4669.

5. J. Niu, T. Yan, W. Guo, et al., "The COPS3-FOXO3 Positive Feedback Loop Regulates Autophagy to Promote Cisplatin Resistance in Osteosarcoma," *Autophagy* 19 (2023): 1693–1710.

6. M. C. S. Mancini, A. P. Morelli, M. B. Severino, et al., "Knockout of NRF2 Triggers Prostate Cancer Cells Death Through ROS Modulation and Sensitizes to Cisplatin," *Journal of Cellular Biochemistry* 123 (2022): 2079–2092.

7. C. Tang, M. J. Livingston, R. Safirstein, and Z. Dong, "Cisplatin Nephrotoxicity: New Insights and Therapeutic Implications," *Nature Reviews Nephrology* 19 (2023): 53–72.

8. S. Li, M. J. Livingston, Z. Ma, et al., "Tubular Cell Senescence Promotes Maladaptive Kidney Repair and Chronic Kidney Disease After Cisplatin Nephrotoxicity," *JCI Insight* 8 (2023): e166643.

9. J. Zhang, Z. W. Ye, K. D. Tew, and D. M. Townsend, "Cisplatin Chemotherapy and Renal Function," *Advances in Cancer Research* 152 (2021): 305–327.

10. S. Xu, P. Jia, Y. Fang, et al., "Nuclear Farnesoid X Receptor Attenuates Acute Kidney Injury Through Fatty Acid Oxidation," *Kidney International* 101 (2022): 987–1002.

11. M. Gu, M. Tan, L. Zhou, et al., "Protein Phosphatase 2Acα Modulates Fatty Acid Oxidation and Glycolysis to Determine Tubular Cell Fate and Kidney Injury," *Kidney International* 102 (2022): 321–336.

12. S. E. Piret, A. A. Attallah, X. Gu, et al., "Loss of Proximal Tubular Transcription Factor Krüppel-Like Factor 15 Exacerbates Kidney Injury Through Loss of Fatty Acid Oxidation," *Kidney International* 100 (2021): 1250–1267.

13. B. Wang, J. Xu, Q. Ren, et al., "Fatty Acid-Binding Protein 4 Is a Therapeutic Target for Septic Acute Kidney Injury by Regulating Inflammatory Response and Cell Apoptosis," *Cell Death & Disease* 13 (2022): 333.

14. Y. Chen, Y. Dai, K. Song, et al., "Pre-Emptive Pharmacological Inhibition of Fatty Acid–Binding Protein 4 Attenuates Kidney Fibrosis by Reprogramming Tubular Lipid Metabolism," *Cell Death & Disease* 12 (2021): 572.

15. L. Liu, X. Ning, L. Wei, et al., "Twist1 Downregulation of PGC-1α Decreases Fatty Acid Oxidation in Tubular Epithelial Cells, Leading to Kidney Fibrosis: Erratum," *Theranostics* 13 (2023): 1419–1420.

16. L. Wang, Y. Xie, B. Xiao, et al., "Isorhamnetin Alleviates Cisplatin-Induced Acute Kidney Injury via Enhancing Fatty Acid Oxidation," *Free Radical Biology and Medicine* 212 (2024): 22–33.

17. Y. Hou, E. Tan, H. Shi, et al., "Mitochondrial Oxidative Damage Reprograms Lipid Metabolism of Renal Tubular Epithelial Cells in the Diabetic Kidney," *Cellular and Molecular Life Sciences* 81 (2024): 23.

18. M. D. M. Chagas, C. J. Behrens, G. X. M. Moragas-Tellis, A. R. Penedo, C. F. Silva, and C. Gonçalves-de-Albuquerque, "Flavonols and Flavones as Potential Anti-Inflammatory, Antioxidant, and Antibacterial Compounds," *Oxidative Medicine and Cellular Longevity* 2022 (2022): 9966750.

19. C. Fang, D. Lou, L. Zhou, et al., "Natural Products: Potential Treatments for Cisplatin-Induced Nephrotoxicity," *Acta Pharmacologica Sinica* 42 (2021): 1951–1969.

20. Y. Shao, B. Tang, Y. Ding, et al., "Kaempferide Ameliorates Cisplatin-Induced Nephrotoxicity via Inhibiting Oxidative Stress and Inducing Autophagy," *Acta Pharmacologica Sinica* 44 (2023): 1442–1454.

21. L. Xu, L. Zhang, S. Zhang, et al., "Taxifolin Inhibits Melanoma Proliferation/Migration Impeding USP18/Rac1/JNK/β-Catenin Oncogenic Signaling," *Phytomedicine* 123 (2024): 155199.

22. M. Iwasa, H. Kato, K. Iwashita, et al., "Taxifolin Suppresses Inflammatory Responses of High-Glucose-Stimulated Mouse Microglia by Attenuating the TXNIP–NLRP3 Axis," *Nutrients* 15 (2023): 2738.

23. W. Li, L. Zhang, Q. Xu, et al., "Taxifolin Alleviates DSS-Induced Ulcerative Colitis by Acting on Gut Microbiome to Produce Butyric Acid," *Nutrients* 14 (2022): 1069.

24. Z. Hu, L. Xuan, T. Wu, et al., "Taxifolin Attenuates Neuroinflammation and Microglial Pyroptosis via the PI3K/Akt Signaling Pathway After Spinal Cord Injury," *International Immunopharmacology* 114 (2023): 109616.

25. H. Jiang, C. Lin, T. Cai, et al., "Taxifolin-Mediated Nrf2 Activation Ameliorates Oxidative Stress and Apoptosis for the Treatment of Glucocorticoid-Induced Osteonecrosis of the Femoral Head," *Phytotherapy Research* 38 (2024): 156–173.

26. T. Inoue, B. Fu, M. Nishio, et al., "Novel Therapeutic Potentials of Taxifolin for Obesity-Induced Hepatic Steatosis, Fibrogenesis, and Tumorigenesis," *Nutrients* 15 (2023): 350.

27. S. Yuceli, B. Suleyman, G. N. Yazici, et al., "Effect of Taxifolin on Ischemia/Reperfusion-Induced Oxidative Injury of Sciatic Nerve in Rats," *Transplantation Proceedings* 53 (2021): 3087–3092.

28. J. Islam, A. Shree, A. Vafa, S. M. Afzal, and S. Sultana, "Taxifolin Ameliorates Benzo[A]Pyrene-Induced Lung Injury Possibly via Stimulating the Nrf2 Signalling Pathway," *International Immunopharmacology* 96 (2021): 107566.

29. N. Akbaş, B. Suleyman, R. Mammadov, G. N. Yazıcı, S. Bulut, and H. Süleyman, "Effect of Taxifolin on Cyclophosphamide-Induced Oxidative and Inflammatory Bladder Injury in Rats," *Experimental Animals* 71 (2022): 460–467.

30. A. Mitrofanova, S. Merscher, and A. Fornoni, "Kidney Lipid Dysmetabolism and Lipid Droplet Accumulation in Chronic Kidney Disease," *Nature Reviews Nephrology* 19 (2023): 629–645.

31. L. Zhou, Y. Luo, Y. Liu, et al., "Fatty Acid Oxidation Mediated by Malonyl-CoA Decarboxylase Represses Renal Cell Carcinoma Progression," *Cancer Research* 83 (2023): 3920–3939.

32. Y. Mori, A. K. Ajay, J. H. Chang, et al., "KIM-1 Mediates Fatty Acid Uptake by Renal Tubular Cells to Promote Progressive Diabetic Kidney Disease," *Cell Metabolism* 33 (2021): 1042–1061.

33. M. Li, C. Li, Z. Ye, et al., "Sirt3 Modulates Fatty Acid Oxidation and Attenuates Cisplatin-Induced AKI in Mice," *Journal of Cellular and Molecular Medicine* 24 (2020): 5109–5121.

34. J. Lv, Y. Hu, L. Li, et al., "Targeting FABP4 in Elderly Mice Rejuvenates Liver Metabolism and Ameliorates Aging-Associated Metabolic Disorders," *Metabolism: Clinical and Experimental* 142 (2023): 155528.

35. M. Zhang, L. Hou, W. Tang, et al., "Oridonin Attenuates Atherosclerosis by Inhibiting Foam Macrophage Formation and Inflammation Through FABP4/PPARγ Signalling," *Journal of Cellular and Molecular Medicine* 27 (2023): 4155–4170.

36. X. Wu, L. Y. Cheong, L. Yuan, et al., "Islet-Resident Memory T Cells Orchestrate the Immunopathogenesis of Type 1 Diabetes Through the FABP4-CXCL10 Axis," *Advanced Science* 11 (2024): e2308461.

37. Z. Ma, A. Y. W. Lee, C. H. Kot, P. S. H. Yung, S. Chen, and P. P. Y. Lui, "Upregulation of FABP4 Induced Inflammation in the Pathogenesis of Chronic Tendinopathy," *Journal of Orthopaedic Translation* 47 (2024): 105–115.

38. Y. Gao, Y. Wang, X. Wang, et al., "FABP4 Regulates Cell Proliferation, Stemness, Apoptosis, and Glycolysis in Colorectal Cancer via Modulating ROS/ERK/mTOR Pathway," *Discovery Medicine* 35 (2023): 361–371.

39. Y. Huang, A. V. Hertzel, S. R. Fish, et al., "TP53/p53 Facilitates Stress-Induced Exosome and Protein Secretion by Adipocytes," *Diabetes* 72 (2023): 1560–1573.

40. W. Lai, M. Shi, R. Huang, P. Fu, and L. Ma, "Fatty Acid-Binding Protein 4 in Kidney Diseases: From Mechanisms to Clinics," *European Journal of Pharmacology* 931 (2022): 175224.

41. J. Liu, R. Huang, X. Li, et al., "Genetic Inhibition of FABP4 Attenuated Endoplasmic Reticulum Stress and Mitochondrial Dysfunction in Rhabdomyolysis-Induced Acute Kidney Injury," *Life Sciences* 268 (2021): 119023.