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Review Article

EXPERIMENTAL NEPHROTOXICITY MODELS FOR SCREENING NEPHROPROTECTIVE AGENTS: COMPARATIVE EVALUATION, BIOMARKERS, TRANSLATIONAL RELEVANCE, AND EMERGING ALTERNATIVES

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ABSTRACT

Background: Nephrotoxicity is a significant contributor to both acute and long-term renal damage, and it continues to be a significant obstacle to the creation of successful nephroprotective treatments. The translation of experimental nephrotoxicity models to human disease may be limited by variations in pathophysiology, biomarkers, pharmacokinetics, and species responses. However, these models are frequently employed to study mechanisms of renal injury and assess possible nephroprotective drugs. Objective: This review aimed to critically evaluate established experimental nephrotoxicity models used for screening nephroprotective agents, compare their pathological and translational relevance, evaluate conventional and emerging biomarkers of renal injury, and assess advanced human-relevant alternatives for improving the predictive value of preclinical nephrotoxicity studies. Methods: Using PubMed/MEDLINE, Scopus, Web of Science, and Embase, a systematic literature search was carried out in accordance with PRISMA 2020 standards. Peer-reviewed English-language research released between 2000 and 2026 was taken into account. Renal damage biomarkers, nephroprotective treatments, experimental nephrotoxicity models, and new nephrotoxicity evaluation platforms were all assessed. A qualitative synthesis was made of pertinent data on nephrotoxicants, model features, mechanisms of injury, biomarkers, histopathological results, and nephroprotective effects. Animal studies were methodologically evaluated using the SYRCLE Risk of Bias tool. Drug-induced, chemical-induced, disease-based, surgical, and advanced in vitro platforms were the categories into which the models were divided. Results: Drug-induced models, particularly cisplatin- and gentamicin-induced nephrotoxicity, provide reproducible systems for investigating acute tubular injury and screening nephroprotective compounds, whereas disease-based models such as diabetic and hypertensive nephropathy better represent chronic and progressive renal injury. Although surgical models offer valuable methods for examining ischemia and obstructive renal injury, they may have drawbacks in terms of clinical extrapolation and technical complexity. While kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), cystatin-C, clusterin, osteopontin, microRNAs, proteomic, and metabolomic markers offer complementary information regarding structural, functional, and molecular changes, conventional biomarkers like serum creatinine and blood urea nitrogen have limited sensitivity for early renal injury. Conclusion: Although no single model can accurately replicate the complexities of human kidney disease, experimental nephrotoxicity models are crucial for examining renal damage pathways and evaluating nephroprotective medicines.

Key words: Nephrotoxicity, Nephroprotective Agents, experimental nephrotoxicity models, chronic renal injury.

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1. INTRODUCTION

Kidney diseases are a significant global health concern and are becoming more widely acknowledged as significant global contributors to morbidity, mortality, and healthcare costs. Collectively, drug-induced nephrotoxicity, chronic kidney disease (CKD), and acute kidney injury (AKI) affect hundreds of millions of people and place a significant strain on healthcare systems, particularly in low- and middle-income countries (LMICs) (Zhang et al., 2023; Francis et al., 2024) The key characteristic of acute kidney injury (AKI) is a sudden reduction in renal function, which causes problems with fluid, electrolyte, and acid-base homeostasis. Hospitalized and critically ill patients often suffer from AKI, a clinical consequence that is linked to longer hospital stays, higher medical expenses, and higher death. According to current estimates, 13.3 million people globally acquire AKI each year, which results in about 1.7 million deaths (Cerda et al., 2026; Batte et al., 2023). In line with epidemiological research, almost 50% of critically sick patients in to intensive care units may develop AKI, which affects 20–22% of hospitalized adults .Furthermore, AKI has become known as both an acute occurrence and a major risk factor for the development of end-stage renal disease and chronic kidney disease later on (Chen et al., 2026)Chronic kidney disease (CKD) is defined as persistent abnormalities of kidney structure or function for at least three months and is one of the fastest growing non-communicable diseases globally. Based on the analysis of the Global Burden of Disease (GBD) 2021 (Deng et al., 2025), 673.7 million people worldwide were living with CKD in 2021, and more than 1.5 million deaths were directly caused by CKD. In addition, epidemiological studies have shown an upsurge in CKD prevalence over the past 30 years, which is linked to an aging population, diabetes mellitus, hypertension, obesity and other metabolic disorders (Qin et al., 2024). Recent global estimates show that the prevalence of CKD has increased by approximately one-third since 1990 and is projected to become one of the leading causes of years of life lost globally in the coming decades (Francis et al., 2024). In addition to its direct impact on renal function, CKD is linked with a markedly increased risk of cardiovascular disease, hospitalization, impaired quality of life and early death (Francis et al., 2024).

Furthermore, epidemiological studies have shown that the aging population, diabetes mellitus, hypertension, obesity, and other metabolic illnesses are the main causes of the rising trend in CKD prevalence during the past 30 years (Qin et al., 2024). According to recent assessments conducted worldwide, the prevalence of chronic kidney disease (CKD) has risen by approximately one-third since 1990 and is predicted to rank among the top causes of years of life lost worldwide in the upcoming decades. Chronic kidney disease (CKD) is linked to a much higher risk of cardiovascular disease, hospitalization, poor quality of life, and early death in addition to its direct effects on renal function (Francis et al., 2024). Furthermore, epidemiological studies have shown that the aging population, diabetes mellitus, hypertension, obesity, and other metabolic illnesses are the main causes of the rising trend in CKD prevalence during the last 30 years (Qin et al., 2024). According to recent assessments conducted worldwide, the prevalence of chronic kidney disease (CKD) has risen by about one-third since 1990 and is predicted to rank among the top causes of years of life lost worldwide in the upcoming decades. Chronic kidney disease (CKD) is linked to a much higher risk of cardiovascular disease, hospitalization, poor quality of life, and early mortality in addition to its direct effects on renal function (Francis et al., 2024).

The increasing worldwide burden of acute kidney injury (AKI), chronic kidney disease (CKD) and drug-induced nephrotoxicity has led to an increasing demand for effective nephroprotective therapies. Much progress has been made in understanding renal pathophysiology, but treatment options available for prevention or reversal of kidney injury are limited. As a result, preclinical nephroprotection studies have become an important part of renal drug discovery and development, providing valuable information on disease mechanisms and therapeutic efficacy prior to clinical evaluation (Perazella, 2019; Griffin et al., 2019).For examining the pathophysiology of renal injury and locating possible nephroprotective medicines, experimental nephrotoxicity models are essential resources. These models facilitate mechanistic studies that are challenging to conduct in clinical settings by allowing researchers to replicate particular pathological aspects of human kidney illnesses under controlled laboratory circumstances (Faria et al., 2019). Researchers can assess the role of oxidative stress, inflammation, apoptosis, mitochondrial dysfunction, fibrosis, and vascular injury in renal damage using chemically produced, drug-driven, disease-based, and surgical models (Awdishu & Mehta, 2017). One of the main advantages of experimental models is the possibility to provide a repeatable platform for screening new nephroprotective substances. Drug-induced nephrotoxicity is a common cause of hospital-acquired AKI and is often linked to agents such as gentamicin, cisplatin, cyclosporine and nonsteroidal anti-inflammatory medications (Awdishu & Mehta, 2017). Experimental models that mimic these toxic challenges can be employed to test the protective effects of natural products, synthetic compounds, nanomedicines, and repurposed drugs that can precede expensive clinical trials (Mody et al., 2020). Doing preclinical work early reduces the risk of clinical failure and helps to identify viable treatment options. Experimental models are also necessary for biomarker identification and validation. Traditional indicators for renal impairment, BUN and serum creatinine, are sometimes unable to detect early kidney injury. Animal and cell-based models have been utilized to study the development of biomarkers like neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin-C, clusterin and urine microRNAs (Vaidya et al., 2008). These biomarkers may improve preclinical and clinical evaluation of nephroprotective medicines and have shown greater sensitivity for subclinical renal injury detection.

The assessment of molecular targets involved in nephrotoxicity is another significant use of experimental

models. Nuclear factor-kappa B (NF-κB), transforming growth factor-beta (TGF-β), mitogen-activated protein kinase (MAPK), nuclear factor erythroid 2-related factor 2 (Nrf2), and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathways are among the signaling pathways linked to renal injury that have been thoroughly studied thanks to advances in molecular biology (Griffin et al., 2019). Comprehending how nephroprotective drugs alter these pathways can aid in the creation of precision medicine techniques and targeted treatment plans. Traditional animal models have intrinsic limitations despite their usefulness. The translational applicability of results to human patients may be diminished by species-specific variations in renal anatomy, physiology, pharmacokinetics, and immunological responses (Griffin et al., 2019). Additionally, alternative methods such as kidney organoids, three-dimensional (3D) cell cultures, microphysiological systems, and kidney-on-a-chip technologies have been developed due to ethical concerns about animal experiments (Low et al., 2019). These new platforms promote the replacement, reduction, and refinement (3Rs) of biomedical research while providing chances to increase the predictive accuracy of preclinical nephrotoxicity testing.

In general, nephroprotection research still relies heavily on experimental nephrotoxicity models. They offer a controlled setting for determining therapeutic targets, confirming biomarkers, clarifying disease mechanisms, and assessing the effectiveness and safety of nephroprotective treatments. It is anticipated that the translational success of upcoming nephroprotective drug development initiatives will be improved by the ongoing improvement of these models and the incorporation of cutting-edge human-relevant technologies.

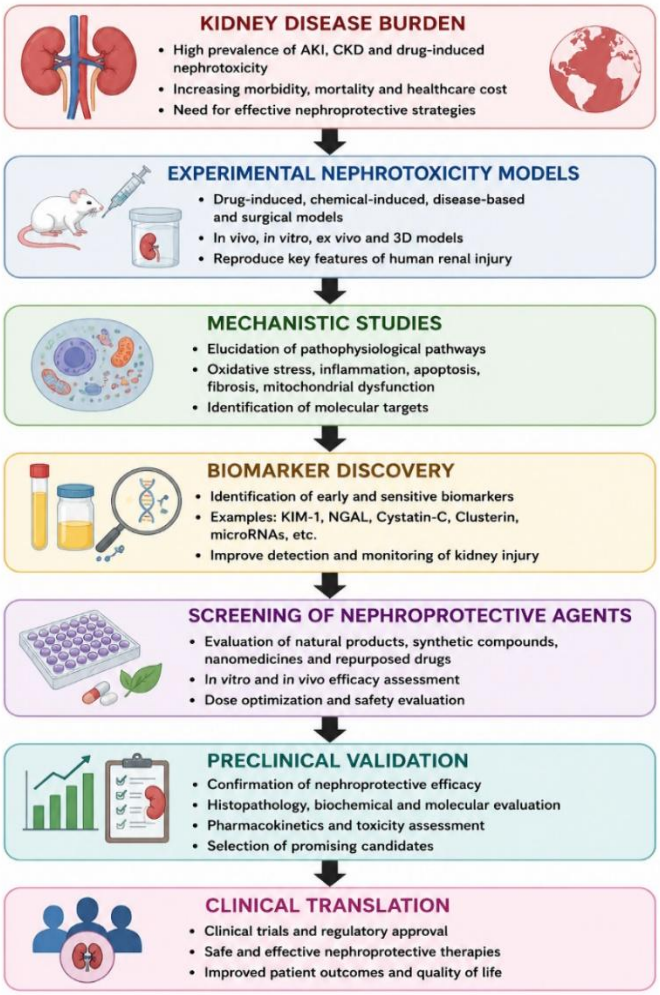

Figure 1. Role of Experimental Models in Nephroprotective Drug Development

The research and pharmaceutical sectors face a hurdle in translating preclinical data into clinically viable nephroprotective medicines, even though experimental nephrotoxicity models are crucial for drug development and mechanistic studies. A number of medicines demonstrating encouraging nephroprotective properties in animal studies have not transferred to human therapy in a comparable fashion in clinical trials. Numerous reasons, such as species differences, the limitations of currently available biomarkers, and significant heterogeneity associated with experimental models, contribute to this lack of translational efficacy to people (Griffin et al., 2019; Mak et al., 2014).

Review objective

Early research and development of medications with nephroprotective qualities are aided by nephrotoxicity

models. Over the past few decades, numerous models that mimic various aspects of acute kidney injury (AKI), chronic kidney disease (CKD), and other forms of renal dysfunction have been developed. These models are based on the application of drugs or chemicals, a particular clinical state, or surgery. Despite the widespread usage of these models, significant variation has been noted in terms of pathophysiological relevance, predictive capability, biomarker responsiveness, and model application to kidney illnesses in humans (Griffin et al., 2019; Faria et al., 2019). Additionally, the growing awareness of the drawbacks of conventional animal models has prompted the creation of substitute methods, such as kidney organoids, micro physiological systems, kidney-on-a-chip technologies, and sophisticated in vitro platforms. While addressing the ethical and translational issues related to animal testing, these new tools have the potential to increase the predicted accuracy of nephrotoxicity evaluation (Low et al., 2019; Jang et al., 2013). Thus, this systematic review's main goal is evaluate experimental nephrotoxicity models that are already in use for screening nephroprotective drugs. This review specifically aims to:

1.Examine experimental nephrotoxicity models according to their injury processes, pathological features, reproducibility, benefits, drawbacks, and appropriateness for nephroprotective medication screening.

2.Examine both established and new biomarkers for nephroprotection and renal damage, paying special attention to their translational usefulness, sensitivity, and specificity.

3.Examine how well frequently used nephrotoxicity models replicate actual renal pathophysiology and forecast clinical outcomes to determine their translational significance.

4.Examine new options for improving translational success in nephroprotection research, such as kidney organoids, kidney-on-a-chip systems, three-dimensional (3D) cell cultures, and artificial intelligence-assisted nephrotoxicity prediction platforms.

Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines (Page et al., 2021). To find research on experimental nephrotoxicity models and nephroprotective modalities, a thorough literature search was conducted utilizing the PubMed/MEDLINE, Scopus, Web of Science, and Embase databases. Combinations of keywords like "nephrotoxicity model," "renal injury model," "nephroprotective," "renoprotective," "animal model," and "experimental model" were among the search results. Original, peer-reviewed research that was published in English between 2000 and 2026 was deemed qualified. Clinical research, conference papers, editorials, and duplicate articles were removed, but studies incorporating animal models, in vitro systems, nephroprotective medicines, biomarkers of renal injury, and new nephrotoxicity assessment platforms were included. Relevant information about nephrotoxic substances, biomarkers, histopathological results, nephroprotective outcomes, and model characteristics was extracted and qualitatively summarized. The SYRCLE Risk of Bias tool was used to evaluate the methodological quality of the included animal research (Hooijmans et al., 2014). To enable comparative assessment of their translational relevance and usefulness in nephroprotective drug screening, experimental models were then divided into drug-induced, chemical-induced, disease-based, surgical, and advanced in vitro platforms.

Pathophysiological Basis of Experimental Nephrotoxicity

Key molecular and physiological pathways driving kidney injury in humans are replicated in experimental nephrotoxicity models. Oxidative stress, inflammation, apoptosis, and fibrosis are common pathophysiological processes that contribute to renal dysfunction, even though different nephrotoxic drugs cause kidney damage through diverse pathways (Perazella, 2019; Awdishu & Mehta, 2017). Determining possible therapeutic targets and assessing the effectiveness of nephroprotective measures depend on an understanding of these mechanisms.

Oxidative stress

When the amount of reactive oxygen species (ROS) produced surpasses the endogenous antioxidant defence systems' capabilities, oxidative stress results. Nephrotoxic substances as cisplatin, gentamicin, potassium dichromate, and mercuric chloride cause excessive ROS production in renal tubular cells during experimental nephrotoxicity (Perazella, 2019). Superoxide anion ($O_2\bullet^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), which can harm cellular proteins, lipids, and nucleic acids, are the main ROS implicated (Li et al., 2019). Lipid peroxidation, a process in which free radicals damage membrane phospholipids and cause loss of membrane integrity and cellular malfunction, is one of the most significant effects of ROS overproduction. In nephrotoxicity research, malondialdehyde (MDA) is frequently employed as a biomarker of lipid peroxidation (Ayala et al., 2014). Oxidative damage is exacerbated by the simultaneous depletion of antioxidant defenses including reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) (Fang et al., 2002).Prolonged oxidative stress is linked to pro-inflammatory and pro-fibrotic signalling pathway activation, tubular necrosis, and mitochondrial malfunction.

Inflammation

Another important factor that contributes to the development of nephrotoxicity is inflammation. Many pro-inflammatory mediators are released by activated tubular epithelial cells, macrophages, and infiltrating immune cells after renal injury, which exacerbate tissue damage and advance the course of the disease (Ramesh & Reeves, 2004). Long-term functional impairment, chronic renal fibrosis, and acute kidney damage are all caused by persistent inflammation. One important pro-inflammatory cytokine implicated in nephrotoxicity is tumor necrosis factor-alpha

(TNF-α). There have been reports of elevated TNF-α expression in a number of experimental models, such as renal damage caused by gentamicin and cisplatin (Faria et al., 2019). TNF-α exacerbates kidney injury by promoting leukocyte recruitment, oxidative stress, apoptosis, and endothelial dysfunction (Gupta et al., 2000). A key transcription factor that controls the expression of several inflammatory genes, such as TNF-α, IL-1β, and IL-6, is nuclear factor-kappa B (NF-κB) (Lawrence, 2009). Numerous nephrotoxicity models exhibit activation of NF-κB signaling, which amplifies inflammatory responses in renal tissues (Markó et al., 2016). By inhibiting NF-κB activation, a number of nephroprotective drugs reduce cytokine production and prevent tissue damage (Sun, 2017). Together, oxidative stress and inflammation form a network of interrelated processes that propel the onset and development of nephrotoxicity. Developing successful nephroprotective treatments still heavily relies on targeting these mechanisms.

Apoptosis

In both acute and chronic kidney disorders, apoptosis, also known as programmed cell death, plays a major role in renal tubular damage and is a key mechanism underpinning nephrotoxicity. Numerous experimental nephrotoxicity models, such as those caused by cisplatin, gentamicin, and ischemia-reperfusion, have been shown to cause excessive apoptosis of renal tubular epithelial cells (Pabla & Dong, 2008; Servais et al., 2008). In contrast to necrosis, apoptosis is an energy-dependent process that results in DNA fragmentation, chromatin condensation, cell shrinkage, and the creation of apoptotic bodies without causing a noticeable inflammatory reaction (Elmore, 2007).

Apoptosis is mediated by a class of cysteine-dependent proteases called caspases. Both internal (mitochondrial) and extrinsic (death receptor-mediated) apoptotic pathways are triggered by nephrotoxic insults, which ultimately result in the activation of executioner caspases, including caspase-3 (Galluzzi et al., 2017). Increased production of caspase-3, caspase-8, and caspase-9 is commonly utilized as a molecular marker of renal apoptosis and has been consistently seen in nephrotoxic experimental models (Kaushal & Shah, 2016). When these enzymes are activated, structural and regulatory proteins are cleaved, causing irreversible cellular damage and renal function loss.

Mitochondria are essential for controlling apoptosis and cellular viability. Nephrotoxic substances cause mitochondrial dysfunction by opening mitochondrial permeability transition pores, disrupting the potential of the mitochondrial membrane, producing excessive amounts of reactive oxygen species (ROS), and impairing ATP synthesis (Zorov et al., 2014). These changes make it easier for pro-apoptotic proteins such cytochrome c to be released into the cytoplasm, which triggers the caspase cascade and ultimately results in cell death (Green & Reed, 1998). During nephrotoxic damage, the ratio of pro-apoptotic proteins (Bax and Bak) to anti-apoptotic proteins (Bcl-2) is crucial for determining cell fate (Youle & Strasser, 2008). Therefore, by maintaining mitochondrial integrity and inhibiting apoptotic signalling pathways, several nephroprotective drugs have positive benefits.

Fibrosis

Chronic kidney disease (CKD) is characterized by fibrosis, which is the last frequent pathway of increasing kidney injury. Excessive extracellular matrix (ECM) protein accumulation is triggered by oxidative stress, persistent inflammation, and recurrent cellular damage, which ultimately results in structural remodelling and irreversible loss of renal function (Humphreys, 2018). In experimental models including diabetic nephropathy, unilateral ureteral obstruction (UUO), and chronic drug-induced nephrotoxicity, renal fibrosis is frequently seen (Liu, 2011).

Renal fibrosis is known to be efficiently regulated by transforming growth factor-beta (TGF-β). TGF-β signaling stimulates fibroblast activation, extracellular matrix formation, epithelial-to-mesenchymal transition (EMT), and inhibits matrix breakdown after kidney damage (Meng et al., 2016). Numerous nephrotoxicity models have shown elevated TGF-β expression, which is closely correlated with the severity of fibrosis and the course of the disease (Lan, 2011). As a result, TGF-β signalling suppression is thought to be a promising therapeutic approach for avoiding chronic kidney injury.

The primary cells that produce extracellular matrix during fibrosis are activated myofibroblasts, and alpha-smooth muscle actin (α-SMA) is a commonly utilized marker of these cells (Hinz, 2007). Fibroblast activation and the advancement of fibrotic remodelling are indicated by elevated α-SMA expression. Gene expression analysis or α-SMA immunohistochemistry are commonly used in experimental research to assess the antifibrotic effectiveness of nephroprotective treatments (LeBleu et al., 2013).

Renal fibrosis is characterized by excessive extracellular matrix protein deposition, especially collagen types I, III, and IV (Eddy, 2014). In addition to impairing glomerular and tubular function and causing irreversible kidney injury, progressive collagen accumulation also disturbs normal renal architecture. In experimental nephrotoxicity models, collagen deposition is frequently measured using histological methods including Masson's trichrome staining and Sirius Red staining (Farris & Colvin, 2012). Collagen build-up reduction is frequently regarded as a crucial sign of nephroprotective and antifibrotic action. Collectively, fibrosis and apoptosis are important processes that propel nephrotoxicity from acute injury to chronic kidney disease. Experimental nephroprotection studies have demonstrated significant promise for therapeutic approaches that target caspase activation, mitochondrial failure, TGF-β signalling, myofibroblast activation, and extracellular matrix deposition.

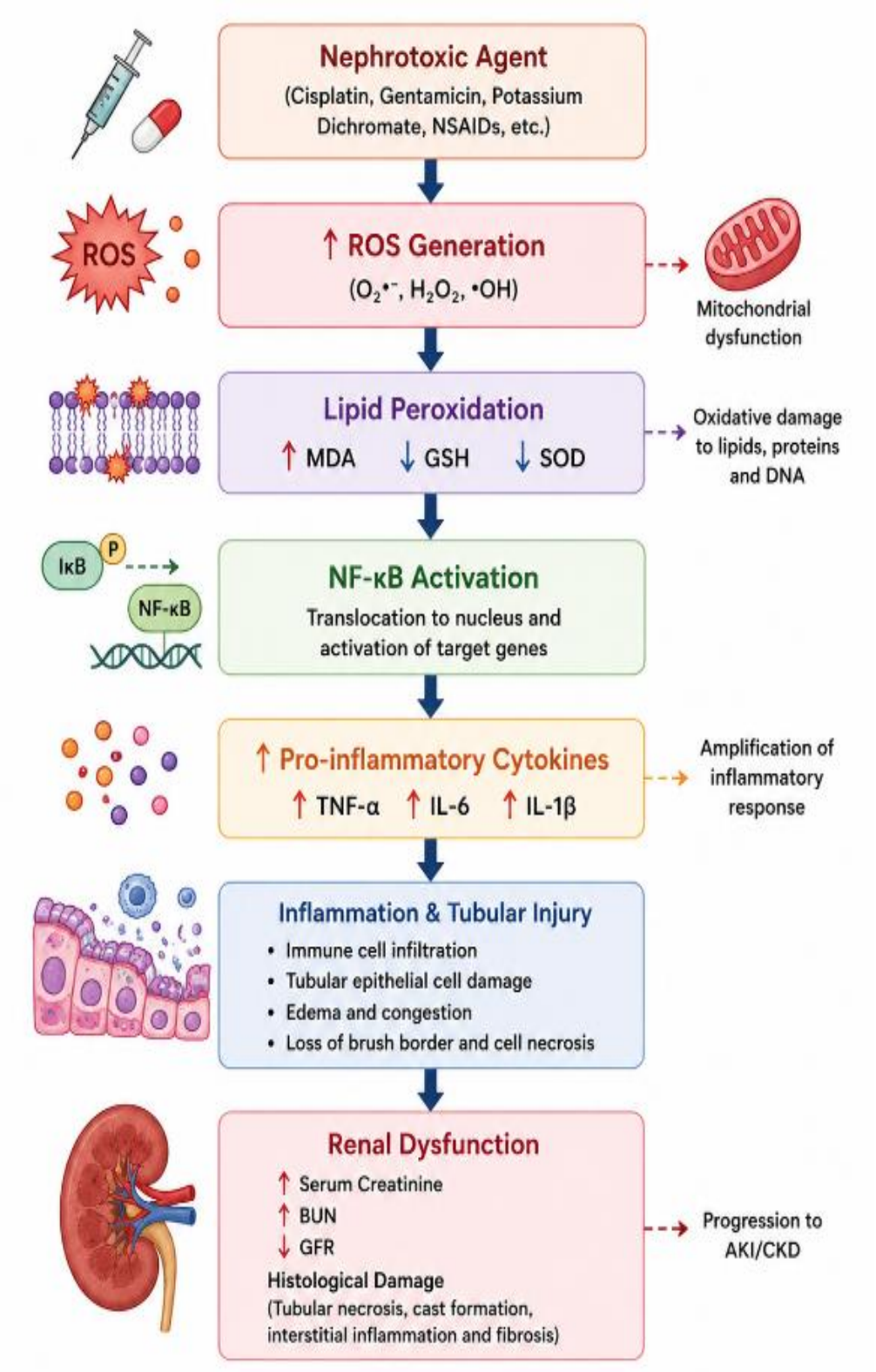

Figure 2. Oxidative Stress–Inflammation Axis in Experimental Nephrotoxicity

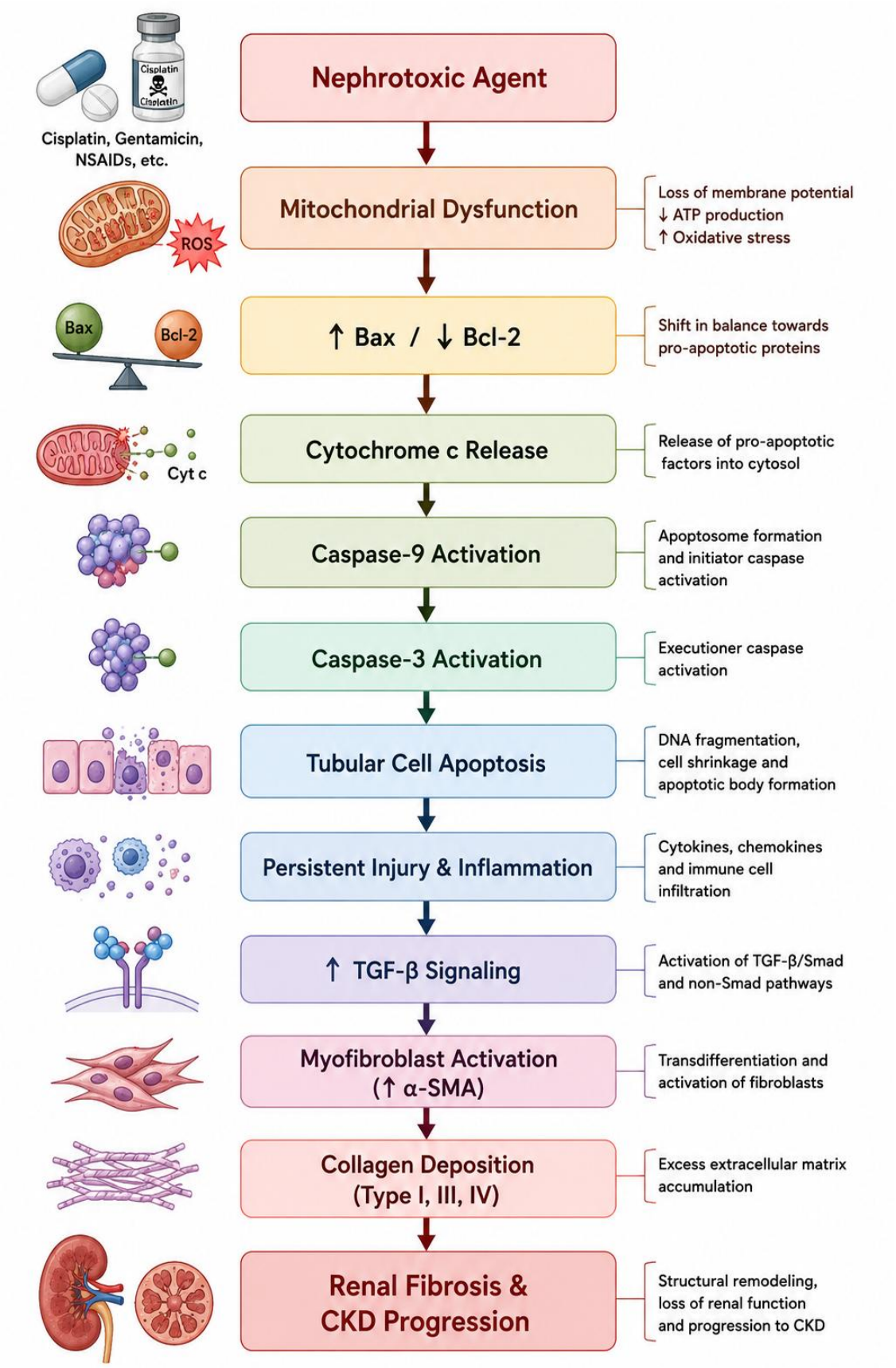

Figure 3. Apoptosis–Fibrosis Axis in Experimental Nephrotoxicity

Abbreviations: ROS, reactive oxygen species; MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; NF-κB, nuclear factor-kappa B; TNF-α, tumor necrosis factor-alpha; IL, interleukin; TGF-β, transforming growth factor-beta; α-SMA, alpha-smooth muscle actin; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; BUN, blood urea nitrogen.

Table 1. Major Pathophysiological Mechanisms and Biomarkers in Experimental Nephrotoxicity Models

Pathophysiol-ogical Mechanism	Key Mediators/Markers	Biological Effects	Common Assessment Parameters	Relevance in Nephrotoxicity Models
Oxidative Stress	ROS ($O_2\bullet^-$, H_2O_2, •OH), MDA	Oxidative damage to lipids, proteins, and DNA	MDA ↑, ROS ↑, GSH ↓, SOD ↓, Catalase ↓	Central mechanism in gentamicin-, cisplatin-, potassium dichromate-, and paracetamol-induced nephrotoxicity
Lipid Peroxidation	Malondialdehyde (MDA), 4-HNE	Membrane damage and loss of cellular integrity	MDA levels, TBARS assay	Indicator of oxidative renal injury
Inflammation	TNF-α, IL-1β, IL-6, MCP-1	Recruitment of inflammatory cells and amplification of tissue injury	ELISA, qPCR, Western blotting	Common in AKI and CKD models
NF-κB Activation	NF-κB p65, IκBα	Upregulation of pro-inflammatory cytokines and chemokines	NF-κB expression, nuclear translocation studies	Major inflammatory signaling pathway in nephrotoxicity
Apoptosis	Caspase-3, Caspase-8, Caspase-9	Programmed cell death of tubular epithelial cells	Caspase activity assays, TUNEL staining	Frequently observed in cisplatin- and gentamicin-induced nephrotoxicity
Mitochondrial Dysfunction	Cytochrome c, Bax, Bcl-2	ATP depletion, ROS generation, initiation of apoptosis	Mitochondrial Dysfunction	Cytochrome c, Bax, Bcl-2
Fibrosis	TGF-β1, Smad2/3, CTGF	Excess extracellular matrix production and tissue remodelling	Fibrosis	TGF-β1, Smad2/3, CTGF
Myofibroblast Activation	α-SMA	Transformation of fibroblasts into matrix-producing cells	α-SMA immunohistochemistry, Western blotting	Hallmark of progressive renal fibrosis
Collagen Deposition	Collagen I, III, IV, Fibronectin	Structural remodeling and irreversible renal damage	Masson's trichrome staining, Sirius Red staining	Marker of advanced fibrosis
Tubular Injury	KIM-1, NGAL, NAG	Loss of tubular integrity and renal function	Urinary KIM-1, NGAL, histopathology	Early indicator of nephrotoxicity
Renal Dysfunction	Serum Creatinine, BUN, Cystatin-C	Reduced glomerular filtration and impaired renal function	Biochemical assays	Standard endpoint in nephroprotection studies

Drug-Induced Experimental Nephrotoxicity Models

Drug-induced nephrotoxicity models are among the most frequently employed experimental systems for evaluating nephroprotective agents because they closely mimic clinically relevant renal injuries caused by therapeutic drugs. These models make it easier to study common pathways that underlie kidney injury, such as oxidative stress, inflammation, apoptosis, mitochondrial dysfunction, and fibrosis. Additionally, they offer repeatable platforms for evaluating the effectiveness of new nephroprotective substances prior to clinical testing (Perazella, 2019; Awdishu & Mehta, 2017).

Gentamicin Induced Nephrotoxicity

For researching drug-induced acute kidney damage (AKI), gentamicin is one of the most widely used models. Gentamicin causes excessive production of reactive oxygen species (ROS), mitochondrial dysfunction, lysosomal damage, and activation of apoptotic pathways in proximal tubular epithelial cells through receptor-mediated endocytosis (Walker & Shah, 1988). The model's histopathological features include interstitial inflammation, tubular necrosis, and epithelial desquamation. Antioxidant and anti-inflammatory nephroprotective drugs are frequently screened using the gentamicin model because of its ease of use, affordability, and good reproducibility. Nevertheless, it does not accurately reflect the intricate pathophysiology of chronic kidney disease (CKD) and instead predominantly represents acute tubular injury (Ali, 1995).

Cisplatin Induced Nephrotoxicity

Since nephrotoxicity is still a significant dose-limiting side effect of cisplatin chemotherapy, cisplatin-induced nephrotoxicity is considered one of the most clinically relevant experimental models. Through organic cation transporters, cisplatin builds up in renal tubular cells and causes oxidative stress, apoptosis, inflammation, mitochondrial dysfunction, and DNA damage (Pabla & Dong, 2008). Renal dysfunction and tubular cell death are largely caused by activation of the p53, NF-κB, and caspase signaling pathways. This model is especially helpful for examining the molecular mechanisms of nephroprotection and closely resembles human chemotherapy-associated AKI. However, overdosing can result in systemic toxicity and raise animal mortality, therefore careful dose optimization is required (Yao et al., 2007).

Cyclosporine Induced Nephrotoxicity

It is one of the calcineurin inhibitor that is frequently used in autoimmune diseases and organ transplantation is cyclosporine. Renal vasoconstriction, oxidative stress, endothelial dysfunction, activation of transforming growth factor-beta (TGF-β), and progressive interstitial fibrosis are all ways that chronic administration causes nephrotoxicity (Naesens et al., 2009). In contrast to gentamicin and cisplatin models, cyclosporine nephrotoxicity is similar to the fibrosis and persistent kidney damage seen in transplant recipients. As a result, it is a useful model for assessing vasoprotective and antifibrotic nephroprotective treatments. However, establishing severe kidney impairment frequently requires longer treatment periods, which could lengthen and raise the expense of the experiment (Naesens et al., 2009).

Doxorubicin Induced Nephrotoxicity

Doxorubicin-induced nephrotoxicity offers a valuable model for researching glomerular injury caused by oxidative stress. Excessive ROS generation, lipid peroxidation, mitochondrial damage, and activation of apoptotic pathways are the main causes of doxorubicin's nephrotoxic effects (Tacar et al., 2013). Doxorubicin is especially useful for studying glomerular pathology since it often causes podocyte damage, proteinuria, and glomerulosclerosis, in contrast to gentamicin and cisplatin, which mainly impact renal tubules. However, doxorubicin administration-related concomitant cardiotoxicity may affect systemic physiological responses and contribute confounding variables into nephroprotection research (Tacar et al., 2013).

Acetaminophene Induced Nephrotoxicity

A well-known toxicological model that represents acute renal damage brought on by drug overdose is paracetamol-induced nephrotoxicity. N-acetyl-p-benzoquinone imine (NAPQI), a reactive molecule that causes intracellular glutathione depletion, oxidative stress, mitochondrial dysfunction, and tubular necrosis, is the main cause of renal injury (McGill & Jaeschke, 2019). This paradigm is still appealing for initial screening of antioxidant nephroprotective chemicals because of its ease of use, low cost, and quick induction of damage. Concomitant hepatotoxicity, however, is often seen and can make it more difficult to evaluate renoprotective efficacy, especially when assessing substances with systemic antioxidant qualities (McGill & Jaeschke, 2019).

Tenofovir Induced Nephrotoxicity

Tenofovir's extensive clinical use in the treatment of HIV and hepatitis B has raised awareness of enofovir-induced nephrotoxicity. The main cause of the nephrotoxic consequences is intracellular accumulation in proximal tubular cells, which leads to tubular dysfunction, oxidative stress, mitochondrial DNA depletion, and decreased ATP generation (Hall et al., 2011). Fanconi syndrome, tubular proteinuria, and a progressive loss of renal function are among the clinically significant symptoms that this animal replicates. Tenofovir nephrotoxicity offers a useful platform for studying chronic tubular injury and mitochondrial-targeted nephroprotective approaches when compared to acute injury models. However, susceptibility may be affected and direct extrapolation to humans may be limited by species-dependent variations in transporter expression (Ailabouni & Prasad, 2025).

Each model primarily represents different features of kidney pathology, even though all drug-induced nephrotoxicity models eventually converge on oxidative stress, inflammation, apoptosis, and renal failure. Models of

gentamicin and cisplatin are excellent for researching oxidative stress-mediated processes and acute tubular damage. Tenofovir and cyclosporine models, on the other hand, more accurately depict progressive fibrosis and chronic nephrotoxicity. While paracetamol is a useful model for examining toxicant-induced oxidative renal damage, doxorubicin is especially useful for assessing glomerular injury and proteinuria. As a result, no one model can accurately mimic human nephrotoxicity, and the particular pathophysiological mechanism being studied as well as the intended translational application should be taken into consideration when selecting a model (Perazella, 2019; Awdishu& Mehta, 2017).

Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; ROS, reactive oxygen species; MDA, malondialdehyde; GSH, reduced glutathione; BUN, blood urea nitrogen; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; TGF-β, transforming growth factor-beta; α-SMA, alpha-smooth muscle actin.

Table 2. Comparative Evaluation of Drug-Induced Experimental Nephrotoxicity Models

Model	Primary Target Site	Major Mechanism of Injury	Histopathological Features	Key Biomarkers	Advantages	Limitations	Translational Relevance
Gentamicin	Proximal tubular epithelial cells	ROS generation, lipid peroxidation, mitochondrial dysfunction, apoptosis	Tubular necrosis, epithelial degeneration, inflammatory infiltration	Serum creatinine, BUN, MDA, GSH, KIM-1	Highly reproducible, inexpensive, rapid induction of injury	Primarily acute injury; limited relevance to CKD	High for drug-induced AKI
Cisplatin	Proximal tubules	DNA damage, oxidative stress, inflammation, apoptosis, mitochondrial dysfunction	Acute tubular necrosis, tubular dilation, inflammatory cell infiltration	Creatinine, BUN, NGAL, KIM-1, TNF-α, IL-6	Clinically relevant chemotherapy-induced nephrotoxicity model	Systemic toxicity and mortality at high doses	Very High
Cyclosporine	Tubulointerstitial compartment	Vasoconstriction, oxidative stress, TGF-β activation, fibrosis	Tubular atrophy, arteriolar hyalinosis, interstitial fibrosis	TGF-β, α-SMA, Collagen I, Creatinine	Excellent model for chronic nephrotoxicity and fibrosis	Long induction period and hemodynamic confounding factors	High
Doxorubicin	Glomeruli and podocytes	ROS generation, lipid peroxidation, apoptosis, mitochondrial dysfunction	Proteinuria, glomerulosclerosis, podocyte injury	Proteinuria, MDA, Caspase-3, Creatinine	Useful for studying glomerular injury and oxidative stress	Concurrent cardiotoxicity may influence outcomes	Moderate–High
Paracetamol	Renal tubular cells	NAPQI formation, GSH depletion, oxidative stress, necrosis	Tubular necrosis, epithelial degeneration	Creatinine, BUN, MDA, GSH	Simple, rapid, and cost-effective model	Associated hepatotoxicity may complicate interpre	Moderate

						tation	
Tenofovir	Proximal tubular epithelial cells	Mitochondrial toxicity, ATP depletion, oxidative stress, tubular dysfunction	Tubular atrophy, proximal tubular damage	KIM-1, NGAL, Cystatin-C, Creatinine	Clinically relevant model of antiviral drug nephrotoxicity	Slow development of injury and species differences	High

Chemical-Induced Experimental Nephrotoxicity Models

Preclinical nephrology research frequently uses chemical-induced nephrotoxicity models because they offer repeatable and mechanistically unique platforms for examining kidney damage and assessing nephroprotective medications. These systems, in contrast to drug-induced models, frequently produce severe oxidative stress, tubular necrosis, crystal nephropathy, or heavy metal toxicity, allowing researchers to examine certain kidney damage mechanisms. Because they can cause consistent biochemical, histological, and molecular changes that closely resemble some types of human nephrotoxicity, chemical nephrotoxins like potassium dichromate, mercuric chloride, carbon tetrachloride, and ethylene glycol have been widely used (Perazella, 2019; Awdishu & Mehta, 2017).

Potassium Dichromate-Induced Nephrotoxicity

Potassium dichromate ($K_2Cr_2O_7$) is a hexavalent chromium compound commonly used to induce acute tubular injury in experimental animals. After systemic exposure, chromium primarily builds up in proximal tubular epithelial cells. In these cells, intracellular reduction of Cr(VI) produces an excess of reactive oxygen species (ROS), which causes oxidative stress, lipid peroxidation, mitochondrial dysfunction, DNA damage, and apoptosis (Cerda et al., 2026). Tubular degeneration, tubular necrosis, glomerular congestion, and inflammatory cell infiltration are common histopathological findings. This model is especially helpful for assessing antioxidant and anti-inflammatory nephroprotective drugs because oxidative stress plays a significant role. However, the degree of toxicity may differ according on the dosage and mode of administration, which could have an impact on repeatability (Molina-Jijón et al., 2011).

Mercuric Chloride-Induced Nephrotoxicity

Mercuric chloride ($HgCl_2$) is a classical heavy metal nephrotoxin that selectively targets proximal tubular epithelial cells. Mercury exhibits a strong affinity for sulfhydryl groups of cellular proteins, resulting in enzyme inhibition, oxidative stress, mitochondrial injury, calcium dysregulation, and tubular cell necrosis (Bridges & Zalups, 2017). Acute exposure produces marked elevations in serum creatinine and blood urea nitrogen (BUN), accompanied by extensive tubular degeneration and inflammatory responses. This model is highly reproducible and has been widely utilized to investigate mechanisms of heavy metal toxicity and nephroprotection. Nevertheless, the rapid onset and severity of renal injury may not accurately reflect the progressive nature of many human kidney diseases (Zalups, 2000).

Carbon Tetrachloride-Induced Nephrotoxicity

Although carbon tetrachloride (CCl_4) has long been known to be a hepatotoxic toxin, oxidative stress-mediated processes also cause severe kidney damage. Highly reactive trichloromethyl ($\bullet CCl_3$) and trichloromethyl peroxy ($\bullet OOCCl_3$) radicals are produced when cytochrome P450 enzymes metabolically activate CCl_4. These radicals cause lipid peroxidation and damage to cellular membranes (Weber et al., 2003). These processes cause oxidative stress, tubular degeneration, glomerular changes, and inflammatory reactions in the kidney. The model is especially useful for assessing substances that have membrane-stabilizing and antioxidant qualities. However, concomitant hepatotoxicity is a significant drawback of this model and may make it more difficult to evaluate nephroprotective effects (Weber et al., 2003).

Ethylene Glycol-Induced Nephrotoxicity

A common experimental paradigm for nephrolithiasis and crystal-induced kidney damage is ethylene glycol-induced nephrotoxicity. After consumption, ethylene glycol is converted to oxalic acid, glycolic acid, and glyoxylic acid, which causes calcium oxalate crystal deposition in the renal tubules and hyperoxaluria (Khan, 2004). Tubular blockage, oxidative stress, inflammation, and gradual renal failure are all facilitated by crystal accumulation. The model's histological features include interstitial inflammation, tubular dilatation, significant calcium oxalate crystal deposition, and epithelium damage. This model is especially helpful for assessing antiurolithiatic and nephroprotective treatments since it closely resembles human calcium oxalate nephrolithiasis. However, the results of experiments may be affected by variations in crystal formation and reliance on dietary variables (Atmani & Khan, 2000).

The mechanisms of harm and translational applicability of chemical-induced nephrotoxicity models vary significantly. Models of potassium dichromate and mercuric chloride are useful for researching antioxidant and cytoprotective therapies because they primarily cause acute tubular injury through oxidative stress and direct cytotoxicity. Although concomitant liver toxicity limits its usefulness, carbon tetrachloride is especially useful for studying oxidative stress-mediated damage. Ethylene glycol, on the other hand, reproduces crystal nephropathy and nephrolithiasis in a unique way, offering a crucial platform for testing anti-crystallization and anti-inflammatory

treatments. Consequently, the particular disease process and therapeutic mechanism being studied should be taken into consideration when choosing a chemical-induced model (Perazella, 2019; Awdishu & Mehta, 2017).

Table 3. Comparative Evaluation of Chemical-Induced Experimental Nephrotoxicity Models

Model	Primary Target Site	Major Mechanism of Injury	Histopathological Features	Key Biomarkers	Advantages	Limitations	Translational Relevance
Potassium Dichromate	Proximal tubules	ROS generation, lipid peroxidation, mitochondrial dysfunction, apoptosis	Tubular necrosis, tubular degeneration, glomerular congestion	Creatinine, BUN, MDA, GSH, SOD	Highly reproducible oxidative stress model	Toxicity varies with dose and route	High
Mercuric Chloride	Proximal tubular epithelial cells	Sulfhydryl binding, oxidative stress, mitochondrial injury, necrosis	Acute tubular necrosis, epithelial desquamation	Creatinine, BUN, KIM-1, NGAL	Excellent model of heavy metal nephrotoxicity	Severe acute toxicity	High
Carbon Tetrachloride (CCl4)	Tubular and glomerular cells	Free radical generation, lipid peroxidation, inflammation	Tubular degeneration, glomerular injury	MDA, GSH, Creatinine, TNF-α	Useful for antioxidant screening	Concurrent hepatotoxicity	Moderate
Ethylene Glycol	Renal tubules	Hyperoxaluria, calcium oxalate crystal deposition, oxidative stress	Crystal deposition, tubular dilation, inflammation	Oxalate, Creatinine, BUN, MDA	Mimics nephrolithiasis and crystal nephropathy	Variability in crystal formation	Very High

While ethylene glycol is still the model of choice for nephrolithiasis and crystal nephropathy, potassium dichromate and mercuric chloride are particularly helpful for examining acute oxidative and heavy metal-induced kidney damage. Due to concurrent hepatic damage, carbon tetrachloride should be interpreted cautiously despite providing important insights into oxidative stress-mediated nephrotoxicity. When combined, these models enhance drug-induced systems and expand the field of preclinical nephroprotection studies.

Disease-Based Experimental Nephrotoxicity Models

For investigating the pathophysiological mechanisms underlying chronic kidney disease (CKD) and assessing nephroprotective therapies in settings that closely mimic real renal diseases, disease-based nephrotoxicity models are crucial. Disease-based models, in contrast to acute toxicological models, usually cause progressive renal impairment marked by oxidative stress, inflammation, apoptosis, glomerulosclerosis, and tubulointerstitial fibrosis. As a result, these models are frequently used in preclinical nephroprotection research and have higher translational value for chronic nephropathies (Breyer et al., 2005; Eddy et al., 2012).

Streptozotocin-Induced Diabetic Nephropathy

One of the most popular models for researching diabetes-related kidney damage is streptozotocin (STZ)-induced diabetic nephropathy. Through oxidative stress and DNA alkylation, streptozotocin specifically kills pancreatic β-cells, causing long-term hyperglycemia and metabolic problems (Furman, 2021). In the end, chronic hyperglycemia causes glomerular hypertrophy, mesangial expansion, thickening of the basement membrane, advanced glycation end-product (AGE) formation, activation of protein kinase C (PKC), oxidative stress, inflammation, and transforming growth factor-beta (TGF-β)-mediated fibrosis (Forbes & Cooper, 2013). This model is very useful for assessing antidiabetic and nephroprotective medications because histopathological results closely reflect early-stage diabetic nephropathy seen in humans. Reproducibility may be impacted by the strain, dosage, and duration of hyperglycemia, which can affect the severity of the disease (Tesch & Lim, 2011).

Hypertensive Nephrotoxicity

Spontaneously hypertensive rats (SHR), deoxycorticosterone acetate (DOCA)-salt models, and angiotensin II infusion models are frequently used to

simulate hypertension nephropathy. Increased intraglomerular pressure, endothelial dysfunction, oxidative stress, renin-angiotensin-aldosterone system (RAAS) activation, inflammation, and fibrosis are all consequences of persistent hypertension (Crowley & Coffman, 2012). Renal function is eventually hampered by progressive damage, which causes glomerulosclerosis, vascular remodelling, tubular atrophy, and interstitial fibrosis. When assessing antihypertensive, anti-inflammatory, and antifibrotic treatments meant to delay the course of CKD, these models are especially helpful. However, direct translational extrapolation may be limited by variations in animal and human cardiovascular physiology and blood pressure responses (Crowley & Coffman, 2012).

Obesity Associated Nephrotoxicity

Since obesity is a significant independent risk factor for chronic kidney disease globally, obesity-associated nephropathy has become a significant model. Metabolic syndrome models, high-fat diets, and hereditary obesity models are frequently used to produce experimental obesity(Kovesdy et al., 2017).Insulin resistance, dyslipidemia, oxidative stress, persistent low-grade inflammation, and the activation of profibrotic pathways are all facilitated by excess adiposity. Glomerular hyperfiltration, podocyte damage, mesangial enlargement, proteinuria, and progressive renal fibrosis are the outcomes of these changes (D'Agati et al., 2016). The histological features of obesity-associated nephropathy include tubular damage, extracellular matrix buildup, glomerulomegaly, and localized segmental glomerulosclerosis. This model, which is increasingly being used to assess therapies aimed at obesity-related renal impairment, closely resembles metabolic kidney disease seen in humans. However, establishing severe pathology frequently necessitates longer experimental periods, which lengthens the study's duration and raises its cost (DeClèves & Sharma, 2015).

Compared to many acute toxicological models, disease-based nephropathy models offer greater translational value since they replicate the chronic pathological processes associated with human chronic kidney disease. The gold standard model for diabetic kidney disease is still streptozotocin-induced diabetic nephropathy, which is especially useful for examining hyperglycemia-mediated renal injury. While models of hypertensive nephropathy are helpful for researching hemodynamic stress, RAAS activation, and vascular remodelling, models of obesity-associated nephropathy allow for the study of the inflammatory and metabolic processes that connect obesity to chronic kidney disease. These models are more biologically variable than acute nephrotoxicity models and typically need longer induction times, despite their clinical relevance.

Abbreviations: AGE, advanced glycation end-products; PKC, protein kinase C; RAAS, renin–angiotensin–aldosterone system; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6.

Model	Primary Etiology	Major Mechanisms	Histopathological Features	Key Biomarkers	Advantages	Limitations	Translational Relevance
Streptozotocin-Induced Diabetic Nephropathy	Hyperglycemia due to β-cell destruction	AGE formation, oxidative stress, PKC activation, inflammation, fibrosis	Mesangial expansion, glomerular hypertrophy, basement membrane thickening	HbA1c, Albuminuria, Creatinine, TGF-β, Collagen IV	Gold-standard diabetic nephropathy model	Variable disease severity and progression	Very High
Hypertensive Nephropathy	Persistent hypertension	RAAS activation, oxidative stress, endothelial dysfunction, fibrosis	Glomerulosclerosis, vascular remodeling, tubular atrophy	Blood pressure, Creatinine, Angiotensin II, TGF-β	Mimics hypertensive CKD progression	Species-specific cardiovascular differences	High
Obesity-Associated Nephropathy	Obesity and metabolic syndrome	Insulin resistance, oxidative stress, inflammation, lipotoxicity, fibrosis	Glomerulomegaly, proteinuria, focal segmental glomerulosclerosis	Proteinuria, TNF-α, IL-6, Leptin, Adiponectin	Closely resembles metabolic kidney disease	Long induction period and high variability	High

Table 4.Comparative evaluation of disease-based experimental nephropathy models highlighting their etiological factors, pathophysiological mechanisms, histopathological characteristics, biomarkers, advantages, limitations, and translational relevance for nephroprotection research.

Biomarkers used in Nephroprotection studies

In nephroprotection research, biomarkers are essential because they make it possible to identify, track, and predict kidney damage early on. Although traditional biomarkers like blood urea nitrogen (BUN) and serum creatinine are still commonly utilized, their incapacity to identify early kidney impairment has prompted the creation of more sensitive and targeted biomarkers. The translational success of nephroprotective therapies may be enhanced by emerging and molecular biomarkers, which offer important insights into renal pathophysiology (Vaidya et al., 2008; Dieterle et al., 2010).

Conventional Biomarkers

Serum Creatinine

]The most widely used measure of renal function is serum creatinine, which represents glomerular filtration rate (GFR). Both preclinical and clinical research frequently use elevated serum creatinine levels, which are indicative of compromised kidney function. Serum creatinine, however, is regarded as an insensitive test due to the possibility of substantial nephron loss prior to detectable alterations (Waikar & Bonventre, 2009).

Blood Urea Nitrogen (BUN)

Another conventional metric for evaluating renal function is blood urea nitrogen (BUN). Reduced renal clearance and compromised kidney function are typically indicated by elevated BUN values. However, the specificity of BUN can be limited by factors unrelated to kidney disease, such as dietary protein intake, liver function, and hydration (Stevens & Levey, 2005).

Limitations of Conventional Biomarkers

Serum creatinine and BUN are still common markers of renal failure, but they usually don't rise until significant kidney damage has taken place. Moreover, they are insufficient for early nephrotoxicity identification since they mostly represent functional impairment rather than direct tissue damage (Vaidya et al., 2008; Waikar & Bonventre, 2009).

Emerging Biomarkers

Kidney Injury Molecule-1 (KIM-1)

A transmembrane glycoprotein called KIM-1 is significantly expressed in proximal tubular cells that have been damaged. It has been approved by regulatory bodies for preclinical nephrotoxicity evaluation and is regarded as one of the most sensitive biomarkers of acute tubular injury (Bonventre, 2009).

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Within hours of kidney damage, NGAL can be found in both urine and plasma due to its quick release from injured tubular epithelial cells. As an early indicator of acute kidney damage (AKI), it is well known (Devarajan, 2010).

Cystatin-C

Cystatin-C is a low-molecular-weight protein which is filtered by the glomerulus and reabsorbed by proximal tubules. It provides a more accurate assessment of GFR and is less affected by age, sex, and muscle mass as Compared with creatinine (Laterza et al., 2002).

N-Acetyl-β-D-Glucosaminidase (NAG)

It is a lysosomal enzyme that is released from damaged proximal tubular cells. Elevated urinary levels of NAG indicates tubular injury which have been extensively used in experimental nephrotoxicity studies (Price, 1992).

Clusterin

Clusterin is a glycoprotein that helps with tissue remodelling and cellular defense. Elevated urine clusterin levels have demonstrated potential as an early nephrotoxicity biomarker and are linked to renal tubular damage (Dieterle et al., 2010).

Osteopontin

Osteopontin is an inflammatory glycoprotein that plays a role in tissue remodeling and immune cell recruitment. Increased levels are associated with renal fibrosis, inflammation, and the advancement of kidney damage (Lorenzen & Thum, 2012).

Molecular Biomarkers

MicroRNAs

Small non-coding RNAs known as microRNAs (miRNAs) control gene expression and are crucial for oxidative stress, renal inflammation, fibrosis, and apoptosis. Certain miRNAs, such as miR-21 and miR-192, have been identified as possible indicators of kidney damage and responsiveness to treatment (Kölling et al., 2017).

Proteomics

Proteomic methods allow for a thorough examination of the patterns of protein expression linked to nephrotoxicity. Proteomic biomarkers may enhance the mechanistic knowledge of nephroprotective therapies and aid in the early diagnosis of renal damage (Good et al., 2010).

Metabolomics

Metabolomics assesses changes in endogenous metabolites brought on by renal damage. Before traditional biomarkers become aberrant, alterations in amino acids, lipids, and energy metabolic pathways can offer early signs of nephrotoxicity (van Norman, 2019).

Biomarker	Sensitivity	Specificity	Early Detection	Clinical Translation
Serum Creatinine	Low	Moderate	Poor	Excellent
BUN	Low	Low–Moderate	Poor	Excellent
KIM-1	High	High	Excellent	High
NGAL	High	High	Excellent	High
Cystatin-C	High	High	Good	High
NAG	Moderate–High	Moderate	Good	Moderate
Clusterin	High	Moderate–High	Good	Moderate
Osteopontin	Moderate–High	Moderate	Good	Emerging
microRNAs	Very High	High	Excellent	Emerging

Data co

Table 5. Comparative performance of conventional, emerging, and molecular biomarkers used for detection and monitoring of nephrotoxicity and evaluation of nephroprotective interventions.

Translational Relevance of Experimental Nephrotoxicity Models

The ultimate goal of experimental nephrotoxicity models is to make it easier to convert preclinical discoveries into successful clinical treatments for kidney damage prevention or treatment. There has been little successful transfer from animal research to clinical treatment, despite significant advancements in our knowledge of renal pathophysiology and the discovery of multiple nephroprotective drugs. The complexity, variability, and multivariate character of kidney disorders in humans cannot be adequately replicated by a single experimental model, which presents a significant challenge. Thus, while choosing experimental models and interpreting preclinical results, translational relevance must be carefully taken into account (Mak et al., 2014; van Norman, 2019).

The ability of experimental nephrotoxicity models to mimic human renal diseases varies widely. Nephroprotective substances are frequently screened using drug-induced models, such as cisplatin and gentamicin nephrotoxicity, which closely match acute tubular injury seen in clinical settings. Because they replicate the persistent metabolic and hemodynamic disorders that cause renal fibrosis and functional decline, disease-based models—such as streptozotocin-induced diabetic nephropathy and hypertensive nephropathy—better capture the chronic and progressive nature of human chronic kidney disease (CKD) (Pabla & Dong, 2008; Breyer et al., 2005). However, human kidney disorders usually develop gradually over months or years, whereas experimental models frequently use high dosages of nephrotoxic drugs and cause renal impairment over relatively short periods of time. Moreover, various comorbidities such diabetes, hypertension, obesity, cardiovascular disease, age, and polypharmacy are often present in clinical nephropathies; these conditions are rarely portrayed concurrently in animal models. Preclinical efficacy may therefore overstate clinical benefit (Eddy et al., 2012).

Clinical failure of promising nephroprotective agents

Many drugs failed during clinical development while showing notable nephroprotective benefits in preclinical investigations. This translational gap is caused by a number of variables. First, while people with kidney disease are highly variable and may present with numerous underlying diseases, preclinical research often uses young, healthy animals with little biological heterogeneity. Second, while treatment in clinical practice typically starts after severe kidney damage has already occurred, nephroprotective drugs are frequently given before or just after renal injury induction in experimental research. Third, disparities between preclinical and clinical results may be caused by variations in study design, endpoint selection, and methodological quality (Perazella, 2018; Himmelfarb & Ikizler, 2007). Additionally, preferential reporting of favorable results due to publication bias may exaggerate the perceived effectiveness of nephroprotective therapies. As a result, many drugs that seem to be very successful in lab conditions do not show clinically significant advantages in human studies (Sena et al., 2010).

Species Differences

One of the biggest obstacles to effective translation is species-specific variations. Humans and frequently used lab animals differ significantly in terms of renal anatomy, physiology, immunological responses, transporter expression, and nephrotoxin susceptibility. For instance, variations in the expression of the organic cation transporter (OCT) and organic anion transporter (OAT) can affect the renal absorption and toxicity of medications like tenofovir and cisplatin (Ciarimboli, 2014).

Pharmacokinetic Differences

Pharmacokinetic differences between experimental animals and humans represent an important limitation in translating nephrotoxicity findings to clinical settings. The concentration of nephrotoxic substances that reach renal tissues can be affected by variations in absorption, distribution, metabolism, and excretion, which can change systemic and renal drug exposure. Tubular drug uptake and elimination are significantly influenced by renal drug transporters, such as organic anion transporters (OAT1 and OAT3), organic cation transporter 2 (OCT2), and multidrug and toxin extrusion transporters (MATE1 and MATE2-K). However, these transporters' expression and function differ significantly between human and animal species, which may have an impact on intracellular accumulation and the

degree of renal damage (Zou et al., 2018). Examples of how species-dependent transporter activity might impact renal toxicity include tenofovir and related antiviral drugs, while variations in renal organic cation transport may also have an impact on cisplatin nephrotoxicity. Direct comparison of animal doses with human therapeutic doses is further complicated by variations in renal clearance, plasma protein binding, and systemic exposure. Therefore, when interpreting experimental nephrotoxicity investigations, pharmacokinetic characteristics such area under the concentration–time curve (AUC), maximum plasma concentration (Cmax), renal clearance, urine excretion, and renal tissue concentrations should be taken into account(Basit et al., 2019). The prediction of human nephrotoxicity may be improved by combining pharmacokinetic data with kidney biomarkers, histopathological results, and human-relevant in vitro models. Therefore, in order to properly choose and understand experimental nephrotoxicity models and to increase their translational relevance, species-specific pharmacokinetic features must be taken into account(Jansen et al., 2020; Ailabouni & Prasad, 2025).

Biomarker translation challenges

As traditional markers like serum creatinine and blood urea nitrogen (BUN) may only rise after significant loss of renal function, biomarkers are being utilized more frequently for the early identification of renal damage (Vaidya et al., 2008). Tubular injury and cellular stress can be detected earlier by novel biomarkers such as kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and tissue inhibitor of metalloproteinase-2/insulin-like growth factor-binding protein 7 (TIMP-2/IGFBP7) (Vaidya et al., 2008; Schmidt-Ott & Paragas, 2012; Devarajan, 2010).

Emerging Alternatives to Traditional Animal Models.
Kidney Organoids

To improve the human relevance of nephrotoxicity testing and get over the drawbacks of traditional animal models, new alternatives are being created. Aspects of renal tubular and glomerular architecture can be replicated in three-dimensional (3D) kidney cultures and human kidney organoids, which also enable assessment of drug-induced damage in human-derived cells (Low et al., 2019; Takasato et al., 2015). More physiologically relevant evaluation of nephrotoxicity is made possible by kidney-on-a-chip and renal microphysiological systems, which offer regulated microenvironments with fluid flow and cell-cell interactions (Devarajan, 2010; Vaidya et al., 2008). Renal cells and organoids produced from human induced pluripotent stem cells (iPSCs) offer patient-relevant platforms for researching medication responses and disease processes (Devarajan, 2010; Bonventre et al., 2010). Furthermore, it is challenging to ascertain if biomarker changes appropriately reflect structural kidney injury because the majority of clinical investigations do not show a direct association between biomarker concentrations and renal histology (Bonventre et al., 2010; Dieterle et al., 2010). Comparing different research is additionally hampered by variations in sample duration, assay platforms, normalization techniques, and diagnostic cut-off values (Vaidya et al., 2008; Bonventre et al., 2010). Moreover, a single biomarker would not fully capture the complexity of renal damage since nephrotoxicity comprises several interrelated pathways (Devarajan, 2010; Bonventre et al., 2010). Better sensitivity and translational relevance could be achieved using a multimarker strategy that includes functional, structural, and mechanistic biomarkers (Schmidt-Ott & Paragas, 2012; Devarajan, 2010). Although more extensive clinical validation is still needed, regulatory approval of biomarkers like KIM-1 has supported their usage in preclinical and drug-development investigations (Devarajan, 2010; Dieterle et al., 2010). Therefore, improved biomarker translation requires standardized analytical techniques, clinically relevant cut-off values, longitudinal validation, and connection with significant clinical outcomes (Dieterle et al., 2010; Ozer et al., 2010). The predictive efficacy of nephrotoxicity studies may be further enhanced by combining biomarkers with pharmacokinetic data, histological evaluation, and human-relevant experimental models (Vaidya et al., 2008; Bonventre et al., 2010).

Kidney-on-a-Chip: Microfluidic Systems

Microfluidic platforms called kidney-on-a-chip devices are made to mimic key elements of human kidney physiology in carefully regulated lab settings. Human renal tubular epithelial cells grown in microfluidic channels that offer constant fluid flow and mechanical stimulation are frequently used in these systems (Jang & Suh, 2010; Jang et al., 2013). Microfluidic systems can more accurately simulate tubular architecture, shear stress, cell-cell interactions, and kidney transport processes than traditional static cell cultures (Jang & Suh, 2010; Musah et al., 2017). Drug-induced nephrotoxicity, namely tubular damage and alterations in renal transport and barrier function, has been studied using kidney-on-a-chip models (Jang et al., 2013; Wilmer et al., 2016). They may also make it possible to measure indicators of kidney injury and track cellular reactions in real time. It may be possible to decrease reliance on animal models and increase species relevance by using human or patient-derived cells (Musah et al., 2017; Wilmer et al., 2016). However, there are obstacles, such as the necessity for validated methods, cell-source heterogeneity, insufficient uniformity across platforms, and limitations in replicating the entire complexity of the kidney (Wilmer et al., 2016). Notwithstanding these drawbacks, kidney-on-a-chip technology offers a viable substitute or supplement to conventional animal models for drug development and nephrotoxicity screening.

3D Bioprinting

A new technology called 3D bioprinting creates tissue-like kidney structures by depositing biomaterials and living cells under computer control. Compared to traditional two-dimensional cell cultures, it can offer a three-dimensional microenvironment that more closely mimics the native kidney (Murphy & Atala, 2014; Kacarevic et al., 2018). It

may be possible to replicate elements of nephron architecture and cell-cell interactions in printed constructions by incorporating several renal cell types, such as tubular epithelial cells, endothelial cells, and podocytes (Kacarevic et al., 2018; Homan et al., 2016). Researching drug-induced nephrotoxicity, cellular reactions, and causes of renal injury may benefit from the use of 3D bioprinted renal tissues (Homan et al., 2016; Mao et al., 2020). Additionally, the technique allows for the controlled spatial arrangement of extracellular matrix components and cells, which may enhance the physiological relevance of experimental models (Murphy & Atala, 2014; Mao et al., 2020). These systems' applicability for customized drug testing can be further increased by using human and patient-derived cells (Kacarevic et al., 2018; Murphy & Atala, 2014). Achieving proper vascularization, preserving long-term cell viability and differentiation, choosing appropriate bioinks, and replicating the intricate structure and function of the kidney are obstacles, albeit (Homan et al., 2016; Murphy & Atala, 2014). Prior to broad use, standardization, repeatability, and validation against recognized nephrotoxicity models are also necessary. All things considered, 3D bioprinting is a potentially useful supplementary method for creating renal models that are applicable to humans and lessening reliance on conventional animal testing.

Humanized Models

In order to increase animal models' relevance to human disease and treatment reactions, humanized models are experimental systems that include human cells, tissues, genes, or physiological traits (Shultz et al., 2014). Humanized models can be used in nephrotoxicity studies to address species-specific variations in renal transporters, metabolism, immunological responses, and medication susceptibility (Prasad et al., 2016). To study drug-induced kidney damage and toxicity pathways, human renal cells or tissues can be added to appropriate experimental systems (Takebe et al., 2018). Compared to traditional animal models, these models may offer more clinically relevant data on drug disposition, transporter-mediated uptake, and cellular responses (Shultz et al., 2014; Freedman et al., 2015). Interactions between human renal tissues and systemic variables can also be investigated using humanized mice and other chimera systems (Takebe et al., 2018). The predictive capability of human-derived cells may be further enhanced by the integration of organoid and microphysiological technologies (Freedman et al., 2015; Wilmer et al., 2016). High cost, technological complexity, inadequate humanization, model variability, and challenges in replicating the entire human renal environment are some of the shortcomings, though (Shultz et al., 2014; Wilmer et al., 2016). Regulatory and ethical issues must also be taken into account. All things considered, humanized models offer a viable supplementary strategy forenhancing the translational significance of experimental nephrotoxicity investigations while lessening some of the drawbacks of traditional animal models.

Artificial Intelligence and Machine Learning

Emerging computational methods like artificial intelligence (AI) and machine learning (ML) can help identify substances with potential kidney toxicity and forecast drug-induced nephrotoxicity. To find intricate patterns linked to kidney damage, these techniques can combine sizable datasets that include chemical structures, pharmacokinetic traits, molecular targets, biomarkers, and experimental toxicity data (Freedman et al., 2015; Ekins et al., 2019). Prior to comprehensive experimental testing, nephrotoxic potential can be predicted using machine-learning algorithms trained on previously identified substances. Important biochemical pathways, toxicity-associated biomarkers, and drug-target interactions implicated in renal injury may also be identified with the help of AI-based models (Ekins et al., 2019; Vora et al., 2023). Predicting human nephrotoxicity may be further improved by integrating AI with omics data, kidney organoids, high-throughput screening, and kidney-on-a-chip platforms (Vora et al., 2023; Wilmer et al., 2016). These methods can help prioritize substances for additional research and minimize the number of experimental animals needed. However, the size, diversity, and quality of the accessible datasets have a significant impact on their accuracy (Freedman et al., 2015; Wilmer et al., 2016). Model interpretability, discrepancies between training and real-world datasets, and the requirement for independent experimental and clinical validation are further constraints. Therefore, rather than serving as whole substitutes for experimental nephrotoxicity models, AI and ML should now be viewed as complimentary techniques. Future nephrotoxicity screening may be more effective and have greater translational potential if computational predictions are combined with validated biomarkers and human-relevant in vitro models.

Research Gaps and Future Directions

There are still a number of significant gaps in experimental nephrotoxicity research, which restricts the findings' repeatability and therapeutic use. Comparing research is challenging due to inconsistencies in animal species, nephrotoxicant dosage, exposure length, sampling time, outcome measurements, and biomarker selection (Hooijmans et al., 2014; Mahadeo et al., 2022). Variations in experimental procedures, cell sources, culture conditions, analytical techniques, and reporting standards are also linked to poor reproducibility. The necessity for standardized experimental and reporting frameworks is highlighted by recent evidence from human proximal-tubule in-vitro investigations that demonstrates significant variance in biomarker selection and reporting (Rodríguez-Marcano et al., 2026). Another significant limitation is the lack of adequate biomarkers. While more recent biomarkers like KIM-1, NGAL, and TIMP-2/IGFBP7 may have issues with specificity, timing, and disease context, more traditional markers like serum creatinine and BUN may detect renal dysfunction relatively late (Vaidya et al., 2008; Bonventre et al., 2010). Moreover, a large number of biomarkers found in research on animals have not received enough validation in

human populations (Ostermann et al., 2020). Interspecies variations in renal morphology, drug transporters, pharmacokinetics, immunological responses, and vulnerability to nephrotoxic damage lead to limited human translation (Qin et al., 2024). Therefore, standardized procedures, validated multimarker panels, kidney-on-a-chip systems, organoids, human-derived kidney models, and the integration of pharmacokinetic and molecular data should be the main areas of future research. Human nephrotoxicity prediction and repeatability may be enhanced by combining complimentary models rather than depending on a single experimental system (Weber et al., 2017; Wilmer et al., 2016).

2. CONCLUSION

Although no single model can accurately replicate the complexities of human kidney disease, experimental nephrotoxicity models are crucial for examining renal damage pathways and evaluating nephroprotective medicines. While disease-based models like diabetic and hypertensive nephropathy are better suited for chronic renal injury, drug-induced models like cisplatin and gentamicin are helpful for acute tubular injury. While KIM-1, NGAL, cystatin-C, and other new molecular biomarkers offer complementary information, conventional biomarkers like blood creatinine and BUN have low sensitivity for early damage. As a result, the particular kind and mechanism of renal injury under investigation should be taken into consideration while choosing models and biomarkers.

Future research on nephroprotection should incorporate pharmacokinetic analysis, histopathology, and multimarker evaluation while concentrating on consistent and repeatable experimental procedures. To increase translational relevance and uphold the 3Rs principle, human-relevant techniques such as kidney organoids, 3D cultures, kidney-on-a-chip systems, humanized models, 3D bioprinting, and artificial intelligence-based prediction should be used more frequently. An integrated approach that combines proven biomarkers with complementing animal and human-relevant models may help close the gap between preclinical research and human application and enhance the prediction of clinical nephroprotective efficacy.

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