J Nephropharmacol. 2027; 16(1): e12792.

https://jnephropharmacology.com DOI: 10.34172/npj.12792

Journal of Nephropharmacology

Therapeutic effect of semapimod on renal ischemia/reperfusion injury via necroptosis pathway inhibition in male rats; an experimental animal study

Ali F. Jaber[1], **Ali M. Janabi**[2*]

[1]Imam Hussain Medical City, Karbala Health Directorate, Karbala, Iraq
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq

ARTICLE INFO

Article Type:
Original

Article History:
Received: 4 May 2025
Revised: 24 Oct. 2025
Accepted: 26 Jun. 2026
ePublished: 17 Aug. 2026

Keywords:
Semapimod
Kidney injury
Inflammation
Necroptosis
Tumor necrosis factor-alpha
Kidney injury molecule-1

ABSTRACT

Introduction: Renal ischemia-reperfusion (I/R) injury is a critical contributor to acute kidney injury (AKI) and chronic kidney disease (CKD), driven by oxidative stress, inflammation, and regulated cell death pathways such as necroptosis.

Objectives: This study investigates the therapeutic potential of semapimod, a macrophage inhibitor and cholinergic anti-inflammatory agent, in mitigating renal I/R injury by targeting necroptosis in male rats.

Materials and Methods: In this experimental animal study at the university of Kufa, Iraq, 24 male Sprague Dawley rats (about 8 weeks old, ~200 g) were housed under controlled conditions and randomly assigned to sham, I/R, vehicle+I/R (veh + I/R), and semapimod hydrochloride HCl + I/R (SPD + I/R) groups. In this study, I/R, veh+I/R, and SPD + I/R groups underwent a model of I/R consisting of 30 minutes of ischemia and 24 hours of reperfusion. The SPD+I/R group receives 10 mg/kg of semapimod HCl, and the veh+I/R group receives the solvent of semapimod HCl by intraperitoneal route, one hour before the induction of ischemia. The sham group underwent laparotomy I/R and received nothing. After anesthesia rates were sacrificed and kidneys were collected for histopathology, immunohistochemistry (mixed lineage kinase domain-like pseudokinase [MLKL]), and molecular analyses for kidney injury molecule-1 (KIM-1), tumor necrosis factor-α (TNF-α), caspase-3, and receptor-interacting protein kinase 1 (RIPK1), with all assessments were conducted to evaluate renal injury, inflammation, and necroptosis/apoptosis markers. Data were collected and compared between four groups.

Results: The results demonstrated that semapimod pretreatment attenuates renal I/R injury through multimodal modulation of inflammatory, necroptotic, and apoptotic pathways. Inflammatory markers KIM-1 and TNF-α were markedly elevated in I/R and vehicle-treated groups, with semapimod significantly reducing both biomarkers. Necroptotic markers RIPK1 and MLKL exhibited robust upregulation in I/R injury, which persisted in vehicle-treated rats but were substantially normalized by semapimod. Similarly, caspase-3 activity and histopathological damage scores, reflective of apoptotic and structural injury, peaked in I/R and vehicle groups, while semapimod restored caspase-3 to near-sham levels and mitigated tubular necrosis, though residual histological damage persisted.

Conclusion: These results collectively position semapimod as a promising therapeutic agent that concurrently dampens necroptosis, apoptosis, and inflammation, with normalization of injury biomarkers, emphasizing its efficacy in preserving renal architecture and function post-I/R insult.

Implication for health policy/practice/research/medical education:
In this experimental animal study, semapimod emerged as a multifunctional therapeutic agent that effectively attenuates renal ischemia-reperfusion injury by simultaneously modulating inflammatory responses, necroptotic signaling, and apoptotic pathways.

Please cite this paper as: Jaber AF, Janabi AM. Therapeutic effect of semapimod on renal ischemia/reperfusion injury via necroptosis pathway inhibition in male rats; an experimental animal study. J Nephropharmacol. 2027;16(1):e12792. DOI: 10.34172/npj.12792.

***Corresponding author**: Ali M. Janabi, Email: alim.hashim@uokufa.edu.iq

Original

Introduction

Acute kidney injury (AKI) represents a critical clinical challenge affecting millions of patients worldwide, with an estimated 13 million people impacted annually on a global scale (1). This heterogeneous syndrome encompasses a broad spectrum of renal function deterioration, ranging from subtle changes in serum creatinine levels to severe conditions requiring renal replacement therapy (2). In critically ill patients, AKI occurs with alarming frequency, affecting 30-70% of patients and representing one of the most common complications following hospital admission (1). The clinical significance of AKI extends far beyond immediate morbidity, as patients experiencing AKI face substantially elevated mortality rates, with studies demonstrating mortality rates of 41.8% in patients with AKI compared to 14% in those without this complication (3). Furthermore, AKI survivors are at increased risk for long-term adverse outcomes, including the development or progression of chronic kidney disease (CKD), cardiovascular events, and reduced overall survival (4,5).

Ischemia-reperfusion (I/R) injury stands as one of the predominant mechanisms underlying AKI, accounting for approximately 66% of all AKI cases through acute tubular necrosis. This pathophysiological process is characterized by temporary oxygen deficiency resulting from restricted blood flow, followed by the sudden restoration of oxygen supply, which paradoxically exacerbates tissue damage (6). Renal I/R injury involves a complex cascade of events, including robust inflammatory responses, oxidative stress, microvascular dysregulation, and cellular injury that collectively disturb organ function (7). The injury occurs in various clinical scenarios such as shock, major surgery, organ transplantation, and other conditions where blood flow to the kidney is temporarily compromised (2). Despite advances in understanding the molecular mechanisms of I/R injury, including the recognition that damage primarily affects proximal tubular epithelial cells, effective therapeutic interventions remain limited, with current management largely consisting of conservative measures such as removing causative factors, managing fluid and electrolyte imbalances, and providing supportive care (2,8).

Semapimod, also known as CNI-1493 (9), represents a promising therapeutic compound with demonstrated anti-inflammatory properties that may offer novel treatment approaches for I/R injury. This tetravalent guanylhydrazone molecule has been shown to inhibit systemic inflammation and stimulate the cholinergic anti-inflammatory pathway, effectively attenuating tumor necrosis factor synthesis through interactions with nicotinic acetylcholine receptors on macrophages (10). The drug's selective interference with macrophage and microglial function has been demonstrated in experimental models, where it potently inhibits inflammatory cell-mediated tissue damage (11). Given the central role of inflammation and immune dysregulation in the pathogenesis of renal I/R injury, and the emerging understanding that regulated cell death pathways, including necroptosis, contribute significantly to I/R injury-induced AKI (8), semapimod's anti-inflammatory mechanisms present a compelling therapeutic target for mitigating renal I/R injury through modulation of these critical pathophysiological processes.

Objectives

The objective of this study was to investigate the protective effects of semapimod against renal I/R injury in a rat model, specifically by evaluating its ability to modulate inflammatory, necroptotic, and apoptotic pathways through the analysis of biomarker profiles and histopathological changes.

Materials and Methods

Study design and samples

In this experimental animal study, twenty-four male Sprague Dawley rats, each approximately 8 weeks of age and weighing around 200 g, were procured from the Faculty of Sciences at the University of Kufa, Iraq, in 2024, for use in the present study. These animals were selected based on their standardized age and weight to ensure consistency and minimize variability in experimental outcomes.

Animal preparation and experimental design

Rats were maintained in individual cages, with unrestricted access to water and food under regulated conditions at a temperature of 25 ± 2 °C. Calculation of the sample size was conducted by the resource equation method (12). After the end of the one-week acclimatization phase, rats were randomly grouped into sham, I/R, vehicle+I/R (veh + I/R), and semapimod hydrochloride HCl + I/R (SPD + I/R) groups. In this study, I/R, veh+I/R, and SPD+I/R groups underwent a model of I/R consisting of 30 minutes of ischemia and 24 hours of reperfusion. The SPD+I/R group receives 10 mg/kg of semapimod HCl (13) (CAS No.: 164301-51-3, MedChemExpress, USA), and the veh+I/R group receives the solvent of semapimod HCl (10% of dimethyl sulfoxide (DMSO) (CAS No.: 67-68-5, MW: 78.13, CDH, India)) by intraperitoneal route, one hour before the induction of ischemia. The sham group underwent laparotomy I/R and received nothing.

Ischemia/reperfusion injury procedure

The renal I/R injury model was conducted according to established protocols (14). Under pathogen-free conditions, rats were anesthetized via intraperitoneal injection of ketamine (100 mg/kg; Alfasan, Netherlands) and xylazine (10 mg/kg; Alfasan, Netherlands) (15). Following midline laparotomy, bilateral renal ischemia was induced by applying non-traumatic microvascular clamps to both renal pedicles. Ischemia was maintained for 30 minutes, after which clamps were removed to restore blood flow, initiating a 24-hour reperfusion period.

All surgical procedures were performed under aseptic conditions to minimize infection risk. After completing the reperfusion phase, rats were humanely euthanized under deep anesthesia following institutional animal care guidelines (16).

Tissue collection

For this study, both kidneys were harvested immediately following the experimental protocol. Renal tissues intended for immunohistochemistry (IHC) and hematoxylin and eosin (H&E) staining were fixed in 10% neutral buffered formalin to preserve cellular architecture. In contrast, tissues designated for quantitative reverse transcription polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA) analysis were rapidly frozen in liquid nitrogen and stored at ultra-low temperatures to maintain RNA and protein integrity for subsequent measurements and molecular assessments.

ELISA technique

For the analysis of key renal injury and inflammatory markers, the supernatant was collected and used to quantify the levels of kidney injury molecule-1 (KIM-1), tumor necrosis factor-α (TNF-α), and caspase-3 using commercially available rat-specific ELISA kits (KIM-1: Cat. No. SL0433Ra; TNF-α: Cat. No. SL0722Ra; caspase-3: Cat. No. SL0152Ra; Sunlong, China). Each ELISA was performed according to the manufacturer's instructions, enabling sensitive and specific detection of these proteins in the renal samples.

RT-qPCR analysis

Total RNA was extracted from renal tissues using the Easy-spin™ RNA purification kit (Intron, Cat. No. 17221), followed by cDNA synthesis with the AddScript cDNA Synthesis Kit (Addbio, Cat. No. 22701). Quantitative reverse transcription PCR (RT-qPCR) analysis was performed using the GoTaq® RT-qPCR System (Promega, Cat. No. A6020) to measure receptor-interacting protein kinase 1 (RIPK1) mRNA expression levels, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) serving as the endogenous control for data normalization. Relative gene expression quantification was calculated using the $2^{-\Delta\Delta CT}$ method, which compares threshold cycle values between experimental and control groups to determine fold changes in transcriptional activity.

HE and IHC examination

Histopathological examination by H&E staining was conducted through multiple tissue processing stages for the formalin-fixed renal tissue according to Yahiya et al study (17). For preparing IHC staining, paraffin-embedded tissue was deparaffinized using xylene, rehydrated with descending ethanol concentration, and washed with distilled water. Next, antigen retrieval was done by incubating the slides with the retrieval solution, cooled, and washed with the buffer solution. To prevent nonspecific binding, a blocking agent (peroxidase) was used, followed by adding the post-protein block. The primary anti mixed lineage kinase domain-like pseudokinase (MLKL) (Cat. No. E-AB-67102, dilution 1:100, Elabscience, China) was added, incubated, and then washed. Subsequently, the secondary antibody was applied, followed by adding horseradish peroxidase, chromogen, and the counterstain (hematoxylin). The damage score for the histopathological examination was measured as in Hassoon et al study (18), while H-score for IHC was assessed as in Charafe-Jauffret et al (19) and Parris et al (20) studies. A histopathologist blinded to the study examined the slides and evaluated the scores.

Outcomes

The primary outcomes of this study included assessment of renal injury severity through histopathological scoring, measurement of inflammatory biomarkers such as KIM-1 and TNF-α, and evaluation of necroptosis pathway modulation via RIPK1 and MLKL expression. Secondary outcomes focused on apoptotic activity as indicated by caspase-3 levels, comparative efficacy of semapimod in attenuating necroptotic versus apoptotic signaling, and the persistence of residual structural damage despite treatment. Together, these outcomes were chosen to comprehensively evaluate semapimod's protective effects on renal I/R injury by targeting multiple interconnected pathways involved in inflammation, programmed cell death, and tissue preservation.

Statistical analysis

Statistical analysis was carried out by GraphPad Prism version 10.0 and IBM SPSS Statistics version 27.0 (IBM Corp, USA). Data were initially tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Bartlett's test. Parametric data were examined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. In contrast, nonparametric data (histopathological scores) were analyzed using the Kruskal–Wallis test, and pairwise comparisons were conducted by the Mann–Whitney U test. Results are presented as mean ± standard deviation (SD), and a P value < 0.05 was considered statistically significant. Bar graphs were generated by GraphPad Prism 10.0.

Results

This study included twenty-four male Sprague Dawley rats, each weighing approximately 200 grams and about eight weeks old. The rats were divided into four groups of six; sham, I/R, I/R+ vehicle, and I/R+semapimod. The comparative analysis of renal damage and inflammatory markers across experimental groups revealed distinct patterns in both KIM-1 and TNF-α levels. For KIM-1, the sham group exhibited the lowest expression, while I/R and I/R+vehicle groups demonstrated significantly

elevated levels, with no marked difference between these two injury groups. In contrast, I/R+semapimod group showed a substantial reduction in KIM-1 compared to I/R and vehicle-treated groups, though levels remained higher than the sham group. Similarly, TNF-α levels were lowest in the sham group and elevated in both I/R and I/R+vehicle cohorts, which were statistically indistinguishable. The semapimod-treated group displayed a pronounced decrease in TNF-α relative to the I/R and vehicle groups, approaching sham levels without full normalization. Notably, the effect of semapimod on TNF-α attenuation was less pronounced compared to its impact on KIM-1, suggesting differential modulation of inflammatory and direct injury pathways. These trends highlight semapimod's partial but significant mitigation of renal damage and inflammation, particularly in attenuating injury-associated biomarker elevation (Table 1 and Figure 1).

The comparative analysis of necroptosis pathway markers across experimental groups revealed distinct modulation patterns in RIPK1 and MLKL expression. For RIPK1, the sham group exhibited baseline expression levels, which were significantly elevated in the I/R group. Pretreatment with semapimod (I/R+SPD) substantially attenuated RIPK1 expression compared to both I/R and I/R+vehicle groups, though levels remained above sham values. Notably, the vehicle-treated group showed no meaningful reduction in RIPK1 relative to untreated I/R, underscoring semapimod's targeted inhibitory effect. A similar trend was observed for MLKL, a downstream effector of necroptosis, with sham animals demonstrating minimal staining intensity. The I/R group displayed a pronounced increase in MLKL activation, which persisted in the vehicle-treated cohort but was markedly reduced by semapimod pretreatment, nearly normalizing to sham levels (Table 2, Figures 2 & 3).

The analysis of cellular death markers across experimental groups revealed distinct patterns in caspase-3 activity and histopathological renal damage. Caspase-3 levels were lowest in the sham group, with a marked elevation observed in both I/R and I/R+vehicle cohorts, which were statistically significant. Semapimod pretreatment (I/R+SPD) significantly attenuated caspase-3 activity compared to untreated and vehicle-treated I/R groups, restoring levels to near-sham values. Histopathological scoring mirrored these trends, with sham animals showing no structural abnormalities, while I/R and vehicle groups exhibited severe tubular injury characterized by increasing histopathological scores. Semapimod pretreatment substantially reduced histopathological damage, though residual injury persisted compared to sham controls (Table 3 and Figure 4).

In the sham group, histopathological staining showed that rat renal tubules exhibited normal histology (Figure 5A). In the I/R group, histopathological staining indicated that rat renal tubules exhibited score 4 damage, affecting 80% of the studied tubules (Figure 5B). In the Veh + I/R group, histopathological staining demonstrated that rat

Table 1. Comparative analysis of renal damage and inflammatory markers among treatment groups

	Group		Mean	SD	*P* value*
KIM-1 (pg/mL)	Sham		1310.67	85.89	<0.001
	IR		1988.28	177.94	
	I/R + Veh		2109.73	169.09	
	I/R + SPD		1695.06	101.98	
	Group		**Mean difference**		***P* value****
	Sham	I/R	677.61		<0.001
		I/R + Veh	799.06		<0.001
		I/R + SPD	384.38		<0.001
	IR	I/R + Veh	121.45		0.453
		I/R + SPD	293.22		0.008
	I/R + Veh	I/R + SPD	414.67		<0.001
	Group		**Mean**	**SD**	***P* value***
TNF-α (ng/L)	Sham		367.09	69.06	<0.001
	IR		514.74	24.07	
	I/R + Veh		507.87	25.81	
	I/R + SPD		411.99	62.86	
	Group		**Mean difference**		***P* value****
	Sham	I/R	147.65		<0.001
		I/R + Veh	140.78		<0.001
		I/R + SPD	44.90		0.424
	IR	I/R + Veh	6.87		0.995
		I/R + SPD	102.75		0.010
	I/R + Veh	I/R + SPD	140.78		0.016

Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod; SD: Standard deviation; KIM-1: Kidney injury molecule-1; TNF-α: Tumor necrosis factor alpha. *One-way ANOVA, **Post hoc Tukey test.

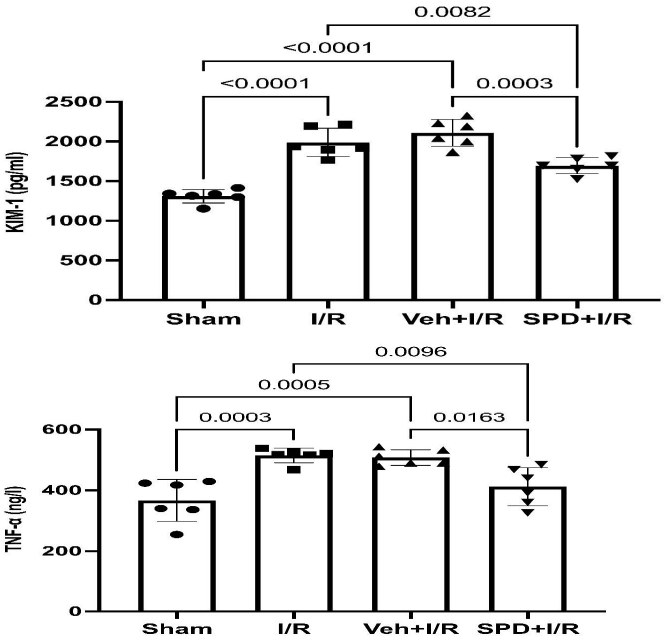


Figure 1. Comparison of the renal damage and inflammatory markers among the treatment groups. Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod; KIM-1: Kidney injury molecule-1; TNF-α: Tumor necrosis factor alpha.

renal tubules exhibited score 4 damage, which affected 80% of the studied tubules (Figure 5C). In the SPD+I/R group, histopathological staining found that rat renal tubules exhibited score 1 damage, which affected 25% of the studied tubules (Figure 5D).

Discussion

The findings indicated that semapimod pretreatment effectively reduces renal I/R injury by targeting multiple pathways, including inflammation, necroptosis, and apoptosis. Inflammatory markers such as KIM-1 and

Table 2. Comparative analysis of necroptosis pathway markers among treatment groups

	Group		Mean	SD	*P* value*
RIPK1 ($2^{-\Delta\Delta CT}$)	Sham		1.00	0.00	<0.001
	IR		4.14	0.31	
	I/R + Veh		3.36	0.91	
	I/R + SPD		2.34	0.46	
	Group		**Mean difference**		***P* value****
	Sham	I/R	3.14		<0.001
		I/R + Veh	2.36		<0.001
		I/R + SPD	1.34		0.002
	IR	I/R + Veh	0.77		0.088
		I/R + SPD	1.80		<0.001
	I/R + Veh	I/R + SPD	1.02		0.016
	Group		**Mean**	**SD**	***P* value***
MLKL (H-score)	Sham		35.50	17.40	<0.001
	IR		177.16	45.69	
	I/R + Veh		160.66	37.45	
	I/R + SPD		60.50	52.87	
	Group		**Mean difference**		***P* value****
	Sham	I/R	141.66		<0.001
		I/R + Veh	125.16		<0.001
		I/R + SPD	25.00		0.713
	IR	I/R + Veh	16.50		0.894
		I/R + SPD	116.66		<0.001
	I/R + Veh	I/R + SPD	100.16		0.002

Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod; SD: Standard deviation; RIPK1: Receptor-interacting protein kinase 1; MIKL: Mixed lineage kinase domain-like pseudokinase. *One-way ANOVA, **Post hoc Tukey test

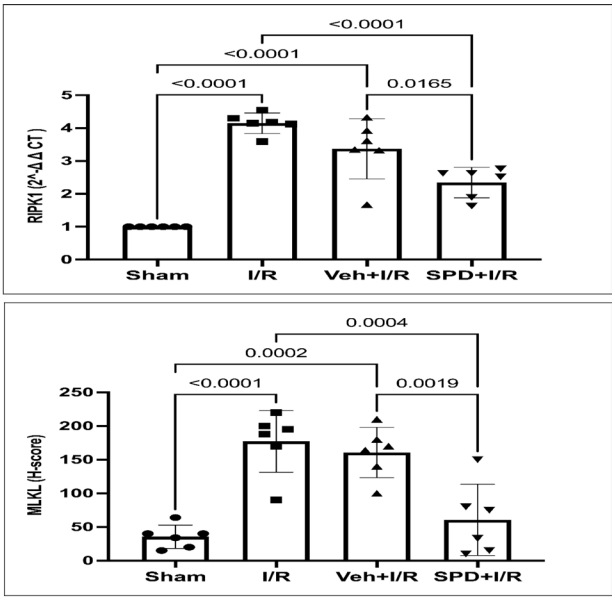
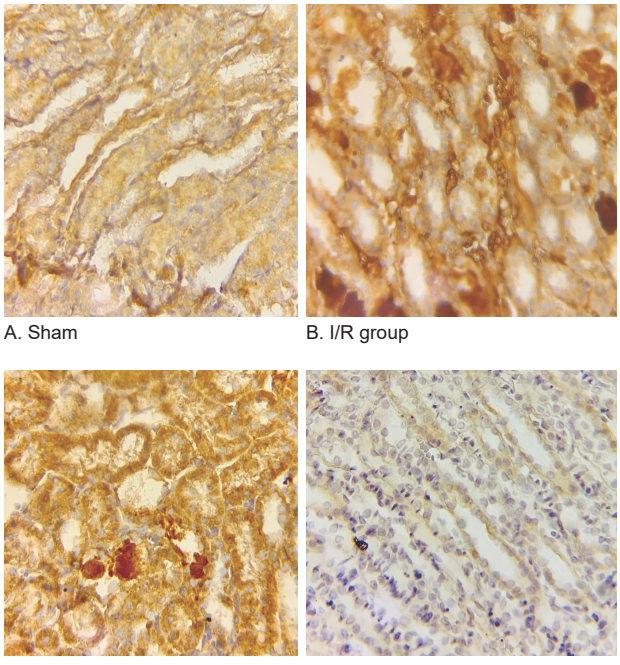


Figure 2. Comparison of pathway markers among treatment groups. Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod; RIPK1: Receptor-interacting protein kinase 1; MIKL: Mixed lineage kinase domain-like pseudokinase.

TNF-α were significantly elevated in both the I/R and vehicle-treated groups, but semapimod treatment led to a notable decrease in these biomarkers. Necroptosis-related proteins RIPK1 and MLKL were strongly upregulated following I/R injury and remained high in the vehicle group; however, semapimod treatment substantially normalized their expression. Likewise, caspase-3 activity and histopathological damage scores, which reflect apoptotic activity and tissue injury, were elevated in the I/R and vehicle groups. However, semapimod administration reduced caspase-3 levels to nearly those observed in sham controls and lessened tubular necrosis, although some residual histological damage was still present.



A. Sham B. I/R group

C. Veh + I/R group D. SPD + I/R group.

Figure 3. MLKL IHC staining of renal tissue. Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod.

The observed reduction in KIM-1 and TNF-α levels following semapimod treatment aligns with previous investigations into renal I/R injury pathobiology and therapeutic interventions. The significant elevation of KIM-1 in untreated I/R models corroborates findings from an experimental study by Dase et al, demonstrating its rapid upregulation within two hours of reperfusion as a sensitive biomarker of proximal tubular damage (21). This consistency across models reinforces KIM-1's role as a reliable indicator of renal parenchymal injury during ischemic events. The attenuation of TNF-α levels with semapimod parallels results observed with valproic acid administration, which reduced IL-1β and TNF-α (22,23). However, semapimod's mechanism appears distinct from other anti-inflammatory agents like mefunidone, which primarily targets extracellular signal-regulated kinase (ERK) and signal transducer and activator of transcription 3 (STAT3) phosphorylation pathways rather than direct cytokine suppression (24,25). The drug's efficacy in mitigating both biomarkers is different from dexamethasone, which showed impact on TNF-α-mediated c-Jun N-terminal kinase (JNK) activation in non-renal models (26,27), and contrasts with interventions like dexketoprofen that reduced pain without affecting cytokine levels (28). These differential effects suggest semapimod's unique dual action on both epithelial injury and inflammatory markers. The collective evidence positions semapimod as a promising therapeutic candidate comparable to established agents in

Table 3. Comparative analysis of cellular death markers among treatment groups

	Group		Mean	SD	*P* value*
Caspase-3 (ng/mL)	Sham		4.36	0.68	<0.001
	IR		5.91	0.49	
	I/R + Veh		6.38	0.48	
	I/R + SPD		4.58	0.28	
	Group		**Mean difference**		***P* value****
	Sham	I/R	1.55		<0.001
		I/R + Veh	2.02		<0.001
		I/R + SPD	0.22		0.870
	IR	I/R + Veh	0.47		0.400
		I/R + SPD	1.32		0.001
	I/R + Veh	I/R + SPD	1.79		<0.001
	Group		**Mean**	**SD**	***P* value*****
Histopathological score	Sham		0	0	<0.001
	IR		3.83	0.40	
	I/R + Veh		4.00	0.00	
	I/R + SPD		2.33	0.51	
	Group		**Mean difference**		***P* value******
	Sham	I/R	3.83		<0.001
		I/R + Veh	4.00		<0.001
		I/R + SPD	2.33		<0.001
	IR	I/R + Veh	0.16		0.817
		I/R + SPD	1.50		<0.001
	I/R + Veh	I/R + SPD	1.66		<0.001

Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod. *One-way ANOVA, **Post hoc Tukey test, *** Kruskal–Wallis test, ****Mann–Whitney U.

ameliorating I/R injury-associated inflammation, while its specific targeting of regulated cell death mechanisms may offer advantages over broad-spectrum anti-inflammatory approaches.

The observed upregulation of RIPK1 and MLKL following I/R injury aligns with prior investigations demonstrating necroptosis pathway activation in renal tubular epithelial cells during AKI, where RIPK1 phosphorylation initiates MLKL oligomerization and plasma membrane permeabilization (29-31). These findings corroborate studies showing that genetic ablation of augmenter of liver regeneration (ALR) exacerbates renal I/R injury while increasing RIPK1 and MLKL expression (31), and that RIPK1 inhibition via necrostatin-1 attenuates

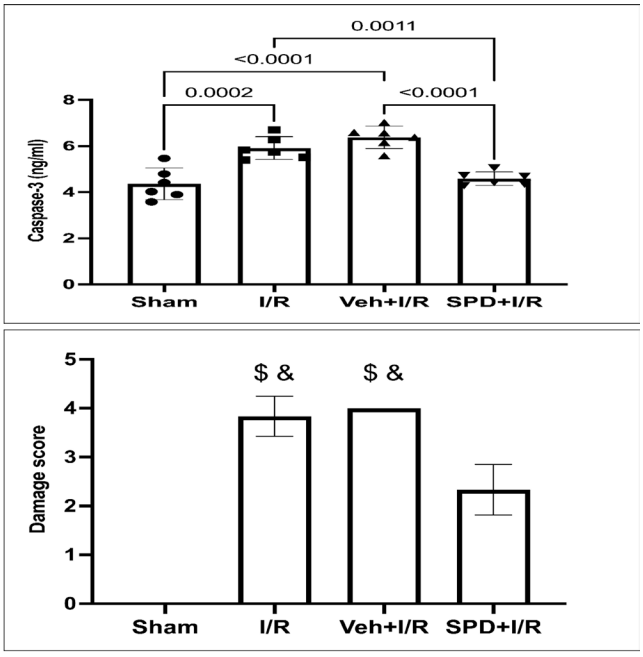


Figure 4. Comparison of cellular death markers among treatment groups. Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod.

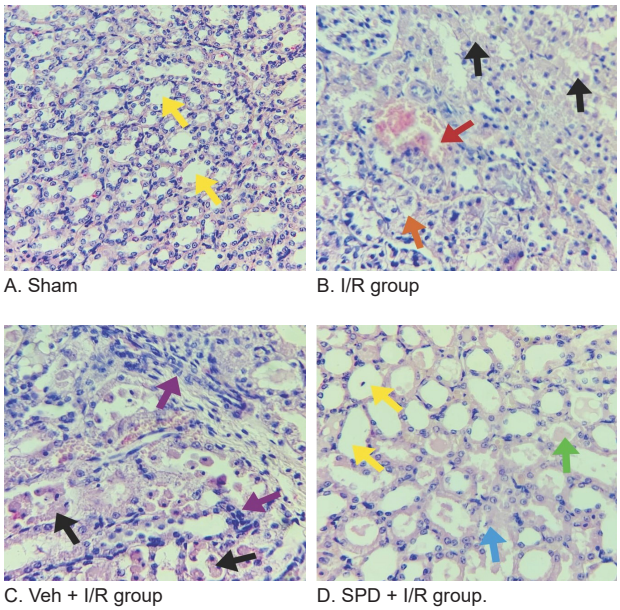


Figure 5. Histopathological staining of renal tissue. Sham: Healthy control; I/R: Ischemia/reperfusion; Veh: Vehicle; SPD: Semapimod. *Note*: **Yellow** arrow: normal tubule. **Black** arrow: cytoplasmic eosinophilia. **Red** arrow: hemorrhage. **Orange** arrow: cytoplasmic vacuoles. **Purple** arrow: inflammation. Green arrow: eosinophilic cast. **Blue** arrow: damaged tubule.

tubular necrosis and improves renal function in murine models (29). However, semapimod's normalization of RIPK1/MLKL expression demonstrates comparable efficacy compared to pathway-specific inhibitors like ponatinib and pazopanib (32). The compound's dual modulation of both kinases surpasses the effects of S100A8/A9 blockade, which reduces inflammation (33), and contrasts with ALR overexpression strategies that require genetic manipulation (31). These results position semapimod as a multifunctional therapeutic agent capable of simultaneously targeting multiple nodes in the necroptosis cascade, offering advantages over single-target approaches in managing I/R injury's complex pathophysiology. The findings underscore the clinical potential of pharmacological necroptosis inhibition while highlighting the need for further investigation into semapimod's precise molecular targets and long-term renal outcomes compared to existing anti-necroptotic therapies.

The observed reduction in caspase-3 activity and histopathological damage following semapimod administration aligns with previous investigations into renal I/R injury mechanisms and therapeutic interventions, while revealing distinct advantages over existing approaches. The elevation of caspase-3 in untreated I/R models corroborates studies demonstrating its central role in executing apoptosis through cleavage of cellular substrates and promotion of secondary necrosis via gasdermin E activation, processes implicated in both acute and chronic kidney injury (34, 35). Semapimod's efficacy in normalizing caspase-3 activity parallels findings with fibroblast growth factor 10 (FGF10), which attenuated renal I/R injury by suppressing caspase-3 activation and Bax expression (36), and exceeds the limited protective effects observed with pan-caspase inhibitors like zVAD-fmk that failed to mitigate kidney damage despite caspase inhibition (29). The compound's dual attenuation of both apoptotic markers and tubular necrosis contrasts with necrostatin-1's selective inhibition of receptor-interacting protein kinase 1 (RIPK1)-mediated necroptosis without affecting caspase-3 activity (37), suggesting semapimod's broader modulation of cell death pathways. Similar caspase-3 inhibiting effects were seen in a combination of erythropoietin-derived therapies and caspase-3 siRNA (38). The results also resolve contradictory observations from studies where caspase-3 knockout unexpectedly exacerbated DNA damage-induced necrosis in non-renal models (39), demonstrating that pharmacological caspase-3 modulation rather than complete inhibition preserves cellular homeostasis in I/R contexts. This therapeutic profile suggests semapimod may circumvent limitations of direct caspase inhibitors like Ac-DNLD-CHO, which showed strict target specificity for caspase-3 (40), while offering advantages over neuroprotective agents that reduce caspase-3 activity indirectly through endoplasmic reticulum stress modulation (41). The collective evidence establishes semapimod as a multifaceted therapeutic agent capable of concurrently addressing apoptotic and inflammatory components of renal I/R injury, representing a significant advancement over previous mono-mechanistic approaches.

Conclusion

The findings underscore semapimod as a multifaceted therapeutic agent capable of mitigating renal I/R injury through concurrent modulation of inflammatory, necroptotic, and apoptotic pathways. Pretreatment with semapimod significantly reduced elevated levels of KIM-1 and TNF-α, markers of renal inflammation. Similarly, semapimod normalized the overexpression of RIPK1 and MLKL, key mediators of necroptosis, in I/R-injured kidneys, effectively disrupting this cell death cascade. Concurrently, semapimod attenuated caspase-3 activation and histopathological damage, indicating suppression of apoptosis and structural degeneration, albeit with residual tubular injury persisting relative to sham control. These results collectively position semapimod as a promising candidate for limiting acute renal I/R injury, though its incomplete restoration of histological integrity highlights the need for adjunct therapies to address residual damage. Future studies should explore combinatorial approaches targeting complementary pathways to enhance renal recovery.

Limitations of the study

This study has several important limitations: First, achieving optimal effectiveness may require increasing

the dosing frequency, which could impact the practicality and feasibility of using this treatment. Second, semapimod needed to be administered both before and after ischemia to evaluate its preventive and therapeutic effects fully. Lastly, the study was conducted exclusively on male rats; therefore, future research should include both male and female subjects to address possible sex-related differences in drug response and treatment outcomes.

Acknowledgments

The authors gratefully acknowledge the University of Kufa, Iraq, for facilities essential to this study. We extend our sincere appreciation to the Central Committee of Bioethics at Kufa University for their ethical oversight and approval.

Authors' contribution

Conceptualization: All authors.
Data curation: All authors.
Formal analysis: Ali F. Jaber.
Investigation: Ali F. Jaber.
Methodology: All authors.
Project Management: Ali M. Janabi.
Resources: All authors.
Supervision: Ali M. Janabi.
Validation: Ali M. Janabi
Writing–original draft: All authors.
Writing–review and editing: All authors.

Conflicts of interest

The authors declare no conflict of interest.

Ethical issues

The research and the protocol of this study followed the guidelines of animal studies and were approved by the Ethics Committee of the Central Committee of Bioethics at Kufa University, Iraq, with the approval number 20554 registered on August 29, 2024. We also followed the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978). Besides, the authors have ultimately observed ethical issues (including plagiarism, data fabrication, and double publication).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of generative artificial intelligence (AI) and AI-assisted technologies in the writing process

While preparing this work, the authors utilized AI (Perplexity.ai) to refine grammar points and language style. Subsequently, they thoroughly reviewed and edited the content as necessary, assuming full responsibility for the publication's content.

Funding/Support

The authors did not receive any source of funding.

References

1. Wang H, Lambourg E, Guthrie B, Morales DR, Donnan PT, Bell S. Patient outcomes following AKI and AKD: a population-based cohort study. BMC Med. 2022;20:229. doi: 10.1186/s12916-022-02428-8.
2. Choi HS, Mathew AP, Uthaman S, Vasukutty A, Kim IJ, Suh SH, et al. Inflammation-sensing catalase-mimicking nanozymes alleviate acute kidney injury via reversing local oxidative stress. J Nanobiotechnol. 2022;20:205. doi: 10.1186/s12951-022-01410-z.
3. Tejera D, Varela F, Acosta D, Figueroa S, Benencio S, Verdaguer C, et al. Epidemiology of acute kidney injury and chronic kidney disease in the intensive care unit. Rev Bras Ter Intensiva. 2017;29:444–52. doi: 10.5935/0103-507x.20170061.
4. Fortrie G, de Geus HRH, Betjes MGH. The aftermath of acute kidney injury: a narrative review of long-term mortality and renal function. Crit Care. 2019;23:24. doi: 10.1186/s13054-019-2314-z.
5. Ikizler TA, Parikh CR, Himmelfarb J, Chinchilli VM, Liu KD, Coca SG, et al. A prospective cohort study of acute kidney injury and kidney outcomes, cardiovascular events, and death. Kidney Int. 2021;99:456–65. doi: 10.1016/j.kint.2020.06.032.
6. Park M, Kwon CH, Ha HK, Han M, Song SH. RNA-Seq identifies condition-specific biological signatures of ischemia-reperfusion injury in the human kidney. BMC Nephrol. 2020;21:398. doi: 10.1186/s12882-020-02025-y.
7. Chen H, Busse LW. Novel Therapies for Acute Kidney Injury. Kidney Int Rep. 2017;2:785–99. doi: 10.1016/j.ekir.2017.06.020.
8. Li C, Yu Y, Zhu S, Hu Y, Ling X, Xu L, et al. The emerging role of regulated cell death in ischemia and reperfusion-induced acute kidney injury: current evidence and future perspectives. Cell Death Discov. 2024;10:216. doi: 10.1038/s41420-024-01979-4.
9. Hesketh AJ, Maloney C, Behr CA, Edelman MC, Glick RD, Al-Abed Y, et al. The Macrophage Inhibitor CNI-1493 Blocks Metastasis in a Mouse Model of Ewing Sarcoma through Inhibition of Extravasation. PLoS One. 2015;10:e0145197. doi: 10.1371/journal.pone.0145197.
10. Bernik TR, Friedman SG, Ochani M, DiRaimo R, Ulloa L, Yang H, et al. Pharmacological stimulation of the cholinergic antiinflammatory pathway. J Exp Med. 2002;195:781–8. doi: 10.1084/jem.20011714.
11. Miller IS, Didier S, Murray DW, Turner TH, Issaivanan M, Ruggieri R, et al. Semapimod sensitizes glioblastoma tumors to ionizing radiation by targeting microglia. PLoS One. 2014;9:e95885. doi: 10.1371/journal.pone.0095885.
12. Arifin WN, Zahiruddin WM. Sample Size Calculation in Animal Studies Using Resource Equation Approach. Malays J Med Sci. 2017;24:101–5. doi: 10.21315/mjms2017.24.5.11.
13. dos Santos CC, Shan Y, Akram A, Slutsky AS, Haitsma JJ. Neuroimmune regulation of ventilator-induced lung injury. Am J Respir Crit Care Med. 2011;183:471–82. doi: 10.1164/rccm.201002-0314OC.
14. Alsaaty EH, Janabi AM. Moexipril Improves Renal Ischemia/Reperfusion Injury in Adult Male Rats. J Contemp Med Sci. 2024;10:25–30.
15. Alaasam ER, Janabi AM, Al-Buthabhak KM, Almudhafar RH, Hadi NR, Alexiou A, et al. Nephroprotective role of resveratrol in renal ischemia-reperfusion injury: a preclinical study in Sprague-Dawley rats. BMC Pharmacol Toxicol. 2024;25:82. doi: 10.1186/s40360-024-00809-8.
16. Alkhafaji GA, Janabi A. GIP/GLP-1 dual agonist tirzepatide ameliorates renal ischemia/reperfusion damage in rats. Int J App Pharm. 2025;17:165–73. doi: 10.22159/ijap.2025v17i2.53156.
17. Yahiya YI, Hadi NR, Abu Raghif A, Al Habooby NGS. Protective effect of IAXO-102 on renal ischemia-reperfusion injury in rats. J Med Life. 2023;16:623–30. doi: 10.25122/jml-2022-0280.
18. Hassoon MF, Hadi NR, Mahboba W, Jawad D, Hussein YA, Abdulkhaleq MA. Nephroprotective potential effect of Canagliflozin in renal ischemia reperfusion injury in rat model: Role of Nrf2 pathway. Syst Rev Pharm. 2019;10:P60. doi: 10.5530/srp.2019.2.11.

19. Charafe-Jauffret E, Tarpin C, Bardou VJ, Bertucci F, Ginestier C, Braud AC, et al. Immunophenotypic analysis of inflammatory breast cancers: identification of an 'inflammatory signature'. J Pathol. 2004;202:265–73. doi: 10.1002/path.1515.
20. Parris TZ, Aziz L, Kovács A, Hajizadeh S, Nemes S, Semaan M, et al. Clinical relevance of breast cancer-related genes as potential biomarkers for oral squamous cell carcinoma. BMC Cancer. 2014;14:324. doi: 10.1186/1471-2407-14-324.
21. Dase J, Rasyid H, Masadah R, Cangara MH, Bukhari A, Dwiyanti R, et al. Analysis of mRNA and protein kidney injury Molecule-1 (KIM-1) expression in a kidney model during the initiation phase of ischemia reperfusion injury. Ann Med Surg (Lond). 2022;75:103373. doi: 10.1016/j.amsu.2022.103373.
22. Speir RW, Stallings JD, Andrews JM, Gelnett MS, Brand TC, Salgar SK. Effects of valproic acid and dexamethasone administration on early bio-markers and gene expression profile in acute kidney ischemia-reperfusion injury in the rat. PLoS One. 2015;10:e0126622. doi: 10.1371/journal.pone.0126622.
23. Costalonga EC, Silva FM, Noronha IL. Valproic Acid Prevents Renal Dysfunction and Inflammation in the Ischemia-Reperfusion Injury Model. Biomed Res Int. 2016;2016:5985903. doi: 10.1155/2016/5985903.
24. Li J, Jiang Y, Dai Q, Yu Y, Lv X, Zhang Y, et al. Protective effects of mefunidone on ischemia-reperfusion injury/Folic acid-induced acute kidney injury. Front Pharmacol. 2022;13:1043945. doi: 10.3389/fphar.2022.1043945.
25. Liu C, Mei W, Tang J, Yuan Q, Huang L, Lu M, et al. Mefunidone attenuates tubulointerstitial fibrosis in a rat model of unilateral ureteral obstruction. PLoS One. 2015;10:e0129283. doi: 10.1371/journal.pone.0129283.
26. Lu YC, Evans CH, Grodzinsky AJ. Effects of short-term glucocorticoid treatment on changes in cartilage matrix degradation and chondrocyte gene expression induced by mechanical injury and inflammatory cytokines. Arthritis Res Ther. 2011;13:R142. doi: 10.1186/ar3456.
27. González MV, Jiménez B, Berciano MT, González-Sancho JM, Caelles C, Lafarga M, et al. Glucocorticoids antagonize AP-1 by inhibiting the Activation/phosphorylation of JNK without affecting its subcellular distribution. J Cell Biol. 2000;150:1199–208. doi: 10.1083/jcb.150.5.1199.
28. Rambe AS, Sjahrir H, Machfoed MH. Tumour Necrosis Factor-A, Interleukin-1 and Interleukin-6 Serum Levels and Its Correlation with Pain Severity in Chronic Tension-Type Headache Patients: Analysing Effect of Dexketoprofen Administration. Open Access Maced J Med Sci. 2017;5:54–7. doi: 10.3889/oamjms.2017.024.
29. Price PM, Hodeify R. A possible mechanism of renal cell death after ischemia/reperfusion. Kidney Int. 2012;81:720–1. doi: 10.1038/ki.2011.495.
30. Pefanis A, Bongoni AK, McRae JL, Salvaris EJ, Fisicaro N, Murphy JM, et al. Dynamics of necroptosis in kidney ischemia-reperfusion injury. Front Immunol. 2023;14:1251452. doi: 10.3389/fimmu.2023.1251452.
31. Liao YJ, Ma YX, Huang LL, Zhang Z, Tan FY, Deng LL, et al. Augmenter of liver regeneration protects the kidney against ischemia-reperfusion injury by inhibiting necroptosis. Bioengineered. 2022;13:5152–67. doi: 10.1080/21655979.2022.2037248.
32. Fauster A, Rebsamen M, Huber KV, Bigenzahn JW, Stukalov A, Lardeau CH, et al. A cellular screen identifies ponatinib and pazopanib as inhibitors of necroptosis. Cell Death Dis. 2015;6:e1767. doi: 10.1038/cddis.2015.130.
33. Yao W, Chen Y, Li Z, Ji J, You A, Jin S, et al. Single Cell RNA Sequencing Identifies a Unique Inflammatory Macrophage Subset as a Druggable Target for Alleviating Acute Kidney Injury. Adv Sci (Weinh). 2022;9:e2103675. doi: 10.1002/advs.202103675.
34. Deng C, Li J, Li L, Sun F, Xie J. Effects of hypoxia ischemia on caspase-3 expression and neuronal apoptosis in the brain of neonatal mice. Exp Ther Med. 2019;17:4517–21. doi: 10.3892/etm.2019.7487.
35. Wen S, Wang ZH, Zhang CX, Yang Y, Fan QL. Caspase-3 Promotes Diabetic Kidney Disease Through Gasdermin E-Mediated Progression to Secondary Necrosis During Apoptosis. Diabetes Metab Syndr Obes. 2020;13:313–23. doi: 10.2147/dmso.S242136.
36. Tan X, Yu L, Yang R, Tao Q, Xiang L, Xiao J, et al. Fibroblast Growth Factor 10 Attenuates Renal Damage by Regulating Endoplasmic Reticulum Stress After Ischemia-Reperfusion Injury. Front Pharmacol. 2020;11:39. doi: 10.3389/fphar.2020.00039.
37. Liu H, Zhang MZ, Liu YF, Dong XH, Hao Y, Wang YB. Necroptosis was found in a rat ischemia/reperfusion injury flap model. Chin Med J (Engl). 2019;132:42–50. doi: 10.1097/cm9.0000000000000005.
38. Zhang Y, Wu Y, Wang W, Liu F, Zhang Y, Yang C, et al. Long-Term Protection of CHBP Against Combinational Renal Injury Induced by Both Ischemia-Reperfusion and Cyclosporine A in Mice. Front Immunol. 2021;12:697751. doi: 10.3389/fimmu.2021.697751.
39. Brown MF, Leibowitz BJ, Chen D, He K, Zou F, Sobol RW, et al. Loss of caspase-3 sensitizes colon cancer cells to genotoxic stress via RIP1-dependent necrosis. Cell Death Dis. 2015;6:e1729. doi: 10.1038/cddis.2015.104.
40. Yoshimori A, Sakai J, Sunaga S, Kobayashi T, Takahashi S, Okita N, et al. Structural and functional definition of the specificity of a novel caspase-3 inhibitor, Ac-DNLD-CHO. BMC Pharmacol. 2007;7:8. doi: 10.1186/1471-2210-7-8.
41. Yang JW, Hu ZP. Neuroprotective effects of atorvastatin against cerebral ischemia/reperfusion injury through the inhibition of endoplasmic reticulum stress. Neural Regen Res. 2015;10:1239–44. doi: 10.4103/1673-5374.162755.