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UNIVERSITAS INDONESIA

THE EFFECT OF N-ACETYLCYSTEINE ON INFLAMMATION AND ACUTE KIDNEY INJURY IN PATIENTS WITH LEPTOSPIROSIS: A RANDOMIZED CONTROLLED TRIAL

RESEARCH RESULTS

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JAKARTA

2026

APPROVAL SHEET FOR RESEARCH RESULTS

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Judul Proposal : **Efek N-Acetylcystein terhadap Biomarker Stres Oksidatif, Inflamasi, dan Cedera Ginjal Akut pada pasien Leptospirosis: Sebuah Uji Klinis Acak Terkontrol**

Telah berhasil dipertahankan di Divisi Ginjal Hipertensi dan telah disetujui untuk maju Ujian Tertutup Hasil Penelitian di tingkat Program Studi Subspesialis Ilmu Penyakit Dalam Fakultas Kedokteran Universitas Indonesia.

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ABSTRACT

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Title : The Effect of N-Acetylcysteine on Inflammation and Acute Kidney Injury in Patients with Leptospirosis: A Randomized Controlled Trial

Background: Leptospirosis is a zoonotic disease that remains a major public health problem in tropical countries and may cause acute kidney injury (AKI) through complex mechanisms involving inflammation, oxidative stress, and kidney tubular injury. Assessment of kidney function based solely on serum creatinine has limited sensitivity for the early detection of kidney injury, highlighting the need for more sensitive biomarkers, such as Kidney Injury Molecule-1 (KIM-1), Reactive Oxygen Species (ROS), and C-Reactive Protein (CRP). N-acetylcysteine (NAC) possesses antioxidant and anti-inflammatory properties that may confer renoprotective effects in patients with leptospirosis. However, evidence regarding its effects on these three biomarkers remains limited.

Objective: To evaluate the effect of oral N-acetylcysteine on changes in urinary KIM-1, ROS, and CRP levels in patients with leptospirosis.

Methods: This double-blind, randomized, placebo-controlled clinical trial enrolled adult patients with confirmed leptospirosis who met the predefined inclusion criteria. Participants were randomly assigned to receive either oral NAC 800 mg three times daily or placebo for five days in addition to standard therapy. Urinary KIM-1, ROS, and CRP levels were measured on Day 1 and Day 5. Data were analyzed according to the intention-to-treat principle. Changes in biomarker levels between groups were compared using appropriate statistical analyses.

Results: NAC administration resulted in greater reductions in ROS and CRP levels than placebo after adjustment for baseline values, whereas the change in urinary KIM-1 levels was not statistically different between the two groups. These findings indicate that NAC exerts biological effects by reducing oxidative stress and systemic inflammation in patients with

leptospirosis but did not demonstrate a significant effect on the biomarker of kidney tubular injury during the five-day intervention period.

Conclusion: Adjunctive treatment with NAC effectively reduced biomarkers of oxidative stress and systemic inflammation in patients with leptospirosis but did not significantly affect urinary KIM-1 levels. These findings support the potential role of NAC as an adjunctive therapy for modulating the biological pathways underlying kidney injury in leptospirosis. Nevertheless, further studies with larger sample sizes and longer follow-up periods are warranted to determine its long-term clinical benefits.

Keywords: Leptospirosis; acute kidney injury; N-acetylcysteine; Kidney Injury Molecule-1; Reactive Oxygen Species; C-Reactive Protein; inflammation; oxidative stress.

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CHAPTER 1

INTRODUCTION

1.1 Background

Leptospirosis is a zoonotic disease caused by pathogenic bacteria of the genus Leptospira and remains a public health problem, particularly in tropical and subtropical regions.[1] Indonesia is one of the countries in which leptospirosis is endemic, with environmental conditions that facilitate disease transmission, including high rainfall, flooding, high population density, suboptimal sanitation, and exposure to animal reservoirs.[1,2] Leptospirosis in Indonesia continues to be associated with substantial mortality, particularly among patients with multiorgan involvement.[3]

The kidneys are among the major organs affected by leptospirosis. Kidney involvement encompasses a broad spectrum, ranging from tubular dysfunction to acute kidney injury (AKI).[4–6] The pathogenesis of kidney injury in leptospirosis is complex and is not solely attributable to hemodynamic disturbances. Direct invasion of kidney tissue by Leptospira, tubulointerstitial injury, activation of immune and inflammatory responses, microcirculatory disturbances, and increased oxidative stress act concurrently in the development of kidney injury.[4–7]

One of the mechanisms that plays an important role in the pathogenesis of leptospirosis is excessive formation of reactive oxygen species (ROS).[7–9] An imbalance between ROS production and endogenous antioxidant capacity results in oxidative stress, which may subsequently damage lipids, proteins, and nucleic acids and exacerbate cellular and tissue dysfunction. In the kidneys, oxidative stress may contribute to tubular epithelial cell injury and amplify the inflammatory response.[4,9]

Systemic inflammation is also an important component of the clinical course of leptospirosis. C-reactive protein (CRP) is an acute-phase protein widely used as a marker of systemic inflammatory activity. Elevated CRP levels in leptospirosis reflect activation of the inflammatory response and may be associated with disease severity and organ involvement. The interaction between inflammation and oxidative stress may establish a mutually reinforcing pathological cycle that contributes to progressive tissue injury.[4,7,9]

Conventional assessment of kidney injury continues to rely primarily on serum creatinine and urine output. However, creatinine is a marker of altered kidney function, and its elevation may occur only after kidney injury has already developed.[9–12] Therefore, biomarkers capable of providing information on structural kidney injury are needed. Kidney Injury Molecule-1 (KIM-1) is a transmembrane protein whose expression increases in proximal tubular epithelial cells following injury. Urinary KIM-1 levels may reflect tubular injury and may provide information complementary to conventional kidney function tests.[13–15]

The involvement of oxidative stress, inflammation, and tubular injury in the pathogenesis of leptospirosis provides a biological rationale for investigating adjunctive therapies targeting these mechanisms.[16,17] N-acetylcysteine (NAC) has antioxidant activity through its role as a glutathione precursor and its ability to modulate cellular redox balance. NAC has also been reported to exert anti-inflammatory effects through modulation of several signaling pathways and inflammatory mediators.[18]

NAC has been investigated in various conditions involving oxidative stress and kidney injury.[18] Previous studies in patients with leptospirosis have suggested a potential benefit of NAC for kidney function recovery. However, evidence regarding the effects of NAC on the biological mechanisms underlying kidney injury in leptospirosis remains limited. In particular, it remains unclear whether NAC can simultaneously influence markers of kidney tubular injury, systemic inflammation, and oxidative stress in patients with leptospirosis.

Measurement of urinary KIM-1, ROS, and CRP enables evaluation of three interrelated biological components: tubular injury, inflammation, and oxidative stress. Assessment of changes in these three biomarkers before and after the intervention may provide a more comprehensive evaluation of the biological effects of NAC than conventional kidney function parameters alone. Accordingly, this double-blind randomized controlled trial was conducted to evaluate the effect of five days of oral NAC administration on changes in urinary KIM-1, ROS, and CRP levels in patients with leptospirosis. This study is expected to provide evidence regarding the biological effects of NAC as adjunctive therapy for leptospirosis and to provide a basis for further studies evaluating the relationship between biomarker changes and clinical outcomes.

1.2 Problem Identification and Research Questions

1.2.1 Problem Identification

Leptospirosis may involve the kidneys through complex mechanisms, including tubular injury, inflammation, and oxidative stress. Serum creatinine, as a conventional kidney function parameter, has limitations in directly reflecting structural kidney injury. Urinary KIM-1 may reflect tubular injury, whereas ROS and CRP represent oxidative stress and systemic inflammation, respectively.

NAC has antioxidant activity and may modulate the inflammatory response. Although NAC has been investigated in various conditions involving oxidative stress and kidney injury, evidence regarding its simultaneous effects on urinary KIM-1, ROS, and CRP in patients with leptospirosis remains limited. No randomized controlled trial in an Indonesian population has evaluated changes in all three biomarkers following NAC administration. Therefore, a study is needed to determine whether NAC produces changes in urinary KIM-1, ROS, and CRP levels that differ from those observed with placebo in patients with leptospirosis.

1.2.2 Research Questions

1. Is there a difference in the change in KIM-1 levels from Day 1 to Day 5 between the NAC group and the placebo group in patients with leptospirosis?

2. Is there a difference in the change in ROS levels from Day 1 to Day 5 between the NAC group and the placebo group in patients with leptospirosis?

3. Is there a difference in the change in CRP levels from Day 1 to Day 5 between the NAC group and the placebo group in patients with leptospirosis?

1.3 Research Hypotheses

Based on the identified problems and research questions, the following hypotheses were formulated:

1. The change in KIM-1 levels between Day 1 and Day 5 differs between patients receiving NAC and those receiving placebo.

2. The change in ROS levels between Day 1 and Day 5 differs between patients receiving NAC and those receiving placebo.

3. The change in CRP levels between Day 1 and Day 5 differs between patients receiving NAC and those receiving placebo.

1.4 Research Objectives

1.4.1 General Objective

To determine the effect of NAC administration on changes in biomarkers of kidney tubular injury, inflammation, and oxidative stress in patients with leptospirosis.

1.4.2 Specific Objectives

1. To assess the difference in the change in urinary KIM-1 levels from Day 1 to Day 5 between patients receiving NAC and those receiving placebo.

2. To assess the difference in the change in ROS levels from Day 1 to Day 5 between patients receiving NAC and those receiving placebo.

3. To assess the difference in the change in CRP levels from Day 1 to Day 5 between patients receiving NAC and those receiving placebo.

1.5 Significance of the Study

1.5.1 Academic Significance

This study is expected to add to the scientific evidence regarding the roles of oxidative stress, inflammation, and tubular injury in the course of leptospirosis and the biological effects of NAC on urinary KIM-1, CRP, and ROS. The findings may contribute to the development of knowledge in nephrology, tropical and infectious diseases, and adjunctive pharmacotherapy.

1.5.2 Significance for Health Care

This study is expected to provide preliminary information regarding the biological effects of NAC as adjunctive therapy in patients with leptospirosis. The findings may provide a scientific basis for further research before the effects of NAC on clinical outcomes and its role in routine management can be established.

1.5.3 Significance for Research

This study is expected to provide a basis for further studies with larger sample sizes, longer observation periods, and clinical outcomes such as AKI occurrence, kidney replacement therapy requirements, length of hospital stay, mortality, and long-term kidney function.

CHAPTER 2

LITERATURE REVIEW

2.1 Leptospirosis

Leptospirosis is a zoonotic disease caused by pathogenic bacteria of the genus Leptospira. These organisms are aerobic spirochetes with active motility that facilitates tissue penetration and hematogenous dissemination. Various mammals, particularly rodents, serve as reservoirs and may excrete Leptospira in urine into the environment.[1–3] Human transmission occurs through direct or indirect contact with urine from infected animals or with contaminated environments. The bacteria may enter the body through abraded skin or mucous membranes. Environmental conditions characterized by high rainfall, flooding, inadequate sanitation, and substantial exposure to reservoir animals contribute to leptospirosis transmission.[2,4]

2.1.1 Epidemiology and Transmission

Leptospirosis is a zoonotic disease caused by pathogenic Leptospira spp. Leptospira are spiral-shaped aerobic bacteria (spirochetes) belonging to the family Leptospiraceae.[2] The organisms measure approximately 0.15–0.3 μm in diameter and 6–20 μm in length. The Leptospira bacterial structure includes a lipopolysaccharide layer and the organisms are classified into pathogenic, intermediate, and non-pathogenic groups. Leptospira possess endoflagella that facilitate motility.[12]

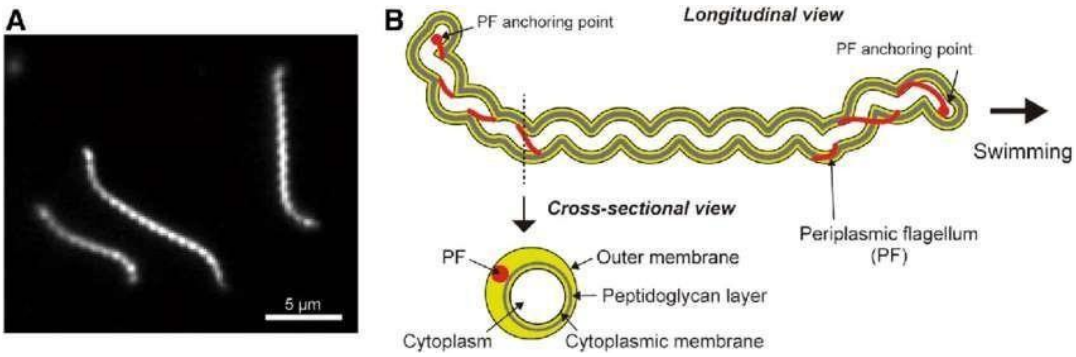


Figure 2.1 Structure of Leptospira spp.[19]

Leptospirosis occurs in tropical and subtropical regions as well as in temperate climates. According to the Leptospirosis Burden Epidemiology Reference Group (LERG) in 2010, the incidence of leptospirosis was 5 cases per 100,000 population. According to the World

Health Organization (WHO), the reported incidence of leptospirosis from highest to lowest was in Africa, the Western Pacific, the Americas, Southeast Asia, and Europe, with median incidences of 95.5 (62.8–160.2), 66.4 (1.1–975.0), 12.5 (0.1–306.2), 4.8 (0.3–7.3), and 0.5 (0.1–15.8) cases per 100,000 population, respectively.[2,20]

Approximately 10% of patients with leptospirosis progress to a second phase, Weil's syndrome, with more severe manifestations lasting 4–30 days. The kidney is one of the target organs of Leptospira, and kidney injury may occur in 20–85% of patients.[21] AKI is an early manifestation of severe leptospirosis, with an incidence ranging from 10% to 60% of cases. Contributing mechanisms include bacterial invasion of renal tubular cells with nephrotoxic effects, dehydration, rhabdomyolysis, and hemodynamic alterations. In severe leptospirosis, approximately 31.6% of cases develop reduced glomerular filtration rate and severe electrolyte disturbances requiring renal replacement therapy (RRT). Kidney function may recover over several months; however, AKI in severe leptospirosis may progress to chronic kidney disease (CKD).[12]

The principal reservoirs and carriers include several mammalian species such as rodents, cattle, pigs, domestic animals, and wildlife. Transmission occurs through direct animal-to-animal contact.[4] Individuals at increased risk include those with occupations involving direct exposure to these animals, such as farmers, veterinarians, fishermen, miners, sugarcane workers, and military personnel. Recreational activities including rafting, canoeing, and triathlons, as well as natural disasters such as floods, are also risk factors.[1,3,4] Human infection occurs after direct contact with infected animals, contaminated water, or urine. Following penetration through the skin or mucosa, organisms traverse tissues through intercellular junctions. Leptospira can be detected in the bloodstream shortly after penetration and in several organs within three days after infection.[2]

2.1.2 Clinical Manifestations and Kidney Involvement

The incubation period of Leptospira is 5–14 days. The clinical spectrum is broad, ranging from asymptomatic infection and mild disease to severe and fatal illness. Leptospirosis classically follows a biphasic pattern consisting of an infective (leptospiremic) phase during the first week, followed by an immune phase with complications during the second week. During the infective phase, most patients experience only mild symptoms such as sudden-

onset fever accompanied by nonspecific manifestations including headache, myalgia, abdominal pain, sore throat, conjunctival suffusion, chills, diarrhea, and rash.[5,6,9] During this period, Leptospira may be identified in blood and cerebrospinal fluid.[2,12]

The immune phase is characterized by detection of Leptospira in urine culture and the appearance of Leptospira immunoglobulin M (IgM) antibodies in the bloodstream. Complications including jaundice, AKI, pulmonary hemorrhage, acute respiratory distress syndrome (ARDS), myocarditis, aseptic meningitis, and shock may occur in 5–10% of patients; this severe form is known as Weil's disease.[22] Certain complications, such as uveitis, may appear several months later without detectable clinical manifestations during the acute phase.[2,13,22]

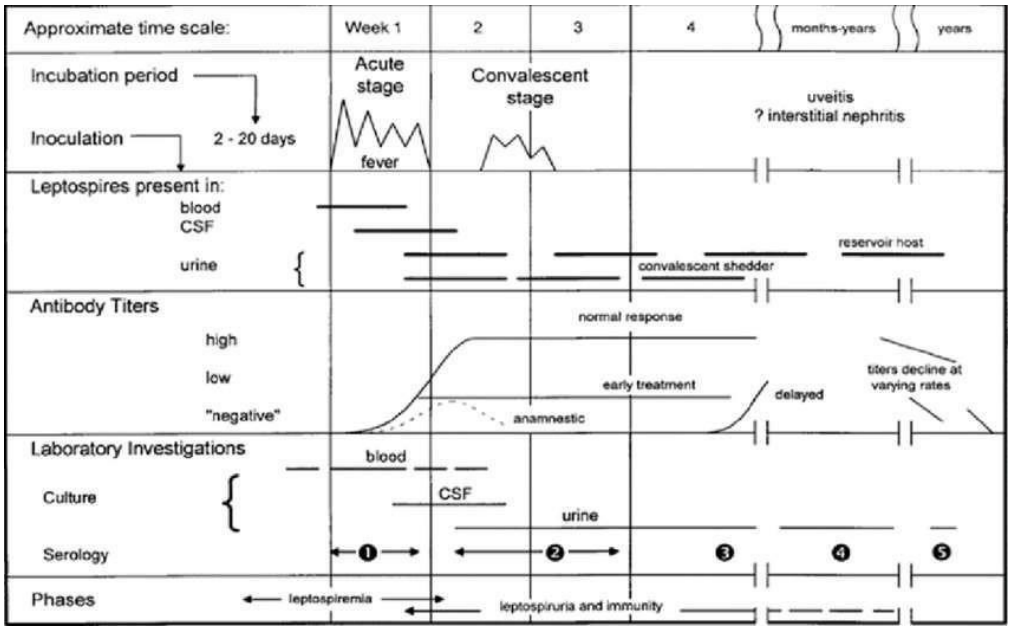


Figure 2.2 Clinical Course of Leptospirosis[23]

Leptospirosis has a wide clinical spectrum, ranging from mild uncomplicated infection to severe disease with multiorgan dysfunction. According to WHO guidance, leptospirosis is clinically categorized as non-severe leptospirosis and severe leptospirosis (Weil's disease). Non-severe leptospirosis is the most common form and is generally characterized by acute fever with constitutional symptoms such as headache, myalgia, arthralgia, malaise, nausea, vomiting, and conjunctival hyperemia. Severe organ dysfunction or life-threatening complications are absent in this group. Most patients improve with appropriate antibiotic and supportive therapy.

Severe leptospirosis or Weil's disease is characterized by major organ involvement related to systemic inflammatory responses and vascular endothelial injury. Clinical manifestations may include jaundice with acute kidney failure, pulmonary hemorrhage or severe hemorrhagic syndrome, respiratory failure, shock, myocarditis, meningitis or encephalitis, and multiorgan dysfunction requiring intensive care. Mortality is substantially higher than in non-severe disease, particularly when AKI and pulmonary hemorrhage are present.

Kidney involvement may occur in severe leptospirosis. In mild cases, abnormalities may include urinary sediment abnormalities, albuminuria, microscopic hematuria, and pyuria. In severe cases, urine output may range from non-oliguric to oliguric or anuric. Renal insufficiency commonly occurs together with jaundice on days 3–4 of illness, followed by marked increases in urea and creatinine.[2] Histopathological examination may demonstrate acute interstitial nephritis and acute tubular necrosis (ATIN), with acute inflammatory cells in renal tubules, histiocytic and mononuclear cell infiltration, and spirochetal fragments in tubular cells, tubular basement membranes, tubular lumina, and the interstitium.[24]

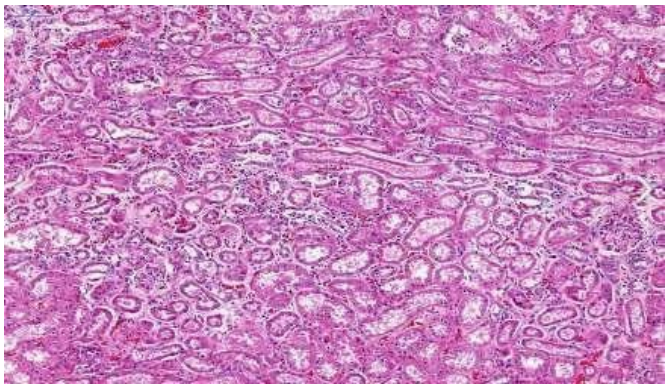


Figure 2.3 Acute Tubular Necrosis and Acute Interstitial Nephritis (ATIN) with Inflammatory Infiltrates and Vascular Congestion[25]

Leptospirosis causes AKI at reported frequencies ranging from 10% to 88%. According to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) criteria, AKI is defined as an increase in serum creatinine (SCr) of more than 0.3 mg/dL within 48 hours, an increase in SCr to 1.5 times baseline within the preceding 7 days, or reduced urine output. A retrospective study by Teles et al. evaluated 205 patients with leptospirosis-associated AKI and classified patients according to AKI severity, including KDIGO stages 1–3.[26]

According to KDIGO, AKI is diagnosed when serum creatinine increases by ≥0.3 mg/dL within 48 hours, increases to ≥1.5 times baseline known or presumed to have occurred within the prior 7 days, or urine output is <0.5 mL/kg/hour for 6 hours. AKI severity has also been described using the AKIN (Acute Kidney Injury Network) and RIFLE (Risk, Injury, Failure, Loss, End-stage Kidney Disease) classifications.[27,28]

Table 2.1 AKI Severity According to KDIGO/AKIN and RIFLE[27,28]

AKIN	RIFLE
Stage 1:	***Risk*** **(Risiko):**
SCr increase ≥0.3 mg/dL (≥26.5 μmol/L) or 1.5–1.9 times baseline;	SCr 1.5 times baseline or GFR decrease >25%. Urine output <0.5 mL/kg/hour for >6 to ≤12 hours.
Stage 2:	***Injury*** **(Cedera):**
SCr increase 2.0–2.9 times baseline;	SCr 2 times baseline or GFR decrease >50%. Urine output <0.5 mL/kg/hour for ≥12 hours.
Stage 3:	***Failure*** **(Gagal):**
SCr increase ≥3 times baseline or SCr ≥4.0 mg/dL (≥353.6 μmol/L);	SCr 3 times baseline or SCr ≥4 mg/dL with an acute increase ≥0.5 mg/dL. Urine output <0.3 mL/kg/hour for ≥24 hours or anuria for ≥12 hours.
	Loss **(Kehilangan):**
	Persistent kidney failure requiring kidney replacement therapy for >4 weeks.
	End-stage kidney disease: Requirement for kidney replacement therapy for >3 months.

2.2 Pathogenesis of Acute Kidney Injury in Leptospirosis

The pathogenesis of kidney injury in leptospirosis is multifactorial. Interactions among the pathogen, host immune response, microcirculatory disturbances, inflammation, and oxidative stress contribute to renal cell injury, particularly tubular epithelial injury.[28,29]

2.2.1 Leptospiral Invasion and Tubulointerstitial Injury

After entering the body, Leptospira can disseminate hematogenously and reach multiple organs, including the kidneys. The organisms exhibit tropism for renal tissue and can interact with tubular epithelium. Responses to the bacteria and their components may trigger immune-cell activation, release of inflammatory mediators, and alterations in tubular epithelial function and integrity.[29]

Tubulointerstitial injury is a major feature of renal involvement in leptospirosis. Tubular epithelial damage may impair reabsorptive and secretory functions, while interstitial inflammation may further aggravate kidney dysfunction. Renal colonization by Leptospira spp. can cause acute tubular necrosis and, if persistent, may contribute to CKD. After entering the kidney, the bacteria interfere with the sodium/phosphate cotransporter in renal tubules, leading to increased ammonia and urinary pH. Renal tubular epithelial cells are among the structures most affected by Leptospira spp. Outer-membrane components including OmpL1, LipL41, LipL32, and lipopolysaccharide can adhere to and infiltrate tubular epithelial cells, activate complement, and directly contribute to kidney injury.[29,30] During the acute phase, the organism may also promote a cytokine storm with accumulation of proinflammatory cytokines and macrophage migration.[12]

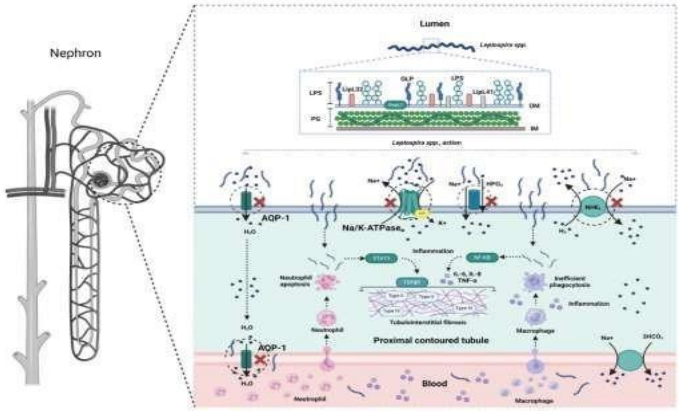


Figure 2.4 Biomolecular Mechanisms of Leptospira spp. in Renal Tubules

The proximal tubule is another major target of leptospiral infection. Leptospira can impair Na/K-ATPase affinity and activity and reduce expression or function of the sodium/hydrogen exchanger and aquaporin-1 (AQP-1) at apical and basolateral membranes.[29,31] These changes lead to sodium and phosphate wasting, while free fluid accumulates in the tubular lumen, contributing to polyuria, hypotension, and secondary hypovolemia.[12]

The thick ascending limb of the loop of Henle is also affected. Infection of tubular cells is associated with nitric oxide (NO) release, which decreases systemic vascular resistance, reduces renal blood flow, and lowers glomerular filtration rate. Reduced expression of the Na/K/2Cl cotransporter (NKCC2) promotes urinary sodium and potassium losses. Loss of the lumen-positive electrical gradient further reduces calcium and magnesium reabsorption.[30] Ion-pump dysregulation causing hyponatremia, hypokalemia, hypomagnesemia, and non-oliguric AKI forms part of the spectrum of leptospiral nephropathy.[12]

Glycoprotein (GLP), an endotoxin with fatty-acid-binding properties, also contributes to the effects of Leptospira. GLP shows specificity for Na/K-ATPase on the cytosolic surface of cell membranes and interacts with oleic acid, which is associated with impaired albumin adsorption.[32] GLP also induces inflammatory responses by increasing IL-6, IL-8, and TNF-α and by influencing mononuclear-cell expression of CD69 and HLA-DR, leukotriene B4, PGE2, and NO, thereby contributing to more extensive tissue injury.[12]

Myoglobinuria secondary to rhabdomyolysis is another factor that may precipitate AKI through nephrotoxicity, tubular obstruction, and renal vasoconstriction. Persistence of Leptospira in the kidney may also cause interstitial inflammation, extracellular-matrix accumulation, and tubular epithelial injury. Fibrotic mediators, including STAT3-related pathways, TGF-β, and type III and IV collagen production, may contribute to progression of kidney damage.[33] Following injury, nephrons undergo maladaptive functional changes as compensation for injured glomeruli and tubules. If inflammation persists, immune-cell invasion, abnormal fibroblast migration, and renal fibrosis may occur and ultimately contribute to CKD.[12]

2.2.2 Inflammatory Response

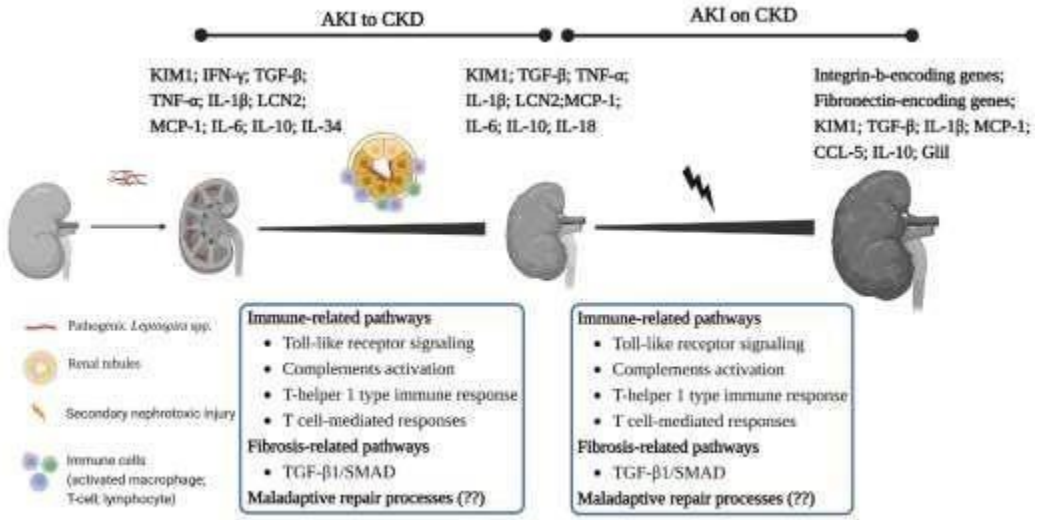


Figure 2.5 AKI-to-CKD and AKI-on-CKD Processes in Leptospirosis-Associated Kidney Disease[34]

Leptospirosis complicated by AKI carries a long-term risk of CKD. Progression from AKI to CKD is influenced by AKI severity, nephron loss, and maladaptive compensatory mechanisms in the remaining nephrons. Maladaptive repair promotes inflammation, infiltrating immune cells, and interstitial fibrosis.[35] Mechanisms include cell-cycle arrest in response to DNA damage, aberrant epigenetic modification, mitochondrial dysfunction caused by oxidative stress, defects in phagocyte differentiation and migration, and dysregulated immune responses.[34]

KIM-1 is a proximal tubular biomarker that may participate in maladaptive repair and renal fibrosis. In mice infected with Leptospira spp., HAVCR1, the gene encoding KIM-1, is upregulated on day 7 of acute infection and day 28 of chronic infection. Previous studies have shown tubular epithelial cell-cycle arrest to be important in maladaptive repair and fibrosis. G2/M arrest impairs tubular repair and promotes fibrosis through release of proinflammatory cytokines and growth factors that contribute to CKD progression.[34]

During AKI, JNK signaling may be activated through a cyclin-dependent kinase (CDK) inhibitor p21-dependent pathway, with production of TGF-β and connective tissue growth factor (CTGF).[36] CDK inhibitors can protect damaged DNA by facilitating repair and limiting cell-cycle progression; however, persistent p21 induction in chronic or advanced

kidney injury may lead to cell-cycle arrest and production of profibrotic factors, promoting renal fibrosis.[26,34]

IL-34 is another inflammatory cytokine implicated in the AKI-to-CKD transition. It is secreted by tubular epithelial cells and increases on day 7 after acute infection and day 28 after chronic infection. Post-AKI IL-34 expression is associated with CKD progression through tubular epithelial destruction and macrophage proliferation and infiltration.[37] Toll-like receptor 2 (TLR2), mediated in part by human defensin-2, has also been implicated in renal tubular tissue injury in leptospirosis.[26,34,38]

2.2.3 Oxidative Stress

Reactive oxygen species (ROS) are reactive molecules generated during cellular metabolism and immune responses. Under physiological conditions, their production is controlled by endogenous antioxidant systems. Oxidative stress occurs when ROS generation exceeds antioxidant capacity.[34]

In leptospirosis, activation of neutrophils and macrophages, mitochondrial dysfunction, hypoperfusion, and tissue hypoxia may increase ROS production. Excessive ROS causes lipid peroxidation, protein modification, nucleic-acid damage, and mitochondrial dysfunction. Renal tubular epithelial cells are particularly susceptible to oxidative injury because of their high energy requirements. Mitochondrial damage may further increase ROS production, creating a self-perpetuating cycle of injury.[34]

AKI-on-CKD involves pathological processes including oxidative stress, mitochondrial dysfunction, abnormal autophagy, chronic inflammation, and vascular dysfunction. Activation of TGF-β, p53, hypoxia-inducible factor, and cellular signaling in injured tubular cells during CKD progression may increase susceptibility to AKI. Activated p53 can stimulate TGF-β expression during renal tubulointerstitial fibrogenesis. Hypoxia-inducible factor signaling may promote renal hypoxia and fibrosis through extracellular-matrix remodeling and interaction with TGF-β in epithelial-to-mesenchymal transition.[34] Chronic leptospiral colonization is nephrotoxic and has been associated with fibronectin and type IV collagen α1 (Col4a1) regulation, excessive protein expression, and fibrosis. Hedgehog signaling, including upregulation of Gli1, has also been implicated in renal injury and fibrosis.[34]

2.2.4 Interaction Among Inflammation, Oxidative Stress, and Tubular Injury

Inflammation and oxidative stress are not independent processes. Immune-cell activation increases ROS formation, while ROS can activate multiple inflammatory pathways. This interaction may amplify endothelial dysfunction, microcirculatory impairment, and tubular epithelial injury. Kidney injury in leptospirosis can therefore be viewed as the result of interaction among three major biological components: inflammation, oxidative stress, and structural tubular injury. These three components provide the rationale for selecting CRP, ROS, and KIM-1 as biomarkers in this study.

2.3 Biomarkers of Acute Kidney Injury

A biomarker is an endogenous molecule that can be detected quantitatively or qualitatively to identify pathophysiological processes and responses to pharmacotherapy in kidney disease. Urea and creatinine were among the earliest biomarkers used to identify kidney dysfunction; however, biomarker science has expanded to include markers that more specifically reflect nephron morphology and physiology, including renal function and endothelial integrity.[20]

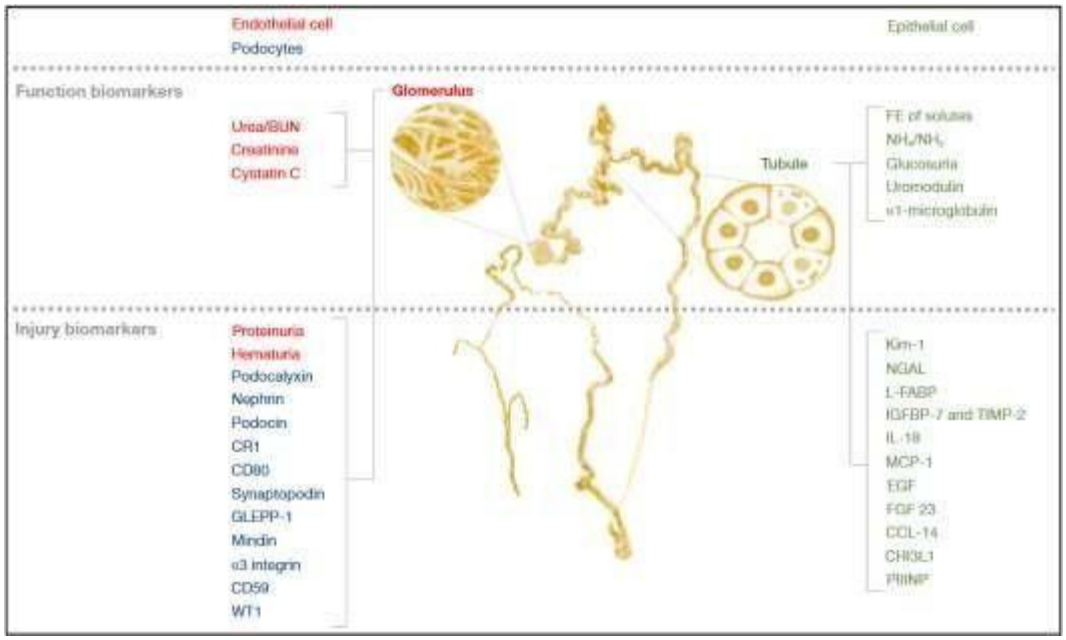


Figure 2.6 Biomarkers for the Detection of Kidney Disease[48]

Biomarkers are important for detecting and diagnosing AKI. AKI biomarkers may be categorized as stress markers, damage markers, and functional markers. Stress markers reflect cellular stress that may resolve or worsen. Damage markers indicate structural injury that may occur with or without a reduction in kidney function. Functional markers reflect

abnormalities in glomerular filtration. These biomarker categories may enable a more precise approach to AKI diagnosis and management than serum creatinine and urine output alone.[48]

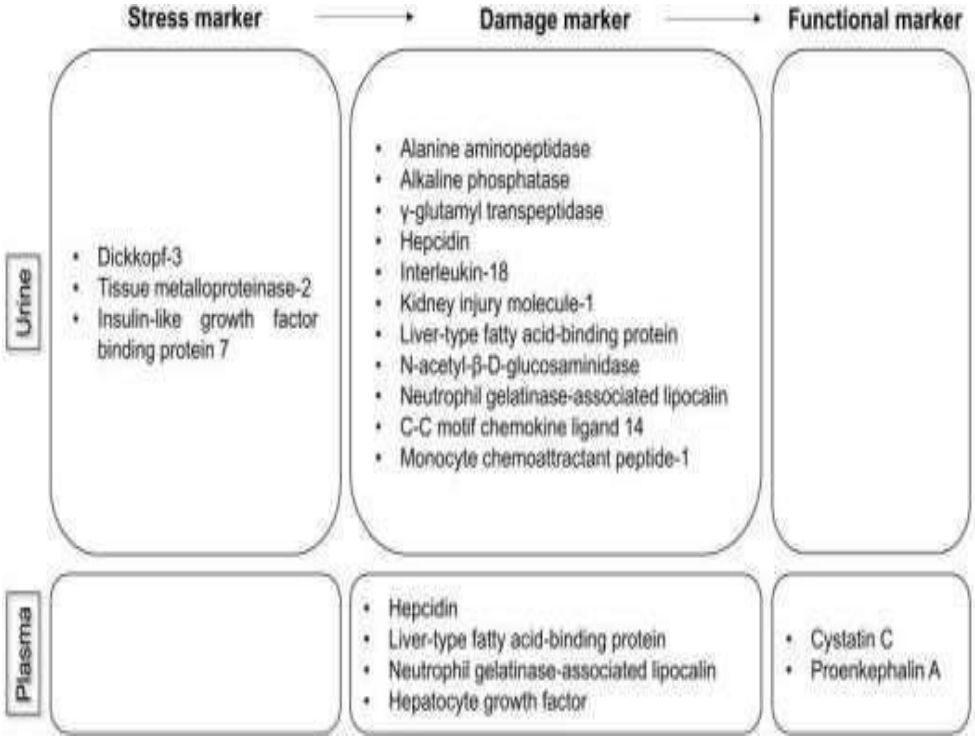


Figure 2.7 Biomarkers in AKI[48]

2.4 Biomarkers Evaluated in This Study

The biomarkers used in this study were selected to represent three distinct but interrelated biological processes. Urinary KIM-1 was used as a marker of tubular injury, CRP as a marker of systemic inflammation, and ROS as a marker of oxidative stress.

2.4.1 Reactive Oxygen Species (ROS) as a Marker of Oxidative Stress

ROS comprise various reactive molecules generated during metabolism and immune responses. During infection, ROS formation is part of host defense against pathogens; however, excessive production can cause cellular and tissue injury. In leptospirosis, increased ROS may result from immune-cell activation, mitochondrial dysfunction, hypoxia, and microcirculatory disturbances. ROS may therefore act not only as biomarkers but also as mediators of cellular injury. Because NAC has antioxidant activity and contributes to maintenance of the glutathione system, changes in ROS levels are relevant for evaluating the biological effects of the intervention.

2.4.1.1 ROS Structure

Inflammation and oxidative stress are closely interconnected. Immune-cell activation increases ROS production, while ROS may activate inflammatory pathways, thereby amplifying endothelial dysfunction, microcirculatory disturbances, and tubular epithelial injury.

ROS are reactive oxygen-derived molecules generated in multiple metabolic processes. A cohort study by Araujo et al. reported high ROS levels in patients with leptospirosis both with and without AKI.[39] ROS include superoxide (O_2•−), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), organic hydroperoxides (ROOH), and hydroxyl radicals (•OH). Sequential monovalent electron transfer to molecular oxygen generates superoxide, which can subse

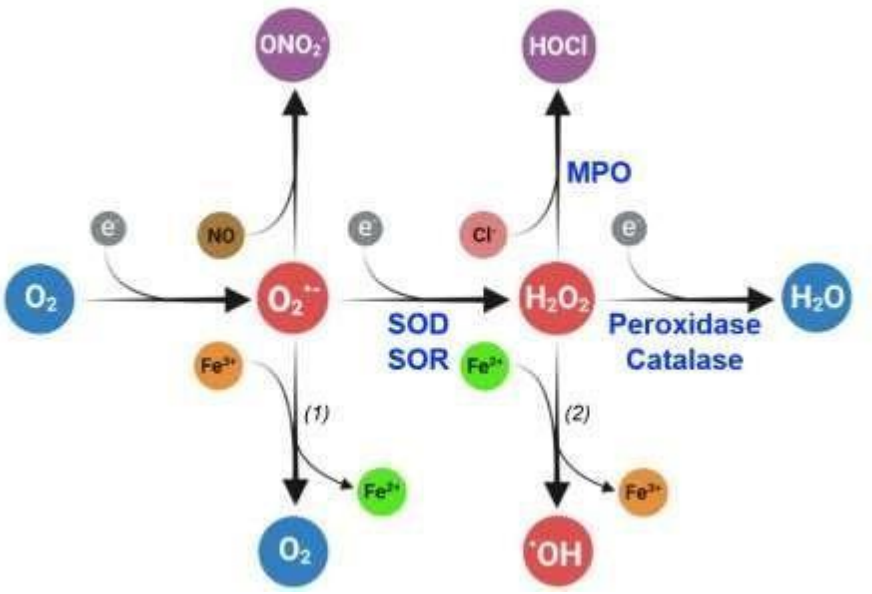


Figure 2.8 Structure of ROS[41]

Peroxidase and catalase catalyze the conversion of hydrogen peroxide to water. Superoxide can react with nitric oxide (NO) to form peroxynitrite (ONOO−), while myeloperoxidase-related reactions can generate hypochlorous acid. Haber–Weiss and Fenton reactions further contribute to reactive species formation. ROS differ in reactivity; the hydroxyl radical (•OH) is particularly reactive and can interact with proteins, DNA, and lipids, whereas O_2•− and H_2O_2 are less reactive and participate in more selective oxidative reactions.[41,42]

2.4.1.2 Sources of ROS in Leptospira spp.

ROS in Leptospira arise from endogenous and exogenous sources. Endogenous sources include flavin- and quinone-containing enzymes in bacterial electron-transfer chains, where electron leakage generates $O_2^{\bullet -}$. Superoxide may be converted to H_2O_2, while release of iron from heme groups, iron–sulfur clusters, or mononuclear iron enzymes can facilitate Fenton chemistry and formation of •OH from H_2O_2. Inactivation of H_2O_2-detoxifying enzymes can increase ROS concentrations and produce growth defects.[18,41]

Exogenous bacterial exposure to ROS may arise from environmental factors such as ionizing radiation, ultraviolet radiation, and chemicals such as paraquat/methyl viologen. Within the host, macrophages and neutrophils containing NADPH-dependent oxidase (Phox) generate superoxide in response to pathogen invasion. Superoxide can interact with NO to form peroxynitrite. NO and peroxynitrite are reactive nitrogen species with oxidative properties analogous to ROS. NADPH oxidase-related activity has been described in renal, pulmonary, and ocular epithelial and endothelial cells and can also be detected in urine and blood.[41]

Within mitochondria, ROS may arise from electron leakage across respiratory-chain complexes I–IV, resulting in incomplete oxygen reduction and superoxide generation. Mitochondrial ROS production may also be stimulated by pathogen-associated molecular patterns (PAMPs). Host-derived ROS exert bactericidal activity but also participate in other antimicrobial pathways, including innate immune responses, neutrophil extracellular trap formation (NETosis), and production of proinflammatory molecules.[41]

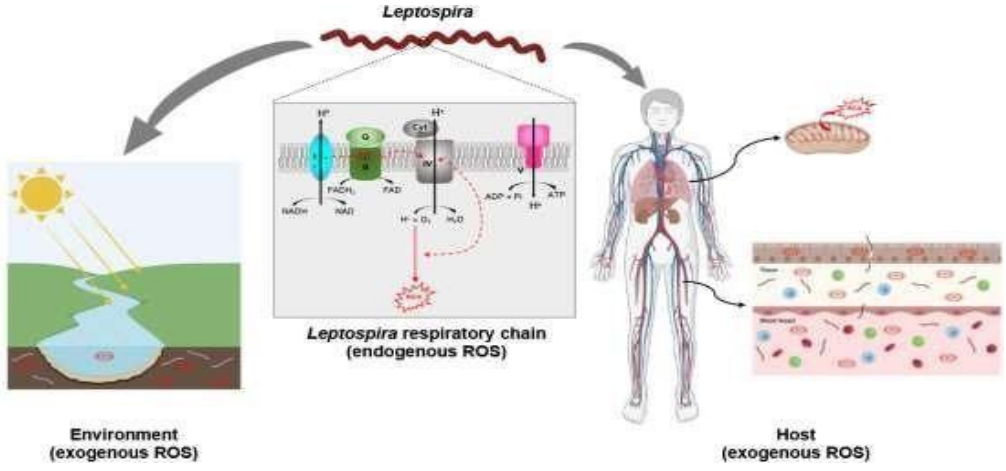


Figure 2.9 Exogenous Sources of ROS in Leptospira[41]

In individuals without specific antimicrobial immunity, phagocytosis is important for pathogen elimination. Shijun et al. reported that mononuclear macrophages can kill Leptospira. During phagocytosis of pathogenic microorganisms, phagocytes generate large amounts of ROS, inducing cellular oxidative stress and enhancing antimicrobial responses. ROS contribute to resistance against leptospiral infection in activated macrophages. Hu W et al. reported that increased ROS generation could be inhibited by the NADPH oxidase inhibitor apocynin.[43]

2.4.1.3 Regulation of ROS Defense Mechanisms in Leptospira spp.

A protein with cysteine characteristics and SoxR homology has been identified in Leptospira spp. Two regulatory proteins, PerRA and PerRB, participate in ROS regulation. PerRA controls expression of defenses against H_2O_2. PerRB acts as an ROS sensor that dissociates from DNA in the presence of ROS, resulting in transcriptional upregulation, and functions as a repressor of genes involved in superoxide detoxification.[41,44]

The H_2O_2 transcriptional response in Leptospira includes catalase and peroxidase genes (katE and CCP) regulated by PerRA, among the genes most responsive to H_2O_2. Catalase constitutes a first-line defense when Leptospira are exposed to H_2O_2 and is located in the leptospiral periplasm, where it rapidly eliminates H_2O_2. At high H_2O_2 exposure, additional oxidative-stress and redox-response genes, including thiol peroxidase and thioredoxin disulfide reductase, as well as DNA-repair pathways, are activated. Leptospira can rapidly degrade H_2O_2 before it is converted to highly reactive •OH and possess multiple repair mechanisms for oxidative protein and DNA damage when H_2O_2-detoxifying systems are overwhelmed.[41,45]

2.4.1.4 ROS Production in Severe Leptospirosis

A study by Araujo et al. evaluated ROS production and glutathione (GSH) levels in relation to complications among hospitalized patients with leptospirosis. Twelve patients and nine healthy controls were included; seven patients developed AKI and five did not. Blood samples were analyzed for ROS production and GSH using chemiluminescence and absorbance assays. ROS production was higher and GSH levels lower in patients with leptospirosis than in healthy individuals. Low GSH was associated with thrombocytopenia and higher serum creatinine, nitric oxide, and potassium levels.[18]

Patients were categorized into AKI, non-AKI, and healthy-control groups. The AKI group had a higher frequency of oliguria (43% vs 20%), lower hematocrit (27.9% vs 37.4%), lower platelet counts (median 53,000/μL vs 142,000/μL), and a greater need for hemodialysis (42.9% vs 0%) than hospitalized patients without AKI. Patients with severe leptospirosis with and without AKI had higher ROS production than healthy participants (P=0.0012 and P=0.0043, respectively), but ROS production did not differ significantly between AKI and non-AKI patients. GSH levels were lower in severe leptospirosis with and without AKI than in healthy controls (P=0.0290 and P=0.0002, respectively), without a significant difference between the two leptospirosis groups.[18]

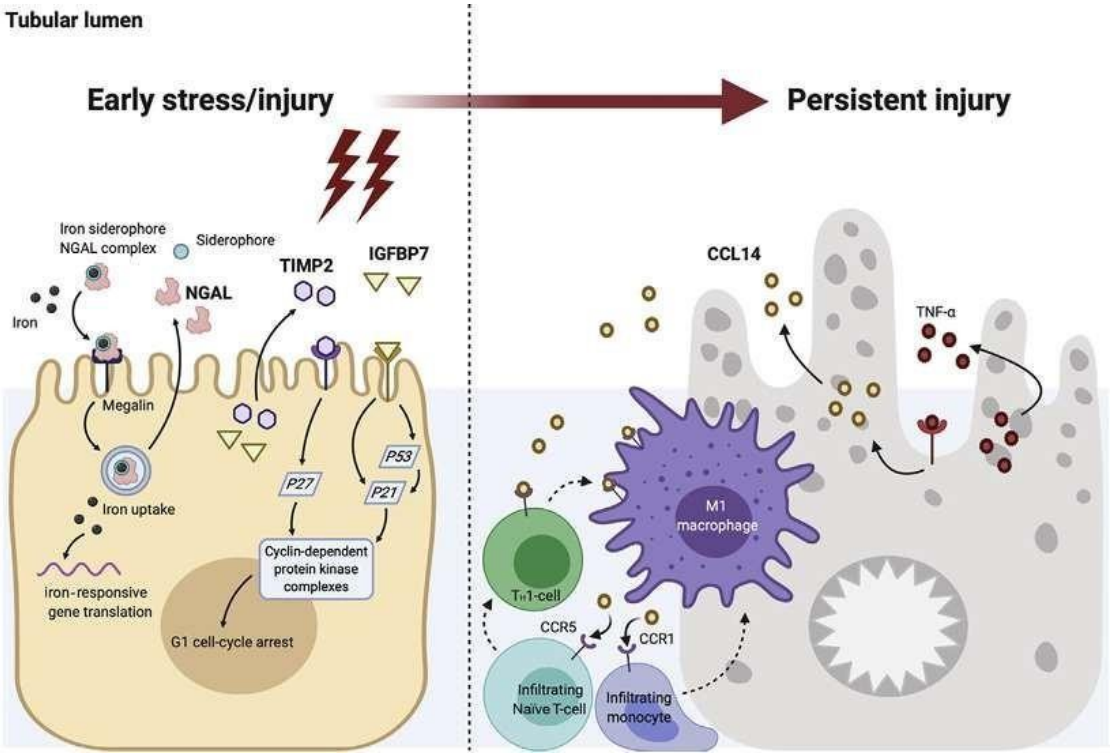


Figure 2.10 Mechanisms of AKI[59]

2.4.2 C-Reactive Protein (CRP) as a Marker of Systemic Inflammation

CRP is an acute-phase protein produced primarily by hepatocytes in response to inflammatory mediators, particularly interleukin-6. CRP concentrations increase in many infectious and inflammatory conditions. In leptospirosis, elevated CRP reflects systemic inflammatory activity but is not specific to kidney injury. Accordingly, CRP was used in this study as a marker of the inflammatory component rather than as a specific biomarker of AKI.

NAC may modulate inflammatory responses through interactions with redox balance and inflammatory signaling pathways. Changes in CRP during the intervention period may therefore provide information regarding the effect of NAC on systemic inflammation.

AKI involves inflammation, endothelial dysfunction, tubular injury, and hemodynamic alterations. Recent studies have emphasized the importance of inflammation in AKI pathogenesis.[17] CRP is a pentameric protein composed of five identical subunits and functions in acute responses to infection and inflammation.[14,60] CRP is synthesized in the liver; during acute infection, secretion begins within approximately 4–10 hours after onset, peaks at about 48 hours, and has a half-life of approximately 19 hours.[60,61]

In AKI, CRP may be expressed by inflammatory cells such as macrophages and intrinsic renal cells, including tubular and endothelial cells. In CKD or end-stage renal disease, CRP may contribute to chronic inflammation. CRP has proinflammatory and anti-inflammatory properties related to two isoforms, pentameric CRP (pCRP) and monomeric CRP (mCRP), which participate in inflammation, fibrosis, and ischemia–reperfusion injury in kidney disease.[62]

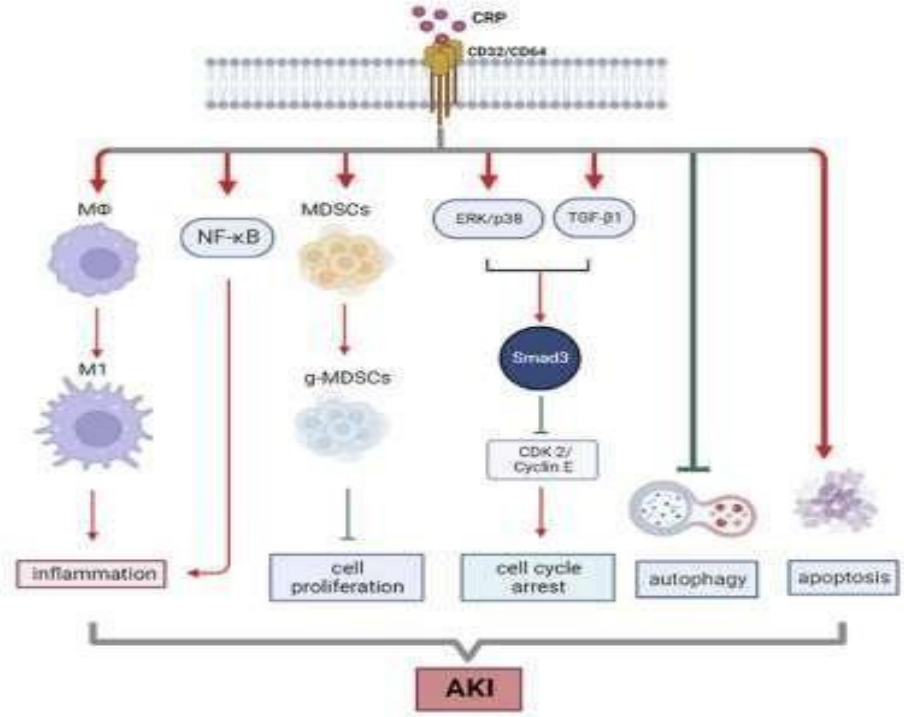


Figure 2.11 Mechanisms of CRP in AKI[63]

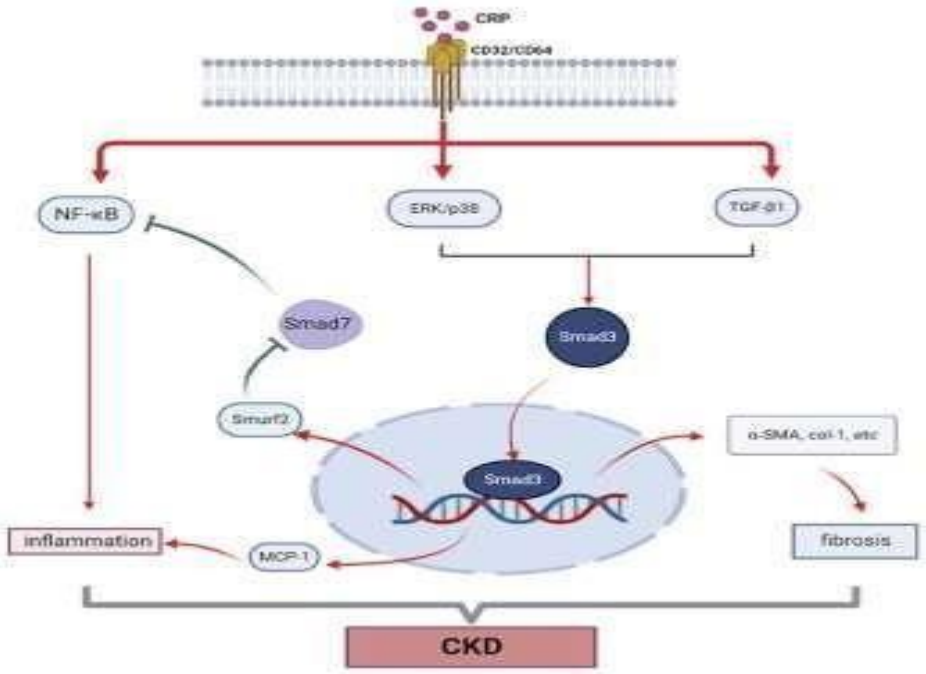


Figure 2.12 Mechanisms of CRP in CKD[63]

Mechanisms through which CRP may participate in AKI and CKD include M1 macrophage activation, NF-κB signaling, and Smad3 signaling.[63]

1. CRP and M1 macrophage activation. M1 and M2 macrophages have important roles in AKI and CKD. M1 macrophages infiltrate renal tubules and release proinflammatory mediators that may exacerbate kidney injury. M2 macrophages contribute to control of inflammation and tissue repair during AKI, although some studies suggest that they may also participate in the transition from AKI to CKD. CRP can induce an M1 phenotype and promote M2-to-M1 conversion in vitro. M1 activation and FcγR gene expression contribute to ischemia–reperfusion-associated AKI.[63]

2. CRP and NF-κB signaling. CRP activates NF-κB signaling and induces monocyte chemoattractant protein-1 (MCP-1), promoting macrophage infiltration and severe renal inflammation. CRP may also induce membrane glycoproteins and DPP4, which regulate NF-κB activation in diabetic nephropathy.[63]

3. CRP and Smad3 signaling. Smad3 is an important mediator of TGF-β signaling and interacts with other pathways, including NF-κB and mTOR, to promote inflammation and fibrosis in CKD. High CRP levels may contribute to renal necrosis and fibrosis through

Smad3 signaling. Following binding to CD32, CRP may activate Smad3 directly and indirectly through TGF-β1 and ERK/p38 MAPK–Smad crosstalk, leading to cell death through Smad3–p27-dependent G1 cell-cycle arrest.[63]

4. Other mechanisms. Myeloid-derived suppressor cells, autophagy, and apoptosis represent additional mechanisms through which CRP may contribute to AKI and CKD. CRP may increase accumulation of myeloid-derived suppressor cells during AKI. CRP overexpression can dysregulate autophagy and activate apoptosis in HK-2 cells, increasing susceptibility to ischemia–reperfusion-associated AKI.

2.4.3 Kidney Injury Molecule-1 (KIM-1) as a Marker of Tubular Injury

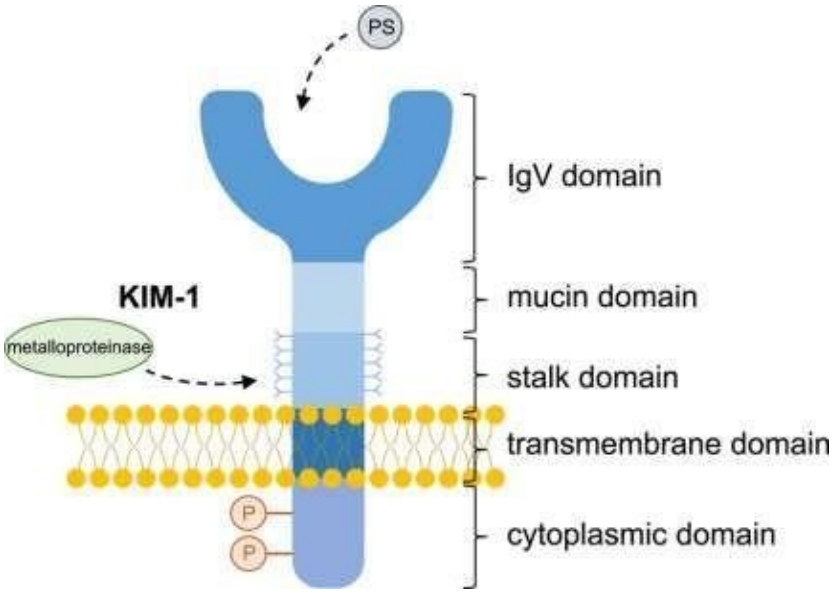


Figure 2.13 Structure of KIM-1[64]

Kidney Injury Molecule-1 (KIM-1), previously known as T-cell immunoglobulin mucin-1 (TIM-1) or hepatitis A virus cellular receptor 1 (HAVCR1), is a transmembrane protein expressed at the apical surface of proximal tubular cells. Its structure includes an O-glycosylated mucin domain and a six-cysteine immunoglobulin-like domain.[65] KIM-1 is a 38.7-kDa immunoglobulin-like molecule that functions as an adhesion molecule and receptor involved in phagocytosis of apoptotic epithelial-cell debris after ischemic or toxic injury.[64]

KIM-1 is expressed at very low levels in normal kidneys and can be detected in urine within 24–48 hours after tubular injury.[57] In healthy individuals, urinary KIM-1 levels are very low

(<1 ng/mL). Quantitative urinary measurements indicate that KIM-1 may increase linearly with age even in healthy individuals.[66] KIM-1 can serve as an early biomarker of proximal tubular epithelial injury and proliferation and has demonstrated useful sensitivity and specificity for AKI detection. It may also have value in chronic kidney disease and in studies of drug nephrotoxicity.[48,59,67]

During AKI, the extracellular domain (ectodomain) of KIM-1 is shed into urine and levels may increase markedly. KIM-1 rises early, including within approximately 24 hours after renal ischemia–reperfusion injury.

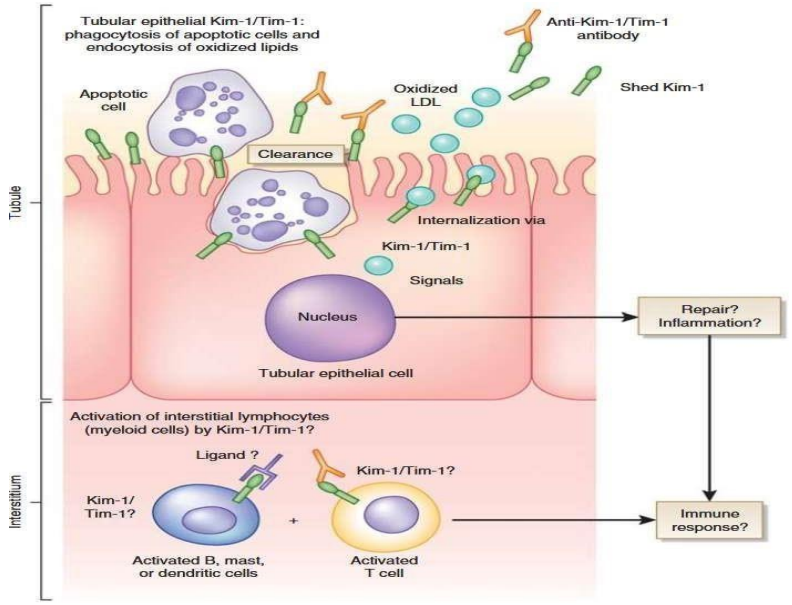


Figure 2.14 Pathophysiology of KIM-1/TIM-1 Expression in Injured Proximal Tubular Epithelium[65]

After injury, proximal tubular cells become KIM-1-positive and much of KIM-1 is released into the extracellular space, including urine.[58] A small proportion of activated T cells may also express KIM-1/TIM-1. KIM-1 activation occurs together with lymphocyte and myeloid-cell activation during injury-related immune responses. KIM-1/TIM-1 antibodies may influence receptor activation and ligand binding and may also interact with low-density lipoprotein-related pathways.[65]

KIM-1 has shown sensitivity for early detection of kidney injury in both animals and humans. A meta-analysis by Shao et al. reported diagnostic sensitivity of 74% and specificity of 86%. Elevated KIM-1 levels have been associated with inflammation and fibrosis on

histopathological examination. Literature also suggests that KIM-1 may provide prognostic information regarding progression from AKI to CKD and has been evaluated in populations after cardiac surgery, in liver cirrhosis, diabetic nephropathy, and nephrotoxic exposures such as cisplatin.[68]

Dias et al. investigated KIM-1 for AKI detection in dogs with leptospirosis. Thirty animals were divided into a leptospirosis group (n=25) and healthy controls (n=5). Diagnosis used urinary PCR and the microscopic agglutination test (MAT) during days 3–7 of symptoms. All samples in the leptospirosis group were reactive by MAT (titer ≥100), 56% (14/25) had high antibody titers (≥800), and 72% (18/25) were PCR-positive. Common findings included anemia, leukocytosis, and azotemia. Urinary KIM-1 concentrations differed significantly between animals with and without leptospirosis ($P<0.01$), supporting an association between KIM-1, hypoxia, inflammatory responses, and kidney injury.[69]

A Sri Lankan study by Thilini et al. evaluated KIM-1 and MCP-1 in leptospirosis-associated AKI. Among 170 patients with suspected leptospirosis, 79% had confirmed leptospirosis and 24.05% developed AKI according to KDIGO criteria. Serum KIM-1, urinary KIM-1, serum MCP-1, and urinary MCP-1 were higher in patients with leptospirosis-associated AKI than in those without AKI. The study supported KIM-1 as a kidney-injury biomarker and MCP-1 as a marker related to ischemic AKI. During infection, hematogenous leptospiral dissemination to the kidney can injure tubular capillaries and the proximal tubular lumen, triggering KIM-1 release. Serum KIM-1 was detectable early and peaked later during the febrile course.[68,70,71]

Quantitative KIM-1 assays have been developed using microbead-based KIM-1 ELISA and lateral/laminar-flow dipstick approaches. These methods support the concept that KIM-1 may increase earlier than serum creatinine and blood urea nitrogen after renal ischemia.[49] Urinary KIM-1 may therefore complement conventional measurements such as serum creatinine for earlier AKI detection.[68]

2.4.4 Kinetics and Relevance of CRP, ROS, and KIM-1 in Leptospirosis

CRP is produced by hepatocytes in response to IL-6, IL-1β, and TNF-α released by macrophages and endothelial cells. CRP functions as a pattern-recognition molecule that recognizes phosphocholine on microorganisms and dead cells and facilitates opsonization and complement activation through the classical pathway. During the leptospiremic phase

(days 1–4), CRP begins to increase as innate immunity is activated. During the immune phase (days 5–10), CRP may peak, particularly in severe complications such as AKI or Weil's syndrome. During resolution, CRP declines in parallel with pathogen clearance and attenuation of inflammation. Clinically, CRP correlates with disease severity but is not kidney-specific. In leptospirosis-associated AKI, high CRP indicates systemic inflammation that may activate profibrotic cytokines and renal endothelial injury but does not specifically quantify renal parenchymal damage.[69,70]

ROS are generated by NADPH oxidase complexes in neutrophils and macrophages and by renal tubular mitochondria. In leptospirosis, ROS generation may be triggered by TLR2/4 activation by leptospiral lipoproteins, followed by endothelial permeability changes, hypoperfusion, tubular hypoxia, and mitochondrial ROS production. Persistent immune dysregulation may produce a prolonged oxidative burst. During early infection (days 1–2), NOX activation increases local and systemic ROS; levels may peak during days 3–7, particularly with endothelial dysfunction and pro-oxidative tubular injury. After day 7, ROS may remain elevated if inflammation is uncontrolled or tubulointerstitial fibrosis develops. ROS are not merely markers but may mediate tubular apoptosis or necrosis, NLRP3 inflammasome activation with amplification of IL-1β and IL-18, and mitochondrial dysfunction that aggravates AKI.[69,71]

KIM-1 is a transmembrane protein minimally expressed in normal kidneys but strongly induced in injured proximal tubular cells. It is released into urine as soluble KIM-1 through proteolytic shedding involving ADAM metalloproteinases. In leptospirosis-associated AKI, KIM-1 may begin to rise when proximal tubular injury develops on approximately days 2–3. Urinary KIM-1 expression may peak during days 4–7, reflecting tubular injury, regeneration, and phagocytosis of debris. During days 7–14, levels may decline with resolution but remain elevated in persistent tubulointerstitial injury. KIM-1 is considered a sensitive structural AKI biomarker and has been associated with histopathological tubular injury and risk of non-recovery or progression to CKD.[70,71]

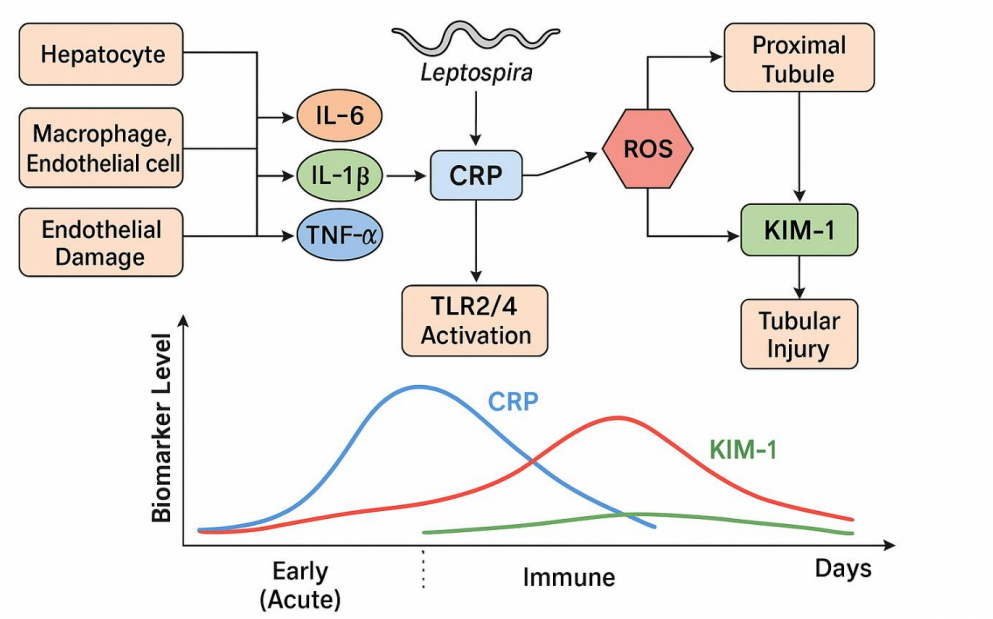


Figure 2.15 Kinetics of CRP, ROS, and KIM-1 Biomarkers in Leptospirosis

2.5 N-Acetylcysteine (NAC)

2.5.1 Structure and Pharmacology

N-acetylcysteine (NAC) is an acetylated derivative of the amino acid L-cysteine containing a thiol group. It has long been used in clinical practice and has several biological effects related to cellular redox systems. Following administration, NAC serves as a source of cysteine for glutathione synthesis. Its thiol group also enables interaction with several oxidant species.

NAC ($C_5H_9NO_3S$) is the N-acetyl derivative of L-cysteine. Its sulfhydryl (–SH) group contributes to glutathione (GSH) synthesis. NAC acts as an antioxidant in conditions of oxidative stress. When antioxidant capacity and ROS generation become imbalanced, oxidative stress can damage cellular components and impair cellular function.[53, 73, 74]

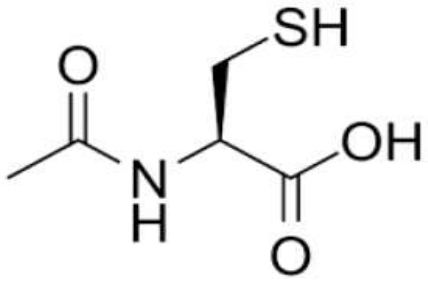


Figure 2.16 Chemical Structure of NAC[75]

2.5.2 Antioxidant Mechanisms

The antioxidant effects of NAC are primarily related to its ability to support glutathione synthesis, one of the major intracellular antioxidant systems. Increased cysteine availability helps maintain glutathione capacity during increased oxidative burden. By modulating redox balance, NAC may reduce oxidative damage to lipids, proteins, and other cellular components. This mechanism is relevant to leptospirosis because oxidative stress is an important component of its pathogenesis.

NAC is absorbed through the small intestine and undergoes first-pass metabolism, particularly in the liver. After cellular uptake, it contributes to the GSH synthesis cycle.[77,78] L-cysteine is rapidly oxidized to cystine and used by the cystine/glutamate antiporter (system Xc−). Cystine enters cells in exchange for glutamate and is reduced intracellularly to cysteine, increasing GSH synthesis.[73,76,79,80] NAC may also interact with ROS and increase total antioxidant capacity. It may inhibit inflammatory mediators such as IL-8, IL-6, and TNF-α. Its antioxidant effects include direct activity through the thiol group and indirect activity through increased intracellular cysteine as a GSH precursor.[81]

NAC is a thiol compound and acetylated precursor of L-cysteine capable of reducing several radicals by donating electrons or acting as a nucleophile. Its sulfhydryl group and acetyl group contribute to metabolic, antioxidant, and mucolytic actions.[77]

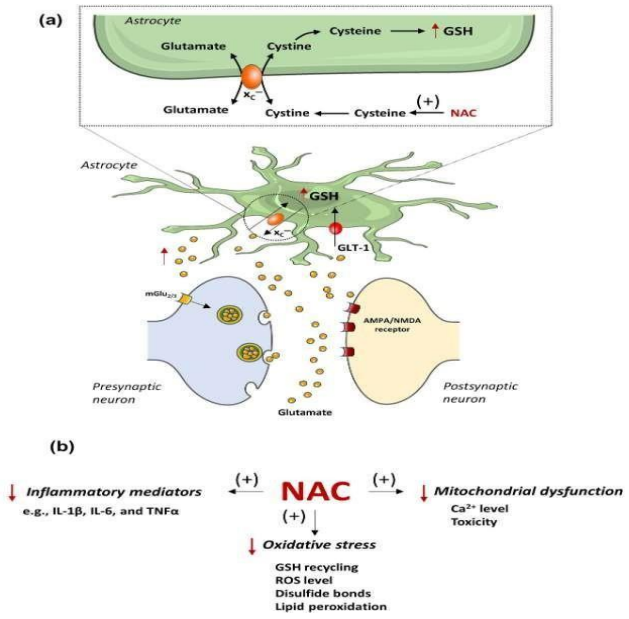


Figure 2.17 Mechanism of Action of NAC[76]

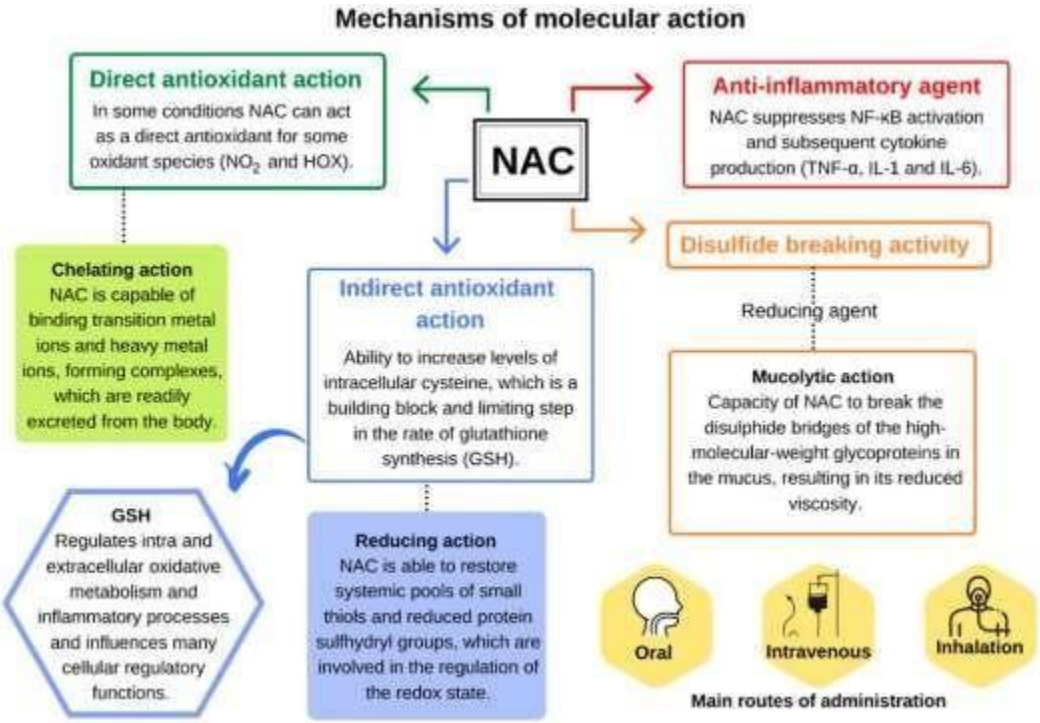


Figure 2.18 Molecular Mechanisms of Action of NAC[77]

Direct antioxidant activity is related to the free thiol group, which reacts with reactive oxygen and nitrogen species (RONS), including hydroxyl radicals, nitrogen dioxide, carbonate radicals, superoxide, hydrogen peroxide, and peroxynitrite. Experimental data indicate that NAC reacts rapidly with highly reactive radical species, whereas reactions with superoxide, hydrogen peroxide, and peroxynitrite are relatively slow.[77]

Table 2.2 Reaction Rate Constants of NAC with Various Compounds in Experimental Systems[77]

The original thesis presents reaction-rate constants for NAC with several reactive oxygen and nitrogen species. The numerical values and experimental conditions should be retained exactly as in the source table when the final integrated thesis is typeset.

GSH is the most abundant non-protein thiol in the body and is a major antioxidant that maintains cellular redox status through direct reactions with reactive species and by acting as a cofactor or substrate for antioxidant enzymes. Intracellular cysteine concentrations are lower and may become rate-limiting for GSH biosynthesis, explaining the role of NAC as a source of cysteine and a means of restoring intracellular GSH.[77]

Administration of GSH or cysteine alone is less effective for increasing intracellular GSH, making NAC an important strategy for limiting oxidative injury in xenobiotic poisoning such as acetaminophen toxicity and in other conditions associated with GSH depletion.[77]

Reactive Species	Rate Constant ($M^{-1}\cdot s^{-1}$)	Experimental Conditions
$^{\bullet}OH$	1.36×10^{10}	pH 7, rt
$^{\bullet}NO_2$	$\approx 2.4 \times 10^{8}$ [a] $\approx 1.0 \times 10^{7}$ [b]	pH > pKa, rt pH 7.4, rt
$CO_3^{\bullet-}$	$\approx 1.0 \times 10^{7}$ 1.8×10^{8}	pH 7, rt pH 12, rt
HNO	5×10^{5}	pH 7.4, 37 °C
$O_2^{\bullet-}$	$68 \pm 6 \times 10^{3}$	pH 7, rt pH 7.4, 25 °C
H_2O_2	0.16 ± 0.01 0.85 ± 0.09	pH 7.4, 37 °C pH 7.4, 25 °C
$ONOO^-$	415 ± 10	pH 7.4, 37 °C

Figure 2.19 Chemical Formula of NAC and Its Conversion to Glutathione[77]

2.5.3 Anti-inflammatory Mechanisms and Potential Renoprotective Effects

Redox balance is closely linked to regulation of inflammatory pathways. By modifying the intracellular redox environment, NAC may influence activation of inflammatory signaling pathways and production of proinflammatory mediators. In the kidney, attenuation of oxidative stress and inflammation may help preserve tubular epithelial integrity and limit amplification of injury. These mechanisms represent a biological rationale and potential mechanism, rather than proof that NAC prevents AKI in leptospirosis.

NAC can reduce cysteinylated extracellular proteins and release free thiol groups with antioxidant capacity, thereby supporting GSH biosynthesis. Its indirect antioxidant activity also includes restoration of low-molecular-weight thiols and protein sulfhydryl groups in their reduced form. Mercaptoalbumin is one important antioxidant in plasma and extracellular fluid.[77]

Experimental studies indicate that NAC may alter protein structure or function, including reducing angiotensin II receptor-binding affinity in vascular smooth-muscle cells, modifying TNF-α receptor affinity for cytokines, and reducing the ability of TGF-β1 to bind the type III receptor (TβRIII/betaglycan).[77]

NAC also has anti-inflammatory activity through inhibition of NF-κB, an important transcription factor in inflammatory cascades and immune responses to oxidative stress. NAC may prevent nuclear translocation and activation of NF-κB and thereby suppress expression of proinflammatory genes. Studies have reported reduced release of TNF-α, IL-1β, and IL-6 from lipopolysaccharide-activated macrophages.[77]

Pharmacodynamically, NAC increases cellular antioxidant capacity by improving redox balance through increased GSH, neutralizing ROS, and inhibiting inflammatory pathways such as NF-κB and MAPK involved in IL-6, IL-8, and TNF-α production.[77] In experimental models of ischemic and septic kidney injury, reductions in oxidative-stress and inflammatory biomarkers may be detectable within 24–48 hours after intervention.[77,82]

NAC acts directly through its thiol group and indirectly by increasing intracellular glutathione synthesis. The source thesis describes rapid onset of direct antioxidant effects after intravenous administration and a later onset after oral administration, with anti-inflammatory effects developing over the subsequent hours.[77,82]

The acetyl group of NAC is removed to generate free cysteine. NAC-deacetylating acylase activity is high in the kidney and liver, and after oral administration deacetylation also occurs in the intestinal mucosa. GSH is synthesized in the liver using cysteine and other sulfur-containing amino acids delivered through the portal circulation. Hepatic GSH is then released into the systemic circulation. GSH is a major intracellular reducing agent, closely related to the GSH:GSSG ratio and mitochondrial redox status. Approximately half of plasma GSH is taken up by the kidney, an organ highly dependent on GSH because of the high aerobic energy requirements of tubular cells. Renal GSH is obtained through basolateral uptake and endogenous synthesis from cysteine.[82]

The integrity of GSH synthesis pathways influences the effectiveness of NAC as a GSH precursor. Advanced CKD has been associated with lower GSH concentrations in mononuclear cells and plasma than in healthy individuals and patients with earlier CKD. Nrf2 regulates enzymes and transporters involved in GSH metabolism, including glutamate-cysteine ligase and amino-acid transporters such as SLC6A9 and SLC7A11. Nrf2 also induces gamma-glutamyltransferase, increasing cysteine availability for GSH synthesis, particularly in proximal tubular epithelium. Nrf2 and its target genes may become

dysfunctional in advanced CKD and AKI, potentially contributing to variation in response to NAC among patient groups.[82]

2.5.4 Pharmacokinetics of Oral NAC and Rationale for Dose Selection

In healthy individuals, the intestinal mucosa is an important site of first-pass NAC metabolism through deacetylation. Total oral bioavailability is approximately 10%, with reported values of 8.3% after a 600-mg effervescent tablet, 11.6% after 1200 mg, and approximately 9.1% after a 400-mg effervescent formulation. Reported maximum total NAC plasma concentrations (Cmax) and time to Cmax vary by formulation.[83]

Oral bioavailability varies substantially according to formulation. Repeated administration has produced variable effects on Cmax across studies. In patients with stage 5 CKD receiving dialysis, plasma NAC levels after oral administration may be considerably higher than in healthy individuals. In peritoneal dialysis patients, repeated NAC administration has also been associated with increased plasma concentrations.[82,83]

Although peak plasma concentrations after oral NAC are lower than after intravenous administration, repeated oral administration may still increase intracellular GSH. Reductions in oxidative-stress and inflammatory biomarkers such as ROS and CRP may become apparent after 48–72 hours of consistent oral therapy, particularly in conditions with high systemic oxidative burden. Oral administration is therefore biologically relevant as an adjunctive strategy in infection-associated AKI such as leptospirosis.[82] Based on biomarker kinetics, CRP and ROS peak during the acute phase, whereas urinary KIM-1 may appear within 24–48 hours and peak later. Measurements on Day 1 and Day 5 were selected to capture changes during the intervention period before natural disease resolution.

Reported steady-state total volume of distribution of NAC is approximately 0.47 L/kg and plasma clearance approximately 0.11 L/hour/kg in healthy participants, although other studies report different clearance and terminal half-life estimates. A fraction of NAC is excreted renally. NAC can also be removed by hemodialysis, with extraction depending on dialysis blood and dialysate flow rates.[83]

Table 2.3 Pharmacokinetic Parameters of Total NAC[83]

Pharmacokinetic parameters	Healthy, NAC p.o.	CKD G5, dialysis, NAC p.o.	Healthy, NAC i.v.	CKD, no KRT, NAC i.v.	CKD G5, dialysis, NAC i.v.
Bioavailability (%)	~10	–	100	100	100
C_{max}	14–17 µmol/l with 600 mg	63 µmol/l with 600 mg	121 µmol/l with 200 mg (applied over 1 min)	430 µmol/l with 150 mg/kg and 2000 µmol/l with 450 mg/kg over 15 min	325 µmol/l with 2 g during 3 h of dialysis
t_{max}	40–70 min after administration; 110 min with sustained release formulation	160 min after administration with sustained release formulation	~at administration	measured 5 min after administration	at end of administration
Repeated dosing	Accumulation possible	Accumulation was shown	–	–	Accumulation was shown
Time to steady state with repeated dosing	–	–	–	–	from 4th dose with 3 dialysis sessions/wk
Total body clearance	0.1–0.9 l/h/kg	0.02–0.06 l/h/kg, Dialytic clearance 0.07 l/h/kg	0.1–0.9 l/h/kg	–	0.02–0.06 l/h/kg, dialytic clearance 0.07 l/h/kg

The source thesis summarizes total NAC pharmacokinetic parameters across healthy individuals and patients with advanced CKD/dialysis. In stage 5 CKD, total body clearance is markedly reduced and terminal half-life is prolonged compared with healthy individuals. These findings highlight the need for further clinical studies to characterize achievable plasma concentrations, time profiles, formulation differences, and steady-state concentrations after repeated administration.[83]

NAC metabolites may contribute to observed biological effects. These include cysteine, glutathione, other sulfhydryl-containing compounds, hydrogen sulfide, and sulfane sulfur species. However, knowledge regarding the pharmacokinetics and tissue uptake of these metabolites remains limited. Studies in healthy participants have demonstrated increases in plasma cysteine and GSH after repeated oral NAC, although findings vary by formulation and timing. In CKD, GSH responses may differ according to disease severity and dialysis status.[83]

2.5.5 Use of NAC in Acute Kidney Injury and Leptospirosis

NAC has been studied in conditions involving oxidative stress, inflammation, and kidney injury. Findings vary according to population, etiology of kidney injury, dose, route, timing of intervention, and outcomes assessed. Experimental evidence supports the ability of NAC to modulate oxidative stress and inflammation; however, translation of these biological effects into consistent clinical benefit has not been demonstrated across all settings. Evidence from other diseases therefore cannot be directly generalized to leptospirosis.

A previous clinical trial in patients with leptospirosis evaluated NAC in relation to recovery from kidney dysfunction. Although that study provided a basis for further research, it did not

simultaneously assess urinary KIM-1, CRP, and ROS as markers of tubular injury, inflammation, and oxidative stress.

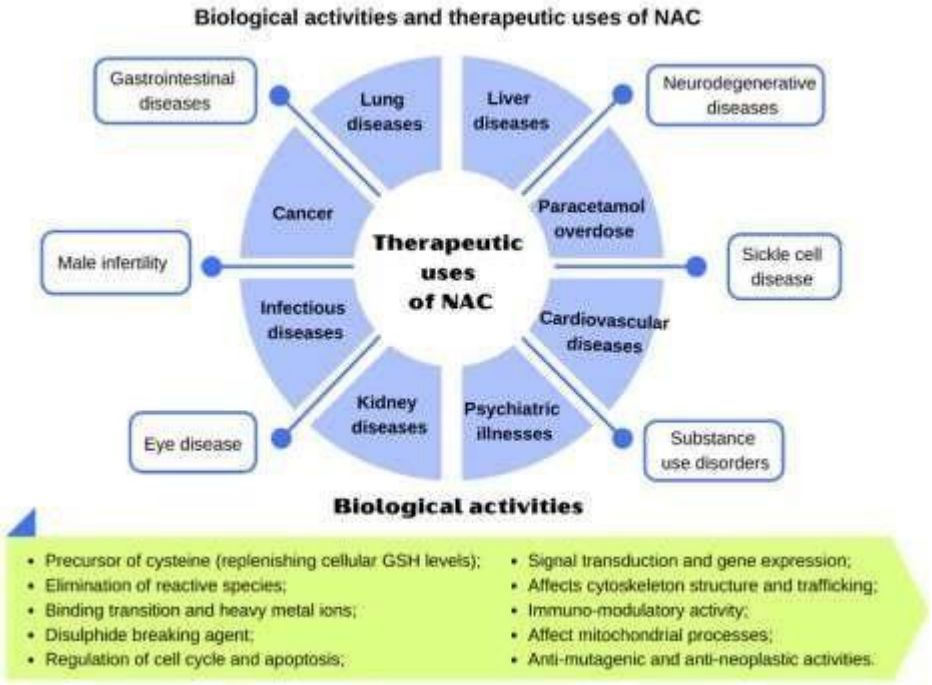


Figure 2.19 Biological Activities and Therapeutic Uses of NAC[77]

Renal cortical and medullary cells are rich in mitochondria and are highly susceptible to oxidative stress. Systemic inflammation, such as that occurring in sepsis, releases multiple inflammatory mediators, while oxidative stress may contribute to AKI through vascular insufficiency, apoptosis, autophagy, and fibrosis. Because renal cellular metabolism is highly dependent on mitochondrial function, ROS generation is an important pathway in AKI pathogenesis.[46]

Ischemia–reperfusion injury is a common experimental model of oxidative kidney injury. NAC contains a thiol group and acts as an antioxidant by supporting detoxification, increasing intracellular glutathione, and counteracting free radicals. Watanabe et al. investigated short- and longer-duration NAC administration in Wistar rats with ischemic AKI using a dose of 150 mg/kg delivered through the renal artery. ROS and RNS were assessed in urine using ferrous oxidation of xylenol orange version 2 (FOX-2) and thiobarbituric acid reactive substances (TBARS).[47]

Table 2.4 Effects of NAC on ROS and RNS After Renal Ischemia–Reperfusion Injury[47]

Groups	Urinary peroxides (nmol/g urinary Cr)	Nitric oxide (nmol/g urinary Cr)	TBARS (nmol/g urinary Cr)
SHAM	1.7 ± 0.1	30.2 ± 2.2	54.4 ± 15.6
SHAM + NAC	1.4 ± 0.4	29.0 ± 5.2	69.8 ± 5.5
ISC 30	5.9 ± 0.2^{ab}	76.6 ± 6.6^{ab}	98.7 ± 12.5^{ab}
ISC 30 + NAC	2.4 ± 0.4^{c}	31.1 ± 2.4^{c}	58.1 ± 16.4^{c}
ISC 45	9.7 ± 1.8^{abcd}	76.2 ± 14.5^{abd}	83.9 ± 8.6^{ad}
ISC 45 + NAC	7.8 ± 1.6^{abcde}	82.9 ± 5.0^{abd}	104.8 ± 13.7^{abd}

Table 2.5 Effects of NAC on Antioxidant Capacity After Renal Ischemia–Reperfusion Injury[47]

Groups	Thiols (nmol/mg total protein)	GSH-Px activity (U/g of renal tissue)
SHAM	204.3 ± 25.3	12.1 ± 4.9
SHAM + NAC	200.8 ± 12.5	11.4 ± 1.6
ISC 30	109.3 ± 9.5^{ab}	7.3 ± 1.7
ISC 30 + NAC	95.6 ± 3.7^{ab}	14.5 ± 3.0^{c}
ISC 45	109.2 ± 35.1^{ab}	8.3 ± 1.3^{d}
ISC 45 + NAC	103.7 ± 20.0^{ab}	13.2 ± 2.8^{c}

NAC reduced urinary peroxide and nitrite excretion and increased antioxidant capacity, as reflected by GSH activity, after short-duration ischemia. High NAC doses may have pro-oxidant effects through reduction of transition metals and enhancement of Fenton reactions. In the experimental model, prolonged ischemia was associated with higher urinary TBARS despite increased renal GSH activity, and prolonged NAC administration did not improve kidney recovery. Nitric oxide and peroxynitrite pathways may contribute to these divergent effects.[47]

2.5.5.1 Preclinical Study of the Anti-inflammatory Effects of NAC in Septic Rats with AKI

Heng Fan et al. evaluated the renoprotective effects of NAC in a cecal ligation and perforation (CLP) model of septic AKI. Male rats aged 10–12 weeks and weighing 300–400 g were allocated to control, control+NAC, CLP, and CLP+NAC groups. NAC 150 mg/kg

was administered for three days before induction of sepsis. Renal inflammatory factors including TNF-α, IL-1β, IL-6, and IL-8 were measured by ELISA.[46]

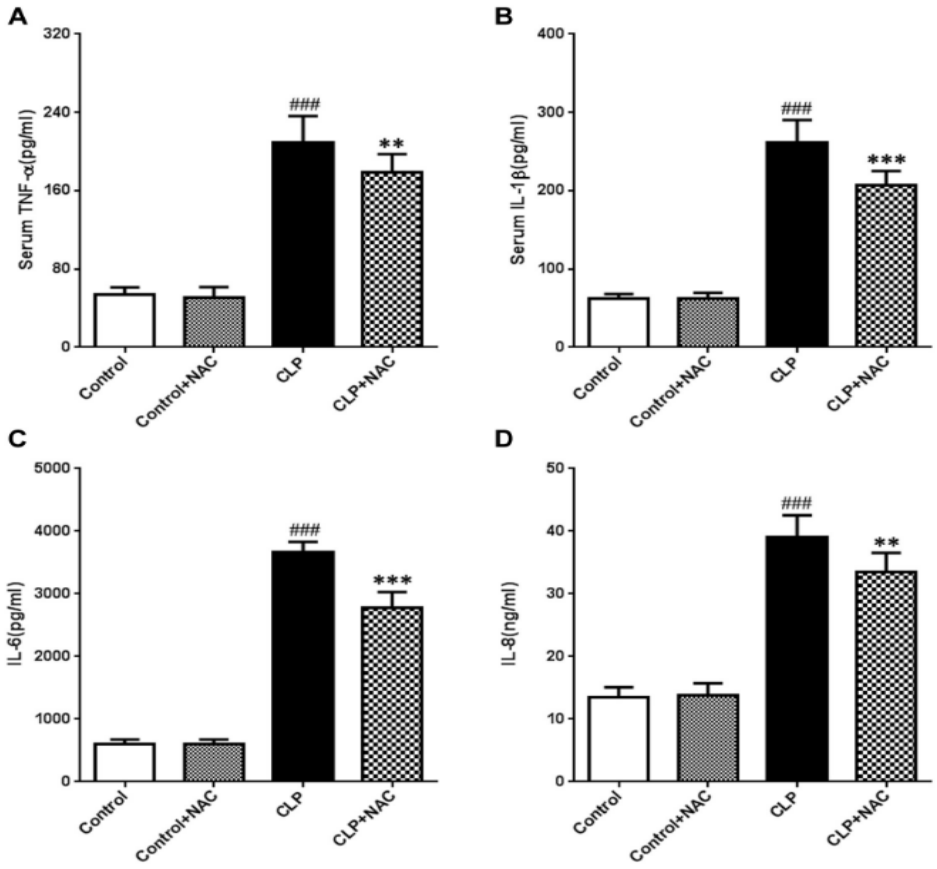


Figure 2.20 Anti-inflammatory Effects of NAC in Renal Tissue[46]

The study supported the importance of increased inflammatory responses in septic AKI. Pretreatment with NAC significantly reduced renal inflammatory mediators, including TNF-α, IL-1β, IL-6, and IL-8, compared with the CLP group. These findings support an anti-inflammatory effect of NAC in this experimental model.[46]

2.5.5.2 Experimental Study of NAC in Vancomycin-Induced Nephrotoxicity

Vancomycin is used for serious infections caused by methicillin-resistant gram-positive organisms, particularly Staphylococcus aureus and Staphylococcus epidermidis. Nephrotoxicity is an important adverse effect. Proposed mechanisms include oxidative stress, mitochondrial dysfunction, apoptosis, and altered intracellular signaling such as mitogen-activated protein kinase pathways.[72]

Ping Yu et al. evaluated NAC in experimental vancomycin nephrotoxicity using renal tubular epithelial-cell culture and rat models. Animals were randomly allocated to treatment groups, and outcome assessment was blinded. Interventions included vancomycin, vitamin C, NAC at different doses, and combinations. Outcomes included creatinine, BUN, KIM-1, NGAL, GSH, SOD, catalase, and MDA.[72]

Histopathological examination showed extensive tubular degeneration, atrophy, dilatation, necrosis, cast formation, interstitial edema, and inflammation after vancomycin exposure. NAC and vitamin C attenuated these changes and improved biochemical indices of kidney dysfunction.[72]

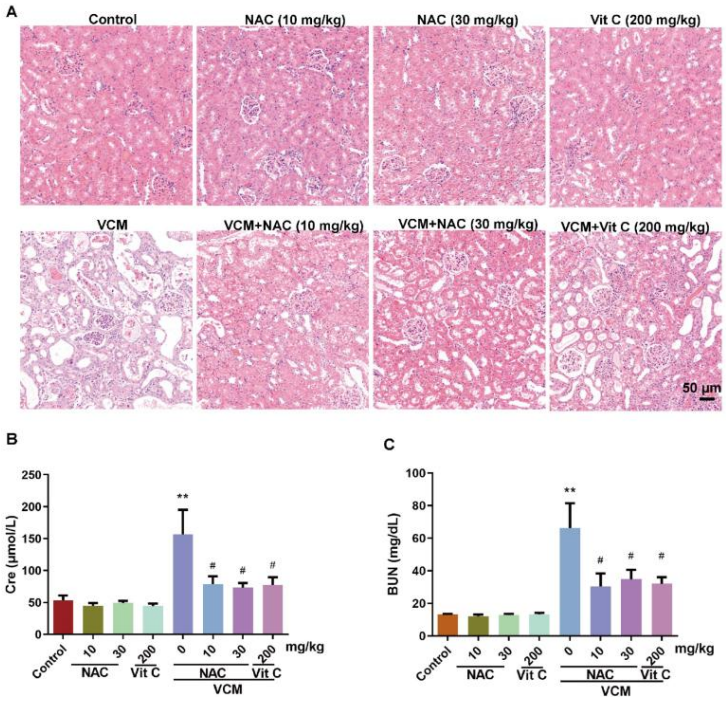


Figure 2.21 Effects of NAC on Vancomycin-Induced Renal Histopathological Changes and Kidney Function in Rats[72]

KIM-1 and NGAL are promising biomarkers of tubular injury. In this experimental study, vancomycin increased KIM-1 and NGAL within 24 hours, whereas NAC or vitamin C reduced their expression. These findings support a protective effect of antioxidants against vancomycin-induced kidney injury.[72]

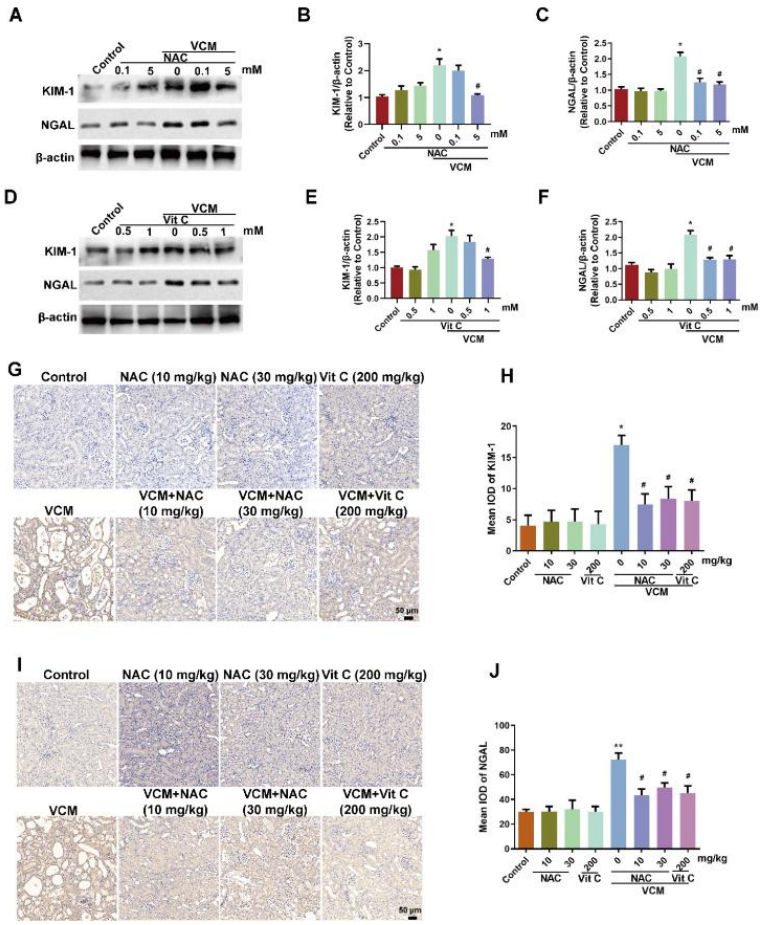


Figure 2.22 Effects of NAC on Increased KIM-1 and NGAL Expression[72]

KIM-1 is a transmembrane glycoprotein located on proximal tubular epithelial cells, while NGAL is expressed in tubular epithelium and is upregulated after kidney injury. Increased expression of both markers has been linked to oxidative stress in several models. Antioxidant administration can suppress their expression. In the vancomycin model, NAC was associated with less histopathological injury, improved kidney function, improved tubular-cell viability, and improved metabolic parameters, consistent with antioxidant and anti-apoptotic effects.[72]

2.5.5.3 Clinical Study of NAC and Recovery of Kidney Failure in Patients with Leptospirosis

Lotfollah et al. evaluated the effectiveness of NAC in reducing serum creatinine among patients with leptospirosis. The study included 64 patients with leptospirosis and AKI confirmed by the microscopic agglutination test (MAT). NAC was administered to patients with eGFR <60 mL/min at a dose of 1200 mg every 12 hours for 48 hours. Thirty-two

patients received routine care and 32 received NAC plus routine care. Serum creatinine was evaluated over several time points, together with other laboratory parameters.[81]

In the NAC group, serum creatinine was 3.76±0.76 mg/dL on Day 1, 3.51±0.73 mg/dL on Day 3, and 2.31±0.46 mg/dL on Day 7. In the control group, mean serum creatinine at hospital admission was 3.53±0.45 mg/dL, followed by 3.33±0.45 mg/dL on Day 1, 2.67±0.34 mg/dL on Day 3, and 2.17±0.33 mg/dL on Day 7. The source thesis reports no consistent significant treatment effect across time points and a non-significant repeated-measures treatment effect (P=0.255).[81]

Most participants in both groups were men. Mean age was 37.88±8.86 years in the intervention group and 35.56±6.18 years in controls, without a significant between-group difference. Length of hospital stay also did not differ significantly. Only a minority required prolonged hospitalization or hemodialysis.[81]

Previous studies suggest that NAC may reduce AKI risk in sepsis and nephrotoxic exposures, but evidence in leptospirosis-associated AKI remains limited.[18] No consensus or guideline currently establishes NAC for kidney disease because available studies vary in quality, dose, and route of administration.[77] The limited clinical evidence provides a rationale for better-designed trials in diverse populations.[18]

2.6 Study Rationale and Evidence Gap

Kidney injury in leptospirosis involves interactions among oxidative stress, inflammation, and tubular injury. These processes can be assessed using biomarkers representing different biological components: ROS, CRP, and urinary KIM-1.

NAC has a biological rationale as an intervention capable of modulating redox balance and inflammatory responses. Although previous studies have evaluated NAC in several forms of kidney injury and one clinical study has evaluated NAC in leptospirosis, evidence regarding simultaneous changes in urinary KIM-1, CRP, and ROS remains limited. Therefore, an evidence gap remains regarding whether NAC administration in patients with leptospirosis produces changes in biomarkers of tubular injury, inflammation, and oxidative stress that differ from placebo. This evidence gap forms the basis for the present study.

Table 2.6 Studies of NAC in Various Clinical Conditions[77]

Disease	Study Type	Dosage	Treatment Duration	Route of Administration
Acute liver failure	Randomized case-control study	150 mg/kg over 1 hour, followed by 12.5 mg/kg/hour for 4 hours and a constant infusion of 6.25 mg/kg/hour for 67 hours	72 hours	Intravenous
—	Systematic review	150 mg/kg over 1 hour, followed by 12.5 mg/kg/hour for 4 hours and a constant infusion of 6.25 mg/kg/hour for 67 hours	72 hours	Intravenous
Non-alcoholic fatty liver disease	Clinical trial	600 mg/day	3 months	Oral
Acute alcoholic hepatitis	Multicenter clinical trial, NCT00863785	Day 1: doses of 150, 50, and 100 mg/kg administered over 30 minutes, 4 hours, and 16 hours, respectively. Days 2–5: 100 mg/kg	28 days	Intravenous
—	Multicenter	300 mg/kg	14 days	Intravenous

Disease	Study Type	Dosage	Treatment Duration	Route of Administration
	clinical trial			
Chronic kidney disease patients undergoing cardiac procedures	Systematic review with meta-analysis	150 mg/kg up to 1200 mg	6 hours to 7 days	Intravenous
Chronic kidney disease	Clinical trial	5000 mg	One hemodialysis session	Intravenous
—	Clinical trial	600 mg/day	2 doses at 1-week intervals	Oral
Helicobacter pylori infection	Clinical trial	600 mg/day	14 days	Oral
—	Clinical trial	800 mg/day	5 days	Oral
Colitis-associated colorectal cancer	Systematic review	8000 mg to 1200 mg/day	7 days to 12 weeks	Oral
Gastrointestinal cancer	Clinical trial	1200 mg/day	2 days before surgery to 5 days after surgery	Parenteral
—	Clinical trial	800 mg/day	12 weeks	Oral
Ulcerative colitis	Clinical trial	800 mg/day	4 weeks	Oral
Chronic hepatitis	Clinical trial	8000 mg/day	28 days	Intravenous

Disease	Study Type	Dosage	Treatment Duration	Route of Administration
B				
Pulmonary tuberculosis	Clinical trial	600 mg/day	2 months	Oral
SARS-CoV-2	Clinical trial, NCT04374461	6000 mg/day	3 weeks	Intravenous

The source thesis summarizes clinical trials and systematic reviews of NAC across chronic obstructive pulmonary disease, cystic fibrosis, idiopathic pulmonary fibrosis, cardiovascular disease and cardiac surgery, schizophrenia, bipolar disorder, depression, Parkinson's disease, Alzheimer's disease, multiple sclerosis, acetaminophen poisoning, acute liver failure, non-alcoholic fatty liver disease, alcoholic hepatitis, CKD, Helicobacter pylori infection, gastrointestinal disorders, tuberculosis, SARS-CoV-2 infection, chorioamnionitis, intrauterine infection/inflammation, acute pyelonephritis, radiotherapy-related conditions, smoking cessation, male infertility, sickle-cell anemia, pre-eclampsia, metabolic syndrome, chronic blepharitis, and meibomian gland dysfunction. Reported doses, treatment durations, and administration routes vary widely and are retained in the original thesis table.[77]

Table 2.7 Clinical Studies of N-Acetylcysteine in Acute Kidney Injury or Chronic Kidney Disease

Akbar H (2023), N=30: uncontrolled RCT; mean age 53 years; baseline SCr 2.12±0.79 mg/dL; AKI; oral NAC 600 mg twice daily every 12 hours for 48 hours; post-treatment SCr 1.28±0.63 mg/dL. The study reported a statistically significant reduction in serum creatinine in patients with predominantly pre-renal or post-renal AKI.
Chiu AH (2023), N=330 (165 NAC, 165 controls): cohort; mean age 73 years; CKD stages III–V; NAC 600 mg twice daily for 36 months. The retrospective study reported a lower incidence of hemodialysis among NAC users, with changes in SCr and eGFR suggesting a

potential role in slowing CKD progression.
Mainra R (2007), N=26: RCT; mean age 67 years; CKD stage III; oral NAC 600 mg in phase 1 and 1200 mg with cimetidine before NAC in phase 2. The study did not detect significant short-term changes in serum creatinine or cystatin C.
Rehman T (2008), N=29: RCT; mean age 65 years; CKD stages III–V; oral NAC 1200 mg every 12 hours for four doses. No meaningful changes in serum creatinine or cystatin C were detected at 4 or 48 hours after the final dose.
Moist L (2010), N=60 (30 NAC, 30 placebo): double-blind RCT; mean age 70 years; CKD stage III; oral NAC 1200 mg for four doses at 12-hour intervals. NAC had no significant short-term effect on serum creatinine.
Larki R (2019), N=40 (21 NAC, 19 placebo): double-blind RCT; mean age 60 years; CKD stage V/ESRD; oral NAC 600 mg twice daily for 8 weeks. Serum creatinine did not differ meaningfully between NAC and placebo groups.
Ahmadi F (2016), N=47 (26 NAC, 21 controls): RCT; mean age 58 years; CKD stage V/ESRD; oral NAC 1200 mg twice daily. After 3 months, the study suggested that NAC may help preserve kidney function in patients undergoing hemodialysis.

2.7 Theoretical Framework

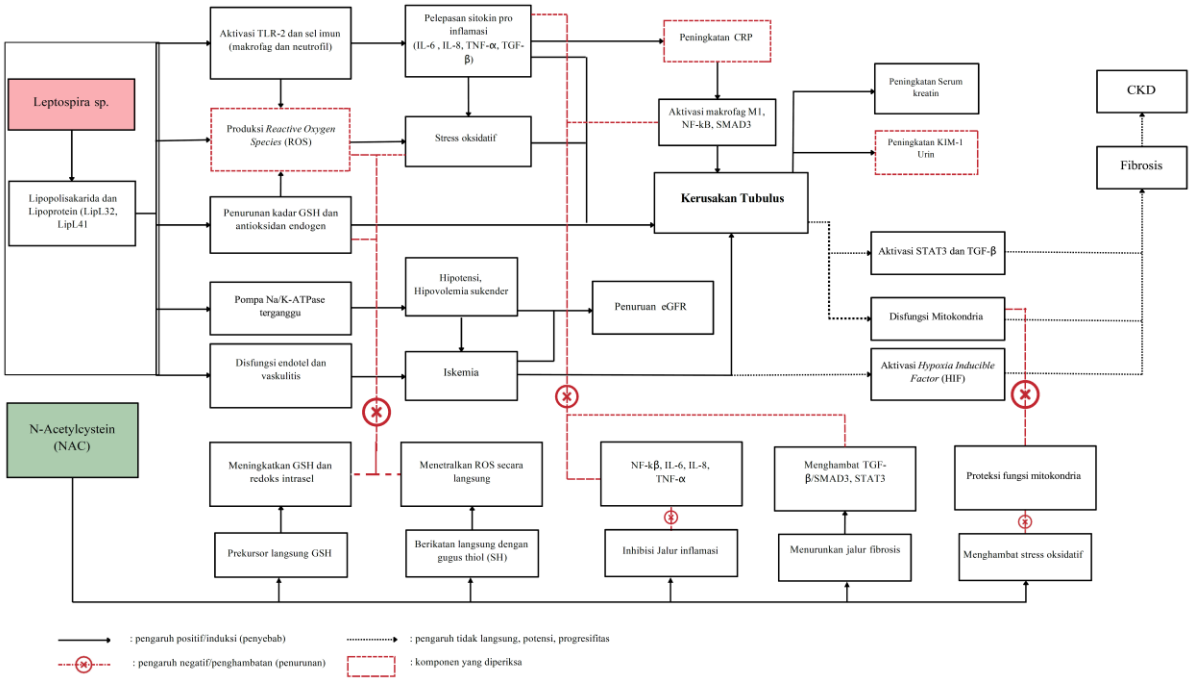


Figure 2.23 Theoretical Framework

The theoretical framework in the source thesis integrates leptospiral invasion, host inflammatory responses, oxidative stress, renal tubular injury, and the proposed antioxidant and anti-inflammatory actions of NAC. The framework links ROS, CRP, and KIM-1 to the principal biological pathways evaluated in the study.

CHAPTER 3

CONCEPTUAL FRAMEWORK AND RESEARCH METHODS

3.1 Conceptual Framework

Acute kidney injury in leptospirosis results from a complex interaction among Leptospira spp. invasion, the systemic inflammatory response, and increased oxidative stress, ultimately leading to renal tubular injury. In leptospirosis, activation of the immune system induces the release of various proinflammatory cytokines and increases the generation of reactive oxygen species (ROS). Excessive ROS production causes oxidative stress, which aggravates cell-membrane damage, mitochondrial dysfunction, and apoptosis of renal tubular epithelial cells. Concurrently, expression of Kidney Injury Molecule-1 (KIM-1) increases as a marker of proximal tubular injury, whereas C-reactive protein (CRP) increases as a reflection of systemic inflammation.

N-acetylcysteine (NAC) is a glutathione precursor with antioxidant and anti-inflammatory activity. By increasing intracellular glutathione stores and scavenging free radicals, NAC is expected to reduce oxidative stress, inhibit activation of inflammatory pathways, and attenuate renal tubular injury. These biological effects were evaluated by changes in serum ROS, serum CRP, and urinary KIM-1 after NAC administration compared with placebo in patients with leptospirosis.

The relationships among the study variables are presented in Figure 3.1.

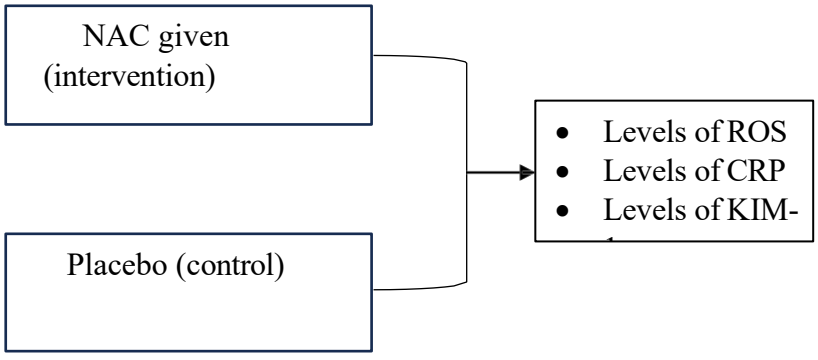


Figure 3.1 Conceptual Framework

3.2 Identification of Variables

3.2.1 Independent Variables

1. N-acetylcysteine (NAC) 800 mg administered three times daily for five days.

2. Oral placebo administered three times daily for five days.

3.2.2 Dependent Variables

1. Change in urinary KIM-1 levels from Day 1 to Day 5.

2. Change in ROS levels from Day 1 to Day 5.

3. Change in CRP levels from Day 1 to Day 5.

3.3 Study Design

This study was a double-blind randomized controlled trial with a two-group parallel design. Participants who met the study criteria were randomly allocated in a 1:1 ratio to the intervention group receiving oral N-acetylcysteine or the control group receiving placebo. All participants continued to receive standard therapy for leptospirosis according to hospital guidelines. Study participants, the principal investigator, and laboratory personnel remained unaware of treatment allocation until completion of data collection. Analysis was performed after database locking and unblinding of the randomization codes.

3.4 Study Setting and Period

The study was conducted at Balaraja Regional General Hospital (RSUD Balaraja), Tangerang Regency, Banten Province, Indonesia. Biomarker measurements were performed at the Molecular Genetics Laboratory, Faculty of Medicine, Universitas Padjadjaran. Participant recruitment was conducted from September 2025 to March 2026. Laboratory examinations were performed after all samples had been collected, in accordance with the research laboratory procedures. Statistical analysis was conducted after all study data were confirmed to be complete and had undergone verification and data cleaning.

3.5 Study Population and Sample

3.5.1 Target Population

All adult patients diagnosed with leptospirosis in Indonesia.

3.5.2 Accessible Population

All adult patients diagnosed with leptospirosis who were hospitalized at RSUD Balaraja during the study period.

3.5.3 Study Sample

Patients with leptospirosis who fulfilled all inclusion criteria, met none of the exclusion criteria, and provided written informed consent to participate in the study. Participants were recruited consecutively until the planned sample size was achieved.

3.6 Participant Selection Criteria

3.6.1 Inclusion Criteria

1. Patients aged ≥18 years.

2. Patients with fever onset on Day 3 to Day 5.

3. Patients with non-severe (mild-to-moderate) leptospirosis according to the WHO classification, serologically confirmed by a positive Leptospira IgM ELISA.

4. No history of chronic kidney disease based on medical history, medical records, or kidney-function assessment before the episode of leptospirosis.

5. Willingness to participate in the study and provision of written informed consent.

6. No prior N-acetylcysteine therapy before randomization.

3.6.2 Exclusion Criteria

1. Severe leptospirosis (severe leptospirosis or Weil's disease) according to the WHO classification.

2. Chronic kidney disease stages G3–G5 according to the KDIGO classification.

3. Diabetes mellitus with diabetic nephropathy.

4. Uncontrolled or malignant hypertension.

5. Glomerulonephritis or other immune-mediated kidney disease.

6. Current use of potentially nephrotoxic medications, including aminoglycosides, non-steroidal anti-inflammatory drugs (NSAIDs), chemotherapeutic agents, or other nephrotoxic drugs.

7. Severe chronic liver disease.

8. Severe heart disease or severe circulatory impairment.

9. Severe chronic pulmonary disease, including severe COPD or uncontrolled severe persistent asthma.

10. Gastrointestinal disorders that could interfere with oral N-acetylcysteine absorption (ileus, persistent vomiting, or severe malabsorption).

11. Pregnancy or breastfeeding.

12. History of hypersensitivity to N-acetylcysteine.

13. Refusal to participate or withdrawal of informed consent.

3.6.3 Withdrawal Criteria

1. Development of a medical emergency during the study.

2. Development of a severe NAC-related adverse effect requiring discontinuation of therapy.

3. Loss to follow-up.

4. Death.

3.7 Sample Size

The study sample was drawn from research participants who met the inclusion criteria and did not meet the exclusion criteria. Sample-size determination was adjusted to the study objectives.

a. For the difference in mean ROS levels between Day 1 and Day 5 after NAC administration, the sample-size formula for comparison of two independent means was used. Type I error was set at 5% with a two-sided hypothesis ($Z\alpha=1.96$), type II error at 20% ($Z\beta=0.842$), the minimum clinically meaningful difference ($X1-X2$) at 1, and the standard deviation at 1.35 based on the literature. The calculated minimum sample size was 29 participants per group.

b. For the difference in mean CRP levels between Day 1 and Day 5 after NAC administration, the sample-size formula for comparison of two independent means was used. Type I error was set at 5% with a one-sided hypothesis; the source thesis reports $Z\alpha=1.96$. Type II error was set at 20% ($Z\beta=0.842$), the minimum meaningful difference at 1.5, and the

standard deviation at 1.32 based on the literature. The calculated minimum sample size was 13 participants per group.

c. For the difference in mean KIM-1 levels between Day 1 and Day 5 after NAC administration, the sample-size formula for comparison of two independent means was used. Type I error was set at 5% with a one-sided hypothesis; the source thesis reports $Z\alpha=1.96$. Type II error was set at 20% ($Z\beta=0.842$), and the minimum meaningful difference ($X1-X2$) at 600. Because no literature data were available for the standard deviation, the investigators estimated the standard deviation at one and a half times the minimum meaningful mean difference; the source thesis states an estimated standard deviation of 1050 ng/mL. The calculated sample size was 28 participants per group.

After allowing for an anticipated 10% dropout rate, the required sample size was 32 participants in each group, for a total of 64 participants. During the study period, 64 participants who fulfilled all study criteria were successfully recruited, and the planned sample size was achieved.

3.8 Sampling Techniquex

Consecutive sampling was used. All patients with leptospirosis who met the inclusion criteria and did not meet the exclusion criteria were recruited sequentially until the required sample size was reached.

3.9 Randomization and Blinding

Participants who fulfilled all inclusion criteria and met none of the exclusion criteria were randomly allocated to either the intervention or control group. Randomization was performed in a 1:1 ratio using a random-number generator in Microsoft Excel.

The randomization sequence was prepared by a research assistant who was not involved in participant recruitment, intervention administration, laboratory testing, or statistical analysis. Allocation numbers were retained by the research assistant and were not disclosed to the principal investigator, study participants, or laboratory personnel during data collection.

This was a double-blind study in which both study participants and the principal investigator were unaware of group allocation. NAC and placebo preparations were made identical in appearance, color, size, and packaging to maintain blinding. Allocation codes were opened

only after completion of data collection and statistical analysis, except when emergency unblinding was required for participant safety.

3.10 Study Intervention

3.10.1 Intervention Group

The intervention group received oral N-acetylcysteine 800 mg three times daily for five consecutive days as adjunctive therapy to standard treatment for leptospirosis.

3.10.2 Control Group

The control group received placebo with an identical dosage form, color, size, and administration schedule to the intervention group for five days. In addition to the study treatment, all participants continued to receive standard therapy for leptospirosis according to RSUD Balaraja management guidelines. Treatment adherence was monitored daily during hospitalization through medication-administration records completed by healthcare personnel. Participants were monitored for adverse events throughout the study; all events were documented in the study forms and evaluated by the principal investigator.

3.11 Study Procedures

The study began with screening of all patients diagnosed with leptospirosis and hospitalized at RSUD Balaraja from September 2025 to March 2026. Leptospirosis was diagnosed based on compatible clinical manifestations and a positive Leptospira IgM serological test.

All patients who met the inclusion criteria were evaluated against the exclusion criteria. Eligible patients received verbal and written explanations regarding the study objectives, potential benefits, study procedures, potential risks, and participant rights. Patients who agreed to participate were asked to sign a written informed consent form.

After consent was obtained, baseline characteristics were recorded, including age, sex, body weight, height, body mass index, comorbidities, vital signs, routine laboratory results, and clinical severity of leptospirosis. Venous blood and urine samples were then collected before administration of the intervention (Day 0) to determine baseline serum ROS, serum CRP, and urinary KIM-1 levels.

Participants were subsequently randomized in a 1:1 ratio to the NAC or placebo group according to the randomization sequence prepared by the independent research assistant. The

intervention group received oral N-acetylcysteine 800 mg three times daily for five days, whereas the control group received an identical placebo at the same frequency. All participants continued to receive standard management for leptospirosis according to RSUD Balaraja guidelines.

Clinical monitoring was performed daily during hospitalization. Adverse events, changes in clinical condition, and discontinuation of the intervention were recorded in the study forms. On Day 5, after completion of the intervention, venous blood and urine samples were collected again using the same procedures as at baseline and processed for serum ROS, serum CRP, and urinary KIM-1 measurements.

All study data underwent verification and data cleaning before statistical analysis. Analysis was conducted after the data were confirmed to be complete and the randomization code was opened following database lock.

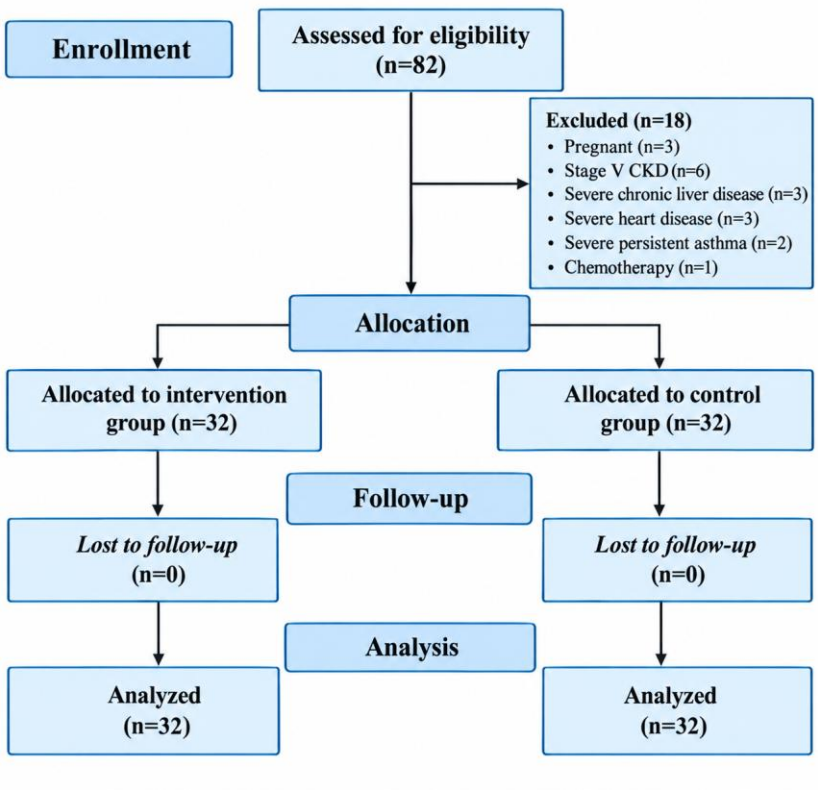


Figure 3.2 Study Flow (CONSORT Flow Diagram)

A total of 82 participants underwent screening during the study period. Eighteen were excluded because they did not meet the study criteria: pregnancy (n=3), stage V chronic

kidney disease (n=6), severe chronic liver disease (n=3), severe heart disease (n=3), severe persistent asthma (n=2), and chemotherapy (n=1). Sixty-four participants were randomized in a 1:1 ratio to the N-acetylcysteine group (n=32) or placebo group (n=32). All participants completed the study according to protocol; there was no loss to follow-up, intervention discontinuation, or exclusion from the analysis.

3.12 Laboratory Examinations

3.12.1 Urinary KIM-1 Measurement

Spot urine samples were collected in sterile containers before administration of the intervention and on Day 5 after treatment. Samples were centrifuged to remove cellular debris, after which the supernatant was transferred to sterile microtubes and stored at −20°C until analysis. Urinary Kidney Injury Molecule-1 (KIM-1) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) at the Molecular Genetics Laboratory, Faculty of Medicine, Universitas Padjadjaran, using the Bioenzym Human Kidney Injury Molecule-1 ELISA Kit, Lot No. ZE805215.

3.12.2 Serum ROS Measurement

Approximately 5 mL of venous blood was collected into tubes without anticoagulant. Samples were allowed to clot and were then centrifuged to obtain serum. Serum was transferred into sterile microtubes and stored at −20°C until analysis. Reactive Oxygen Species (ROS) levels were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Testing was performed at the Molecular Genetics Laboratory, Faculty of Medicine, Universitas Padjadjaran, using the OxiSelect In Vitro Human Reactive Oxygen Species ELISA Kit, Lot No. 20250613.

3.12.3 Serum CRP Measurement

C-reactive protein (CRP) concentrations were measured using serum obtained from venous blood samples. Testing was performed in the same laboratory according to its standard operating procedures. All laboratory measurements were performed by laboratory analysts who were unaware of participants' intervention assignments.

3.12.4 Laboratory Quality Control

All specimens were labeled with study identification codes without indicating intervention assignment, thereby maintaining blinding of laboratory personnel. All assays were performed according to kit instructions and the laboratory's standard operating procedures.

3.13 Operational Definitions

Variable	Definition	Measurement	Scale / Result
Age	Chronological age according to the identity card at registration at RSUD Balaraja.	Medical record data.	Numeric; 18–65 years.
Sex	Sex according to the identity card at registration at RSUD Balaraja.	Medical record data.	Categorical: 1=Male; 2=Female.
Patient with leptospirosis	Patient diagnosed with leptospirosis.	Medical record data.	Categorical: 0=No; 1=Yes.
N-acetylcysteine (NAC)	Generic capsule formulation, 200 mg per capsule.	Dose: 800 mg three times daily for each patient.	Numeric.
Acute Kidney Injury (AKI)	Acute decline in kidney function fulfilling KDIGO 2012 criteria: SCr increase ≥0.3 mg/dL within 48 h, or ≥1.5 times baseline within 7 days, or urine output <0.5 mL/kg/h for ≥6 h.	Hourly urine output and serum urea/creatinine.	Categorical: 0=No AKI; 1=Stage 1; 2=Stage 2; 3=Stage 3.
Reactive Oxygen Species (ROS)	ROS level measured from venous blood before NAC and after 5 days of NAC.	Flow cytometry (as stated in the source operational-definition table).	Numeric.
C-Reactive Protein (CRP)	CRP level measured from venous blood before NAC and after 5 days of NAC.	Immunoturbidimetry (as stated in the source operational-definition table).	Numeric.
Urinary KIM-1	Kidney Injury Molecule-1 measured in urine before NAC and after 5 days of NAC.	ELISA.	Numeric.

3.14 Statistical Analysis

Patient data were recorded in research forms or case report forms and subsequently entered into electronic storage. Data were analyzed using SPSS version 30. General descriptive analyses were performed using univariate methods to summarize participant demographic characteristics. Categorical data were presented as counts and percentages. Normally distributed numerical data were presented as mean and standard deviation, whereas non-normally distributed numerical data were presented as median and interquartile range.

Data normality was assessed using the Shapiro–Wilk test. Within-group comparisons before and after treatment in the NAC and control groups were performed using the paired t-test or Wilcoxon signed-rank test according to data distribution. Correlation between ROS and CRP was analyzed using Pearson correlation for normally distributed data or Spearman correlation for non-normally distributed data. Associations of KIM-1 and CRP with AKI were assessed using the chi-square test or Fisher's exact test as appropriate. Risk factors for AKI were evaluated using bivariate and multivariable logistic regression analyses. Comparison of two AUC curves for ROS and CRP in patients with leptospirosis was performed using an AUC comparison test. Statistical significance for all hypotheses was defined as $p<0.05$ with a 95% confidence interval. Participants who dropped out, declined to continue participation, or died during the study period were not included in the study analysis.

3.15 Research Ethics

The study received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia–Cipto Mangunkusumo Hospital (FKUI–RSCM), approval No. KET-1176/UN2.F1/ETIK/PPM.00.02/2025, before participant recruitment began. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, International Council for Harmonisation–Good Clinical Practice (ICH-GCP), and applicable laws and regulations in Indonesia.

All participants received verbal and written information regarding the study objectives, procedures, expected benefits, potential risks, and their right to refuse participation or withdraw from the study at any time without affecting the medical care they received. After understanding the information provided, each participant signed a written informed consent form before any study procedures were undertaken.

Participant identity and personal data were kept confidential using study identification numbers so that no identifying information was used during data collection, analysis, or reporting. Study data were accessible only to authorized investigators and research-team members and were used solely for scientific purposes.

The study was also registered on ClinicalTrials.gov as part of the commitment to transparency in clinical-trial conduct and reporting.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Study Characteristics

This double-blind randomized controlled trial evaluated N-acetylcysteine (NAC) as adjunctive therapy for biomarkers of oxidative stress, inflammation, and renal tubular injury in patients with leptospirosis. Eligible patients were recruited consecutively and randomized 1:1 to NAC or placebo in addition to standard therapy. Reporting of participant flow followed CONSORT. Of 82 patients assessed for eligibility, 18 were excluded and 64 were randomized (NAC, n=32; placebo, n=32). All participants completed the five-day intervention, there was no loss to follow-up, and all randomized participants were included in the final analysis. The 100% retention rate reduced attrition bias and strengthened internal validity. Interpretation nevertheless considered baseline imbalances between groups.

4.2 Baseline Characteristics of Study Participants

The 64 participants were equally allocated to the intervention and control groups. Most demographic, anthropometric, and baseline laboratory characteristics were relatively balanced. Sex, body mass index (BMI), urea, creatinine, and CRP did not differ significantly. Mean age, ROS, and urinary KIM-1, however, differed significantly at baseline and were therefore important considerations in subsequent interpretation.

Table 4.1 Baseline characteristics of study participants

Variable	NAC (n=32)	Placebo (n=32)	p
Age, years	50.91 ± 13.23	42.91 ± 13.85	0.032
Male, %	62.5	71.9	0.424
Female, %	37.5	28.1	
BMI, kg/m²	21.90 ± 3.14	22.33 ± 3.02	0.581
Urea*, mg/dL	81.5 (16–354)	77.3 (8–293)	0.773
Creatinine*, mg/dL	2.1 (0.7–8.7)	1.8 (0.6–10.1)	0.804
ROS*, pg/mL	462.24 ± 163.45	290.04 ± 45.51	<0.001
CRP*, mg/L	97.55 ± 65.97	87.71 ± 60.24	0.535
Urinary KIM-1*, ng/mL	2.88 ± 2.26	0.92 ± 0.31	<0.001

*Baseline laboratory results.

4.2.1 Age

Mean age was 50.91 ± 13.23 years in the NAC group and 42.91 ± 13.85 years in the placebo group (p=0.032). Both groups were within the productive adult age range commonly affected by leptospirosis in tropical settings. Older age may increase AKI susceptibility

through nephron loss, reduced renal functional reserve and perfusion, higher basal oxidative stress, and immunosenescence. Nevertheless, the approximately eight-year difference was unlikely to explain all biomarker changes, particularly because baseline creatinine, urea, and CRP were comparable.

4.2.2 Sex

Most participants were male (62.5% in the NAC group and 71.9% in controls; p=0.424), consistent with the epidemiology of leptospirosis and greater occupational and environmental exposure among men. Because sex distribution was balanced, confounding by sex was considered minimal.

4.2.3 Body Mass Index

Mean BMI was 21.90 ± 3.14 versus 22.33 ± 3.02 kg/m² (p=0.581), with no significant difference in BMI categories. Although nutritional status can affect immunity, inflammation, and oxidative stress, mean BMI in both groups was within the normal Asia-Pacific range.

4.2.4 Baseline Kidney Function Parameters

Median urea was 81.5 versus 77.3 mg/dL and median creatinine 2.1 versus 1.8 mg/dL in the NAC and placebo groups, respectively, without significant differences. Serum creatinine reflects kidney function rather than structural injury and may rise later than tubular biomarkers such as KIM-1, supporting the use of KIM-1 as an early study outcome.

4.2.5 Baseline Biomarkers

Baseline CRP was comparable (97.55 ± 65.97 vs 87.71 ± 60.24 mg/L; p=0.535). In contrast, ROS (462.24 ± 163.45 vs 290.04 ± 45.51 pg/mL; p<0.001) and urinary KIM-1 (2.88 ± 2.26 vs 0.92 ± 0.31 ng/mL; p<0.001) were significantly higher in the NAC group, indicating greater oxidative stress and tubular injury before intervention.

4.3 Changes in Kidney Injury Molecule-1 (KIM-1)

4.3.1 Study Results

In the NAC group, KIM-1 decreased from 2.88 ± 2.26 to 1.92 ± 0.50 ng/mL on Day 5; the mean reduction was 0.96 ng/mL (95% CI 0.11–1.82; p=0.006). In controls, KIM-1 changed from 0.92 ± 0.31 to 0.81 ± 0.28 ng/mL; the mean reduction of 0.11 ng/mL was not significant (p=0.07; 95% CI −0.01 to 0.22). The unadjusted mean change was 0.97 ± 2.36

ng/mL with NAC and 0.08 ± 0.29 ng/mL with placebo, with a between-group mean difference of 0.88 ng/mL (95% CI 0.04–1.73; p=0.01).

Tables 4.2–4.4 Summary of KIM-1 results

Comparison	NAC	Control	p / 95% CI
Day 1 → Day 5	2.88±2.26 → 1.92±0.50	0.92±0.31 → 0.81±0.28	NAC p=0.006; control p=0.07
Mean change	0.97 ± 2.36	0.08 ± 0.29	Between-group p=0.01; 95% CI 0.04–1.73

4.3.2 Summary of Main Findings

Five days of NAC was associated with a larger decline in urinary KIM-1 than placebo, supporting a potential effect on tubular epithelial injury. However, the intervention group started with substantially higher KIM-1, providing greater opportunity for absolute decline; this baseline imbalance must be considered.

4.3.3 Biological and Pathophysiological Explanation

Kidney injury in leptospirosis involves direct tubulointerstitial invasion, immune activation, microcirculatory disturbance, and oxidative stress. Proximal tubular epithelial injury markedly increases KIM-1 expression and urinary shedding. KIM-1 also participates in phagocytosis and tissue repair, so declining levels may reflect attenuation of injury and reduced reparative demand. NAC replenishes glutathione and scavenges reactive species, reducing ROS-mediated NF-κB, MAPK, and AP-1 activation, proinflammatory cytokine production, tubular apoptosis, and KIM-1 release.

4.3.4 Comparison with Previous Studies

The source thesis identified no randomized clinical trial specifically evaluating NAC and KIM-1 in leptospirosis. Experimental ischemia-reperfusion, cisplatin, and vancomycin nephrotoxicity models have shown lower KIM-1 after NAC together with reduced oxidative stress and tubular damage. Human NAC studies have been more heterogeneous, particularly when serum creatinine rather than early structural biomarkers was used as the endpoint.

4.3.5 Analysis of Similarities and Differences

The baseline KIM-1 imbalance, modest sample size, multifactorial pathogenesis of leptospirosis, concomitant antimicrobial therapy, and five-day observation period all affect interpretation. KIM-1 may remain elevated during tubular regeneration even after the injurious stimulus has subsided. Larger multicenter studies with longer follow-up and baseline-adjusted analyses are required.

4.3.6 Biological and Clinical Implications

The KIM-1 decline indicates a biological effect on tubular injury but does not directly establish reduced dialysis requirement, length of stay, mortality, or improved long-term kidney recovery. KIM-1 may have value not only for early AKI detection but also for monitoring renoprotective treatment response. These findings are insufficient to support routine NAC use.

4.3.7 Subsection Synthesis

Overall, NAC was associated with a greater KIM-1 reduction than placebo. Despite baseline imbalance, the direction of change was compatible with the antioxidant mechanism of NAC and experimental evidence. The next analysis therefore focused on ROS as an upstream oxidative-stress biomarker.

4.4 Changes in Reactive Oxygen Species (ROS)

4.4.1 Study Results

ROS in the NAC group decreased from 462.24 ± 163.45 to 315.33 ± 136.56 pg/mL, a mean reduction of 146.9 pg/mL (95% CI 96.92–196.88; $p<0.001$). In controls, ROS increased from 290.04 ± 45.51 to 305.01 ± 42.57 pg/mL; the mean difference of −14.96 pg/mL was not significant ($p=0.059$; 95% CI −30.12 to 0.19). The between-group difference in change was 161.87 pg/mL (95% CI 109.96–213.78; $p<0.001$).

Tables 4.5–4.7 Summary of ROS results

Comparison	NAC	Control	p / 95% CI
Day 1 → Day 5	462.24±163.45 → 315.33±136.56	290.04±45.51 → 305.01±42.57	NAC p<0.001; control p=0.059
Mean change	146.90 ± 138.64	−14.96 ± 42.05	Between-group p<0.001; 95% CI 109.96–213.78

4.4.2 Summary of Main Findings

NAC was associated with a substantially greater reduction in ROS than control therapy, supporting modulation of oxidative stress. Baseline ROS was significantly higher in the NAC group, so the magnitude of the absolute reduction should be interpreted cautiously, although the direction of change is strongly consistent with NAC pharmacology.

4.4.3 Biological and Pathophysiological Explanation

Leptospiral infection activates neutrophils, macrophages, and endothelial cells, increasing superoxide, hydrogen peroxide, and hydroxyl radicals. Excess ROS causes lipid

peroxidation, mitochondrial dysfunction, protein and DNA damage, and tubular apoptosis or necrosis. ROS also activates NF-κB, MAPK, and NLRP3 signaling. NAC increases glutathione synthesis and directly scavenges reactive species through its thiol group, potentially preserving mitochondria and limiting tubular injury.

4.4.4 Comparison with Previous Studies

Experimental AKI studies consistently show that NAC reduces ROS, increases glutathione and endogenous antioxidant enzymes, and attenuates tubular damage. Clinical evidence is less consistent because direct oxidative-stress biomarkers are seldom used. By measuring ROS as a primary biological outcome, this study evaluated pharmacological target engagement before overt functional kidney changes.

4.4.5 Analysis of Similarities and Differences

Baseline ROS imbalance, heterogeneous mechanisms of ROS production in leptospirosis, concomitant antibiotics, and the short half-life of ROS all influence interpretation. The observed effect is best regarded as an add-on effect of NAC above standard therapy rather than an isolated drug effect. More frequent serial measurements could better characterize oxidative-stress kinetics.

4.4.6 Biological and Clinical Implications

Reduced ROS suggests modulation of a central pathogenic pathway connecting infection, inflammation, endothelial dysfunction, and kidney injury. The findings support mechanism-based therapy and the potential use of ROS as a pharmacodynamic marker. However, endogenous antioxidant markers such as reduced glutathione, glutathione peroxidase, catalase, and superoxide dismutase were not measured.

4.4.7 Subsection Synthesis

NAC was associated with a greater ROS reduction than control therapy. The concurrent reduction in KIM-1 supports a coherent model in which attenuation of oxidative stress contributes to reduced tubular injury. The subsequent analysis evaluated CRP as a marker of systemic inflammation.

4.5 Changes in C-Reactive Protein (CRP)

4.5.1 Study Results

CRP in the NAC group decreased from 97.55 ± 65.97 to 14.31 ± 12.95 mg/L ($p<0.001$), with a mean reduction of 83.24 mg/L (95% CI 62.13–104.36). Controls also improved, from 87.71 ± 60.24 to 61.37 ± 40.88 mg/L ($p<0.001$), but the mean reduction was only 26.34 mg/L (95% CI 11.72–40.95). Day-5 CRP was significantly lower with NAC, with a mean difference of −47.06 mg/L (95% CI −62.42 to −31.70; $p<0.001$).

Tables 4.8–4.10 Summary of CRP results

Comparison	NAC	Control	p / 95% CI
Day 1 → Day 5	97.55±65.97 → 14.31±12.95	87.71±60.24 → 61.37±40.88	Both p<0.001
Day 5	14.31 ± 12.95	61.37 ± 40.88	p<0.001; mean difference −47.06 (−62.42 to −31.70)

4.5.2 Summary of Main Findings

CRP declined significantly in both groups but substantially more with NAC. Unlike ROS and KIM-1, baseline CRP was balanced, strengthening interpretation of the post-intervention difference. Because all patients also received antibiotics, the NAC effect should still be regarded as adjunctive to standard therapy.

4.5.3 Biological and Pathophysiological Explanation

Leptospiral components activate pattern-recognition receptors, including TLR2 and TLR4, triggering NF-κB and MAPK signaling and increasing TNF-α, IL-1β, IL-6, and IL-8. IL-6 stimulates hepatic CRP synthesis. Oxidative stress and inflammation form a positive-feedback loop: ROS activates inflammatory signaling, while cytokines further increase ROS. NAC may interrupt this cycle by replenishing glutathione, reducing ROS, suppressing NF-κB and IL-6, and thereby lowering CRP.

4.5.4 Comparison with Previous Studies

Experimental ischemia-reperfusion and nephrotoxicity studies show that NAC reduces inflammatory cytokines and tissue inflammatory-cell infiltration. Clinical studies in sepsis, severe pneumonia, ARDS, and COVID-19 have reported reductions in inflammatory biomarkers, although clinical outcomes have not consistently improved. Thus, the present CRP findings demonstrate biological efficacy rather than definitive clinical effectiveness.

4.5.5 Analysis of Similarities and Differences

Antibiotic-mediated bacterial eradication naturally reduces inflammatory stimulation; NAC therefore represents an adjunctive effect. CRP is nonspecific and has slower kinetics than ROS, typically peaking 24–48 hours after IL-6 stimulation. Variation in symptom duration before treatment may also affect response. Nevertheless, concordant changes in ROS, KIM-1, and CRP support an integrated mechanistic interpretation.

4.6 Integration of Study Findings and Renoprotective Mechanisms of NAC

4.6.1 Integration of Study Findings

ROS, KIM-1, and CRP represent interconnected stages of AKI pathogenesis. NAC was associated with a greater reduction in ROS, followed by lower KIM-1 and CRP. In leptospirosis, ROS generated through phagocyte activation and mitochondrial dysfunction activates NF-κB, MAPK, and NLRP3 and directly damages tubular cells. Persistent inflammatory signaling increases IL-6 and CRP. Although causal mediation cannot be proven by this design, the concordant biomarker changes strengthen biological plausibility.

4.6.2 Proposed Mechanistic Model

NAC acts as a glutathione precursor and sulfhydryl donor, increasing intracellular antioxidant capacity. Reduced ROS suppresses NF-κB and MAPK, decreases IL-6, TNF-α, and IL-1β, preserves mitochondrial function, reduces tubular apoptosis and KIM-1 expression, and lowers IL-6-driven hepatic CRP synthesis. The observed ROS, KIM-1, and CRP changes are consistent with this integrated model.

4.7 Strengths and Limitations

4.7.1 Strengths

Major strengths were the double-blind randomized controlled design; simultaneous assessment of oxidative stress, tubular injury, and systemic inflammation; evaluation of NAC in clinical leptospirosis rather than only experimental AKI; uniform standard therapy in both groups; and complete participant retention with all randomized patients analyzed according to their original allocation.

4.7.2 Limitations

The study was single-center with a relatively small sample, and baseline ROS and KIM-1 differed despite randomization. Follow-up was limited to five days, precluding assessment

of longer-term kidney recovery, chronic kidney disease, kidney replacement therapy, or mortality. Biomarkers rather than patient-important clinical outcomes were primary endpoints. A broader mechanistic panel—including glutathione, SOD, catalase, glutathione peroxidase, IL-6, TNF-α, and NGAL—was not measured. The contribution of antibiotic therapy could not be quantitatively separated from NAC, and genetic or individual determinants of antioxidant response were not evaluated. Larger multicenter studies with longer follow-up are required.

4.8 Study Implications

4.8.1 Implications for Clinical Practice

Adjunctive NAC was associated with reductions in biomarkers of oxidative stress, tubular injury, and systemic inflammation, supporting oxidative-stress control as a potential therapeutic strategy. However, routine NAC use cannot be recommended from this study alone because clinical outcomes such as kidney replacement therapy, length of stay, and mortality were not evaluated.

4.8.2 Implications for Scientific Development

Simultaneous evaluation of ROS, KIM-1, and CRP supports a mechanism-based therapy paradigm and illustrates how molecular biomarkers may provide earlier information about biological response than conventional kidney-function parameters. Biomarkers may serve as surrogate endpoints in early intervention research, although they cannot replace clinical outcomes.

4.8.3 Implications for Future Research

Future studies should be multicenter, larger, and longer, with assessment of kidney recovery, kidney replacement therapy, hospitalization, mortality, and transition to CKD. Broader molecular profiling should include glutathione-related pathways, antioxidant enzymes, inflammatory cytokines, NGAL, and endothelial-injury markers. Multi-omics approaches may help identify patients most likely to benefit from antioxidant therapy and support precision medicine.

4.9 Novelty of the Study

The study contributes to nephrology by evaluating NAC in leptospirosis through an integrated biological approach using ROS, KIM-1, and CRP to represent oxidative stress,

proximal tubular injury, and systemic inflammation. The concordant changes support an integrated pathogenic model and extend evidence for molecular biomarkers as surrogate endpoints in AKI intervention studies. The findings also support mechanism-based therapy, while the relatively small sample, baseline biomarker imbalance, and biomarker-centered outcomes mean that the study should be regarded as a scientific foundation for larger multicenter clinical-outcome trials.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the findings regarding N-acetylcysteine (NAC) as adjunctive therapy in patients with non-severe leptospirosis, the following conclusions were drawn:

1. There was a difference in the change in urinary Kidney Injury Molecule-1 (KIM-1) levels from Day 1 to Day 5 between the NAC and placebo groups.

2. There was a difference in the change in Reactive Oxygen Species (ROS) levels from Day 1 to Day 5 between the NAC and placebo groups.

3. There was a difference in the change in C-Reactive Protein (CRP) levels from Day 1 to Day 5 between the NAC and placebo groups.

4. NAC as adjunctive therapy was associated with changes in biomarkers reflecting renal tubular injury, inflammation, and oxidative stress in patients with non-severe leptospirosis.

5.2 Recommendations

5.2.1 For Clinical Practice

These findings may provide a scientific basis for further investigation of N-acetylcysteine as adjunctive therapy in leptospirosis. However, because the primary outcomes were biological biomarkers, routine incorporation of NAC into clinical management requires evidence from studies evaluating clinically meaningful outcomes.

5.2.2 For Future Research

1. Enroll larger numbers of participants using multicenter designs to strengthen the evidence.

2. Extend follow-up to evaluate kidney-function recovery, AKI events, kidney replacement therapy, length of hospital stay, mortality, and transition to chronic kidney disease.

3. Evaluate additional kidney and inflammatory biomarkers associated with injury and repair to provide a more comprehensive understanding of NAC mechanisms.

4. Determine the most effective NAC dose, timing of administration, and duration of therapy in patients with leptospirosis.

5.2.3 For Scientific Development

This study adds evidence regarding changes in biomarkers of renal tubular injury, inflammation, and oxidative stress following NAC administration in patients with non-severe leptospirosis and may serve as a foundation for subsequent translational research and clinical trials of adjunctive therapy for acute kidney injury associated with leptospirosis.

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APPENDICES

APPENDIX 1. ETHICAL APPROVAL

KOMITE ETIK PENELITIAN KESEHATAN
RSUP NASIONAL DR. CIPTO MANGUNKUSUMO
FAKULTAS KEDOKTERAN UNIVERSITAS INDONESIA
Gedung H Fakultas Kedokteran UI, Jalan Salemba Raya No. 6 Jakarta 10430 PO. Box 1358
Telp: (021) 3157008, website: https://komite-etik.fk.ui.ac.id/

Nomor : KET-1176 /UN2.F1/ETIK/PPM.00.02/2025

KETERANGAN LOLOS KAJI ETIK
ETHICAL APPROVAL

Komite Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Indonesia – RSUPN Dr. Cipto Mangunkusumo dalam upaya melindungi hak asasi dan kesejahteraan subjek penelitian kedokteran, telah mengkaji dengan teliti protokol penelitian yang berjudul:

The Ethics Committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital with regards of the Protection of human rights and welfare in medical research, has carefully reviewed the research entitled:

"Efek N-Acetylcystein terhadap Inflamasi dan Gangguan Ginjal Akut pada Pasien Leptospirosis: Sebuah Uji Klinis Acak Terkontrol."

Protocol Number : **25-08-1192**

Peneliti Utama : **DR. dr. Maruhun Bonar H Marbun, SpPD, KGH**
Principal Investigator

Nama Institusi : **Ilmu Penyakit Dalam FKUI-RSCM**
Name of the Institution

Lokasi Penelitian : **Rumah Sakit Umum Daerah (RSUD) Balaraja**
Site

Tanggal Persetujuan : 22 AUG 2025
Date of Approval ***(valid for one year beginning from the date of approval)***

Dokumen Disetujui : **Proposal Penelitian, Version 0.0 tanggal 31 Juli 2025**
Document Approved **Lembar Penjelasan kepada Calon Subjek, Version 0.0 tanggal 31 Juli 2025**

dan telah menyetujui protokol berikut dokumen terlampir.
and approves the above mentioned protocol including the attached document.

Ditetapkan di : Jakarta
Specified in
Ketua
Chair

Prof. Dr. dr. Ratna Dwi Restuti, SpTHTBKL, Subsp.Oto(K), MPH

** Peneliti berkewajiban
1. Menjaga kerahasiaan identitas subjek penelitian.
2. Memberitahukan status penelitian apabila:
   a. Setelah masa berlakunya keterangan lolos kaji etik, penelitian masih belum selesai, dalam hal ini *ethical approval* harus diperpanjang. Harap pengajuan perpanjangan etik dilakukan 30 hari sebelum masa aktif lolos kaji etik habis.
   b. Penelitian berhenti ditengah jalan.
3. Melaporkan kejadian serius yang tidak diinginkan (*serious adverse events*).
4. Peneliti tidak boleh melakukan tindakan apapun pada subjek sebelum protokol penelitian mendapat lolos kaji etik dan sebelum memperoleh *informed consent* dari subjek penelitian.
5. Menyampaikan laporan akhir, bila penelitian sudah selesai.
6. Cantumkan nomor protokol ID pada setiap komunikasi dengan KEPK FKUI-RSCM.
7. Semua prosedur persetujuan dilakukan sesuai dengan standar ICH-GCP.

" Menolong, memberikan yang terbaik "

JCI
CN.3494.1

APPENDIX 2. INFORMATION SHEET FOR PROSPECTIVE PARTICIPANTS



Komite Etik Penelitian Kesehatan
FKUI-RSCM

LEMBAR PENJELASAN KEPADA CALON SUBJEK

Saya, **DR. dr. M. Bonar HM, SpPD, KGH** dari Divisi Ginjal Hipertensi **Departemen Ilmu Penyakit Dalam, Fakultas Kedokteran Universitas Indonesia dengan tim peneliti dr. Selfie, SpPD,** akan melakukan penelitian dengan judul **Efek N-Acetylcystein Terhadap Inflamasi dan Gangguan Ginjal Akut pada Pasien Leptospirosis: Sebuah Uji Klinis Acak Terkontrol**. Penelitian ini tanpa sponsor.

Saya akan memberikan informasi kepada (Bapak/Ibu/Saudara) mengenai penelitian ini dan mengundang (Bapak/Ibu/Saudara) untuk menjadi bagian dari penelitian ini.

Bapak/Ibu/Saudara dapat berpartisipasi dalam penelitian ini dengan cara menandatangani formulir ini. Jika Bapak/Ibu/Saudara setuju untuk berpartisipasi dalam penelitian ini, Bapak/Ibu/Saudara kapan saja dapat secara bebas mundur dari penelitian ini. Bapak/Ibu/Saudara juga berhak untuk menerima informasi terbaru dari kami mengenai pengobatan yang sedang diujikan, bila ada. Jika Bapak/Ibu/Saudara menolak untuk berpartisipasi atau mundur dari penelitian ini, keputusan tersebut tidak akan mempengaruhi hubungan Bapak/Ibu/Saudara dengan saya dan tidak akan berdampak pada pelayanan yang berlaku di rumah sakit ini.

Jika Bapak/Ibu/Saudara tidak mengerti tiap pernyataan dalam formulir ini, Bapak/Ibu/Saudara dapat menanyakannya kepada saya.

1. **Tujuan penelitian**

   Penelitian ini bertujuan untuk mengetahui pengaruh pemberian obat N-acetylcysteine terhadap kesehatan ginjal pasien yang mengalami infeksi Leptospira.

2. **Partisipasi dalam penelitian**

   Secara keseluruhan, pengambilan data dalam penelitian ini akan berjalan selama 5 hari. Apabila Bapak/Ibu/Saudara memutuskan untuk ikut dalam penelitian ini, Bapak/Ibu/Saudara akan diminta kesediaannya untuk mengikuti jadwal kami dan memastikan bahwa Bapak/Ibu/Saudara dapat mematuhi jadwal tersebut. Penelitian ini akan melibatkan Bapak/Ibu/Saudara dalam beberapa sesi wawancara, pemeriksaan, pengambilan sampel darah, pengambilan sampel urin, dan pengobatan. Setiap sesi wawancara dan pemeriksaan akan berlangsung sekitar 30 menit hingga 1 jam. Pengambilan sampel darah dan urin akan dilakukan sebanyak dua kali, yaitu satu kali sebelum memulai pengobatan dan satu kali setelah lima hari pengobatan. Pengambilan sampel darah dan urin akan berlangsung selama 15 menit hingga 30 menit. Pasien akan mendapatkan obat yang diminum tiga kali sehari selama 5 hari berurutan.

3. Alasan memilih Bapak/Ibu/Saudara

Peneliti memilih Bapak/Ibu/Saudara untuk berpartisipasi dalam penelitian ini karena Bapak/Ibu/Saudara merupakan pasien dewasa yang terdiagnosis Leptospirosis, yang dibuktikan dari pemeriksaan laboratorium serta memiliki kesehatan ginjal dan hati yang baik berdasarkan pemeriksaan laboratorium.

4. Prosedur penelitian

4a. Informasi Obat atau Prosedur Intervensi

1. Anda akan diwawancarai oleh dokter untuk menanyakan: nama, usia, jenis kelamin, tingkat pendidikan, riwayat pekerjaan, riwayat penyakit, riwayat penggunaan obat, riwayat alergi, gejala penyakit serta riwayat terpapar lingkungan yang terkontaminasi bakteri Leptospira atau urin tikus dalam 2 minggu sebelumnya, seperti riwayat mengalami kebanjiran atau kontak dengan air sungai/danau/sawah/kebun tanpa alas kaki, ada banyak tikus di lingkungan rumah atau memelihara hewan ternak.
2. Menjalani pemeriksaan fisik oleh dokter untuk memeriksa status kesehatan.
3. Pengambilan sampel darah dan urin dilakukan sebanyak dua kali selama penelitian, yaitu satu kali sebelum memulai pemberian obat N-Acetylsistein dan satu kali setelah pengobatan lima hari obat N-Acetylsistein.
4. Pengambilan sampel darah dilakukan oleh perawat yang berpengalaman melakukan pengambilan darah, menggunakan jarum suntik yang terhubung pada 2 tabung vakum, antara lain tabung berwarna ungu untuk pemeriksaan darah perifer lengkap dan tabung berwarna hijau untuk pemeriksaan kimia darah. Darah diambil sebanyak ± 3 mL atau sekitar 1 sendok teh pada masing-masing tabung.
5. Pengambilan sampel urin dilakukan dengan meminta anda berkemih di dalam pot urin yang sudah disediakan sebanyak ± 10 mL atau sekitar 2 sendok teh. Cara pengambilan urin adalah membuang pancaran urin awal, kemudian menampung pancaran urin tengah hingga pot urin terisi penuh.
6. Pada hari yang ditentukan anda akan diberi obat dalam bentuk kapsul yang harus diminum dengan air putih. Karena dalam penelitian ini terdapat kelompok yang tidak diberi obat atau perlakuan, ada kemungkinan tablet yang diberikan kepada bapak/ibu/saudara tidak mengandung bahan obat.
7. Anda akan diminta meminum obat selama 5 hari berurutan sebanyak 3 kali sehari.
8. Seluruh identitas subjek studi pada sampel akan dirahasiakan dengan pemberian nomor identitas dan dibuat anonim.

5. Risiko, efek samping dan tatalaksananya

Pemberian obat N-Acetylcysteine sejauh ini sudah banyak digunakan dan tidak memberikan efek samping yang berarti bila dikonsumsi secara diminum, namun pada beberapa orang dapat terjadi alergi obat. Efek samping yang mungkin terjadi adalah mual dan muntah akibat aroma menyengat dari obat ini, gatal – gatal,

dan kemerahan pada kulit. Selama penelitian, peneliti mempersiapkan perlindungan yang diperlukan seandainya terjadi sesuatu yang tidak diinginkan. Perlindungan yang diberikan oleh peneliti adalah akses pemeriksaan dan konsultasi dengan dokter terkait gejala yang diduga terjadi akibat efek samping obat penelitian, serta pemberian pengobatan yang diperlukan untuk meringankan gejala yang dirasakan pasien.

6. Manfaat

Manfaat yang dapat Bapak/Ibu/Saudara dapatkan adalah anda mendapatkan pemeriksaan laboratorium untuk mengetahui keadaan darah, fungsi hati dan fungsi ginjal secara gratis.

7. Kompensasi

Peneliti memberikan kompensasi untuk waktu yang Bapak/Ibu/Saudara luangkan untuk berpartisipasi dalam penelitian ini berupa uang sebesar Rp150.000,00 per orang.

8. Pembiayaan

Biaya yang dibutuhkan dalam penelitian ini ditanggung sepenuhnya oleh pihak peneliti.

9. Kerahasiaan

Semua data yang dikumpulkan dalam penelitian ini akan dijaga kerahasiaannya. Data pribadi dan data medis Bapak/Ibu/Saudara hanya akan digunakan oleh pihak peneliti dan pihak rumah sakit untuk keperluan lain yang menyangkut kesehatan Bapak/Ibu/Saudara selama penelitian berlangsung. Presentasi hasil penelitian dalam pertemuan ilmiah / konferensi dan publikasi dalam jurnal ilmiah tidak akan mencantumkan nama Bapak/Ibu/Saudara. Namun, perwakilan dari sponsor, komite etik, dan badan otoritas nasional yang mengatur penggunaan obat akan memiliki akses terhadap data penelitian untuk verifikasi.

10. Kewajiban subyek penelitian

Sebagai subyek penelitian, bapak/ibu/saudara berkewajiban mengikuti aturan atau petunjuk penelitian sebagaimana arahan dari tim peneliti. Bila ada yang belum jelas, bapak/ibu/saudara bisa bertanya lebih lanjut kepada tim peneliti. Selama penelitian, tidak diperbolehkan meminum obat ataupun jamu selain yang diberikan atau diizinkan oleh tim peneliti.

11. Hak untuk menolak dan mengundurkan diri

Bapak/ Ibu/ Saudara tidak harus berpartisipasi dalam penelitian ini bila tidak berkenan untuk melakukannya. Bapak/ Ibu/ Saudara harus paham bahwa walaupun Bapak/ Ibu/ Saudara menyetujui untuk berpartisipasi, Bapak/ Ibu/ Saudara berhak untuk mundur dari penelitian ini kapanpun selama penelitian ini masih berlangsung. Jika Bapak/ Ibu/ Saudara menolak untuk berpartisipasi atau mundur dari penelitian ini, Keputusan tersebut tidak akan mempengaruhi hubungan Bapak/ Ibu/ Saudara dengan saya dan tidak akan

berdampak pada standar pelayanan yang berlaku di rumah sakit ini. Saya akan memberikan kesempatan pada Bapak/ Ibu/ Saudara pada akhir penjelasan ini untuk dapat mempertimbangkan Keputusan yang akan diambil.

12. Akses pasca penelitian (*Post-trial access)*

Pada akhir penelitian ini, kami akan tetap memberikan Bapak/Ibu/Saudara pengobatan terbaik lanjuran terhadap penyakit Bapak/Ibu/Saudara.

13. Informasi Tambahan

Bapak/ ibu/ saudara diberi kesempatan untuk menanyakan semua hal yang belum jelas sehubungan dengan penelitian ini. Jika Anda ingin berdiskusi lebih lanjut perihal penelitian ini dengan seseorang sebelumnya (atau jika Anda mempunyai pertanyaan sesudahnya), mohon hubungi DR. dr. M. Bonar HM, SpPD, KGH pada nomor HP 08128819565 atau dr Selfie, SpPD pada nomor HP 08111803701.

LEMBAR PERSETUJUAN KEIKUTSERTAAN DALAM PENELITIAN

Semua penjelasan tersebut telah disampaikan kepada saya dan semua pertanyaan saya telah dijawab oleh DR. dr. M. Bonar HM, SpPD, KGH dan tim. Saya mengerti bahwa bila memerlukan penjelasan, saya dapat menanyakan kepada DR. dr. M. Bonar HM, SpPD, KGH dan tim.

Sertifikat Persetujuan (*Consent*)	
Saya telah membaca semua penjelasan tentang penelitian ini. Saya telah diberikan kesempatan untuk bertanya dan semua pertanyaan saya telah dijawab dengan jelas. Saya bersedia untuk berpartisipasi pada studi penelitian ini dengan sukarela. _____ Nama subjek/wali _____ Tanda tangan peserta studi Tanggal_____ hari/bulan/tahun	Saya mengkonfirmasi bahwa peserta telah diberikan kesempatan untuk bertanya mengenai penelitian ini, dan semua pertanyaan telah dijawab dengan benar. Saya mengkonfirmasi bahwa persetujuan telah diberikan dengan sukarela. _____ Nama peneliti/peminta persetujuan _____ Tanda tangan peneliti/peminta persetujuan Tanggal_____ hari/bulan/tahun

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Apabila subjek tuna aksara*:*
Seorang saksi yang tidak tuna aksara harus menandatanganinya (apabila memungkinkan, orang ini harus dipilih oleh subjek/partisipan penelitian, bukan orangtuanya, dan tidak boleh memiliki hubungan dengan tim peneliti). Subjek/partisipan penelitian yang tuna aksara juga harus menyertakan cap sidik jarinya.

Saya telah menyaksikan pembacaan dari lembar persetujuan (*consent*) kepada subjek/partisipan penelitian dengan akurat, dan telah diberikan kesempatan untuk mengajukan pertanyaan. Saya mengkonfirmasi bahwa subjek/partisipan telah memberikan persetujuannya dengan bebas.

Nama saksi _____ **DAN** **Sidik jari subjek penelitian**

Tanda tangan saksi _____

Tanggal _____
tanggal/bulan/tahun

APPENDIX 3. INFORMED CONSENT FORM FOR STUDY PARTICIPATION





Komite Etik Penelitian Kesehatan
FKUI-RSCM

LEMBAR PERSETUJUAN

Untuk subjek penelitian ***(Nama Subjek.....)***
yang ikut dalam penelitian:***[Efek N-Acetylcystein Terhadap Inflamasi dan Gangguan Ginjal Akut Pada Pasien Leptospirosis: Sebuah Uji Klinis Acak Terkontrol*]**

saya telah membaca dan mengerti informasi yang tercantum pada lembar informasi dan telah diberi kesempatan untuk mendiskusikan dan menanyakan hal tersebut. Saya setuju untuk mengijinkan anak/ayah/ibu saya mendapatkan **[*terapi / tindakan sesuai protokol penelitian*]**. Saya mengerti bahwa saya dapat menolak untuk ikut dalam penelitian. Saya sadar bahwa saya dapat mengundurkan diri dari penelitian ini kapan saja saya mau. Saya mengerti bahwa apabula saya tidak mengikuti penelitian ini, anak saya tetap akan menerima perawatan medis yang semestinya **[*sesuai penyakit / kondisi pasien*]**.

Saya, sebagai **ORANG TUA/WALI** dari .....

SETUJU untuk berpartisipasi dalam penelitian ini.

Tanggal **: .....**

Tanda tangan Orang Tua/Wali **: .....**

Nama Orang Tua/Wali : **.....**

Tanda tangan Saksi **: .....**

Nama Saksi **: .....**

The signed/official appendix documents are retained in their original form because they are source regulatory and consent documents. Their appendix titles have been translated for the English thesis.

SUMMARY

Leptospirosis is a zoonotic disease that remains a significant public health problem, particularly in tropical countries, including Indonesia. It is caused by pathogenic bacteria of the genus Leptospira and presents with a wide spectrum of clinical manifestations, ranging from mild self-limiting illness to severe disease with multiorgan involvement. One of the most common and clinically significant complications is acute kidney injury (AKI), which substantially contributes to increased morbidity and mortality among affected patients.

The pathogenesis of AKI in leptospirosis is multifactorial. In addition to the direct invasion of Leptospira into renal tissue, kidney injury is mediated by activation of the host immune response, systemic inflammation, microcirculatory disturbances, and oxidative stress. Excessive production of reactive oxygen species (ROS) results in oxidative damage to lipids, proteins, and nucleic acids, thereby aggravating renal tubular injury. Furthermore, systemic inflammation, reflected by elevated C-reactive protein (CRP) levels, amplifies tissue damage. The interaction between oxidative stress and inflammation establishes a pathological cycle that contributes to the progression of kidney injury in leptospirosis.

Conventional assessment of kidney function primarily relies on serum creatinine concentration and urine output. However, serum creatinine is an insensitive marker of early kidney injury because its elevation generally occurs after substantial nephron damage has already developed. Consequently, more sensitive biomarkers are required to detect structural kidney injury at an earlier stage. Kidney Injury Molecule-1 (KIM-1) is a proximal tubular injury biomarker that is rapidly expressed following tubular epithelial damage and can be detected in urine. Simultaneous assessment of urinary KIM-1, ROS, and CRP provides a more comprehensive evaluation of tubular injury, oxidative stress, and systemic inflammation in patients with leptospirosis.

N-Acetylcysteine (NAC) is a well-established antioxidant and anti-inflammatory agent that acts primarily as a precursor of glutathione while modulating multiple redox-sensitive signaling pathways involved in oxidative stress and inflammation. Previous studies have demonstrated its renoprotective effects in various clinical conditions. Nevertheless, evidence regarding its biological effects on renal injury, oxidative stress, and inflammatory biomarkers in leptospirosis remains limited.

This study aimed to evaluate the effect of oral NAC administration on changes in urinary KIM-1, ROS, and CRP levels in patients with leptospirosis. A randomized, double-blind, placebo-controlled clinical trial was conducted involving adult patients with confirmed leptospirosis who fulfilled the predefined inclusion criteria. Participants were randomly assigned to receive either oral NAC at a dose of 800 mg three times daily or placebo for five days, in addition to standard medical therapy. Urinary KIM-1, ROS, and CRP levels were measured on Day 1 before treatment initiation and on Day 5 after intervention. Data were

analyzed according to the intention-to-treat principle using appropriate statistical methods to compare biomarker changes between the treatment groups.

The study demonstrated that NAC administration produced greater reductions in ROS and CRP levels than placebo after adjustment for baseline values. In contrast, changes in urinary KIM-1 levels were not significantly different between the two groups. These findings suggest that NAC exerts beneficial biological effects by attenuating oxidative stress and systemic inflammation in patients with leptospirosis but does not significantly influence the biomarker of renal tubular injury during the five-day treatment period.

The results support the hypothesis that oxidative stress and inflammation are key mechanisms underlying kidney injury in leptospirosis and that these processes can be modulated by NAC administration. However, the absence of a significant reduction in urinary KIM-1 indicates that structural tubular recovery may require a longer observation period or may be influenced by additional pathogenic mechanisms that cannot be modified by short-term NAC therapy alone.

The strengths of this study include its randomized double-blind placebo-controlled design and the simultaneous evaluation of three complementary biomarkers representing renal tubular injury, oxidative stress, and systemic inflammation. Nevertheless, several limitations should be acknowledged, including the relatively small sample size and the short intervention period of five days, which precluded assessment of long-term renal outcomes and other clinically relevant endpoints.

In conclusion, adjunctive NAC therapy effectively reduced biomarkers of oxidative stress and systemic inflammation in patients with leptospirosis but did not significantly affect urinary KIM-1 levels. These findings provide further evidence supporting the potential role of NAC as an adjunctive therapeutic strategy targeting the major pathophysiological mechanisms involved in leptospirosis-associated kidney injury. Larger randomized clinical trials with longer follow-up periods are warranted to determine whether these biological effects translate into meaningful clinical benefits, including reduced incidence of AKI, decreased need for renal replacement therapy, shorter hospital stay, lower mortality, and improved long-term renal function.