RESEARCH ARTICLE

Renoprotective Effects of Hydroxychloroquine and Folinic Acid via ET-1 and NLRP3 Modulation in Reducing Tubular Injury in A Rabbit Model of Methanol-induced Acute Kidney Injury

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Abstract

BACKGROUND: Methanol intoxication is associated with significant morbidity and mortality, particularly when acute kidney injury (AKI) developed. Emerging evidence implicates Endothelin-1 (ET-1) and Nucleotide-binding domain leucine-rich repeat-containing pyrin receptor 3 (NLRP3) inflammasome in renal injury, but their roles in methanol-induced AKI remain unclear. To date, no studies have examined whether hydroxychloroquine or folinic acid, which are known to modulate ET-1 and NLRP3 signaling, could mitigate renal injury in this setting. This study evaluated their therapeutic effects in a rabbit model of methanol-induced AKI.

METHODS: The animals subjects were randomly assigned to four groups: control group receiving aquabidest, folinic acid group receiving 2 mg/kg body weight (BW) intraperitoneal folinic acid, hydroxychloroquine group receiving 30 mg/kg BW oral hydroxychloroquine phosphate, and combination group receiving both folinic acid and hydroxychloroquine at the same dosages. Histopathological evaluation of tubular injury scores and immunohistochemical analysis of ET-1 and NLRP3 expression were then conducted.

RESULTS: Expressions of ET-1, NLRP3, and tubular injury scores were significantly lower in the hydroxychloroquine, folinic acid, and combination therapy groups compared to the control group (p<0.001). Expression of ET-1 was lowest in folinic acid group (59.38±0.71%), followed by combination group (62.23±1.98%) and hydroxychloroquine group (62.43±1.81%), compared to control group (72.14±1.02%). Expression of NLRP3 was lowest in combination group (58.94±1.05%), followed by folinic acid and hydroxychloroquine group, which showed equal values (60.57±1.38%), compared to control group (72.15±1.02%). Tubular injury scores were also lowest in combination group (27.07±3.16%), followed by hydroxychloroquine group (45.29±1.75%) and folinic acid group (48.38±2.49%), compared to control group (77.15±1.66%).

CONCLUSION: Expression of ET-1 and NLRP3, as well as tubular injury scores, are significantly lower in all treatment groups compared to control, suggesting hydroxychloroquine and folinic acid demonstrated renoprotective effects in methanol-induced AKI, likely through modulation of ET-1 and NLRP3 pathways.

KEYWORDS: methanol intoxication, acute kidney injury, hydroxychloroquine, folinic acid, endothelin-1, NLRP3 inflammasome, experimental animal models, rabbits

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Introduction

Methanol intoxication remains a serious public health problem, particularly in developing countries, with a high case fatality rate that can reach 48% in severe cases. Acute kidney injury (AKI) is a frequent and life-threatening complication, increasing mortality risk significantly. Pathophysiological mechanisms include metabolic acidosis, hypoxia, and nephrotoxic effects of formic acid, which can cause tubular injury and apoptosis.(1–3) Although several studies have explored oxidative stress and mitochondrial dysfunction as key mechanisms in methanol-induced AKI, limited attention has been given to the downstream inflammatory and vasoactive pathways that may mediate sustained tubular damage.(4) Endothelin-1 (ET-1), a potent vasoconstrictor, plays a crucial role in renal hypoperfusion, glomerular permeability, and tubular apoptosis, yet its specific contribution to methanol-induced injury has not been well defined.(5) Similarly, the Nucleotide-binding domain leucine-rich repeat-containing pyrin receptor 3 (NLRP3) inflammasome, a key mediator of inflammatory cell death (pyroptosis), has been implicated in toxin-induced and ischemic AKI, but its activation in response to formic acid accumulation remains underexplored.(6,7) This study is the first to assess the combined modulation of ET-1 and NLRP3 in a methanol-induced AKI model, thereby addressing a significant gap in the mechanistic understanding of this condition.

Current treatments for methanol poisoning, such as fomepizole and hemodialysis, primarily focus on preventing systemic toxicity. However, these approaches may not adequately address the renal-specific damage. Folinic acid, a metabolically active form of folate, accelerates formate elimination and mitigates metabolic acidosis.(8,9) Hydroxychloroquine, traditionally used for autoimmune diseases, is known to exert anti-inflammatory effects.(10) It has also shown promising renoprotective properties in AKI models by targeting Toll-like receptor 9 (TLR-9), inhibiting macrophage activation and polarization, and suppressing proinflammatory cytokines.(11) Hydroxychloroquine reduces macrophage differentiation into both M1-like and M2-like phenotypes, which contribute to tissue injury and fibrosis following AKI. In response to tubular damage, damage-associated molecular patterns (DAMPs) released from injured renal epithelium activate monocytes through the TLR-9/MyD88/mitogen-activated protein kinase (MAPK) signaling pathway, leading to the release of interleukin (IL)-6, tumor nuclear factor (TNF)-α, and transforming growth factor (TGF)-β.(11) Hydroxychloroquine's inhibition of this pathway may attenuate inflammation and fibrotic progression. These mechanisms, also active in ischemia-reperfusion and toxin-induced injury, are relevant to methanol-induced AKI, in which formic acid accumulation causes tubular and mitochondrial damage. Thus, hydroxychloroquine may counteract key inflammatory and apoptotic processes in methanol toxicity, although its specific effects in this setting remain unexplored. In addition, hydroxychloroquine has been shown to inhibit ET-1 signaling and NLRP3 inflammasome activation, further supporting its potential role in this context.(12,13) Despite promising preclinical data, its therapeutic effects in methanol-induced AKI have not been comprehensively evaluated.

Therefore, this study was conducted to investigate the therapeutic effects of hydroxychloroquine and folinic acid in treating methanol-induced AKI. The primary outcomes of this study were tubular injury scores and the expression of ET-1 and NLRP3 in renal tissue. Using a rabbit model, we evaluate their impact on tubular injury and the modulation of ET-1 and NLRP3 expression. Findings from this research are expected to provide insights into novel treatment strategies for methanol-induced renal injury.

Methods

Study Design

This study utilized a randomized, posttest-only control group experimental design to evaluate the therapeutic effects of hydroxychloroquine and folinic acid on methanol-induced AKI in a rabbit model. The study design included methanol administration to induce AKI, followed by treatment with hydroxychloroquine, folinic acid, or a combination of both. Outcomes were assessed through histopathological analysis and immunohistochemistry (IHC).

Animal Model and Ethical Considerations

New Zealand white male rabbits, aged 5–12 months and weighing 2,000–3,000 grams, were used for this study due to their metabolic similarity to humans in processing methanol. The rabbits were housed in well-ventilated cages under controlled conditions, with a temperature of ~22°C and exposure to indirect sunlight. They were fed standardized rabbit pellets containing 17–20% protein, 3–4% fat, and 35–40% carbohydrates, and given water *ad libitum*. Adaptation lasted one week prior to the experiment, and antioxidant-rich vegetables were excluded from their diet three days before methanol administration to minimize

external antioxidant effects. All procedures were conducted in compliance with ethical guidelines and approved by Institutional Review Board of Prof. Dr. I.G.N.G. Ngoerah General Hospital (No: 2458/UN14.2.2.VII.14/LT/2023).

Induction of Methanol-Induced AKI

Methanol-induced AKI was achieved through a single intraperitoneal injection of 40% methanol at a dose of 2 g/kg body weight (BW). This dose was standardized based on pilot studies to reliably induce AKI.(14) The injection was performed using a 23G needle.

Treatment Groups and Administration

The rabbits were randomly assigned into four groups. The control group received aquabidest as a placebo. The folinic acid group was administered folinic acid intraperitoneally at a dose of 2 mg/kg BW, starting 6 hours after methanol administration and repeated every 6 hours for a total of three doses. The hydroxychloroquine group received oral hydroxychloroquine phosphate at 30 mg/kg BW, administered via gavage 6 hours after methanol injection. The combination therapy group received both treatments at the same dosages and intervals. Treatments were diluted appropriately to ensure accurate dosing and tolerability.

Tubular Injury Scores Measurement

Tubular injury was assessed histopathologically using periodic acid-Schiff (PAS) staining (PAS MAD-103.015). Evaluation was conducted on 10 non-overlapping high-power fields (400× magnification) in the cortico-medullary region of the right kidney. A semiquantitative scoring system was applied by a board-certified anatomical pathologist with nephrology subspecialty, who was blinded to group allocation. The injury score was calculated as the percentage of damaged tubules relative to total tubules in each field. The following histopathological features were scored: (1) tubular flattening, (2) tubular dilation, (3) loss of brush border, (4) inflammatory cell infiltration, and (5) presence of intraluminal casts. A tubular injury score >7% was used to define the presence of AKI.

ET-1 and NLRP3 Measurement

The expression of ET-1 and NLRP3 in kidney tissue was evaluated using IHC. Sections were stained with monoclonal antibodies specific to ET-1 (TR.ET.48.5, NB300-526; Novus Biologicals, Centennial, CO, USA) and NLRP3 (Nalpy3-b, NBP1-97601; Novus Biologicals, Centennial, CO, USA). The staining intensity was quantified as a percentage using ImageJ software (NIH, Bethesda, MD, USA).

Euthanasia and Sample Collection

Euthanasia was performed using a combination of 50 mg/kg BW ketamine and 10 mg/kg BW xylazine, followed by cervical dislocation to ensure humane sacrifice. The right kidney was harvested postmortem and immediately fixed in 10% phosphate-buffered formalin for histopathological and IHC analysis.

Histopathological Sample Processing

Kidney tissue samples were processed to prepare histopathological slides. The tissues were fixed in formalin for 24 hours, dehydrated through graded alcohols (70%, 80%, 95%, and absolute), cleared in xylene, and embedded in paraffin at 56–60°C. Sections of 4–6 µm thickness were cut using a microtome and mounted on slides coated with Meyer's egg albumin. Slides were dried on a hot plate and stored in an oven at 30–35°C for at least 12 hours before staining.

IHC Staining and Quantification

Slides underwent deparaffinization in xylene and rehydration in decreasing alcohol concentrations. Antigen retrieval was performed using a pressure cooker at 95°C for 15 minutes. Endogenous peroxidase activity was blocked, and slides were incubated with primary antibodies against ET-1 or NLRP3 for 60 minutes. After washing in phosphate buffered saline (PBS), slides were treated with secondary antibodies and stained with 3,3′-Diaminobenzidine (DAB) chromogen for signal detection. Counterstaining was performed with hematoxylin, followed by dehydration and mounting with entellan.

Quantification of ET-1 and NLRP3 expression was performed using ImageJ software (NIH). Before analysis, the glomerular areas were excluded to ensure the focus remained on tubular structures. Each image was converted to 8-bit grayscale, and a standardized threshold value of 168 was applied uniformly across all samples to optimize detection of positive staining. Area fraction measurements were then conducted and expressed as the percentage of positively stained regions within the cortico-medullary area. For each kidney tissue sample, the analysis was performed on 10 non-overlapping high-power fields at 400× magnification.

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corporation, Armonk, NY, USA) with a significance level set at $p<0.05$. Descriptive statistics, including mean, standard deviation (SD), minimum, and maximum

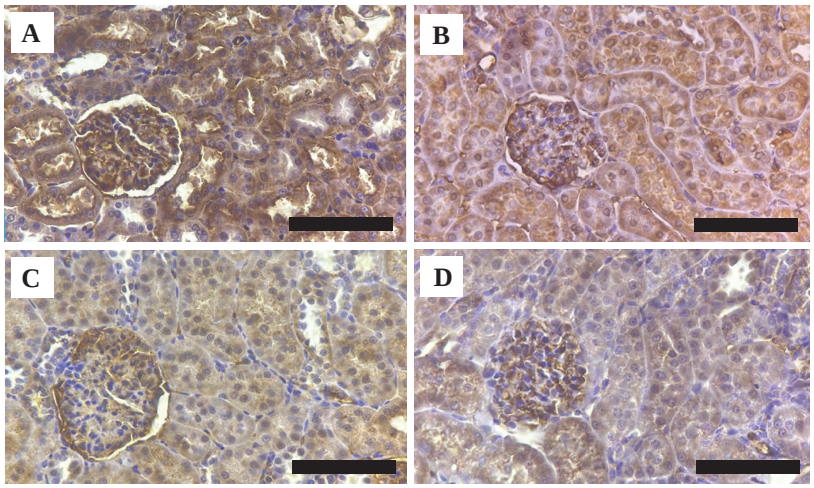

Figure 1. IHC staining of ET-1 expression in rabbit kidney tissue. Control group shows the highest ET-1 expression. The intensity of ET-1 expressions are shown by the brown colors. A: Control group; B: Folinic acid group; C: Hydroxychloroquine group; D: Combination therapy group. Black bar: 1 mm.

values, were used to summarize ET-1 expression, NLRP3 expression, and tubular injury scores for each group. Data distribution was assessed using the Shapiro-Wilk test, with $p>0.05$ indicating normality. Homogeneity of variances across groups was evaluated using Levene's test, with $p>0.05$ indicating homogeneity. For normally distributed and homogeneous data, one-way ANOVA was applied to compare group means, followed by LSD post hoc analysis to identify specific group differences. When data were non-normal or heterogeneous, the Kruskal-Wallis test was used to compare group medians.

Results

The current study evaluated the effects of folinic acid, hydroxychloroquine, and their combination on ET-1 expression, NLRP3 expression, and tubular injury scores in a rabbit model of methanol-induced AKI. Descriptive analysis showed that the experimental data for all variables were normally distributed and homogeneous ($p>0.05$, Shapiro-Wilk and Levene's tests).

ET-1 Expression was Significantly Lower in All Treatment Groups Compared to Control Group

IHC analysis revealed significant differences in ET-1 expression among the groups ($p<0.001$, One-Way ANOVA), following confirmation of normality and homogeneity ($p>0.05$, Shapiro-Wilk and Levene's tests). The expression of ET-1 was significantly lower in all treatment groups compared to the control group (72.14±1.02%). In the folinic acid group, ET-1 expression was 59.38±0.71% (MD: 12.68±2.17%, 95% CI: 8.16–17.20; $p<0.001$); in the hydroxychloroquine group, 62.43±1.81% (MD: 9.63±2.17%, 95% CI: 5.11–14.15; $p<0.001$); and in the combination therapy group, 62.23±1.98% (MD: 9.83±2.17%, 95% CI: 5.31–14.35; $p<0.001$). However, no significant differences were found between the treatment groups: folinic acid *vs.* hydroxychloroquine ($p=0.174$), folinic acid *vs.* combination therapy ($p=0.202$), and hydroxychloroquine *vs.* combination therapy ($p=0.929$). Representative immunohistochemistry images were shown in Figure 1, and group-wise percentage comparisons were provided in Figure 2. These findings indicate that while all treatments significantly reduced ET-1 expression compared to control, combination therapy did not provide additional benefit over monotherapy.

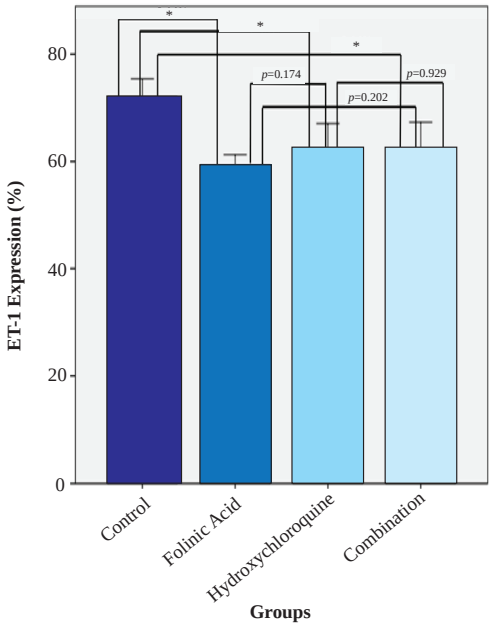

Figure 2. ET-1 expressions in different study groups. ANOVA results of ET-1 expression showed significant reductions in all treatment groups compared to the control group (*$p<0.001$), with no significant differences among the treatment groups.

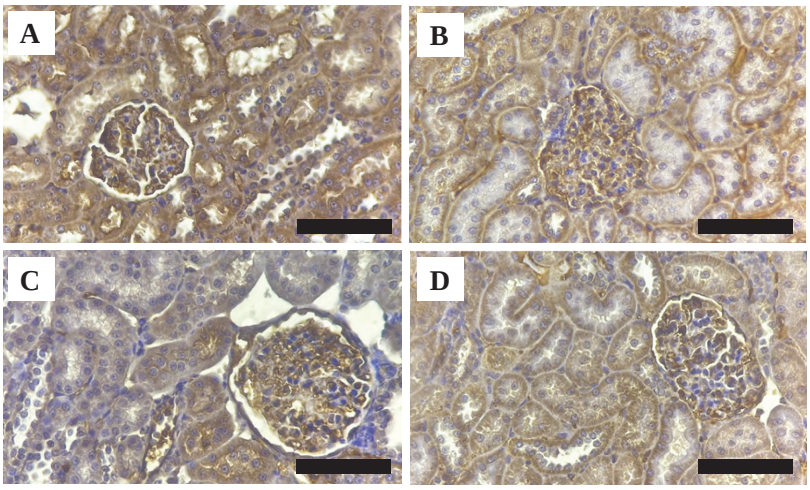

Figure 3. IHC staining of NLRP3 expression in rabbit kidney tissue. Control group shows the highest NLRP expression. The intensity of ET-1 expressions are shown by the brown colors. A: Control group; B: Folinic acid group; C: Hydroxychloroquine group; D: Combination therapy group. Black bar: 1 mm.

NLRP3 Expression was Significantly Lower in All Treatment Groups Compared to Control Group

One-Way ANOVA revealed significant differences in NLRP3 expression across all groups (p<0.001), following confirmation of normality and homogeneity via Shapiro-Wilk and Levene's tests (p>0.05). The expression of NLRP3 was significantly lower in all treatment groups compared to the control group (72.15±1.02%). The folinic acid and hydroxychloroquine groups showed equivalent NLRP3 expression levels of 60.57±1.38% (MD: 11.58±1.69%, 95% CI: 8.05–15.11; p<0.001), while the combination therapy group showed the lowest expression at 58.94±1.05% (MD: 13.21±1.69%, 95% CI: 9.68–16.74; p<0.001). However, no significant differences were observed among the treatment groups: folinic acid *vs.* hydroxychloroquine (p=0.999), folinic acid *vs.* combination (p=0.348), and hydroxychloroquine *vs.* combination (p=0.348). Representative immunohistochemistry images were shown in Figure 3, and group-wise percentage comparisons were presented in Figure 4. These findings indicate that while all treatments significantly reduced NLRP3 expression compared to control, combination therapy did not offer additional benefit over monotherapy.

Combination Therapy Showed the Lowest Tubular Injury Scores

Tubular injury scores, assessed using PAS staining, were significantly different across the study groups (p<0.001, One-Way ANOVA), with data showing normal distribution and homogeneous variance (p>0.05, Shapiro-Wilk and Levene's tests). The tubular injury scores were significantly lower in all treatment groups compared to the control group (77.15±1.66%). The folinic acid group showed a score of 48.38±2.49% (MD: 28.77±3.32%; 95% CI: 21.86–35.69; p<0.001), the hydroxychloroquine group 45.29±1.75% (MD: 31.86±3.32%; 95% CI: 24.94–38.77; p<0.001), and the combination therapy group 27.07±3.16% (MD: 50.08±3.32%; 95% CI: 43.16–56.99; p<0.001). Furthermore, combination therapy group resulted in significantly lower scores than either folinic acid (MD: 21.31±3.32%; 95% CI: 14.39–28.22; p<0.001) or hydroxychloroquine monotherapy (MD: 18.22±3.32%; 95% CI: 11.31–25.14; p<0.001). There was no significant difference between folinic acid and hydroxychloroquine groups (MD: 3.08±3.32%; 95% CI: −3.83 to 10.00; p=0.364). Representative histopathological

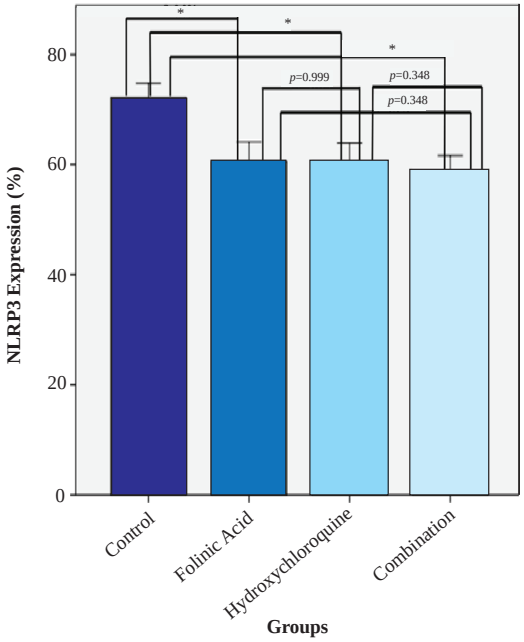

Figure 4. NLRP3 expressions in different study groups. ANOVA results of NLRP3 expression showed significant reductions in all treatment groups compared to the control group (*p<0.001), with no significant differences among the treatment groups.

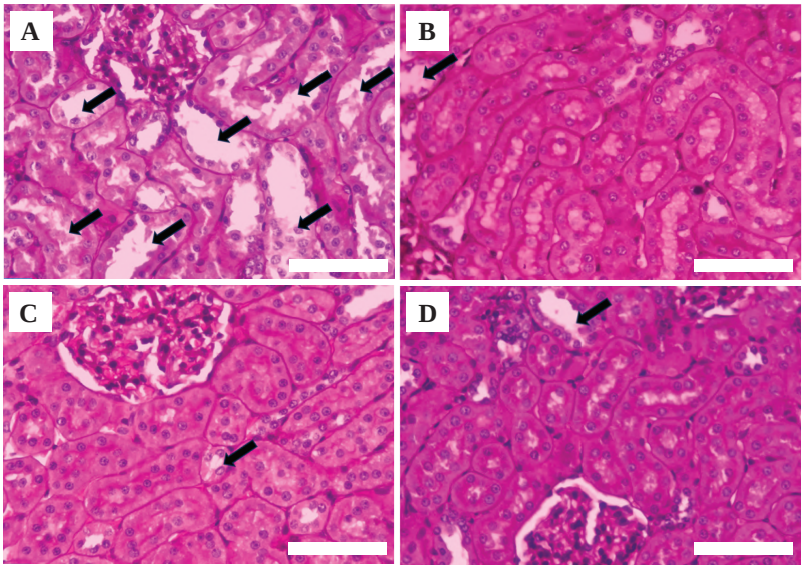

Figure 5. Histopathological appearance of rabbit kidney tissue at 400× magnification with PAS staining. The most prominent tubular injury (black arrow) is observed in the control group. A: Control group; B: Folinic acid group; C: Hydroxychloroquine group; D: Combination therapy group. White bar: 1 mm.

images werre shown in Figure 5, meanwhile the quantitative comparison of tubular injury scores was displayed in the Figure 6.

Discussion

Various cytokines were associated with kidney injury (15), including AKI. This study revealed significantly lower ET-1 expression in all treatment groups (folinic acid, hydroxychloroquine, and their combination) compared to the control. ET-1 is known to mediate renal vasoconstriction, hypoperfusion, and tissue hypoxia, exacerbating tubular injury in methanol-induced AKI.(5,16,17) In methanol-induced kidney injury, oxidative stress and inflammation triggered by formic acid accumulation increase ET-1 production, further damaging renal tissue.(1,3)

Folinic acid plays a critical role in metabolizing formic acid, a toxic byproduct of methanol metabolism, reducing oxidative stress and subsequent ET-1 production. (18) Hydroxychloroquine, as an immunomodulator, inhibits inflammatory pathways such as TLR-9 and nuclear factor kappaB (NF-κB), contributing to reduced ET-1 levels.(19,20) Interestingly, combination therapy did not significantly outperform monotherapy in lowering ET-1 expression, suggesting that either agent alone is sufficient to mitigate ET-1-mediated damage.

NLRP3 inflammasome activation is a hallmark of methanol-induced AKI, driven by oxidative stress and inflammatory signals.(21,22) This study showed significantly lower NLRP3 expression in the treatment groups compared to the control, supporting the role of folinic acid and hydroxychloroquine in suppressing NLRP3-mediated inflammation. Folinic acid reduces oxidative stress by decreasing formic acid levels, while hydroxychloroquine directly inhibits NLRP3 activation by modulating

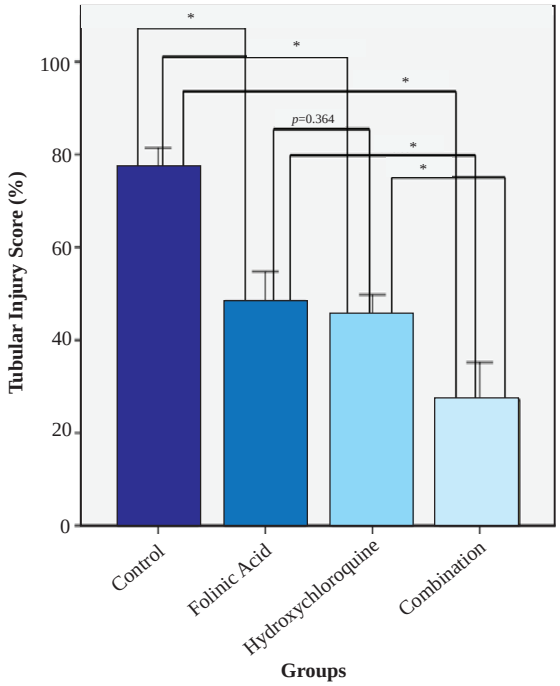

Figure 6. Tubular injury scores in different study groups. ANOVA of tubular injury scores across the study groups demonstrated significant reductions in all treatment groups compared to the control (*p<0.001). Combination therapy showed significantly greater reductions compared to monotherapies (*p<0.001), while no significant differences were observed between the folinic acid and hydroxychloroquine groups.

pathways such as TLR-9 and cathepsin activity.(11,20,23) Combination therapy demonstrated complementary effects, although no additional reduction in NLRP3 expression compared to monotherapy was observed.

Tubular injury scores, a marker of renal structural damage, were significantly lower in all treatment groups, with the combination therapy showing the lowest. Folinic acid mitigates tubular injury by reducing toxic formic acid accumulation, while hydroxychloroquine suppresses inflammation and oxidative stress, key contributors to tubular damage.(3,24,25) The lowest tubular injury scores in the combination therapy group suggesting a possible additive effect of folinic acid and hydroxychloroquine. However, no formal synergy analysis was performed, and this interpretation should be considered descriptive rather than conclusive.

The findings demonstrate the therapeutic potential of folinic acid and hydroxychloroquine in reducing methanol-induced AKI. While combination therapy provided the most significant structural protection, monotherapy with either agent effectively reduced ET-1 and NLRP3 expression. These results suggest that these treatments could form the basis for new therapeutic protocols for methanol poisoning. It is also important to note that while ET-1 and NLRP3 expression were significantly lower in treatment groups, the lack of a dose-response assessment prevents us from conclusively determining whether these reductions were due to direct modulation by the treatments or secondary to reduced tubular injury severity. Further studies incorporating graded dosing and mechanistic inhibition experiments are needed to validate the specificity of these molecular effects.

To better understand the biological mechanisms underlying our findings, we constructed a comprehensive mechanistic diagram (Figure 7), outlining the pathogenesis of methanol-induced AKI and the modulatory roles of folinic acid and hydroxychloroquine. Histologically, methanol-induced AKI is characterized by proximal tubular necrosis without glomerular lesions. This injury is primarily driven by the accumulation of formic acid, a toxic metabolite of methanol. Under physiological conditions, formic acid is further oxidized into carbon dioxide and water, a reaction catalyzed by the enzyme 10-formyl tetrahydrofolate dehydrogenase in the presence of tetrahydrofolate. In methanol toxicity, however, excessive formate production overwhelms the elimination capacity, leading to its accumulation and toxicity.

AKI in this context is mediated by several mechanisms, including severe hypotension (ischemia-reperfusion), metabolic acidosis, myoglobinuria, oxidative stress, and osmotic disturbances in tubular cells. Severe hypotension in methanol toxicity results from reduced stroke volume, decreased cardiac output, and systemic vasodilation, all of which stimulate ET-1 expression. Additionally, metabolic acidosis contributes to hemolysis and myoglobinuria. The nephrotoxic effects of heme proteins during myoglobinuria, including renal vasoconstriction, intraluminal cast formation, and cytotoxicity, which further activate ET-1 and NLRP3.

Mitochondrial injury caused by formate inhibits cytochrome oxidase, promotes anaerobic metabolism, and increases lactate accumulation, contributing to metabolic acidosis and cellular hypoxia. This also disrupts the Krebs cycle and sodium pump, leading to sodium and water influx and subsequent cellular edema, which contributes to tubular oncosis. Mitochondrial damage results in the release of mitochondrial DNA (mtDNA), which activates Toll-like receptor 9 (TLR9), triggering myeloid differentiation primary response protein 88 (MyD88)-dependent NF-κB signaling and the production of inflammatory cytokines including TNF-α, IL-6, and IL-1β. These cytokines promote p53 activation, death receptor signaling (DR4, DR5), and caspase activation (caspase-3, -8, -9), ultimately leading to tubular apoptosis.

Formate and lactic acidosis also stimulate mitochondrial ROS (mROS) production and thioredoxin-interacting protein (TXNIP)-mediated NLRP3 activation. Furthermore, lysosomal stress due to intracellular acidosis causes cathepsin release, contributing to NLRP3 activation. Overactivation of ET-1 and its receptor endothelin A receptor (ETAR), more than endothelin B receptor (ETBR), has been associated with endoplasmic reticulum stress and apoptosis. These combined signals drive cell death via pyroptosis, apoptosis, and necrosis, exacerbating renal damage.

NLRP3 inflammasome activation promotes caspase-1–mediated processing of pro–IL-1β and pro–IL-18, and cleavage of Gasdermin D, forming membrane pores that initiate inflammation and pyroptosis. This signaling contributes to epithelial-mesenchymal transition and fibrosis during AKI progression. The pathway shown in Figure 7 illustrates these interrelated mechanisms and highlights the central roles of ET-1 and NLRP3 in methanol-induced AKI. Folinic acid therapy enhances formate elimination by converting it into CO_2 and water. It also increases antioxidant defenses through Nuclear factor erythroid 2-related factor 2 (Nrf2) activation, enhancing heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and glutathione production, the key enzymes that reduce ROS and mROS. These mechanisms collectively explain the observed reductions in

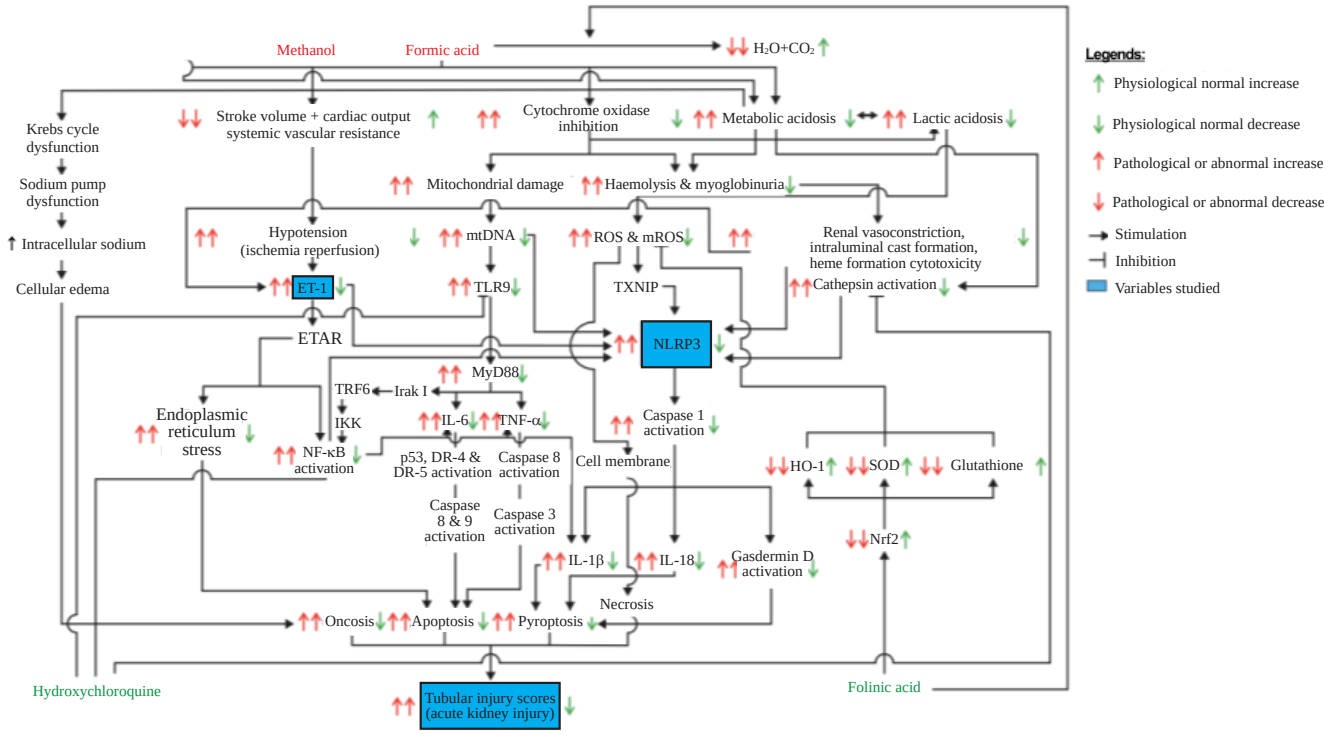

Figure 7. Conceptual diagram of methanol-induced acute kidney injury and the potential protective roles of folinic acid and hydroxychloroquine. This schematic integrates previously reported mechanisms with the specific components measured in this study (ET-1, NLRP3 expression, and tubular injury scores). Other pathways depicted are not directly examined and are included to provide broader mechanistic context for interpretation and future hypothesis generation.

ET-1 and NLRP3 expression following folinic acid therapy. Hydroxychloroquine, known for its anti-inflammatory effects, inhibits multiple pro-inflammatory pathways including ET-1, TLR9, NF-κB, cathepsin, and NLRP3. By suppressing cytokine production (TNF-α, IL-6, IL-1β, IL-18), it reduces tubular inflammation, apoptosis, and pyroptosis.

The novelty of this study lies in its findings, which have not been reported in previous research. First, the study demonstrated significant differences in ET-1 expression among the treatment and control groups, highlighting the impact of folinic acid, hydroxychloroquine, and their combination in reducing this vasoconstrictive mediator. Second, it identified differences in NLRP3 expression across the groups, underscoring the anti-inflammatory effects of the treatments in mitigating inflammasome activation. Third, the study revealed significantly lower tubular injury scores in the treatment groups, with combination therapy showing the greatest reduction, indicating a complementary effect of folinic acid and hydroxychloroquine. Lastly, this research is the first to evaluate the effects of folinic acid and hydroxychloroquine on ET-1 and NLRP3 expression, as well as their impact on tubular injury scores in a methanol-induced AKI rabbit model, offering new insights into potential therapeutic strategies for this condition.

Histopathological differentiation between acute and chronic kidney injury might requires additional staining methods (26), such as Picrosirius Red or Masson's Trichrome, however it was not performed in the current study. Yet, the baseline creatinine levels were measured to exclude pre-existing chronic kidney disease in the study animals. Future studies should incorporate these advanced histological techniques to distinguish more precisely between acute and chronic damage. In addition, translational research using larger animal models or clinical trials in human subjects is recommended to validate the renoprotective effects of folinic acid and hydroxychloroquine observed in this experimental setting.

Conclusion

This study demonstrated that folinic acid and hydroxychloroquine, either as monotherapy or in combination, significantly reduced ET-1 and NLRP3 expressions, as well as tubular injury scores, in an AKI rabbit

model. Combination therapy provided the greatest reduction in tubular injury scores, highlighting the complementary effects of these agents in mitigating methanol-induced renal damage. These findings underscore the potential of folinic acid and hydroxychloroquine as therapeutic options for managing methanol-induced AKI and offer new insights into the underlying mechanisms of renal protection.

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Authors Contribution

P and IGRW were involved in concepting and planning the research. P and YK performed the data acquisition/collection. P and NWW calculated the experimental data and performed the analysis. P, YK, and BP drafted the manuscript and designed the figures. KS and IGRW aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest or competing interests related to the content of this manuscript.

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