Veterinary Letters
ISSN 2581-2416
DOI: https://dx.doi.org/10.29244/avl.10.3.85-86
https://journal.ipb.ac.id/index.php/arshivetlett

Renal lipid peroxidation and tubular lesions following ethylene glycol and ammonium chloride exposure in rats

Retno Wilujeng[1], Eva Harlina[2], Rini Madyastuti Purwono[3,*], Dimas Andrianto[4]

[1] Master Program in Animal Biomedical Sciences, Graduate School, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[2] Division of Pathology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[3] Division of Pharmacy, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[4] Division of Bioanalysis, Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia

ABSTRACT: Nephrolithiasis-induced renal injury involves crystal deposition and oxidative stress. This study evaluated renal malondialdehyde (MDA) levels and histopathological alterations in rats induced with ethylene glycol (EG) and ammonium chloride (NH_4Cl). Ten male Sprague–Dawley rats were assigned to a normal control group and an induction group receiving 0.75% EG and 2% NH_4Cl in drinking water ad libitum for 28 days (n = 5 per group). Renal MDA concentrations were quantified via thiobarbituric acid reactive substances (TBARS) assay, and tissue structural changes were evaluated using hematoxylin and eosin staining. The induction group exhibited a statistically significant elevation in renal MDA levels (11.12 ± 4.45 nmol/mg wet tissue) compared to controls (5.93 ± 0.61 nmol/mg wet tissue). Histopathological examination revealed intratubular crystal deposition, hyaline cast formation, and tubular epithelial cell necrosis. Co-administration of EG and NH_4Cl effectively establishes a nephrolithiasis model characterized by heightened lipid peroxidation and severe nephron structural damage.

Keywords:
hyperoxaluria, nephrocalcinosis, malondialdehyde, oxidative injury, renal histopathology

■ INTRODUCTION

Nephrolithiasis involves crystal formation, growth, aggregation, and retention within the kidney. Calcium oxalate (CaOx) stones occur in humans and companion animals, supporting comparative investigation of stone-associated renal injury (Alford *et al.* 2020). Crystal–epithelium interactions can promote reactive oxygen species (ROS) generation and cellular damage (Khan 2013).

Oxidative degradation of membrane lipids produces malondialdehyde (MDA), a commonly measured indicator of lipid peroxidation (Ito *et al.* 2019). Spectrophotometric thiobarbituric acid-reactive substances (TBARS) assays are not chemically specific for MDA (Tsikas 2017), and their results are therefore expressed as MDA equivalents. Combining this biochemical endpoint with histology provides complementary evidence of tissue injury. This study evaluated renal TBARS and tubular lesions following 28 days of ethylene glycol (EG) and ammonium chloride (NH_4Cl) exposure in male Sprague–Dawley rats.

■ MATERIALS AND METHODS

Animals and experimental design. Ten Sprague Dawley rats (250–350 g) were acclimatized for one week at 23 ± 2°C with 12-hour light/dark cycle and provided with food and water. Animals were divided into groups (n = 5): control (KK), water, and induction (KN), 0.75% ethylene glycol (EG) and 2% ammonium chloride (NH_4Cl) in water for 28 days to induce nephrolithiasis (Purwono *et al.* 2019).

Tissue collection and histopathological examination. On day 29, rats euthanized and kidneys harvested. The tissues were fixed in 10% formalin and paraffin-embedded. Blocks were sectioned at 4–5 µm and H&E-stained. Intratubular crystals, hyaline casts, and tubular epithelial necrosis were documented using a Hayear imaging system.

Biochemical analysis of lipid peroxidation. Renal tissue homogenates were prepared in phosphate-buffered saline (pH 7.4). Lipid peroxidation was quantified using the TBARS spectrophotometric assay. Absorbance at 532 nm was recorded, and MDA concentrations were determined from a standard curve as nmol/mg wet tissue.

Statistical analysis. Data are presented as mean ± standard deviation (SD). The Shapiro–Wilk test confirmed the distribution. Control and induction groups were compared using an independent-samples t-test with Welch's correction. Significance at $p < 0.05$.

Received: 09-06-2026 | **Revised:** 11-07-2026 | **Accepted:** 20-07-2026

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Table 1. Renal TBARS expressed as MDA equivalents after induction.

Group	n	MDA Level (nmol/mg)
Normal (KK)	5	5.93 ± 0.61
Induced (KN)	5	11.12 ± 4.45

Note: Mean ± SD; n = rats. Shapiro–Wilk p: KK, 0.40; KN, 0.86. Approximate two-sided Welch p = 0.059.

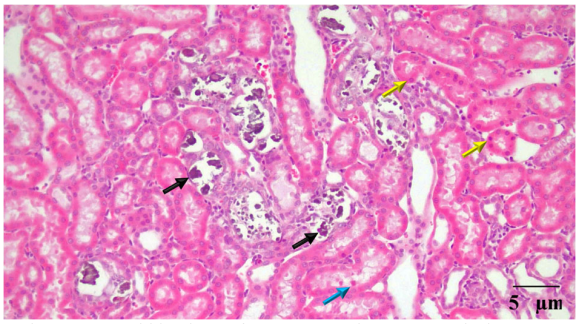


Figure 1. Renal histology after EG–NH_4Cl exposure. Black arrows, intratubular deposits; yellow arrows, tubular epithelial necrosis; blue arrows, hyaline casts. Haematoxylin and eosin.

■ RESULTS AND DISCUSSION

Co-administration of ethylene glycol (EG) and ammonium chloride (NH_4Cl) over 28 days induced marked biochemical alterations in renal tissue. Renal TBARS concentrations in the induction group (KN) reached 11.12 ± 4.45 nmol/mg wet tissue, representing a substantial 1.88-fold increase compared to the uninduced control group (KK: 5.93 ± 0.61 nmol/mg) (Table 1). This pronounced elevation in TBARS levels reflects extensive peroxidative degradation of membrane lipids, establishing a state of heightened renal oxidative stress secondary to chemical induction.

Dual-induction protocol was used. Ethylene glycol is a precursor for metabolic oxalate synthesis (Green *et al.* 2005), whereas ammonium chloride provides acidosis and acidification (Purwono *et al.* 2019). Lowering the urinary pH reduces the solubility of calcium oxalate, thereby accelerating supersaturation within the tubular lumens (Prabhu *et al.* 2016). EG and NH_4Cl are used to model nephrolithiasis-associated tissue injury.

Histopathological analysis confirmed nephrotoxicity, which was consistent with the biochemical findings (Figure 1). Kidneys from animals with induced crystal deposition showed intratubular deposition, hyaline cast formation, and necrosis of tubular epithelial cells. Luminal crystal aggregates cause stress, occlusion, intratubular pressure elevation, ischemic insults, and proteinaceous exudation with compromised nephron clearance.

These lesions delineate crystal-induced toxicity in the kidneys. Crystal adhesion to tubular epithelial cells triggers ROS generation via the calcium-sensing receptor pathway (Li *et al.* 2021). Elevated ROS levels initiate membrane lipid peroxidation, yielding malondialdehyde (MDA), consistent with EG-induced urolithiasis (Zhu *et al.* 2024). Membrane degradation impairs the barrier function and enhances crystal retention (Khan 2013). Crystal deposition triggers NLRP3 inflammasome assembly and interleukin-1β release, driving epithelial necrosis (Mulay *et al.* 2013).

■ CONCLUSION

The co-administration of 0.75% ethylene glycol and 2% ammonium chloride for 28 days established a rat nephrolithiasis model characterized by elevated renal lipid peroxidation and nephron damage. Increased TBARS concentrations with intratubular crystal deposition, hyaline cast formation, and tubular epithelial cell necrosis confirmed that nephrolithiasis-associated tubular injury is driven by lipid peroxidation and cell membrane disruption.

■ AUTHOR INFORMATION

Corresponding Author

*RMP: keyla@apps.ipb.ac.id

Veterinary Teaching Hospital, School of Veterinary Medicine and Biomedical Sciences, IPB University, Jl. Agatis Kampus IPB Dramaga, Bogor, INDONESIA.

■ ETHICS AND DECLARATIONS

Conflict of interest: The authors declare no conflict of interest.
Funding: This report received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.
Data availability: All data are included within the article.

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