Global Health: Journal of Health Sciences, Public Health and Pharmacy

E-ISSN: 3062-8687
P-ISSN: 3063-7465

*Research Article*

# Histomorphometric Analysis of Hyaline Droplet Formation in Renal Tissue of Nephrolithiasis-Induced Rats Treated with Avocado Leaf and Gooseberry Extracts

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**Abstract:** Nephrolithiasis, commonly known as kidney stone disease, can be experimentally induced in animal models using ethylene glycol (EG) in combination with ammonium chloride, which closely mimics calcium oxalate stone formation in humans. This condition is associated with significant renal tissue injury, including glomerular atrophy, tubular necrosis, and hyaline droplet accumulation, ultimately impairing kidney function. The present study aimed to investigate the histopathological changes in the kidneys of EG-induced rats and evaluate the nephroprotective potential of combined avocado (Persea americana) and gooseberry (Physalis angulata) leaf extracts. A total of 30 male Sprague Dawley rats were randomly divided into five groups: a standard control (no induction), a negative control (EG-induced without treatment), a positive control (EG-induced and treated with a commercial nephroprotective agent), and two treatment groups receiving the combined extracts at different doses. Nephrolithiasis induction was performed over 28 days, followed by oral administration of the respective treatments. At the end of the experiment, kidney tissues were collected and processed for histological examination using hematoxylin-eosin staining to assess the extent of tissue damage. The negative control group exhibited the most severe histopathological alterations, with hyaline droplet formation reaching $15.0 \pm 5.25\%$. In contrast, extract-treated groups demonstrated a marked reduction in tissue damage, with the most significant improvement observed in the group receiving 300 mg/kg avocado extract and 100 mg/kg gooseberry extract, which recorded hyaline droplet formation of only $5.27 \pm 2.74\%$, a result comparable to that of the standard control group. These findings suggest that the combination of P. americana and P. angulata leaf extracts confers protective effects against EG-induced renal injury. Therefore, this herbal combination may represent a promising natural nephroprotective agent that warrants further investigation in preclinical and clinical settings.

**Keywords:** Calcium oxalate; Ethylene glycol; Hyalin droplet; Nephrolithiasis; Renal histopathology

**Received: May,17,2025;**
**Revised: May,31,2025;**
**Accepted: June,16,2025;**
**Published: June,30,2025;**
**Curr. Ver.: June,30,2025;**



## 1. Introduction

Nephrolithiasis, commonly referred to as kidney stone disease, is a prevalent urological condition characterized by the formation of crystalline aggregates within the renal system. These deposits, primarily composed of calcium oxalate (CaOx), can obstruct urinary flow, trigger inflammatory responses, and progressively impair renal function if left untreated. Globally, the incidence and recurrence rates of nephrolithiasis have increased, especially in regions with high ambient temperatures, dehydration risks, and limited access to mineral-balanced water [1]. Several risk factors have been implicated, including dietary patterns rich in oxalates and sodium, genetic predisposition, sedentary lifestyle, obesity, and metabolic disturbances such as hyperoxaluria or hypercalciuria [2]; [3].

To better understand the underlying mechanisms of kidney stone formation and explore potential therapeutic strategies, the establishment of reproducible and representative animal models is critical. Among various models, the use of ethylene glycol (EG), often combined with ammonium chloride (AC), is well-documented for inducing calcium oxalate nephrolithiasis in rodents [4]. EG undergoes hepatic metabolism into glycolic and oxalic acids, which then bind to calcium ions, forming insoluble CaOx crystals that deposit within the renal tubules [3]. AC contributes to this process by acidifying the urine, promoting calcium excretion, and enhancing crystal supersaturation [5]. These metabolic and urinary changes accelerate the development of nephrolithiasis, mimicking the human pathological condition with considerable fidelity.

Histopathological evaluations of EG-induced nephropathy consistently reveal hallmark lesions such as glomerular atrophy, tubular epithelial necrosis, and intratubular crystal deposition [6]. Notably, the presence of hyaline droplets—eosinophilic, proteinaceous inclusions within the tubular lumen—has been associated with acute tubular injury, impaired protein reabsorption, and membrane damage [7]. These droplets may also participate in the formation of crystalline-hyaline casts, further aggravating tubular obstruction and inflammation. Despite the growing number of studies targeting nephroprotective therapies, fewer have provided in-depth baseline descriptions of these specific histopathological changes, particularly hyaline droplet formation in untreated models.

Therefore, this study aims to conduct a detailed morphological and quantitative analysis of hyaline droplet formation and associated renal histopathological alterations in rats following ethylene glycol and ammonium chloride administration. The findings may serve as a reference model for future nephrotoxicity research and as a foundational dataset for evaluating plant-based or pharmacological nephroprotective agents.

## 2. Preliminaries or Related Work or Literature Review

Understanding the pathophysiological basis of nephrolithiasis and the accompanying renal histological responses is essential for guiding the development of effective therapeutic strategies. Ethylene glycol (EG) is widely used in nephrotoxicity models due to its ability to induce calcium oxalate (CaOx) crystal formation following metabolic conversion into glycolic and oxalic acids. The accumulation of CaOx crystals within the renal tubules promotes tubular obstruction, inflammation, and oxidative injury, which are histologically characterized by features such as epithelial necrosis, glomerular atrophy, and hyaline droplet formation [8][3]. Although considerable research has focused on pharmacological and nutritional strategies to reduce stone formation, relatively fewer studies have systematically explored the specific histomorphological alterations in untreated models, particularly the role of hyaline droplets as markers of protein leakage and tubular damage [9].

Several natural compounds derived from medicinal plants have been investigated for their potential nephroprotective effects. Natural antioxidants, such as flavonoids, polyphenols, and alkaloids, exert protective effects by reducing oxidative stress, stabilizing cellular membranes, and suppressing inflammatory mediators. *Persea americana* (avocado) leaves contain quercetin and catechins, which have shown strong antioxidant activity in renal injury models [10]. Similarly, *Physalis angulata* (gooseberry) is rich in withanolides and physalins, which have demonstrated anti-inflammatory and immunomodulatory properties relevant in kidney disease [11]. However, studies assessing their combined effects on structural renal repair, especially in terms of hyaline droplet formation and histological improvement, remain limited. This research thus aims to quantitatively assess hyaline droplet reduction and tubular integrity following co-administration of avocado and gooseberry leaf extracts in an EG-induced nephropathy model.

### Pathogenesis of Calcium Oxalate-Induced Renal Injury

Ethylene glycol undergoes hepatic metabolism into glycoaldehyde, glycolic acid, and ultimately oxalic acid. Oxalic acid readily chelates calcium, forming calcium oxalate crystals that precipitate in renal tubules and initiate a cascade of tubular injury, oxidative stress, and inflammatory responses [12];[8]. Ammonium chloride is often co-administered to acidify the urine and enhance oxalate excretion, thereby accelerating crystal supersaturation and deposition [5]. These processes disrupt epithelial integrity and result in the formation of crystalline-hyaline casts, which aggravate tubular obstruction and compromise renal function.

### Nephroprotective Potential of Avocado and Gooseberry Extracts

Herbal nephroprotective approaches are increasingly studied due to concerns about the side effects of synthetic agents. Avocado leaf extract contains antioxidant compounds such as epicatechin and rutin, which have been shown to reduce lipid peroxidation and improve histological architecture in nephrotoxic models [13]. Meanwhile, Physalis angulata extract has demonstrated nephroprotective and anti-inflammatory effects in models of glomerular injury through downregulation of NF-κB and TNF-α expression [14]. Despite promising individual findings, limited studies have evaluated the synergistic or combined impact of these two botanicals on renal histopathology, particularly their role in minimizing hyaline droplet formation, a marker of acute protein leakage and tubular stress. The present study addresses this gap by providing new insight into the histological outcomes of dual extract administration in an experimental nephrotoxic model.

## 3. Proposed Method

This type of research is an experimental study using a complete random design (RAL). This study used 25 male Sprague Dawley rats (250-350g), and acclimatization was carried out for 1 week by feeding and drinking ad libitum. After acclimatization, all treatment groups other than the normal group were given an insider solution of ethylene glycol 0.75% and ammonium chloride 2% ad libitum in drinking water. *Persea americana* Mill. leaves and *Physalis angulata* collected from Biopharmaka Bogor. The plant powders are each macerated with 70% ethanol for 48 hours, and the extracts are concentrated to obtain a semi-solid extract using a rotary evaporator. The temperature of the evaporator is kept below 60 $^{o}$C. The research was conducted in the experimental cage of the Teaching Animal Hospital (RSHP), the Pathology Division, the SKHB IPB University Pharmacy Laboratory, and the Biochemistry Laboratory of the Faculty of Mathematics and Natural Sciences IPB University. It was carried out from April 2024 to December 2024. This research has obtained permission from the ethics commission of the School of Veterinary and Biomedical Medicine IPB University with certificate number 258/KEH/SKE/X/2024.

This study is divided into five groups, namely the control group (KK), the negative group (KN), the positive group (KP) with commercial, the group giving a dose of 300 mg/kg avocado + 100 mg/kg gooseberry (K1), and the group giving a dose of 100 mg/kg avocado + 300 mg/kg gooseberry (K2). The KK group is a control group that is only given equates. The KN, KP, K1, and K2 groups were given an intensive ethylene glycol solution of 0.75% and ammonium chloride of 2% ad libitum in drinking water. Oral administration of the extract once a day lasts for 28 days. After the treatment period, the mice were anesthetized using a ketamine-xylazine combination; then, both kidneys were collected. One kidney was fixed in a formalin solution for histopathological analysis, while the other was stored at −80°C for MDA analysis [15].

### Tools and Materials

The equipment used in this research includes Oregon brand binocular microscopes, a tissue cutting tool (microtome) along with its blades, tissue storage containers (tissue cassettes), and a water bath (floating bath) for the tissue slicing process. Additionally, writing tools such as pencils and label papers, surgical instruments like scissors, tweezers, and probes, as well as other supporting tools like macroscopic knives, glass slides, and cover slips (deck glass), stainless steel containers, measuring cups, timers, and analytical scales were used. The materials used include white rats as test animals, specialized rat feed, media bases made of husk, 0.9% saline solution, and aquadest. For tissue fixation and staining processes, various concentrations of alcohol (70%, 80%, 90%, 96%, and absolute), 10% neutral buffered formalin solution, and histological stains, hematoxylin and eosin, were used.

### Statistical analysis

The acquired data were examined using analysis of variance (ANOVA), and the Duncan test was used to determine whether there were any differences. $P<0.05$ was considered statistically significant.

## 4. Results and Discussion

Hyaline casts are the most common type of casts (cylindrical deposits) formed from Tamm-Horsfall mucoprotein secreted by the tubular epithelial cells of each nephron. Hyaline casts are entirely composed of low molecular weight Tamm–Horsfall protein and have variable morphology, such as parallel sides with rounded ends, cylindrical shapes, a wrinkled appearance, twisting, and being colorless. Low urine flow, concentrated urine, or an acidic urine environment can be contributing factors to the formation of hyaline casts [16]. The results presented in Table 1 indicate significant histopathological changes in the kidneys of rats across treatment groups.

**Table 1. Average percentage of kidney tubules in rats in different treatment groups.**

| Group | Hyaline Droplet of Tubules (%) |
|---|---|
| KK | 6,91 ± 4,27 [a] |
| KN | 15,0 ± 5,25 [b] |
| KP | 6,41 ± 3,63 [a] |
| K1 | 5,27 ± 2,74 [a] |
| K2 | 4,97 ± 2,6 [a] |

Remarks: The data is presented as averages ± standard deviations. The same superscript notation showed that there was no significant difference between groups based on the Duncan post hoc test ($p < 0.05$).

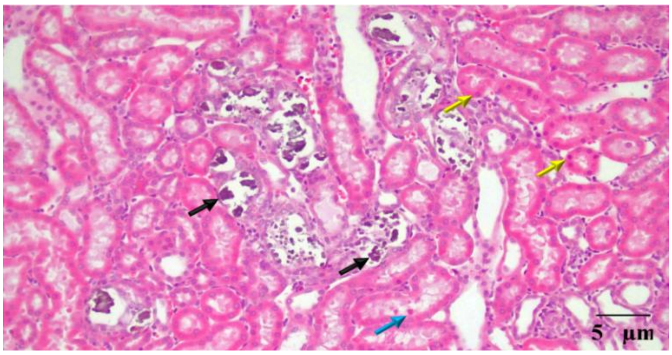


**Figure 1.** Histopathology of the kidneys of rats given ethylene glycol and ammonium chloride for 28 days. Black arrow: calcium deposits in the tubules, yellow arrow: necrotic tubular epithelium, blue arrow: hyaline cast, HE staining, 5 µm.

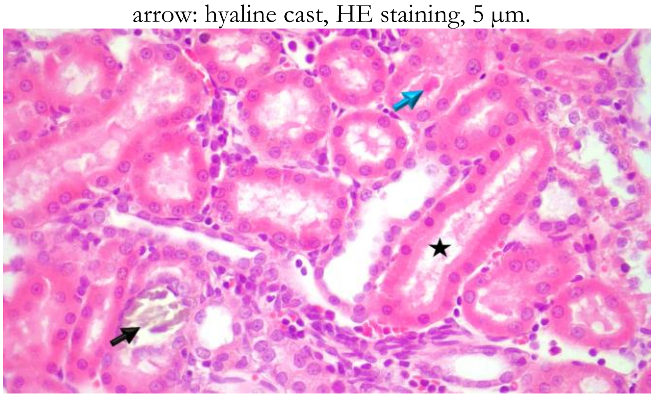

**Figure 2.** Histopathology of the kidney in the negative control group (KN) induced by ethylene glycol. Black arrows: calcium oxalate deposits in the tubules, black stars: necrotic tubular epithelium, blue arrows: hyaline casts. HE staining, magnification 5 µm.

The presence of hyaline droplets or casts in renal tubules is a hallmark of acute tubular injury and was prominently observed in the ethylene glycol-induced nephrolithiasis model, particularly in the negative control (KN) group. As shown in Table 1, the KN group exhibited the highest percentage of hyaline droplets (15.0 ± 5.25%), which was significantly greater than all other groups. This finding aligns with previous studies indicating that ethylene glycol (EG), a nephrotoxic agent, promotes the accumulation of proteinaceous materials within the tubular lumen due to both cellular damage and altered permeability of the glomerular filtration barrier.

The formation of hyaline casts occurs when necrotic debris and leaked plasma proteins coalesce within the tubular lumen, often as a consequence of increased glomerular permeability and tubular epithelial injury [7]. In the context of EG-induced toxicity, this process is exacerbated by the compound's metabolic by-products, namely glycolic acid and oxalic acid, which bind with calcium to form calcium oxalate (CaOx) crystals. These insoluble crystals are deposited within the renal tubules, physically obstructing filtrate flow and inducing localized inflammation, hypoxia, and oxidative stress [17].

The formation of calcium oxalate (CaOx) crystals is central to the nephrotoxic mechanism of ethylene glycol (EG). After ingestion, EG undergoes sequential hepatic metabolism: it is first converted into glycoaldehyde by alcohol dehydrogenase, then into glycolic acid by aldehyde dehydrogenase, and subsequently into glyoxylate by glycolate oxidase. Glyoxylate is finally transformed into oxalic acid by lactate dehydrogenase and transported via the bloodstream to the kidneys [8][3]. In the renal tubules, oxalic acid is filtered through the glomerulus and reacts with calcium to form calcium oxalate, especially under conditions of hyperoxaluria, where oxalate levels are elevated. This leads to supersaturation, initiating the processes of nucleation, crystal growth, and aggregation. The resulting CaOx crystals deposit within the tubular lumen, causing mechanical obstruction, epithelial injury, and inflammatory responses [18]. Moreover, these crystals may interact with leaked proteins and necrotic debris to form crystalline-hyaline casts, which are larger and more damaging. The presence of these crystals is observed in Figure 1, marked by black arrows indicating intratubular deposits consistent with calcium oxalate accumulation.

Ammonium chloride induces mild metabolic acidosis, which enhances calcium excretion in the urine and alters the solubility dynamics of calcium oxalate. Additionally, the shift in urinary pH caused by $NH_4Cl$ plays a role in facilitating the nucleation and growth of calcium oxalate crystals [19]. These crystals are more likely to precipitate and adhere to the tubular epithelium, particularly within the renal cortex, where urine flow is slower and metabolite concentration is higher.

In contrast, the treatment groups, including the commercial nephroprotective agent (KP), K1 (300 mg/kg avocado + 100 mg/kg gooseberry), and K2 (100 mg/kg avocado + 300 mg/kg gooseberry), exhibited markedly lower levels of hyaline droplet formation. The K1 and K2 groups, in particular, showed average hyaline droplet percentages of $5.27 \pm 2.74\%$ and $4.97 \pm 2.6\%$, respectively, which were statistically different from the KN group and not significantly different from the standard control (KK: $6.91 \pm 4.27\%$). This indicates that the combination of extracts is effective in reducing protein accumulation in the tubules, which indicates an improvement in tubular reabsorption function. This protective effect is strongly suspected to originate from the active compounds in avocado (*Persea americana*) and cape gooseberry (*Physalis angulata*), which are known to have antioxidant activity. The active compounds found in avocado leaves include flavonoids, alkaloids, saponins, and tannins, where the flavonoid content acts as an antioxidant [20]. Cape gooseberry contains withanolides, which are known to have strong anti-inflammatory properties and can inhibit the production of pro-inflammatory cytokines such as TNF-α and IL-6 that play a role in the progression of kidney disease [21].

Furthermore, hyaline casts may also play a role in promoting crystal retention. Proteinaceous material within the tubules can act as a scaffold for the nucleation and aggregation of CaOx crystals, enhancing their retention and growth. This interaction between tubular protein accumulation and crystal formation may create a vicious cycle, whereby EG-induced damage facilitates both protein leakage and crystal deposition, further amplifying renal injury [3].

These findings support the hypothesis that the antioxidant and anti-inflammatory properties of bioactive compounds such as flavonoids, withanolides, and polyphenols play a protective role in maintaining tubular epithelial function and preventing protein leakage [20]. Notably, the reduction in hyaline droplet formation may also interrupt the cycle of crystal retention, as proteinaceous material within the tubules can serve as a matrix for calcium oxalate crystal nucleation and aggregation [3]. The significant reduction in hyaline droplet formation among treated groups highlights the potential of these plant-based agents in mitigating EG-induced renal injury, providing not only structural but also functional protection at the tubular level.

## 5. Conclusions

The induction of ethylene glycol and ammonium chloride successfully established a nephrolithiasis model in rats, marked by crystal deposition, tubular necrosis, and significant hyaline droplet formation. The untreated group showed the highest tissue damage, while treatment with commercial agents and combinations of avocado and gooseberry extracts significantly reduced histopathological alterations, particularly hyaline casts. The best outcome was observed in the K1 group (300 mg/kg avocado + 100 mg/kg gooseberry), suggesting its potential nephroprotective effect. These findings highlight the relevance of natural antioxidants in mitigating kidney damage and support further investigation into their therapeutic application.

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