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UROLITHIASIS: EXPERIMENTAL MODELS, ETIOPATHOGENESIS, AND SCREENING APPROACHES FOR DRUG EVALUATION

Dhanenjay Roy[1], Sanuj Muralidharan[1*], Atul Kabra[2]

[1]Department of Dravyaguna Vigyan Shri Dhanwantry Ayurvedic College and Hospital, Chandigarh, India

[2]University Institute of Pharma Sciences, Chandigarh University, Gharuan, Mohali, Punjab, India

ABSTRACT

Urolithiasis, the pathological formation of crystalline deposits within the urinary tract, remains a prevalent global health concern with significant clinical and socio-economic impact. Its multifactorial etiopathogenesis involves complex interactions between genetic predisposition, metabolic abnormalities, dietary influences, and environmental factors, leading to various stone types such as calcium oxalate, calcium phosphate, uric acid, and cystine. This review consolidates current understanding of epidemiology, causative mechanisms, and experimental screening strategies for antiurolithiatic agents, with particular focus on in vivo animal models and in vitro methodologies. Widely employed experimental models include ethylene glycol, sodium oxalate, glyoxylate, hydroxy-L-proline, diet-induced protocols, zinc-disc implantation, and infection-mediated struvite formation, each reproducing specific aspects of stone pathogenesis. We detail the mechanisms underlying these models, standard induction protocols, and their respective strengths and limitations in reflecting human disease. Emerging approaches such as microfluidic organ-on-chip platforms, integration of genomics and metabolomics ("omics"), and advanced imaging technologies offer enhanced physiological relevance and real-time monitoring capabilities. However, translational challenges persist, notably the variability between animal and human urinary biochemistry, ethical considerations, and lack of standardized evaluation criteria. Addressing these gaps will require harmonized methodologies and more representative models integrating host–microbiome interactions and personalized metabolic profiles. By synthesizing conventional and innovative experimental strategies, this review aims to serve as a practical reference for researchers and clinicians, facilitating informed model selection and robust experimental design in the ongoing quest to identify and develop effective antiurolithiatic therapies.

Keywords: Urolithiasis; Calcium oxalate; Ethylene glycol; Glyoxylate; Hydroxy-L-proline; Animal models; Screening assays; Struvite; Cystine; In-vitro models.

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INTRODUCTION

Urolithiasis, or kidney stone disease, remains a pervasive and recurrent urological disorder with significant global health, social, and economic implications. It affects approximately 10–12% of the population, with a lifetime risk estimated to range between 15% and 25%, depending on geography, lifestyle, and socioeconomic status. The rising trend in incidence is attributed to changes in dietary habits, sedentary lifestyle, and climate factors that contribute to dehydration and altered urinary composition. Clinically, urolithiasis presents with severe symptoms such as acute renal colic, hematuria, infection, and obstructive uropathy, and it is associated with recurrent episodes that increase the risk of chronic kidney disease. These recurring and often debilitating episodes significantly diminish quality of life and impose a heavy burden on healthcare systems worldwide [1].

The pathogenesis of stone formation is multifactorial and complex, involving physicochemical, metabolic, genetic, and environmental components. The formation of kidney stones begins with supersaturation of urine by poorly soluble salts like calcium oxalate, calcium phosphate, uric acid, and cystine. This is followed by nucleation, crystal growth, aggregation, and finally retention within the renal tubules or urinary tract. Contributing factors include dietary excesses of oxalate, calcium, sodium, and animal proteins; metabolic abnormalities such as hyperoxaluria, hypercalciuria, or hyperuricosuria; genetic susceptibility; urinary tract infection; and inadequate water intake. Moreover, oxidative stress, inflammation, and alterations in urinary inhibitors such as citrate and magnesium further exacerbate crystallization and stone formation [1], [2]. Given this intricate pathophysiology, preclinical models have become indispensable tools for understanding disease mechanisms and for drug discovery in antiurolithiatic research. Both in vivo and in vitro models provide controlled environments to study biochemical and histopathological changes during lithogenesis. Commonly used animal models, including those induced by ethylene glycol, glyoxylate, sodium oxalate, or hydroxy-L-proline, effectively mimic features of human stone formation. These models help evaluate the therapeutic potential, mechanism of action, and safety profile of candidate compounds before clinical trials. In vitro crystallization assays and cell-based models complement these studies by enabling detailed screening of anti-crystallization and crystal aggregation inhibitors [3], [4]. Collectively, the integration of preclinical models into urolithiasis research remains vital for identifying novel therapeutic strategies and translating laboratory findings into clinically meaningful outcomes.

Epidemiology and Risk Factors

Epidemiological patterns of urolithiasis reveal distinct geographic, gender, and temporal variations, reflecting the interplay between environmental, dietary, and genetic influences. The

prevalence of kidney stone disease is notably higher in regions characterized by elevated ambient temperatures, often referred to as global "stone belts," including parts of South Asia, the Middle East, and the southeastern United States. In such areas, chronic dehydration due to excessive heat contributes to urine concentration and supersaturation, predisposing individuals to crystal formation. Dietary factors further modulate risk, with excessive intake of sodium, oxalate-rich foods, and animal proteins promoting hypercalciuria, hyperoxaluria, and reduced urinary citrate—key determinants of lithogenesis. Obesity and metabolic syndrome have emerged as important contemporary risk factors, linked to insulin resistance and altered urinary pH, which favor uric acid and calcium oxalate stone formation [5], [6].

Gender differences also exist, with men traditionally exhibiting a higher incidence, although recent trends indicate an increasing prevalence in women, possibly due to dietary and lifestyle shifts. Family history remains a strong nonmodifiable determinant, underscoring genetic predisposition. Furthermore, rare monogenic disorders such as primary hyperoxaluria, cystinuria, and Dent's disease contribute to early-onset and recurrent stone formation [7]. Understanding these epidemiological nuances is critical for developing tailored prevention strategies and region-specific public health interventions.

Etiology and Pathophysiology

The etiology and pathophysiology of urolithiasis involve a complex cascade of physicochemical and biological events within the urinary tract. Stone formation begins when the urine becomes supersaturated with lithogenic ions such as calcium, oxalate, phosphate, and uric acid, creating an environment conducive to crystallization. Supersaturation facilitates nucleation—the initial assembly of ions into microscopic crystals—followed by their growth and aggregation into larger structures. Retention of these crystals within the tubules or renal papillae is essential for stone progression, distinguishing transient crystalluria from clinically relevant calculi [8], [9].

Randall's plaques, composed of subepithelial deposits of calcium phosphate, often serve as nidus sites for crystalline adhesion and subsequent overgrowth by calcium oxalate [10]. Various macromolecular inhibitors, including citrate, nephrocalcin, and Tamm–Horsfall protein, normally act to prevent crystallization by chelating ions or modulating crystal surface properties; a reduction or dysfunction in these inhibitors increases lithogenic potential [11]. Tubular epithelial injury and inflammation, frequently driven by oxidative stress, further promote crystal binding and retention by exposing adhesive cellular surfaces. The significance of these processes varies with stone composition and the individual's metabolic milieu-for example, hyperoxaluric states favor calcium oxalate stones, whereas persistently acidic urine promotes uric acid crystallization. Understanding these mechanisms informs targeted preventive and therapeutic strategies.

Experimental Models of Urolithiasis

This section provides an integrated description of the principal experimental induction models, mechanistic basis, typical protocols and their advantages and limitations.

Sodium oxalate induced urolithiasis

Sodium oxalate-induced urolithiasis occurs when there is an excess of oxalate in the bodyleading to the formation of urinary stones. Sodium oxalate is a salt form of oxalate, and its ingestion can contribute to the development of kidney stones. The mechanism of sodium oxalate- induced urolithiasis involves several steps those includes, after ingestion, sodium oxalate is absorbed in the gastrointestinal tract and enters the bloodstream [12]. Secondly, the kidneys filter the blood, removing waste products and excess substances, including oxalate. Oxalate is excreted in the urine by the kidneys. Under normal circumstances, oxalate is present in urine at low concentrations and is typically soluble. In cases where there is an excess intake of oxalate, such as through the ingestion of sodium oxalate or oxalate-rich foods, the concentration of oxalate in the urine can become elevated. Oxalate has a high affinity for calcium ions. When the concentration of oxalate exceeds its solubility limit in urine, it can bind to calcium ions, forming calcium oxalate crystals [13], [14].

Glycolic acid induced urolithiasis

Glycolic acid-induced urolithiasis refers to the formation of urinary stones (uroliths) as a result of exposure to glycolic acid. This condition is associated with certain metabolic disorders, such as primary hyperoxaluria type 1 (PH1), where there is an overproduction of oxalate, leading to its accumulation in the body and subsequent formation of oxalate-based kidney stones [15]. The mechanism by which glycolic acid contributes to urolithiasis involves its metabolism in the liver. Glycolic acid is metabolized to glyoxylate by the enzyme glycolate oxidase. In individuals with PH1 or other disorders of glyoxylate metabolism, there is a deficiency in the enzyme alanine-glyoxylate aminotransferase (AGT), which normally converts glyoxylate into glycine. As a result, glyoxylate accumulates in the body. Glyoxylate has a high affinity for calcium ions, forming calcium oxalate crystals. These crystals can then aggregate and precipitate in the urinary tract, leading to the formation of kidney stones. The formation of these stones can result in symptoms such as severe flank pain, hematuria (blood in the urine), and urinary tract infections [16], [17].

Ethylene glycol induced urolithiasis

Ethylene glycol-induced urolithiasis, also known as ethylene glycol poisoning, is a serious medical condition that occurs when ethylene glycol, a common component of antifreeze, is ingested. Ethylene glycol itself is not directly responsible for urolithiasis, but rather its metabolites are implicated in the formation of urinary stones. The mechanism of ethylene glycol-induced urolithiasis involves the metabolism of ethylene glycol by the liver. Ethylene glycol is metabolized

sequentially by alcohol dehydrogenase and aldehyde dehydrogenase enzymes, leading to the formation of glycolic acid, glyoxylic acid, and oxalic acid. It is the oxalic acid that plays a central role in the formation of urinary stones. Oxalic acid has a high affinity for calcium ions, forming calcium oxalate crystals. These crystals can then aggregate and precipitate in the urinary tract, leading to the formation of kidney stones. The process is similar to the mechanism seen in PH1, where oxalate accumulation leads to the formation of calcium oxalate stones [18]. In addition to urolithiasis, ethylene glycol poisoning can lead to severe systemic toxicity, including metabolic acidosis, renal failure, and central nervous system depression. Prompt medical treatment is essential in cases of ethylene glycol ingestion to prevent these complications and to manage the formation of urinary stones. Treatment typically involves administering antidotes such as fomepizole or ethanol to inhibit the metabolism of ethylene glycol, along with supportive measures to manage acid-base disturbances and renal dysfunction [19], [20].

Hydroxy-L-proline (HLP) and urolithiasis

Hydroxy-L-proline (HLP) is a derivative of the amino acid proline and is a breakdown product of collagen, a protein found in connective tissues. Normally excreted in urine, HLP can play a role in the formation of kidney stones under certain conditions. Chemically, it is (S)-2-amino-4-hydroxy-5-pyrrolidinecarboxylic acid, with the molecular formula $C_5H_9NO_3$, and features a hydroxyl group attached to its pyrrolidine ring. In the urine, HLP can bind to calcium ions, forming soluble complexes. When the levels of HLP and calcium become too high, these complexes may precipitate as crystals. Over time, these crystals can aggregate, forming larger structures that contribute to urinary stones, a condition known as urolithiasis. Factors such as urinary pH, hydration, diet, and underlying health conditions can influence this process. Individuals with high collagen intake or increased collagen turnover are particularly at risk for HLP-related kidney stone formation [21], [22].

Calcium oxalate induced urolithiasis

Calcium oxalate-induced urolithiasis (kidney stones) is the most common type of kidney stone. The mechanism of action involves a series of biochemical and physiological processes that lead to the formation and growth of calcium oxalate crystals in the urinary tract. High concentrations of calcium and oxalate, when the levels of calcium and oxalate in the urine are elevated, the urine becomes supersaturated with these ions. Supersaturation is a critical factor for crystal formation. This occurs when calcium and oxalate ions spontaneously combine to form small, initial crystals without any pre-existing surface. Once nucleated, calcium oxalate crystals grow by attracting more calcium and oxalate ions from the supersaturated urine. Aggregation is facilitated by Tamm-Horsfall protein (THP), also known as uromodulin [23]. This protein is produced by the cells of the thick ascending

limb of the loop of Henle in the kidneys and other macromolecules that act as binding agents. Crystals can adhere to the epithelial cells lining the renal tubules. Factors like renal tubular damage or inflammation can enhance crystal adherence. Crystals can deposit in the renal papillae, leading to the formation of Randall's plaques. These plaques can act as a nidus for further crystal aggregation and stone growth [24], [25].

Ethylene glycol and ammonium chloride induced urolithiasis in rats

The oxidative damage brought on by the high concentration of oxalate produced by 0.75% ethylene glycol must be the primary source of the lithogenic effect. Ethylene glycol is degraded in the liver by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which produces oxalate. Oxalate is not metabolized in animals and is eliminated through the kidneys. At high concentrations, it creates calcium oxalate crystals, which cause nephrotoxicity and oxidative stress [26]. Recent research has highlighted the critical role of oxidative stress in ethylene glycol-induced nephrotoxicity and kidney stone development [27]. A study found that administering *Allium cepa* extract dramatically restored kidney function in rats exposed to ethylene glycol. The treated group had fewer stones, less apoptosis, and lower levels of osteopontin and autophagy-related genes [28]. These studies also investigated several therapies targeted at reducing oxidative damage and avoiding stone formation. So, even though the ethylene glycol rat model's suitability as a generic model to investigate renal stone development may be questioned, it must be thought to be a useful model for assessing the formation of renal papillary stones, at least for those whose origin is connected to oxidative cell damage. Ethylene glycol and ammonium chloride are administered to rats in their drinking water. Ethylene glycol serves as a substrate for calcium oxalate stone formation; the purpose of the 2% ammonium chloride (w/v) was to encourage the kidneys' CaOx deposition and hyperoxaluria. Over time, the rats develop kidney stones due to the accumulation of calcium oxalate crystals in the renal tubules and pelvis. These stones can cause renal damage, urinary obstruction, and inflammation, mimicking certain aspects of kidney stone disease. Assess the severity of urolithiasis by measuring parameters such as stone size, urinary pH, urinary oxalate levels, renal function markers, histological changes in the kidneys, and the incidence of renal calculi. This model has been used to investigate the pathophysiology of kidney stones, evaluate the efficacy of potential therapeutic agents, and explore mechanisms of stone formation and dissolution [29].

Cystine model (cystinuria)

Cystine animal models are widely used to study the formation, prevention, and treatment of cystine kidney stones, which mimic human cystinuria—a genetic disorder that causes recurrent stones due to defective cystine transport in the renal tubules. Common models include mice, rats, dogs, and pigs. In mice, the Slc3a1 and Slc7a9 genes encode subunits of the renal cystine transporter, and

mutations in these genes lead to defective cystine reabsorption and elevated urinary cystine levels. Although only a few knockout mice develop cystine stones, factors like acidified water, high-cystine diets, or dehydration can increase stone formation. Rat models often rely on chemically induced hypercystinuria using cystine analogs or dietary modifications. Dogs are considered highly relevant due to similarities in renal physiology and urine composition with humans, and certain breeds genetically predisposed to cystine stones are valuable for long-term studies of metabolism, recurrence, and therapy.

Different models for renal stone formation have specific advantages and limitations. The ethylene glycol model is well-established for calcium oxalate stones and induces kidney stones rapidly, but its acute toxicity may cause significant renal damage and does not fully replicate chronic stone formation. Combining ethylene glycol with ammonium chloride simulates mixed stones and infection-related urolithiasis but requires precise dosing. Sodium oxalate models focus on oxalate crystals with mild toxicity, while calculi-producing diets mimic dietary causes over long periods but have variable stone composition. Glyoxylate-induced models rapidly produce calcium oxalate crystals but are acute and may not represent chronic formation [30]. Zinc disc-induced urolithiasis is non-toxic and useful for studying crystal growth and testing anti-lithic agents but is artificial and less applicable to chronic or infection-related stones [31]. Each model offers unique insights, helping researchers explore mechanisms and evaluate potential therapies [32].

Struvite model

Struvite stones (magnesium ammonium phosphate stones) are commonly associated with urinary tract infections caused by urease- producing bacteria, such as *Proteus mirabilis*. Animal models are used in research to study the formation, prevention, and treatment of these stones. Rodent models like rats and mice are used. In these models, there are three different methods are employed. Firstly, bacterial inoculation: urease-producing bacteria (*Proteus mirabilis*) are introduced into the urinary tract to induce struvite stone formation [33]. Secondly, through dietary manipulation, high magnesium, ammonium, or phosphate diets may contribute to stone formation. Thirdly, *via* chemical induction *i.e.* injection of ammonium chloride or other stone- promoting substances into the bladder. Other models such as rabbit model, pig model, canine model are existed. Among these models, rabbit model found to be best suited because of their larger bladder size and urinary physiology, which allows for better imaging, surgical procedures, and stone retrieval compared to rodents. Methods of inducing struvite stones in rabbits include bacterial inoculation in which, a urinary catheter is inserted into the rabbit's bladder. A controlled dose of *P. mirabilis* is introduced into the bladder, leading to urine alkalinisation and struvite stone formation [34]. Regular urine pH testing, imaging (X-ray/ultrasound), and urinalysis are carried out during these periods. Struvite

stones form within 1–3 weeks, depending on infection severity X-ray, ultrasound, and CT scans to track stone development. The pH levels, presence of crystals, bacterial cultures. Kidney function and electrolyte balance *etc*. are to be monitored. Bladder tissue for the presence or absence of inflammation/infection is examined.

Glyoxylate induced acute lithiasis

Inducing acute lithiasis in rats using glyoxylate involves administering glyoxylate, a precursor of oxalate, which leads to the formation of calcium oxalate stones in the urinary tract. Acute glyoxylate intoxication-induced glyoxylic lithiasis markedly increased the deposition of the elements that form stones calcium, oxalate, and phosphorus in the kidney problem. Urinary oxalate levels were found to have increased fourfold. The effective oxalate forms are glycollate and glyoxylate [35]. Glyoxylate is the only substance that has been shown to be a direct precursor to oxalate in humans. To cause oxalate stones within 24 hours, the animals were administered 100 mg/kg of sodium glyoxylate intraperitoneally (i.p.) for one day. The dose was given in two separate amounts in the morning and evening. Use imaging techniques such as ultrasound or X- ray to detect the presence of kidney stones in the rats, if applicable. Sacrifice the rats at the end of the experiment or at predetermined time points to collect tissue samples for histological analysis. Examine the kidneys and urinary tract for the presence of stones, tissue damage, or other pathological changes. Analyse the collected data to assess the severity of acute lithiasis, the efficacy of the glyoxylate induction protocol, and the effects of any experimental interventions or treatments.

Zinc disc induced urolithiasis in rats

Urolithiasis in rats using zinc disks involves implanting zinc disks into the bladder or urinary tract, leading to the formation of bladder stones. In the zinc disc implantation model, rats' bladders are surgically filled with zinc disks that are known to weigh and measure [31]. Sodium pentobarbitone (40 mg/kg, i.p.) was used to sedate the rats. The urinary bladder was exposed through a suprabic incision. A little incision was made on the bladder's upper surface. After that, the pee was aseptically aspirated into a sterile container for bacteriological analysis and measurement of pH (using BDH paper with a restricted range). The rats were given a week to heal after having previously weighted, sterilized zinc disks placed into their bladders. The incision was sealed with a single suture using absorbable 4–0 chronic catgut (Ethicon). Urine albumin and creatinine levels significantly increased in the zinc disc-implanted condition.Males saw a larger increase in urinary stones and smooth muscle hypertrophy following 4 and 8 weeks of surgery, respectively, due to the implantation of zinc foreign bodies into the bladder.

Table 1: screening models for urolithiasis

SL no	Screening Model	Description	Mechanism / Induction Method	Advantages	Limitations
1	Sodium Oxalate-Induced Model	Induction by sodium oxalate ingestion leading to calcium oxalate stone formation	Excess oxalate absorbed, forms calcium oxalate crystals in urine	Mimics oxalate stone formation with mild toxicity	Acute model, limited chronic relevance
2	Glycolic Acid-Induced Model	Induction by glycolic acid metabolism disruption, linked to primary hyperoxaluria	Glycolic acid converted to glyoxylate, accumulates causing CaOx crystals	Models genetic/metabolic cause of stones	Acute, specific to metabolic disorder
3	Ethylene Glycol-Induced Model	Induction by ethylene glycol metabolism to oxalate, causing kidney stones	Metabolized to oxalic acid forming calcium oxalate crystals	Well-established, rapid stone formation	Acute toxicity, may cause renal damage
4	Hydroxy-L-Proline Model	Induction using hydroxy-L-proline, a collagen breakdown product influencing Ca crystal formation	Forms complexes with calcium promoting crystal growth	Models inflammation and collagen breakdown effects	Influenced by diet and metabolism
5	Calcium Oxalate-Induced Model	Elevated calcium and oxalate leading to	Spontaneous nucleation,	Most common stone type,	Complex multifactori

		supersaturation and crystal aggregation	crystal growth & aggregation	physiologic al relevance	al factors involved
6	Ethylene Glycol + Ammonium Chloride Model	Combined ethylene glycol and ammonium chloride administration causing mixed stone formation	Oxalate generation from EG, ammonium chloride promotes deposition	Simulates mixed and infection-related stones	Requires dosing precision, acute model
7	Cystine Model (Cystinuria)	Genetic models mimicking human cystinuria causing cystine stones	Mutations affecting cystine reabsorption in kidneys	Genetic relevance, multiple species models available	Limited stone formation in some knockouts
8	Struvite Model	Infection-induced magnesium ammonium phosphate stone formation	Urease producing bacteria (Proteus mirabilis) inoculation	Models infection-related stones, uses multiple animal species	Complex, infection handling required
9	Glyoxylate-Induced Acute Lithiasis	Acute induction of calcium oxalate stones by glyoxylate administration	Glyoxylate precursor leads to CaOx crystal deposition	Rapid stone formation within 24 hours	Acute, less chronic relevance
10	Zinc Disc-Induced Model	Surgical implantation of zinc discs in rat	Foreign body induces crystal	Non-toxic, useful for testing	Artificial, less chronic,

		bladder inducing bladder stones	nucleation and stone growth	crystal growth	focused on bladder stones
11	In Vitro Crystallization Assays	Artificial urine systems to study nucleation, growth, aggregation, dissolution	Controlled chemical environment mimics urine	High throughput, mechanistic study	Lack physiological complexity
12	Cell-Based Assays	Renal epithelial cell models for crystal adherence, oxidative stress, cytotoxicity assessment	Uses renal cell lines (HK-2, MDCK)	Cellular level physiological relevance	In vitro limitations
13	Organ-on-Chip/Microfluidic Systems	Advanced models mimicking dynamic kidney tubular environment and shear stress	Microfluidic flow systems	Better physiological relevance and real-time monitoring	Complex, emerging technology

In-vitro and Ex-vivo Screening Tools

In vitro and ex vivo screening tools play a critical role in complementing animal models for urolithiasis research by offering higher throughput and detailed mechanistic insights. Classical in vitro crystallization assays simulate artificial urine to monitor key stages of stone formation, such as nucleation, crystal growth, aggregation, and dissolution [36]. These assays facilitate rapid screening of antiurolithiatic agents by assessing their ability to inhibit or break down calcium oxalate crystals. Cell-based assays employing renal epithelial cell lines such as HK-2 and MDCK allow evaluation of crystal adherence to renal cells, oxidative stress induction, and cytotoxic effects, providing physiological relevance at the cellular level. Emerging technologies like organ-on-chip and microfluidic flow systems mimic the dynamic tubular architecture and physiological shear

stresses of the kidney, enhancing assay relevance by better replicating the complex microenvironment of the nephron. Together, these tools advance the understanding of urolithiasis mechanisms and accelerate the identification of promising therapeutic candidates.

CONCLUSION

The review consolidates the complex multifactorial etiopathogenesis of urolithiasis involving genetic, metabolic, dietary, and environmental factors leading to different stone types. Despite progress, translational challenges persist due to differences between animal models and human urinary biochemistry, ethical issues, and lack of standardized evaluation criteria. Emerging technologies such as organ-on-chip platforms, integration of multi-omics, and advanced imaging promise better physiological relevance and real-time monitoring. However, there is a need for harmonized methodologies and more representative preclinical models that consider host-microbiome interactions and personalized metabolic profiles. Overall, the review aims to guide researchers and clinicians in selecting appropriate experimental models and designing robust studies to develop effective antiurolithiatic therapies.

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***Corresponding Author:** Sanuj Muralidharan, Department of Dravyaguna Vigyan Shri Dhanwantry Ayurvedic College and Hospital, Chandigarh, India