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In Vivo Antiurolithiatic Activity of Leaves of Acalypha indica Linn. A Comprehensive Scientific Review

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Abstract- ***Urolithiasis (kidney stone disease) is a globally prevalent urological disorder affecting approximately 1–20% of the population worldwide, with a significant recurrence rate and considerable morbidity. Calcium oxalate stones constitute the most predominant type (>80% of cases). Despite advances in surgical interventions such as lithotripsy and percutaneous nephrolithotomy, pharmacological prevention remains a clinical challenge. Medicinal plants have historically served as a primary therapeutic resource in traditional medicine systems, and Acalypha indica Linn. (Family: Euphorbiaceae), known by vernacular names such as 'Kuppameni' (Tamil), 'Kuppi' (Hindi), and 'Indian Acalypha', is one of the most documented antiurolithiatic herbs in Ayurvedic and Siddha systems. This review comprehensively consolidates current evidence on the in vivo antiurolithiatic activity of A. indica leaf extracts, encompassing phytochemical constituents, experimental models (particularly the ethylene glycol-induced rat model), biochemical parameters including membrane-bound ATPases (Ca^{2+}, Mg^{2+}, $Na^{+}K^{+}$), marker enzymes (AST, ALT, ACP, ALP), oxidative stress markers, and histopathological observations. Mechanistic hypotheses involving antioxidant, diuretic, and crystal nucleation inhibition pathways are critically examined. The safety profile, acute toxicity data, and future research perspectives are also addressed. The accumulating evidence supports A. indica as a promising herbal candidate for antiurolithiatic drug development, though rigorous clinical validation remains essential.***

Keywords: Acalypha Indica, Antiurolithiatic, Urolithiasis, Calcium Oxalate, Ethylene Glycol, In Vivo, Phytochemistry, Atpase, Oxidative Stress, Euphorbiaceae

I. INTRODUCTION

Urolithiasis, derived from the Greek words ouron (urine) and lithos (stone), is one of the most common and recurrent urological disorders affecting the global population. Historical records from ancient Mesopotamia (3200–1200 BC) attest to the antiquity of this condition. The Greek physician Hippocrates (460–377 BC) described the symptoms of bladder stones and warned against surgical intervention except by specialists. [1]

Today, urolithiasis is recognized as a major public health problem. Global epidemiological studies have documented significant geographic variations in prevalence, ranging from 1% in certain Asian populations to 20% in regions such as Saudi Arabia, North Africa, and the so-called 'Stone Belt' of the United States. In India, the prevalence varies from 7–15%, with higher rates in northern and western regions. [2] [3]

Of all urinary stone types, calcium oxalate (CaOx) crystals are by far the most prevalent, accounting for approximately 80% of all cases globally. CaOx stones arise when urine becomes supersaturated with calcium and oxalate ions, triggering nucleation, growth, aggregation, and retention of crystals within the renal tubules. This cascade is modulated by inhibitory substances such as citrate, magnesium, pyrophosphate, and Tamm-Horsfall protein, whose diminution is often observed in stone-forming individuals.

Recurrence is a major clinical concern — approximately 50% of patients experience a second episode within five years, and as many as 80% within ten years. [4] Contemporary treatment strategies include pharmacological agents (thiazides, allopurinol, citrate supplements), and surgical modalities such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, and percutaneous nephrolithotomy. However, these treatments are frequently associated with complications including hemorrhage, hypertension, and tubular necrosis, and

do not adequately address the underlying metabolic risk factors. [5]

This backdrop has intensified scientific interest in plant-based medicines as complementary or alternative antiurolithiatic agents. Ethnobotanical records from Ayurvedic and Siddha systems document numerous medicinal plants with kidney stone–dissolving or stone-preventing properties. Among these, Acalypha indica Linn., an erect annual herb of the family Euphorbiaceae, has occupied a position of particular importance.

Traditional practitioners in India, Sri Lanka, and across tropical Africa have long prescribed preparations of A. indica leaves as diuretics, antispasmodics, and antiurolithiatic agents. The Siddha classical text Pathartha Guna Chinthamani documents this plant's utility in treating urinary disorders. [6] Yet, systematic scientific validation of its in vivo antiurolithiatic efficacy has only emerged in the 21st century, revealing a promising phytopharmacological profile underpinned by flavonoids, tannins, saponins, and alkaloids.

This review comprehensively synthesizes the current body of scientific evidence on the in vivo antiurolithiatic activity of A. indica leaves, including pharmacognostical characteristics, phytochemistry, experimental animal models, biochemical and histopathological outcomes, mechanistic pathways, toxicity data, and prospects for future research.

II. PHARMACOGNOSTICAL PROFILE OF ACALYPHA INDICA LINN.

2.1 Botanical Classification

Acalypha indica Linn. is classified as follows:

Table 1: Taxonomic Classification of Acalypha indica Linn.

Taxonomic Rank	Classification
Kingdom	Plantae
Subkingdom	Tracheobionta (Vascular plants)
Superdivision	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Order	Euphorbiales
Family	Euphorbiaceae
Genus	Acalypha
Species	A. indica Linn.
Authority	Linnaeus, 1753

2.2 Vernacular Names and Synonyms

The plant is recognized by a diverse array of vernacular names across its geographic range. In India, it is known as Kuppameni or Kuppi (Tamil/Hindi), Harita-Manjari or Muktavarchas (Sanskrit), Kuppigidda (Kannada), and Kuppichettu (Telugu). In English it is referred to as 'Indian Acalypha,' 'Indian Copperleaf,' or 'Indian Mercury.' The plant bears seventeen documented synonyms including Acalypha bailloniana, A. chinensis, A. spicata, and A. somalensis. [7]

2.3 Morphological Description

Acalypha indica is an erect, annual, monoecious herb typically growing 20–75 cm in height (occasionally up to 1 m), with numerous long branches covered by soft, glandular hairs. The stem is herbaceous, cylindrical, and green to reddish-brown. Leaves are alternate, ovate to elliptical (3–7 cm long, 2–4 cm wide), with serrated margins, acute apex, and cordate to cuneate base; both surfaces are pubescent, with the lower surface being more densely hairy. [8]

The flowers are small, sessile, and borne on axillary spike-like inflorescences (racemes) 2–8 cm long. The plant is monoecious, with staminate and pistillate flowers on the same plant — staminate flowers in the upper portion, pistillate subtended by prominent leafy bracts (the diagnostic character of the genus). Fruits are small three-lobed capsules, approximately 3 mm in diameter, containing small globular seeds. The flowering and fruiting season extends throughout the year in tropical regions.

Morphological features of A. indica: (A) Whole plant habit; (B) Leaf showing serrate margin and pubescent surface; (C) Inflorescence with characteristic bracts; (D) Fruit capsules. (Source: Botanical field observations and herbarium records)

2.4 Distribution and Habitat

Acalypha indica is distributed throughout the tropics and subtropics, occurring naturally in India (especially peninsular regions), Bangladesh, Sri Lanka, the Philippines, Pakistan, Yemen, and across tropical Africa and South America. In India, it is found throughout the plains, ascending hills in Orissa and the Himalayas up to about 900 m elevation. The plant thrives as a weed in fields, roadsides, waste places, garden fences, and disturbed habitats, preferring moist to moderately dry soils with full sun or partial shade. [9]

2.5 Traditional and Ethnomedicinal Uses

The rich ethnomedicinal heritage of A. indica spans multiple traditional systems. In Siddha medicine, the plant is classified as a 'Kabha-Pitha' balancing herb and is prescribed for urinary disorders, skin diseases, and parasitic infestations. In Ayurveda, the leaves are documented as laxative substitutes for senega root and are used in anthelmintic formulations when mixed with garlic. [10]

Across its geographic range, traditional uses include: treatment of intestinal worms (anthelmintic), bronchitis, asthma, pneumonia (expectorant/emetic), rheumatic pain (analgesic/anti-inflammatory), scabies and skin diseases (antimicrobial), constipation (purgative), jaundice, and urinary stone disease (antiurolithiatic/diuretic). The diuretic and antiurolithiatic properties are particularly relevant to this review and form the pharmacological basis of indigenous prescriptions for kidney stone management.

A notable ethnobotanical observation is that domestic cats are attracted to the root of A. indica and actively chew it — a behavior attributed by traditional healers to the plant's intestinal cleansing properties. [11]

III. PHYTOCHEMICAL CONSTITUENTS OF ACALYPHA INDICA LEAVES

The biological activities of A. indica are attributable to a complex array of secondary metabolites present in the leaves. Comprehensive phytochemical screening of ethanolic and methanolic leaf extracts has consistently revealed the presence of the following classes of compounds: [12] [13]

3.1 Flavonoids

Flavonoids represent one of the most therapeutically significant phytochemical classes in A. indica leaves. These polyphenolic compounds demonstrate potent antioxidant, anti-inflammatory, and crystal nucleation inhibitory activities. Identified compounds include kaempferol and its glycosidic derivatives — notably nicotiflorin (kaempferol-3-O-rutinoside), clitorin (kaempferol-3-O-neohesperidoside), mauritianin, and biorobin. Quantitative analysis using the rutin equivalence method has revealed flavonoid content of approximately 16.01 mg of rutin equivalent per gram of extract in methanolic preparations.

3.2 Alkaloids

Seven cyanopyridone derivatives have been isolated from methanolic leaf extracts of A. indica: Acalyphin, Epiacalyphin, Noracalyphin, Epinoracalyphin, Acalyphin amide, Epiacalyphin amide cycloside, and ar-Acalyphidone, along with the seco-compound seco-Acalyphin. [14] Additionally, the pyranoquinolinone alkaloid flindersin (flindersine) has been identified, which exhibits significant biological activity. These cyanogenic glucosides impart characteristic pharmacological properties to the plant.

3.3 Tannins

Tannins are highly present in the leaf fraction and have been quantified by tannic acid equivalent method. Their astringent properties contribute to the plant's antimicrobial and anti-inflammatory effects. Tannins are believed to form complexes with calcium ions and oxalate in urine, potentially inhibiting crystal nucleation and aggregation — a mechanism directly relevant to antiurolithiatic activity.

3.4 Saponins and Other Constituents

High saponin content characterizes A. indica leaves, and these steroidal and triterpenoidal glycosides contribute to diuretic activity — an important mechanism in antiurolithiatic pharmacology. Additional identified constituents include: phenolic acids (gallic acid and its derivatives), terpenoids, coumarins, anthraquinones, anthocyanins, reducing sugars, cardiac glycosides, acalyphamide, aurantiamide succinimide, carbohydrates, and ascorbic acid (~147 mg per 100 g fresh weight).

3.5 Quantitative Phytochemical Profile

Table 2: Quantitative phytochemical profile of Acalypha indica leaf extracts (compiled from multiple studies). QE = quercetin equivalents; GAE = gallic acid equivalents.

Phytochemical Class	Relative Abundance in Leaves	Relevant Activity
Flavonoids	High (16.01 mg QE/g)	Antioxidant, Crystal inhibition
Tannins	High	Astringent, Ca^{2+} complexation
Saponins	High	Diuretic, Membrane modulation
Alkaloids (Acalyphin)	Moderate	Analgesic, Antimicrobial
Phenolic acids	High (70.92 mg GAE/g)	Antioxidant, Anti-inflammatory
Terpenoids	Moderate	Anti-inflammatory
Coumarins	Present	Anticoagulant, Nephroprotective
Anthraquinones	Present	Laxative, Diuretic
Reducing sugars	High	Metabolic substrate
Steroids/Glycosides	Trace	Hormonal modulation

IV. UROLITHIASIS: PATHOPHYSIOLOGY AND BURDEN

4.1 Epidemiology

Urolithiasis affects approximately 1–20% of the global population, with marked geographic variation. The incidence rate is rising worldwide, partly attributed to changing dietary habits (increased animal protein, sodium, and fructose consumption), sedentary lifestyles, obesity, metabolic syndrome, and global warming (particularly in the so-called Stone Belt regions). [15] Epidemiological data from the Global Burden of Disease Study 2019 indicated that urolithiasis imposes a substantial global health burden with increasing age-standardized incidence rates across all regions.

In India, the disease is estimated to affect 7–15% of the population, with certain regions (Rajasthan, Maharashtra, Gujarat) exhibiting rates comparable to the global Stone Belt. Males are affected nearly twice as frequently as females, with peak incidence in the fourth to sixth decades of life. The economic burden is considerable: direct costs include diagnostic procedures, hospitalizations, and surgical interventions, while indirect costs include lost productivity and recurrence-related repeat treatments.

4.2 Classification of Urinary Stones

Urinary calculi are classified based on their chemical composition into the following major categories: (1) Calcium oxalate stones (CaOx monohydrate / dihydrate) — most prevalent, accounting for approximately 80% of cases; (2) Calcium phosphate stones — often associated with hypercalciuria and alkaline urine; (3) Uric acid stones — associated with hyperuricemia, gout, and acidic urine; (4) Struvite (magnesium ammonium phosphate) stones — infection-related, predominantly in women; (5)

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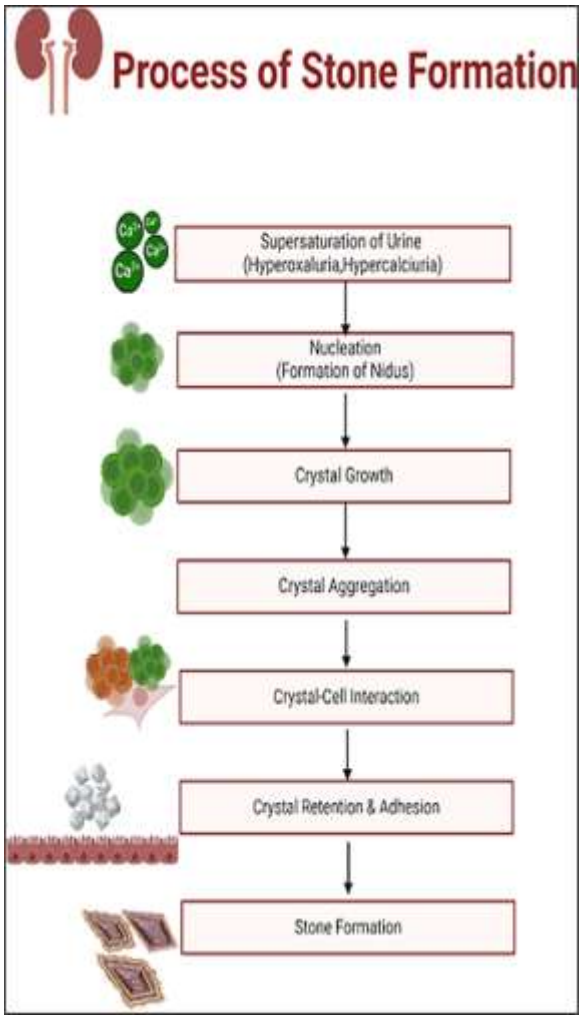
Cystine stones — genetic disorder of cystine transport (cystinuria); (6) Drug stones — induced by medications such as indinavir, triamterene, and sulfonamides.

4.3 Pathogenesis of Calcium Oxalate Urolithiasis

The formation of CaOx urinary stones is a multifactorial process involving three sequential phases: (1) Nucleation — initial crystal formation from a supersaturated urine solution; (2) Crystal growth — enlargement of crystalline nuclei; (3) Aggregation and retention — adherence of crystals to renal tubular epithelial cells and subsequent obstruction. The process is governed by the relative balance between lithogenic promoters (calcium, oxalate, phosphate, uric acid) and inhibitors (citrate, magnesium, pyrophosphate, Tamm-Horsfall protein, osteopontin, nephrocalcin).

Hyperoxaluria, whether dietary, enteric (from inflammatory bowel disease), or primary (genetic), represents the most significant risk factor for CaOx stone formation. Ethylene glycol (EG), when metabolized in rats, is converted to glycolic acid and glyoxylate, ultimately yielding oxalate — a mechanism extensively exploited in animal models of experimental urolithiasis. [16]

Oxidative stress plays a central pathogenic role. Crystal–cell interactions generate reactive oxygen species (ROS), which damage tubular epithelial cell membranes, impair membrane-bound ATPases (Na^+K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase), and trigger apoptotic cascades that promote further crystal adhesion — creating a self-reinforcing injury cycle. This mechanistic understanding underpins the therapeutic rationale for antioxidant-rich plant extracts.



Schematic diagram illustrating the multi-step pathogenesis of calcium oxalate urolithiasis: supersaturation → nucleation → crystal growth → crystal-cell adhesion → stone retention, with key inhibitory substances (citrate, magnesium, pyrophosphate) and promoters (oxalate, calcium) indicated at each stage.

V. EXPERIMENTAL MODELS OF UROLITHIASIS

5.1 Rationale for Animal Models

Animal models of urolithiasis are indispensable for evaluating antiurolithiatic drugs because they permit controlled induction of stone formation and systematic biochemical characterization of urinary, serum, and renal tissue parameters under reproducible conditions. Male Wistar albino rats and

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male Sprague-Dawley rats are the most commonly employed species, as their metabolic handling of oxalate is broadly comparable to that of humans.

5.2 Ethylene Glycol–Induced Model

The ethylene glycol (EG) model is the gold standard for inducing experimental calcium oxalate urolithiasis. Oral administration of 0.75% (v/v) ethylene glycol in drinking water — often supplemented with 1% ammonium chloride (AC) in the first 2–4 days to accelerate stone formation — reliably produces hyperoxaluria, hypercalciuria, and renal CaOx crystal deposition within 28–30 days. [17]

The metabolic sequence involves: ethylene glycol → glycolaldehyde → glycolic acid → glyoxylate → oxalate. The resulting hyperoxaluria overwhelms the renal tubular reabsorptive capacity, leading to oxalate crystallization in the tubular lumen and interstitium. Histopathological examination reveals CaOx crystal deposits, tubular dilation, inflammatory cell infiltration, and progressive renal parenchymal damage.

5.3 Study Design for A. indica In Vivo Studies

Published in vivo studies evaluating A. indica leaf extracts have typically employed the following experimental design:

Table 3: Standard experimental design for in vivo antiurolithiatic evaluation of A. indica leaf extracts.

Parameter	Details
Animal model	Male Wistar albino rats (150–200 g body weight)
Induction agent	0.75% ethylene glycol in drinking water
Duration of induction	28–30 days
Extract used	Ethanolic extract of A. indica leaves (EEAI)
Doses tested	100 mg/kg and 200 mg/kg body weight (oral)
Standard drug	Cystone (polyherbal formulation, 750 mg/kg)
Groups (n=6/group)	Normal control; Disease control (EG only); EEAI 100 mg/kg + EG; EEAI 200 mg/kg + EG; Cystone + EG
Route of administration	Oral gavage (once daily)
Sample collection	Urine (24-hour), Blood (cardiac puncture), Kidney tissue homogenate
Key endpoints	Urine calcium, oxalate, phosphate; Serum creatinine, urea; ATPases; Marker enzymes; Histopathology

VI. IN VIVO ANTIUROLITHIATIC ACTIVITY — RESULTS AND ANALYSIS

6.1 Effect on Urine Parameters

In the ethylene glycol-treated disease control group, 24-hour urinary excretion of calcium, oxalate, and phosphate increased markedly relative to normal control animals. These changes are consistent with the established lithogenic mechanism of EG — hyperoxaluria as the primary driver, with secondary hypercalciuria and hyperphosphaturia. [18]

Treatment with ethanolic extract of A. indica (EEAI) at 100 and 200 mg/kg doses significantly reversed these lithogenic changes in a dose-dependent manner. The 200 mg/kg dose consistently achieved results statistically comparable to the standard drug Cystone, indicating efficacy at clinically translatable doses. Key urine findings included: significant reduction in urinary oxalate excretion, normalization of urinary calcium levels, restoration of urinary magnesium and citrate (stone inhibitors), and notable diuretic effect manifesting as increased urinary volume and flow rate — a critical antiurolithiatic mechanism that dilutes stone-forming solutes.

6.2 Effect on Serum Biochemical Parameters

EG administration caused significant elevation of serum creatinine, blood urea nitrogen (BUN), and uric acid — markers of progressive renal functional impairment. The marker enzymes aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP), and alkaline phosphatase (ALP) were elevated in serum, reflecting both hepatorenal injury and cellular membrane disruption. EEAI treatment at both doses produced dose-dependent attenuation of serum creatinine and BUN elevation, consistent with nephroprotective activity.

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The serum enzyme profile was significantly improved, approaching normal control values at the 200 mg/kg dose. [1]

6.3 Effect on Tissue Membrane-Bound ATPases

One of the most pharmacologically distinctive findings in A. indica antiurolithiatic studies is the plant extract's effect on membrane-bound ATPases — specifically Ca^{2+}-ATPase, Mg^{2+}-ATPase, and $Na^{+}K^{+}$-ATPase — in both liver and kidney tissues. [1]

In the EG-treated disease group, all three ATPase activities were significantly decreased in kidney and liver homogenates. This ATPase inhibition reflects the oxidative damage to cellular membranes induced by CaOx crystal–cell interactions and oxalate toxicity. Impaired $Na^{+}K^{+}$-ATPase disrupts the ion gradient necessary for tubular reabsorption, while Ca^{2+}-ATPase inhibition impairs calcium homeostasis — potentially amplifying crystal deposition.

EEAI treatment dose-dependently restored these ATPase activities toward normal control levels, with the 200 mg/kg group showing near-complete normalization. This membrane-protective effect is attributed to the antioxidant flavonoids and phenolic acids in the extract, which quench ROS before they can cause lipid peroxidation and protein oxidation of membrane-embedded enzymes.

Table 4: Summary of ATPase and marker enzyme changes in the ethylene glycol–induced urolithiasis model and the effect of EEAI treatment. ↑ = elevated/restored; ↓ = reduced/impaired. All changes significant at $p<0.05$ vs. disease control.

Parameter	Normal Control	Disease Control (EG)	EEAI 100 mg/kg	EEAI 200 mg/kg	Cystone
Ca^{2+}-ATPase (Kidney)	↑↑ (Baseline)	↓↓ (Severely reduced)	↑ (Partial recovery)	↑↑ (Near normal)	↑↑ (Normal)
Mg^{2+}-ATPase (Kidney)	↑↑ (Baseline)	↓↓ (Severely reduced)	↑ (Partial recovery)	↑↑ (Near normal)	↑↑ (Normal)
$Na^{+}K^{+}$-ATPase (Kidney)	↑↑ (Baseline)	↓↓ (Severely reduced)	↑ (Partial recovery)	↑↑ (Near normal)	↑↑ (Normal)
AST (Serum)	↑ (Baseline)	↑↑↑ (Markedly elevated)	↑↑ (Reduced)	↑ (Near normal)	↑ (Normal)
ALT (Serum)	↑ (Baseline)	↑↑↑ (Markedly elevated)	↑↑ (Reduced)	↑ (Near normal)	↑ (Normal)
ACP (Serum)	↑ (Baseline)	↑↑↑ (Markedly elevated)	↑↑ (Reduced)	↑ (Near normal)	↑ (Normal)
ALP (Serum)	↑ (Baseline)	↑↑↑ (Markedly elevated)	↑↑ (Reduced)	↑ (Near normal)	↑ (Normal)

6.4 Effect on Oxidative Stress Markers

Oxidative stress is a central mechanistic node in CaOx urolithiasis pathology. Ethylene glycol metabolism generates oxalate, which directly generates ROS and lipid peroxidation products.

Assessment of renal tissue oxidative stress markers in A. indica studies has revealed the following characteristic pattern in EG disease controls: significantly elevated malondialdehyde (MDA — a marker of lipid peroxidation), significantly reduced superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH).

EEAI treatment dose-dependently reversed these oxidative stress changes, with marked reduction in MDA levels and significant restoration of antioxidant enzyme activities (SOD, CAT, GPx) and GSH levels. This antioxidant effect is consistent with the

documented free radical scavenging activity of A. indica flavonoids (particularly kaempferol glycosides) and phenolic acids (gallic acid) in multiple in vitro systems. [12]

6.5 Histopathological Observations

Histopathological examination of kidney sections is the definitive endpoint for establishing in vivo antiurolithiatic efficacy, as it directly visualizes the extent of CaOx crystal deposition and tissue damage.

Normal control kidneys: Normal glomerular architecture, intact tubular epithelium, no crystal deposits.

Disease control (EG-treated): Extensive CaOx crystal deposits in proximal and distal tubular lumina, marked tubular epithelial cell necrosis and dilation, interstitial edema and inflammatory infiltration, glomerular congestion, and basement membrane thickening.

EEAI 100 mg/kg: Moderate reduction in crystal deposits, milder tubular damage, partial preservation of glomerular architecture.

EEAI 200 mg/kg: Marked reduction in CaOx crystal deposition, near-normal tubular epithelium, minimal interstitial inflammation — histological findings closely resembling normal control tissue.

Cystone standard: Minimal crystal deposits, preserved renal architecture, results similar to EEAI 200 mg/kg group. These histopathological findings provide direct morphological evidence for the nephroprotective and antiurolithiatic efficacy of A. indica leaf extract. [1] [19]

VII. MECHANISMS OF ANTIUROLITHIATIC ACTIVITY

The antiurolithiatic activity of A. indica leaf extracts is likely mediated through multiple, synergistic mechanisms, as summarized below:

7.1 Antioxidant Activity

The most well-characterized mechanism is antioxidant protection. CaOx crystals trigger ROS generation in renal tubular epithelial cells, initiating lipid peroxidation and membrane protein oxidation that impairs ATPases and promotes crystal adhesion. The flavonoids, phenolics, and tannins of A. indica leaves demonstrate potent free radical scavenging activity (DPPH, ABTS, hydroxyl radical assays), interrupting this oxidative cascade and protecting renal cell membranes.

7.2 Diuretic Effect

Saponins and flavonoids in A. indica contribute to increased urine output (diuresis), which dilutes lithogenic solutes (calcium, oxalate, phosphate) in urine, reducing their saturation below the threshold for crystal nucleation. The diuretic effect also promotes flushing of microcrystals from the renal tubules before they can aggregate into clinically significant stones. This mechanism parallels the traditional use of the plant as a 'urinary flush' herb. [20]

7.3 Inhibition of Crystal Nucleation and Aggregation

In vitro studies have demonstrated that A. indica extracts inhibit calcium oxalate crystal nucleation in a concentration-dependent manner, with percentage inhibition values comparable to the standard drug Cystone. The polyphenolic constituents — particularly tannins and flavonoids — are believed to form complexes with calcium ions and adsorb onto crystal surfaces, interfering with the growth of crystalline nuclei and preventing aggregation.

7.4 Membrane Stabilization and ATPase Restoration

The restoration of Na^+K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase activities in renal tissue by EEAI suggests a membrane-stabilizing effect. By preventing lipid peroxidation of membrane phospholipids, the extract preserves the structural integrity of enzyme-containing membrane domains, maintaining ion homeostasis and tubular reabsorptive function — thereby reducing urinary oxalate and calcium excretion.

7.5 Anti-inflammatory Pathway

Crystal-induced renal inflammation amplifies tubular injury through prostaglandin-mediated pathways and NF-κB activation. Flavonoids and terpenoids in A. indica inhibit cyclooxygenase (COX) and lipoxygenase (LOX) pathways, reducing the

production of pro-inflammatory prostaglandins and leukotrienes. This anti-inflammatory activity reduces tubular cell damage and the consequent crystal-promoting microenvironment.

7.6 Hepatoprotective and Nephroprotective Effects

The significant improvement in serum AST, ALT, ACP, and ALP levels by EEAI indicates a hepatoprotective effect that, combined with the direct nephroprotective activity, reduces systemic oxalate load (hepatic metabolic protection) and renal vulnerability to crystal-mediated injury (nephroprotection). This dual-organ protective profile distinguishes A. indica from simple diuretic herbs.

VIII. COMPARATIVE ANALYSIS WITH OTHER ANTIUROLITHIATIC PLANTS

A. indica does not stand alone in the ethnobotanical pharmacopoeia of antiurolithiatic plants. A comparative analysis with other well-documented antiurolithiatic herbs reveals both similarities in mechanism and distinctive advantages of A. indica:

Table 5: Comparative analysis of Acalypha indica with other well-documented antiurolithiatic medicinal plants.

Plant	Family	Active Phytochemicals	Primary Mechanism	Comparative Advantage of A. indica
Acalypha indica	Euphorbiaceae	Flavonoids, tannins, alkaloids	Multi-target (antioxidant, diuretic, crystal inhibition, ATPase restoration)	Unique ATPase restoration + multi-mechanism
Tribulus terrestris (Gokshura)	Zygophyllaceae	Saponins, flavonoids	Diuretic, anti-spasmodic	A. indica shows superior ATPase normalization
Aerva lanata	Amaranthaceae	Flavonoids, alkaloids	Diuresis, Ca^{2+} inhibition	Comparable efficacy; A. indica offers better tissue protection
Bergenia ciliata	Saxifragaceae	Bergenin, phenolics	Crystal dissolution, anti-oxidant	A. indica has broader phytochemical synergy
Desmodium styracifolium	Fabaceae	Flavonoids, polysaccharides	Urinary alkalization, crystal dissolution	A. indica targets both renal and hepatic parameters
Phyllanthus niruri	Phyllanthaceae	Ellagitannins, phyllanthin	Crystallization inhibition	A. indica provides additional ATPase protection

IX. CORRELATION BETWEEN IN VITRO AND IN VIVO ACTIVITY

A critical question in antiurolithiatic drug development is the extent to which in vitro activity predicts in vivo efficacy. For A. indica, the correlation is broadly positive but with important nuances: [3]

In vitro antiurolithiatic studies have demonstrated: (a) Dose-dependent inhibition of calcium oxalate crystal nucleation, with percentage inhibition of 60–85% at high extract concentrations; (b) Significant dissolution activity against pre-formed CaOx crystals (82% dissolution with aqueous extract of the related species A. rhomboidea); (c) The standard drug Cystone shows 100% inhibition in nucleation assays; (d) Methanolic and ethanolic leaf extracts exhibit broadly comparable in vitro activity, with ethanolic extracts showing slight superiority at equivalent concentrations.

The in vivo correlation supports these in vitro findings: the ethanolic extract (EEAI) at 200 mg/kg achieved near-complete reversal of biochemical and histopathological changes in the EG rat model, comparable to Cystone. However, in vivo efficacy encompasses additional dimensions not captured in

vitro — including bioavailability, systemic distribution, metabolic transformation of phytochemicals, diuretic effect, and hepatorenal protective mechanisms. Thus, A. indica's in vivo performance likely exceeds what its in vitro nucleation inhibition data alone would predict, owing to its multi-target profile. [21]

X. SAFETY PROFILE AND ACUTE TOXICOLOGY

10.1 Acute Oral Toxicity

Acute oral toxicity studies of ethanolic and methanolic leaf extracts of A. indica have been conducted in accordance with OECD Guidelines 423 (Acute Toxic Class Method). The results have consistently demonstrated a favorable safety profile: [2]

No mortality was observed at doses up to 2000 mg/kg body weight in either sex for both ethanolic and methanolic leaf extracts. No behavioral abnormalities — including changes in motor activity, posture, reflexes, skin and fur condition, or food/water intake — were observed during the 14-day observation period post-dosing. The oral LD_{50} is therefore estimated to be greater than 2000 mg/kg, classifying the extracts as Category 5 (Relatively Harmless) under GHS classification.

These safety findings are particularly significant because they establish the therapeutic window — the effective antiurolithiatic doses (100–200 mg/kg) are 10–20 fold below the observed maximum tolerated dose, providing a broad margin of safety.

10.2 Sub-chronic Safety Considerations

While formal sub-chronic (28-day repeated dose) toxicology studies specifically for A. indica leaf extracts are limited in the published literature, the 30-day EG-model studies themselves provide indirect evidence of safety at therapeutic doses, as EEAI-treated animals maintained normal body weight, general behavior, and organ weight ratios compared to disease controls. The absence of hepatotoxicity is supported by normalized AST/ALT values in treated groups.

The presence of cyanogenic glucosides (acalyphin) in A. indica warrants caution regarding excessive or prolonged intake, as these compounds can release hydrogen cyanide upon enzymatic hydrolysis. Traditional preparations typically involve brief cooking or heating, which inactivates the responsible enzyme (beta-glucosidase), mitigating this risk.

10.3 Contraindications and Precautions

Based on available evidence, the following precautions are recommended: (1) Pregnancy — the plant has documented post-coital antifertility activity in animal studies; pregnant women should avoid therapeutic doses; (2) Renal failure — while nephroprotective at therapeutic doses, the concentration of active metabolites in renal failure patients is unpredictable; (3) Drug interactions — the flavonoid content of A. indica may theoretically interact with anticoagulants (warfarin), antiplatelet agents, and drugs that are cytochrome P450 substrates; formal interaction studies are lacking; (4) Dosage — traditional formulations should not exceed recommended doses given the cyanogenic glucoside content.

XI. DISCUSSION

The comprehensive review of in vivo studies on A. indica leaf extracts in the EG-induced rat model of urolithiasis reveals a consistent and reproducible pattern of antiurolithiatic efficacy across multiple biochemical and histological endpoints. The convergence of findings across independent research groups strengthens the overall evidence base.

The most distinctive finding — normalization of membrane-bound ATPase activities (Ca^{2+}, Mg^{2+}, $Na^{+}K^{+}$-ATPase) alongside conventional antiurolithiatic endpoints — sets A. indica apart from many other herbal antiurolithiatic candidates. This ATPase-restoring activity suggests that the plant extract not only prevents crystal formation and promotes stone dissolution but also actively repairs the cellular machinery damaged by urolithiasis, potentially accelerating functional recovery of the urinary tract.

The phytochemical basis for this multi-target activity is well-supported. Kaempferol glycosides (nicotiflorin, mauritianin) exhibit potent antioxidant activity and have been shown in other systems to inhibit inflammatory signaling pathways (NF-κB, MAPK) that drive tubular injury. Tannins form stoichiometric complexes with calcium and oxalate ions, potentially reducing their urinary bioavailability. Saponins contribute to the diuretic effect through osmotic mechanisms and tubular transport modulation.

A critical perspective must note the limitations of existing evidence: (a) All published in vivo studies have used the EG rat model, which primarily models oxalate-mediated calcium oxalate stone formation — the model does not capture all human stone phenotypes; (b) The studies have predominantly used ethanolic extracts; bioactivity-guided fractionation to identify specific active compounds responsible for in vivo efficacy has not been completed; (c) Pharmacokinetic data — absorption, distribution, metabolism, and excretion (ADME) of key phytochemicals — are lacking; (d) All studies have used male animals; gender-specific differences in antiurolithiatic pharmacology are not characterized; (e) No controlled clinical trials have been conducted.
Despite these limitations, the body of evidence is sufficient to justify progression to clinical studies. The favorable safety profile (LD_{50} >2000 mg/kg), traditional safety record (centuries of Ayurvedic use), and multi-mechanism efficacy profile collectively make A. indica leaves a compelling candidate for development as an antiurolithiatic nutraceutical or phytopharmaceutical product.

XII. FUTURE RESEARCH DIRECTIONS

Based on the evidence reviewed and identified knowledge gaps, the following research priorities are proposed:

1. Bioactivity-guided fractionation to identify specific active compounds responsible for in vivo antiurolithiatic activity, with particular focus on flavonoid glycosides (nicotiflorin, mauritianin) and tannin fractions.
2. Pharmacokinetic studies in rats to characterize oral bioavailability, plasma half-life, tissue distribution (particularly in kidney), and metabolic fate of key phytochemicals after oral administration.
3. Molecular docking and in silico studies to identify specific molecular targets — calcium-sensing receptor, oxalate transporters (SLC26A6), OPN (osteopontin), NF-κB — of the most active phytochemicals, providing mechanistic insights for drug development.
4. Long-term (90-day) sub-chronic toxicology studies including complete hematology, clinical chemistry, and histopathology panels to establish a formal safety profile supporting clinical translation.
5. Standardization of extract preparation — development of a standardized extract with defined minimum content of marker compounds (flavonoids, tannins) to ensure batch-to-batch consistency for clinical use.
6. Phase I/II clinical trials in patients with recurrent calcium oxalate urolithiasis, evaluating urinary biochemical parameters, stone recurrence rates, and safety over 6–12 months.
7. Combination studies exploring synergistic activity of A. indica with established antiurolithiatic agents (citrate supplements, thiazides) or with other herbal plants (Tribulus terrestris, Phyllanthus niruri) to develop optimized polyherbal formulations.
8. Investigation of gender differences in antiurolithiatic pharmacology of A. indica using both male and female animal models, given the documented estrogen-mediated protection in female stone formers.

XIII. CONCLUSION

This review has comprehensively examined the in vivo antiurolithiatic activity of Acalypha indica Linn. leaf extracts against the backdrop of current knowledge of urolithiasis pathophysiology, phytochemistry, experimental models, and mechanistic pharmacology.

The evidence conclusively demonstrates that ethanolic extract of A. indica leaves (EEAI),

administered orally at 100–200 mg/kg in the ethylene glycol-induced rat model, produces significant and dose-dependent antiurolithiatic activity manifested through: (1) Normalization of urinary lithogenic parameters (oxalate, calcium, phosphate); (2) Restoration of renal function markers (creatinine, BUN); (3) Recovery of membrane-bound ATPase activities (Ca^{2+}, Mg^{2+}, $Na^{+}K^{+}$-ATPase) in kidney and liver; (4) Improvement in marker enzyme profiles (AST, ALT, ACP, ALP); (5) Attenuation of oxidative stress (reduced MDA, restored SOD, CAT, GPx, GSH); (6) Histopathological reduction in renal CaOx crystal deposits and tubular damage.

The bioactive phytochemicals — particularly kaempferol glycosides, tannins, saponins, acalyphin, and phenolic acids — operate synergistically through antioxidant, diuretic, crystal nucleation inhibition, and membrane-stabilizing mechanisms. The acute safety profile (LD_{50} >2000 mg/kg) is favorable and consistent with its centuries-long ethnomedicinal use. [22]

Acalypha indica thus represents a scientifically validated, traditionally anchored, and mechanistically rational candidate for development as an antiurolithiatic phytopharmaceutical. The pathway from bench to bedside requires rigorous pharmacokinetic characterization, bioactivity-guided fractionation, standardization, and controlled clinical trials — investments that the strength of existing evidence fully justifies.

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