AMERICAN JOURNAL OF MEDICAL SCIENCE AND CHEMICAL RESEARCH

JOURNALOMS EST 2019

ISSN 2689-3495 (E) / 2689-3509 (P)

Volume:05; Issue:07 (2023)

Available online at: www.journaloms.com

EVALUATION OF THE ANTI-UROLITHIATIC POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *CLITORIA TERNATEA* LINN.

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]

[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

*Corresponding Author, **Email ID**: raginibundela34@gmail.com

Received date: 30-04-2023	Accepted Date: 28-05-2023	Publication date: 08-07-2023

ABSTRACT

The present study investigates the phytochemical composition and anti-lithiatic potential of the hydroalcoholic extract of *Clitoria ternatea* Linn. The whole plant was subjected to sequential extraction using petroleum ether, chloroform, benzene, and a hydroalcoholic solvent via Soxhlet apparatus and hot continuous percolation methods. Qualitative phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, phytosterols, fixed oils, fats, and saponins, while carbohydrates, proteins, gums, mucilage, starch, and tannins were absent. Acute toxicity studies in Wistar albino rats demonstrated the extract's safety at doses up to 2 g/kg, with no observed mortality or toxic effects over 14 days. The anti-lithiatic efficacy was evaluated using an ethylene glycol-induced urolithiasis model in rats. Treatment with hydroalcoholic extracts (200 and 400 mg/kg) significantly ameliorated ethylene glycol-induced alterations in urinary biochemical parameters, including reductions in urinary calcium, oxalate, protein, and uric acid levels, along with restoration of magnesium and phosphate levels. Histopathological examination confirmed a reduction in renal crystal deposition. These findings suggest that the hydroalcoholic extract of *Clitoria ternatea* possesses significant prophylactic and curative effects against urolithiasis, likely attributable to its diverse phytoconstituents. The extract shows potential as a natural therapeutic agent for the prevention and treatment of kidney stones.

KEYWORDS: *Clitoria Ternatea , Anti-Lithiatic Potential , Phytochemical Composition , Acute Toxicity*

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]

[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

1. Introduction

Kidney stones, medically termed urolithiasis or nephrolithiasis, represent a common and significant health problem characterized by the formation of solid concretions or crystals within the kidneys. These stones develop when certain substances in the urine, such as calcium, oxalate, uric acid, cystine, or phosphate, become excessively concentrated, leading to supersaturation and crystallization. The presence of these stones can cause severe pain, urinary obstruction, and sometimes infection or kidney damage if left untreated **(Mwambete et al., 2009).**

The process of stone formation is multifactorial, involving a complex interplay between genetic predisposition, metabolic abnormalities, dietary habits, environmental factors, and certain medical conditions. For instance, individuals with high urinary calcium (hypercalciuria), excessive oxalate levels (hyperoxaluria), or acidic urine are at increased risk of developing stones. Dietary influences such as high protein intake or low fluid consumption also play a crucial role in stone pathogenesis. Additionally, infections with certain bacteria can promote the formation of specific types of stones like struvite stones **(Dawson et al., 2010).**

Kidney stones vary in their chemical composition, with calcium-containing stones being the most common, followed by struvite, uric acid, and cystine stones. Each type has distinct causes and clinical implications, making accurate diagnosis important for effective management and prevention of recurrence. The prevalence of kidney stones has been increasing globally, partly due to changes in diet, lifestyle, and rising rates of obesity and metabolic syndrome **(Dawson et al., 2010).**

Clinically, kidney stones often present with sudden, severe, colicky flank pain radiating to the groin, accompanied by nausea and vomiting. Smaller stones may pass spontaneously, but larger stones can obstruct urinary flow, requiring medical intervention. Understanding the underlying factors and mechanisms that lead to stone formation is vital in developing strategies to prevent new stones and manage existing ones **(Ryall et al., 1981).**

This comprehensive overview aims to explore the key factors inducing kidney stone formation, describe the various types of stones, outline the anatomy and physiology relevant to stone pathogenesis, and discuss the clinical and epidemiological aspects of this common urological disorder.

2. MATERIALS AND METHODS

2.1 Extraction of *Clitoria ternatea Linn.*

The whole plant of *Clitoria ternatea* (L.) was shade-dried, pulverized, and sieved (mesh no. 60) to obtain a coarse powder. The powdered material underwent sequential extraction using petroleum ether (40–60°C) in a Soxhlet apparatus for 72 hours, followed by chloroform and benzene extraction for 40 hours each. Finally, it was extracted with a hydroalcoholic solvent (30:70) at 70–80°C for 40 hours. The solvent was removed under vacuum to yield a greenish-brown dry extract (9.8% w/w). The hydroalcoholic extract was then subjected to qualitative phytochemical analysis and pharmacological screening **(Vidya & Varalakshmi et al., 2000).**

2.2 Preparation of Extracts of *Clitoria ternatea* by Hot Continuous Percolation Method

Approximately 200 g of dried *Clitoria ternatea* powder was packed in Whatman filter paper (grade no. 1) and placed in a thimble within a Soxhlet apparatus. Sequential extraction was carried out using solvents of increasing polarity—petroleum ether (60–80 °C), chloroform, and hydroalcohol—each under its respective controlled temperature. In this hot continuous percolation method, solvent vapors condensed and

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]
[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

continuously percolated through the plant material, allowing for efficient extraction of active constituents. The extraction process was continued until all soluble compounds were exhausted. The resulting extracts were concentrated under reduced pressure using a rotary evaporator to obtain semi-solid residues, which were stored in a desiccator. This procedure was repeated as necessary to achieve the desired yield of extract (Jeong et al., 2005).

2.3 Qualitative Phytochemical Analysis of *Clitoria ternatea* Hydroalcoholic Extracts

The hydroalcoholic extracts of *Clitoria ternatea* were subjected to standard qualitative tests to detect the presence of various phytochemical constituents **(Halabe et al., 2003).** The results of the analysis are summarized below:

Absent Constituents:

- **Carbohydrates:** All standard tests (Molisch's, Fehling's, Benedict's, Barfoed's, and iodine test) were negative, indicating absence.
- **Gums and Mucilage:** Negative results in alcohol precipitation and Molisch's test.
- **Proteins and Amino Acids:** Tests including Ninhydrin, Biuret, Millon's, and Xanthoprotein were negative.
- **Tannins:** Tests using ferric chloride, lead acetate, and gelatin showed no presence.
- **Starch:** No bluish-black coloration observed with iodine.

Present Constituents:

- **Fixed Oils and Fats:** Positive results in spot test and saponification test.
- **Alkaloids:** Positive reactions with Mayer's, Dragendorff's, Wagner's, and Hager's reagents confirmed presence.
- **Glycosides:** Legal's, Baljet's, Borntrager's, and Keller-Killani tests all indicated the presence of glycosides.
- **Phytosterols:** Libermann-Burchard's and Salkowski's tests gave positive results.
- **Flavonoids:** Confirmed through ferric chloride, Shinoda's, flavones reactions, and alkali-acid tests.
- **Saponins:** Foam formation test indicated presence.

This comprehensive screening indicates that *Clitoria ternatea* hydroalcoholic extract contains alkaloids, glycosides, phytosterols, flavonoids, fixed oils and fats, and saponins, but lacks carbohydrates, proteins, gums, mucilage, starch, and tannins **(Malini et al., 2000).**

2.4 Pharmacological evaluation

2.4.1 Approval of Experimental Work from IAEC/CPCSEA

The protocol of the animal experiments involved in this research work has been approved by IAEC/CPCSEA constituted for this purpose **(Viel et al., 1999).**

2.5 Acute Toxicity Study

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. The Acute Toxic Class Method (OECD guidelines, 2000) was followed to determine the maximum safety dose of the drug extracts.

Three Wistar strain female albino rats (8–12 weeks old, 180–200 g body weight) were used in each group. A single dose (2 g/kg) of the extracts was orally administered to overnight fasted animals (food but not water was withheld), while control animals received the vehicle (0.3% w/v CMC).

Animals were observed individually after dosing—at least once during the first 30 minutes, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a total of 14 days. Body weights of the animals were recorded **(Shoemaker et al., 1997).**

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]

[1]PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[2]Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[3]Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[5]Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India

Other observations included changes in skin, fur, eyes, and mucous membranes, as well as respiratory, circulatory, autonomic and central nervous system responses, somatomotor activity, and behavioral patterns. At the end of 14 days, all animals were subjected to gross necropsy.

2.6 Evaluation of Anti-Lithiatic Activity: Experimental Models

For the study of urolithiasis, an animal model was adopted that would satisfy the following conditions:

- The animal should develop urolithiasis rapidly and reproducibly
- Pathological changes in the bladder should result from stone formation
- The symptoms should be ameliorated or prevented by drug treatment effective in human beings
- The drug tested should be administered orally.
- Drug dosages should approximate the optimum therapeutic range for humans, scaled to test animal weight **(Lemann et al., 1991).**

2.6.1 Induction of Lithiasis

Lithiasis can be experimentally induced by different methods.

1. Diet
2. Chemicals
3. Foreign body insertion
4. Infection

2.6.2 Chemically Induced:

Chemically Induced Urolithiasis Models:

- ➢ Literature shows that 0.75% ethylene glycol for 28 days, or a combination of 0.5% ethylene glycol and 0.5 mg 1-alpha (OH) D3 in drinking water for 4 weeks, produces calcium oxalate urolithiasis.
- It is also reported that calcium oxalate urolithiasis can be induced by administration of 1% or 0.75% ethylene glycol in drinking water.
- Feeding rats with 3% glycolic acid in feed for 45 days results in stone formation
- Uninephrectomy enhances urolithiasis in ethylene glycol-treated male Sprague–Dawley rats, producing calcium oxalate stones.
- Nephrolithiasis has been experimentally induced by administering both gentamicin (40 mg/kg body weight, i.p.) and ammonium oxalate (2%, p.o.)
- A stone-forming animal model was developed involving renal tubular injury using a cyclooxygenase-2 selective inhibitor. In this model, male Sprague–Dawley rats were fed chow containing 3% sodium oxalate with or without 100 mg/kg celecoxib.
- Exposure to 3–5% terephthalic acid (TPA) or 1–3% dimethyl terephthalate in the diet for less than two weeks induced urinary tract calculi in weaning Fischer-344 rats **(Low et al., 1997).**

2.6.3 Foreign Body Insertion Technique:

A sterile zinc disc (4 mm diameter) is surgically inserted into the bladder through a small incision, which is then closed with a silk suture. After seven days, stone deposition occurs, and the stone weight is compared with control animals. Calcium oxalate crystals can also be directly implanted into the urinary bladder of adults to induce stone formation.

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]
[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

2.6.4 Infection-Induced Stone Formation:

- Stones form in the presence of infections by urea-splitting bacteria such as *Staphylococcus*, *Streptococcus*, *Proteus*, *Klebsiella*, *Serratia*, and *Mycoplasma*. These organisms increase urine alkalinity, promoting the formation of magnesium ammonium phosphate (struvite) stones.
- Nanobacteria have been isolated from kidney stones and are suggested to act as nuclei initiating renal stone formation.
- Bladder stones have been induced in rats using human urinary stones. Additionally, D-penicillamine increases hepatic oxalate production and urinary oxalate excretion, contributing to stone formation **(Ryall et al., 1981)**.

2.6.5 In Vivo Method Selection & Acclimatization of Animals

- Hydroalcoholic extracts of *Clitoria ternatea* were screened for their anti-lithiatic activity.
- Adult male albino Wistar rats weighing between 180–200 g were selected and fed with a standard pellet diet and water ad libitum. They were housed in well-ventilated cages (3 to 4 animals per cage) and maintained at room temperature under a 12-hour light–dark cycle. The animals were acclimatized to laboratory conditions for one week **(Khan et al., 1997).**

2.6.6 Induction of Lithiasis by Ethylene Glycol (1%)

- Ethylene glycol (1%) was administered orally in drinking water for 28 days to all groups of animals except the normal control group **(Malini et al., 2000).**

2.6.7 Treatment Protocol

The grouped animals received the treatments as follows:

Prophylactic Study:

- **Group I:** Received normal diet and served as control.
- **Group II:** Lithiatic control: Animals were given normal diet and 1% ethylene glycol in drinking water for 28 days.
- **Group III:** Received 1% ethylene glycol in drinking water and then treated with hydroalcoholic extract of *Clitoria ternatea* at a dose of 200 mg/kg.
- **Group IV:** Received 1% ethylene glycol in drinking water and then treated with hydroalcoholic extract of *Clitoria ternatea* at a dose of 400 mg/kg.
- **Group V:** Received 1% ethylene glycol in drinking water and then treated with Cystone at a dose of 750 mg/kg.

Curative Study:

- **Group I:** Received normal diet and served as curative normal controls.
- **Group II:** Lithiatic control: Animals were given normal diet and 1% ethylene glycol in drinking water for 28 days, followed by ordinary water for 15 days.
- **Group III:** Received 1% ethylene glycol in drinking water for 28 days and then treated with hydroalcoholic extract of *Clitoria ternatea* at a dose of 200 mg/kg for the next 15 days.
- **Group IV:** Received 1% ethylene glycol in drinking water for 28 days and then treated with hydroalcoholic extract of *Clitoria ternatea* at a dose of 400 mg/kg for the next 15 days.
- **Group V:** Received 1% ethylene glycol in drinking water for 28 days and then treated with Cystone at a dose of 750 mg/kg for the next 15 days. **(Halabe et al., 2003).**

2.6.8 Biochemical Analysis Summary:

Amit Mishra[1], **Dr. Sourabh Jain**[2*], **Ragini Bundela**[3*], **Dr. Karunakar Shukla**[4], **Dr. Neha Jain**[5]
[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

At the end of the experiment, 24-hour urine samples were collected from rats for analysis of key urinary components like calcium, magnesium, phosphate, oxalate, uric acid, creatinine, and protein. Blood samples were taken via the retro-orbital plexus, and serum was separated to measure similar biochemical parameters using laboratory kits. Finally, kidneys were harvested, fixed, and examined histologically under a microscope to confirm crystal deposits and lithiasis **(Grases et al., 1989).**

2.7 Assay Method

i. Estimation of Serum Calcium

Procedure:

Measure 2 ml of serum, 2 ml of water, and 1 ml of 4% ammonium oxalate in a centrifuge tube. Mix thoroughly and let it stand for at least 30 minutes. Mix again and centrifuge at 1500 rpm for 15 minutes. Pour off the supernatant and drain the tube by inverting it on filter paper for a few minutes. Wipe the mouth of the tube dry with filter paper. Add 3 ml of 2% ammonia, shake, centrifuge, and drain as before. Then add 3 ml of 1N sulfuric acid and shake vigorously. Place the tube in a boiling water bath, shaking intermittently until the precipitate dissolves completely. While still hot, titrate with 0.01N potassium permanganate exactly as before. Record the volume (y ml) of potassium permanganate used.

Formula:

Serum calcium(mg/100ml)= (x–y)X0.2X100/2 =(x–y)X10

ii. Estimation of Urine Calcium

Urine is diluted 1:10 with water. The calcium content in the diluted urine is estimated using the same procedure as serum calcium estimation, and the result is multiplied by 10.

iii. Estimation of Serum Creatinine

To estimate serum creatinine, 7 ml of water is measured into a test tube, followed by the addition of 1 ml of serum and 1 ml of 10% sodium tungstate. The mixture is thoroughly mixed, and 1 ml of 2/3 N sulfuric acid is added with constant shaking. After allowing the mixture to stand for a few minutes, it is filtered. From the filtrate, 5 ml is transferred into a test tube labeled "Unknown." Separately, 5 ml of a working standard creatinine solution is taken in a tube labeled "Standard," and 5 ml of water is taken in a tube labeled "Blank." To each of these tubes, 2.5 ml of alkaline picrate solution is added and mixed well. The tubes are then allowed to stand for 10 minutes. Finally, the absorbance of the unknown and standard samples is read against the blank at 520 nm or using a green filter.

Serum Creatinine (mg/100ml) = u/sX0.01/0.5X100 = u/sX2

iv. Estimation of Urine Creatinine

Urine creatinine may be estimated in the same way as serum creatinine after diluting the urine. The urine is diluted according to the urinary output. The urine should be diluted 1 in 100 if the urine volume is 1–2 litres/day. The dilution should be greater if the urine volume is more than 2 litres/day. The result should be multiplied by the number of times the urine was diluted.

v. Estimation of Serum Uric acid-

Procedure:

Measure 7 ml of water in a test tube. Add 1 ml of serum and 1 ml of 10% sodium tungstate. Mix, then add 1 ml of 2/3 N sulphuric acid slowly with constant shaking. Let it stand for a few minutes and filter. Transfer 5 ml of the filtrate into a tube labeled **Unknown**. Measure 5 ml of the working standard uric acid solution in a

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]
[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

tube labeled **Standard**, and 5 ml of water in a tube labeled **Blank**. To each tube, add 1 ml of 10% sodium carbonate and 1 ml of dilute phosphotungstic acid. Mix and keep the tubes in a water bath at 25°C for 30 minutes. Read the unknown and standard against the blank at 700 nm or using a red filter.

vi. Estimation of Urine Uric acid

The urine is diluted 1 in 10 with water. Uric acid in diluted urine is estimated by the above procedure and the result multiplied 1 in 100 with water and 5 ml of the diluted urine taken instead of protein-free filtrate. [(78)]

vi. Estimation of Serum Phosphate

Procedure:

Measure 9 ml of 10% trichloroacetic acid in a test tube. Add 1 ml of serum drop by drop with constant shaking. Filter the mixture and transfer 5 ml of the filtrate to a tube labeled "Unknown." Pipette 5 ml of the working standard phosphorus solution into a tube labeled "Standard" and 5 ml of 10% trichloroacetic acid into a tube labeled "Blank." To each tube, add 1 ml of the molybdate reagent, 0.4 ml of aminonaphtho sulphonic acid, and 3.6 ml of water. Mix well after each addition. Allow the tubes to stand for 5 minutes. Measure the absorbance of the unknown and standard solutions against the blank at 680 nm using a red filter

Serum Phosphate (mg/100ml) = Au/As X0.02/0.5 X 100 =Au/As X4

vii. Estimation of Urine Phosphate

- **Dilution**: Urine is diluted 1:10 with water. If **protein-free**, may be diluted 1:100 and 5 ml used.
- **Estimation**: Use standard phosphate procedure on the diluted urine.
- **Final Result**: Multiply the result by the dilution factor (e.g., ×10 or ×100).

viii. Estimation of Urine Protein

Procedure:

- Mix **2 ml urine** with **2 ml of 3% sulphosalicylic acid** in a glass tube.
- Let stand for **5 minutes** at room temp.
- Compare precipitate visually with **4 ml of sulphosalicylic acid** in a similar tube.

ix. Estimation of Urine Oxalate

Procedure:

1. Acidify urine with **conc. HCl**.
2. Add **0.5 ml urine**, 1.5 ml water, and **pH indicator (bromo-thymol blue)**.
3. Adjust **pH to 7**.
4. Add **2 ml calcium sulphate solution**, then **14 ml ethanol**. Let stand **3 hours**.
5. Centrifuge at **2000 rpm for 10 min**. Discard supernatant.
6. Dissolve precipitate in **2 ml of 2N H_2SO_4**.
7. Add **zinc**, boil 30 min → remove zinc.
8. Wash with **1% chromotropic acid**, then add **5 ml conc. H_2SO_4**, boil again for 30 min.
9. Cool, dilute to **20 ml with 10N H_2SO_4**, and read absorbance at **570 nm**.

- **Calculation**:

x. Estimation of Serum Oxalate

- **Same procedure** as urine oxalate.
- **Serum is diluted** according to its output volume before proceeding with the oxalate estimation.

xi. Estimation of Urine Magnesium

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]

[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

Procedure:

1. **Sample Preparation**:
   - Add **10 ml well-shaken urine** to a 15 ml conical centrifuge tube.
   - Add **2 drops of methyl orange**.
   - Acidify with **conc. HCl** dropwise until red.
   - Add **1 ml of 5% ammonium phosphate**, mix.
2. **Initial Precipitation**:
   - Add **2 ml of 28% ammonium hydroxide**, mix.
   - Let stand for **1 hour**.
3. **Centrifugation & Washing**:
   - Centrifuge, decant supernatant.
   - Wash precipitate **3 times** with **5 ml alcohol wash**.
4. **Dissolution**:
   - Dissolve the precipitate in **0.5 ml of 1:4 HCl**.
   - Transfer to a **10 ml volumetric flask**, dilute to volume.
5. **Aliquot Processing**:
   - Take **5 ml aliquot** from above, add:
     - **1 ml of 2.5% oxalic acid**
     - **Drop of methyl orange**
   - Add **sodium acetate** slowly to adjust pH to ~4 (indicator turns orange).
   - Let stand **4 hours or more** to precipitate **calcium oxalate**.
6. **Separation of Calcium**:
   - Centrifuge, wash precipitate **twice with 3 ml of 2% ammonium hydroxide**.
   - Save **supernatant and washings** for magnesium estimation.
7. **Magnesium Precipitation**:
   - Add **0.5 ml ammonium phosphate solution** and **2 ml strong ammonia** to supernatant.
   - Let stand **1 hour** for magnesium ammonium phosphate precipitation.
8. **Final Steps**:
   - Centrifuge, wash precipitate **twice with 5 ml alcohol**.
- Dissolve in **1:4 HCl**.
- Perform **phosphate analysis colorimetrically** using a green filter (500–570 mμ).

xii. Estimation of Serum Magnesium

- **Same procedure** as urine magnesium.
- **Dilute serum appropriately** based on output volume before analysis.

2.8 Statistical Analysis

- **Results expressed as**: Mean ± SEM.
- **Data analysis**: One-Way ANOVA.
- **Post hoc test**: Newman–Keuls multiple range test.
- **Significance level**: $p < 0.01$ considered statistically significant.

3. RESULTS

3.1 Pharmacological Evaluation: Acute Toxicity Studies

No mortality was observed in rats after a single oral dose of 2 g/kg of the individual herbal extracts, classifying them under acute toxicity class 5 (>2000 mg–5000 mg/kg) according to OECD guidelines (2000). No toxic signs were noted during the 14-day observation period, including behavioral (e.g., sedation, restlessness, aggression), respiratory (e.g., hypopnea, dyspnea), or ocular (e.g., mydriasis, lacrimation)

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]
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[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

symptoms. Additionally, no changes in body weight, food, or water intake were observed, indicating the extracts are safe at high doses.

3.2 Pharmacological Evaluation: Antilithiatic Activity (Prophylactic and Curative)

Chronic administration of 1% ethylene glycol solution to male Wistar rats induced hyperoxaluria. Significant changes were observed in the urinary concentrations of various ions following ethylene glycol treatment.

Table 2: Effect of HAECT on urinary biochemical parameters on the day 14

GP	Protein(mg/dl)	Magnesium(mg/dl)	Calcium(mg/dl)	Uricacid (mg/dl)	Creatinine(mg/dl)	Oxalate(mg/dl)	Phosphate(mg/dl)
G1	66.60± 3.605	4.40± 0.50	5.80± 0.58	3.20± 0.58	0.80± 0.08	15.80± 1.75	32.75± 2.25
G2	152.55± 6.40**(a)	0.98± 0.22**(a)	19.40± 1.88**(a)	12.50± 1.55**(a)	1.52± 0.18**(a)	32.50± 3.45**(a)	73.65± 4.18**(a)
G3	86.25± 4.30**(b)	2.50± 0.35**(b)	9.22± 1.08**(b)	5.45± 0.85**(b)	0.95± 0.14**(b)	23.15± 2.62**(b)	43.35± 3.42**(b)
G4	81.30± 4.05**(b)	2.65± 0.38**(b)	8.80± 0.85**(b)	5.30± 0.75**(b)	0.90± 0.10**(b)	20.18± 2.28**(b)	36.90± 3.30**(b)
G5	78.20± 3.95**(b)	3.98± 0.42**(b)	7.65± 0.62**(b)	4.88± 0.58**(b)	0.84± 0.09**(b)	18.05± 1.78**(b)	33.45± 2.48**(b)

Table 2: Effect of HAECT on urinary biochemical parameters on the 28th day.

GROUP	Protein(mg/dl)	Magnesium(mg/dl)	Calcium(mg/dl)	Uricacid (mg/dl)	Creatinine(mg/dl)	Oxalate(mg/dl)	Phosphate(mg/dl)
G1	72.0 ±3.35	4.45 ±0.68	6.35 ±0.68	3.45 ±0.60	0.88 ±0.06	15.28 ±1.48	32.35 ±2.22
G2	159.45 ±7.32**(a)	1.40 ±0.30**(a)	20.15 ±1.64**(a)	14.55 ±1.40**(a)	1.80 ±0.22**(a)	46.33 ±4.45**(a)	75.45 ±4.12**(a)

Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]
[1]PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[2]Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[3]Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[5]Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India

G3	94.25 ±5.65**(b)	2.85 ±0.38**(b)	10.56 ±0.83*(b)	7.75 ±0.80**(b)	1.14 ±0.11**(b)	24.33 ±2.65**(b)	42.80 ±3.56**(b)
G4	88.20 ±4.95**(b)	3.20 ±0.40**(b)	10.20 ±0.58**(b)	7.18 ±0.56**(b)	1.04 ±0.09**(b)	22.30 ±2.32**(b)	41.40 ±3.12**(b)
G5	86.75 ±3.68**(b)	3.55 ±0.52**(b)	9.18 ±0.45**(b)	6.56 ±0.48**(b)	0.90 ±0.07**(b)	19.15 ±2.18**(b)	38.30 ±2.69**(b)

Table 3 :Effect of HAECT on serum parameters in prophylactic treatment of animals

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uricacid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	4.80 ±0.72	9.45 ±1.30	3.55 ±0.33	0.62 ±0.07	6.64 ±0.58	12.30 ±1.42
G2	1.34 ±0.38**(a)	18.15 ±2.42**(a)	9.82 ±1.12**(a)	1.08 ±0.15**(a)	12.68 ±1.62**(a)	26.08 ±3.20**(a)
G3	3.64 ±0.60**(b)	11.65 ±1.38**(b)	4.38 ±0.56**(b)	0.88 ±0.11**(b)	8.50 ±0.84**(b)	20.15 ±2.60**(b)
G4	3.95 ±0.48**(b)	11.22 ±1.30*(b)	4.20 ±0.42**(b)	0.84 ±0.08**(b)	8.20 ±0.76**(b)	19.75 ±2.08**(b)
G5	4.22 ±0.58**(b)	10.48 ±1.42**(b)	3.92 ±0.36**(b)	0.78 ±0.06**(b)	7.55 ±0.60**(b)	16.33 ±2.05**(b)

Table 4:Effect of HAECT on urinary biochemical parameters in curative reatment of an I MALS

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uricacid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	75.54 ±3.88	4.65 ±0.58	6.505 ±0.68	3.84 ±0.82	14.90 ±2.22	19.24 ±1.78	34.55 ±2.88

Amit Mishra[1], **Dr. Sourabh Jain**[2*], **Ragini Bundela**[3*], **Dr. Karunakar Shukla**[4], **Dr. Neha Jain**[5]
[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[3*]*Assistant Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

G2	172.80 ±7.90**(a)	1.52 ±0.24**(a)	17.88 ±2.14**(a)	15.10 ±2.48**(a)	55.88 ±4.20**(a)	49.95 ±3.48**(a)	80.45 ±4.78**(a)
G3	113.60 ±4.90**(b)	3.24 ±0.42**(b)	11.80 ±1.22**(b)	9.45 ±1.22**(b)	36.85 ±3.18**(b)	26.90 ±2.48**(b)	43.22 ±3.18**(b)
G4	95.40 ±4.65**(b)	3.75 ±0.42**(b)	10.90 ±1.18**(b)	9.22 ±1.24**(b)	34.55 ±3.08**(b)	24.25 ±2.20**(b)	41.74 ±2.92**(b)
G5	90.40 ±3.68**(b)	4.22 ±0.45**(b)	10.24 ±1.06**(b)	8.28 ±1.08**(b)	32.48 ±2.92**(b)	21.16 ±1.88**(b)	38.40 ±2.68**(b)

Table 5: Effect of HAECT on serum parameters in curative treatment of animals

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uricacid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	5.24 ±0.92	10.36 ±1.28	4.65 ±0.92	0.94 ±0.21	8.80 ±0.73	15.64 ±2.05
G2	1.94 ±0.48**(a)	24.14 ±2.46**(a)	12.20 ±2.37**(a)	2.76 ±0.64*(a)	17.38 ±1.24**(a)	29.88 ±3.05**(a)
G3	4.21 ±0.80**(b)	14.88 ±1.56**(b)	7.42 ±1.10**(b)	1.54 ±0.48**(b)	11.08 ±1.06**(b)	20.75 ±2.46**(b)
G4	4.44 ±0.85**(b)	14.54 ±1.26**(b)	7.10 ±0.90**(b)	1.35 ±0.38**(b)	10.36 ±0.83**(b)	18.90 ±2.25**(b)
G5	4.74 ±0.96**(b)	13.28 ±1.08**(b)	6.55 ±0.73**(b)	1.25 ±0.33**(b)	9.58 ±0.78**(b)	17.35 ±2.06**(b)

3.3 Histopathological Studies

In stone-induced models, the following changes were noted:

1. Damage to epithelial cells at the inner layer of the tubules.
2. Dilatation of the tubules.
3. Presence of crystals in the tubules.

Amit Mishra[1], **Dr. Sourabh Jain**[2*], **Ragini Bundela**[3*], **Dr. Karunakar Shukla**[4], **Dr. Neha Jain**[5]
[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[2]*Associate Professor, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
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[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*
[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*

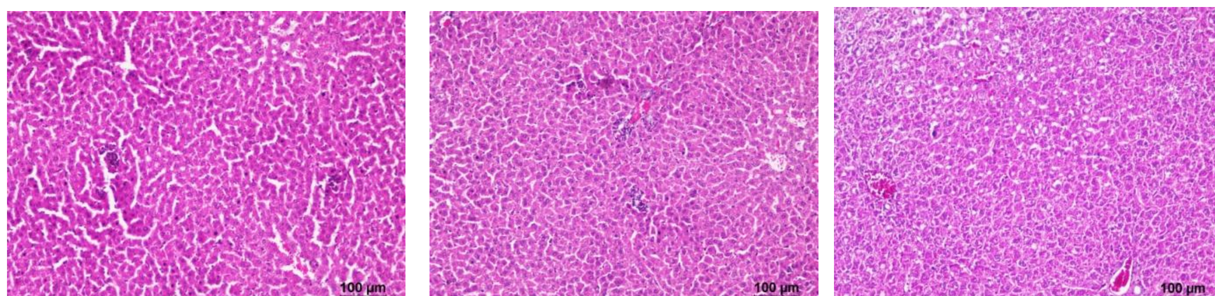

Figure 1: NORMALCONTROL(CURATIVESTUDY), LITHIATICCONTROL (CURATIVECONTROL, HAECT200MG/KG

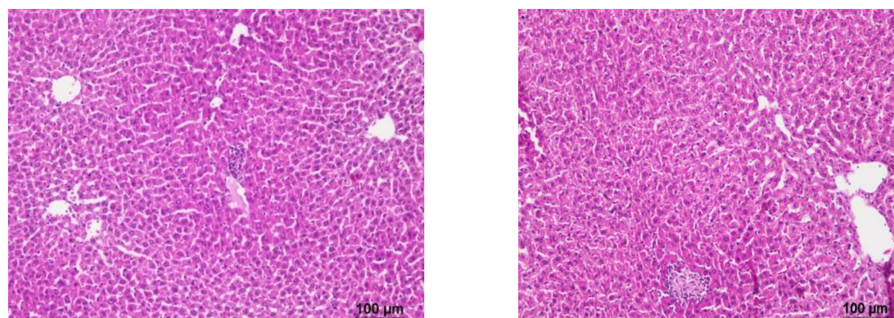

Figure 2:HAECT200MG/KG, HAECT400MG/K

4. CONCLUSION

In conclusion, the presented data indicate that administration of the hydroalcoholic extract of *Clitoria ternatea* to rats in ethylene glycol-induced lithiasis reduced and prevented the growth of urinary stones, supporting the hill tribals' claim regarding the anti-lithiatic activity of the plant.

Earlier studies reported that anti-lithiatic activity might be through antioxidant activity and free radical scavenging principles. The hydroalcoholic extract of *Clitoria ternatea* contains polyphenols such as tyrosol, pyrogallol, saponins, and flavonoids. These components are responsible for the anti-lithiatic activity.

Therefore, treatment with the hydroalcoholic extract of *Clitoria ternatea* may prevent calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation), which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones.

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[4]Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore
[5]Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India

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Amit Mishra[1], Dr. Sourabh Jain[2*], Ragini Bundela[3*], Dr. Karunakar Shukla[4], Dr. Neha Jain[5]

[1]*PG Scholar, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*

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[4]*Professor & Principal, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore*

[5]*Visiting Research Associate, Pinnacle Biomedical Research Institute, Bhopal MP India*