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EVALUATION OF ANTI-OXIDANT AND ANTI-UROLITHIATIC ACTIVITY OF NATHAI CHOORI SEEDS BY ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED UROLITHIASIS IN WISTAR RATS

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Abstract: Kidney stones, also known as urolithiasis, are characterised by the presence of dense mineral deposits in the urinary tract. This study used an albino Wistar rat model of urolithiasis induced by ethylene glycol and ammonium chloride to assess the anti-urolithiatic efficacy of Methanolic extract of *Spermacoce neohispida Goaverts* (Me.Sh) seeds. The rats were split into five groups and given various treatments for a total of 28 days. In comparison to the disease control group, the results demonstrated a considerable improvement in the treatment and standard groups. Creatinine, uric acid, urea, and calcium levels were lower in the treatment group than in the control group, according to serum analysis. Magnesium levels increased, whereas calcium and oxalate levels in the urine were markedly decreased. Positive changes were also seen in urine volume, urine pH. Histopathological examination of kidney sections confirmed that the treatment and standard groups displaying normal renal architecture. According to these findings, Me.Sh shows remarkable anti-urolithiatic potential and may be utilised to prevent and treat urinary stone formation. Further research is necessary to determine whether Nathai choori's therapeutic benefits can be used to treat urolithiasis.

Keywords: Urolithiasis, Spermacoce neohispida Goaverts, Borreria hispida, Nathai choori.

I. INTRODUCTION

Urolithiasis, the formation of urinary stones, has been a prevalent condition in human history, affecting people worldwide regardless of race, culture, or geography. It is considered the third most common urinary system disease, with varying lifelong risks across different regions. Calcium oxalate is the most commonly reported type of stone, accounting for 80% of cases, either alone or in combination with calcium phosphate.(López and Hoppe 2010)

Calcium oxalate crystals exist in two forms: calcium oxalate dihydrate (COD) and calcium oxalate monohydrate (COM). Hyperoxaluria, characterized by high oxalate levels in urine, promotes the formation of COM crystals, which have pointy edges and can cause damage to the epithelial tissue and strongly adhere to the renal epithelium. The preferential formation of dihydrate crystals in urine is suggested to be a defense mechanism against crystal retention, preventing them from attaching to the renal tubular epithelium and inhibiting renal inflammation and stone formation.(Geraghty, Wood, and Sayer 2020)

Renal injury is a predisposing factor for stone formation, as products of cell damage can act as heterogeneous nidus for the retention of calcium oxalate crystals. Supersaturated urine serves as a driving force for crystal precipitation and aggregation. Normally, urine contains stone-inhibiting agents such as citrate and magnesium, but these agents may be masked in individuals at higher risk for developing stones. Studies on animals have shown that urinary crystals and high oxalate concentrations induce inflammation in the renal tubular epithelium, serving as a major risk factor for urolithiasis. Crystalluria and hyperoxaluria trigger the up-regulation of renin and the generation of angiotensin-II, leading to the synthesis of non-phagocytic NADPH oxidase, which increases the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), causing renal inflammation. ROS also play a role in regulating the production of various crystallization modulators involved in the cellular inflammatory cascade. ROS-induced renal injury and fibrosis contribute to cell death and facilitate crystal formation. In the presence of free radicals, crystal inhibitory molecules become defective, resulting in crystal aggregation and growth. Cell death also leads to the formation of new cells to repopulate the epithelial tissue, providing a favorable surface for crystal attachment and retention. ROS and RNS are generated through enzymatic and non-enzymatic redox reactions during cellular metabolism under normal and pathological conditions,

involving various cellular components such as mitochondria, plasma membrane, peroxisomes, and cytosol.(Alelign and Petros 2018)

Current therapeutic approaches, such as extracorporeal shock wave lithotripsy (ESWL) and drug treatments, have improved clinical practice but are limited in reducing the recurrence of urolithiasis and may have unwanted side effects. ESWL, for example, can cause renal impairment, hypertension, and hemorrhage. The effectiveness of pharmaceutical agents like alkali therapy, allopurinol, thiazide, and citrate diuretics in treating kidney stone disease is supported by less convincing scientific evidence.

The use of edible and medicinal plants as alternative medicine has been practiced for centuries, particularly in the subcontinent region. Numerous plants and herbs have been reported to have potential benefits against kidney stone disease. *Spermacoce neohispida Goaverts* (Nathai choori) is one such plant, rich in bioactive compounds, traditionally used in lithiasis treatment. It has been reported to possess antioxidant, antimicrobial, anti-inflammatory, anticancer, hepatoprotective, diuretic, and anti-diabetic properties. However, there is currently no available pharmacological data on the effectiveness of Spermacoce neohispida Goaverts against urolithiasis.("Pharmacological Evaluation of Mentha Piperita Against Urolithiasis: An In Vitro and In Vivo Study - PMC" n.d.)

Therefore, the present study aims to establish the scientific basis for the antiurolithiatic activity of *Spermacoce neohispida Goaverts* through in vitro and in vivo methods, specifically using an ethylene glycol-induced urolithiasis model in male albino Wistar rats.

II. DISEASE PROFILE

Etiology of Urolithiasis

The Urolithiasis, or urinary stone disease, can be caused by various risk factors including diet, personal and family history of stones, environmental factors, medications, and underlying medical conditions. Common risk factors for stone formation include poor oral fluid intake, high intake of animal-derived protein, high oxalate intake, high salt intake, and low calcium intake. Certain medical conditions such as chronic kidney disease, hypertension, gout, diabetes mellitus, hyperlipidemia, obesity, endocrine disorders, and malignancies can also increase the risk of stone development.

Obesity, hyperlipidemia, and type 2 diabetes mellitus are strongly associated with calcium oxalate and uric acid stones. Insulin resistance in obesity and type 2 diabetes promotes metabolic changes that increase urinary calcium and uric acid excretion, contributing to stone formation. Drug-induced urolithiasis is rare but can occur with medications such as protease inhibitors used for HIV treatment and ceftriaxone.

Different types of stones have specific characteristics and causes. Struvite stones, also known as infection stones, form in the presence of urease-producing bacteria, such as Proteus or Klebsiella species, and are associated with elevated urine pH. Uric acid stones are related to low urinary uric acid levels, low urine pH, and low urine volume. Cystine stones are rare and result from an inherited disorder that affects cystine metabolism and transport.(Baatiah et al. 2020)

Preventive measures for urolithiasis include maintaining adequate oral hydration, consuming a balanced diet with moderate animal protein and oxalate intake, and avoiding excessive salt and sugar consumption. Citrate supplementation can help prevent stone formation by inhibiting crystal aggregation. Treatment approaches for urolithiasis depend on stone size, location, composition, and symptom severity and can range from conservative measures to interventions such as lithotripsy or surgical removal.

Table 1: Types of kidney stones and its occurrence

Types of kidney stones	% Occurrence
Calcium oxalate & calcium phosphate	70-80
Struvite	10
Uric acid	10
Cystine	<1

Stone constituents:

The main components of the stone matrix, which make up 2-3% of the total dry weight of urinary stones, consist of macromolecules found in urine. These macromolecules include approximately 64% protein, 9.6% non-amino sugars, 5% hexosamine (such as glucosamine), 10% bound water, and the remaining portion as inorganic ash. Lipids have also been identified as significant components of the stone matrix. Proteins form a discontinuous coat around the crystals, ranging in thickness from 10 to 20 nm. This macromolecular coat may play a role in preventing the dissolution of newly formed crystals during routine urinary pH changes, which could contribute to stone formation.(Aggarwal et al. 2013)

Various urinary molecules have the potential to influence the mass of calcium oxalate (CaOx) deposited from urine and the size of crystal particles produced, thereby impacting the retention of crystalline particles in the renal collecting system and the development of stone disease. Some molecules promote CaOx crystal nucleation in inorganic solutions and concentrated urine, while others, such as albumin, do not significantly affect crystallization in urine. Numerous studies have shown that different urinary proteins can inhibit CaOx crystal growth and aggregation. Interestingly, some proteins can have dual effects, acting as both inhibitors and

promoters of crystal deposition depending on experimental conditions. For example, Tamm-Horsfall mucoprotein can inhibit CaOx crystal aggregation but can also act as a promoter of crystal deposition under certain circumstances. The composition of the stone matrix, particularly the presence of proteins and other macromolecules, plays a role in stone formation by influencing crystal growth, aggregation, and deposition.

Mechanism of stone formation:

Crystal nucleation: Nucleation is the initial step in the transformation of a supersaturated solution from a liquid to a solid phase. In stone formation, nucleation occurs when stone salts combine in solution to form loose clusters that may increase in size. Nuclei are the first crystals that form and have a characteristic lattice pattern. In urine, nuclei usually form on existing surfaces, a process known as heterogeneous nucleation. Various substances such as epithelial cells, urinary casts, red blood cells, and other crystals can act as nucleating centers in urine. Heterogeneous nucleation requires lower saturation levels compared to homogeneous nucleation.

Crystal Growth: Once a crystal nucleus reaches a critical size and the relative supersaturation remains above one, crystal growth occurs. Crystal growth involves adding new components to the nucleus, reducing the overall free energy. Crystal growth is essential for particle formation and stone formation. In the context of stone formation, crystal growth and aggregation play crucial roles. Certain proteins found in the stone matrix, such as human serum albumin, retinol binding protein, transferrin, Tamm-Horsfall glycoprotein, and prothrombin, inhibit the growth of calcium oxalate crystals.

Crystal Aggregation: Crystal aggregation is the process in which crystals in a solution adhere to each other and form larger particles. It is considered the most critical step in stone formation. While crystal growth alone is too slow for crystals to become large enough to obstruct renal tubules, crystal aggregation can lead to the formation of larger particles that can be retained in the kidneys. Viscous binding and the presence of macromolecules, such as self-aggregating Tamm-Horsfall glycoprotein, contribute to crystal aggregation by acting as a glue and attaching to crystal surfaces.

Crystal- Cell interaction: The formation of crystals in the urinary system occurs due to urinary supersaturation. Once the crystals are formed, they can attach to the renal tubular epithelial cells and be internalized through a process called endocytosis. Crystal-cell interactions play a role in this attachment process. Studies investigating crystal-cell interactions in cultured cells have shown that calcium oxalate (COM) crystals rapidly adhere to microvilli on the cell surface and are subsequently taken into the cells.

In the experimental induction of calcium oxalate urolithiasis, the condition starts with hyperoxaluria, followed by the presence of crystals in the urine and their deposition in the kidney. However, certain macromolecules present in the urine can inhibit the attachment of calcium oxalate crystals to renal tubular cells. Specific polyanionic molecules found in urine, such as glycosaminoglycans, glycoproteins, and citrate, have been shown to block the binding of COM crystals to the cell membrane. A common characteristic of these inhibitory molecules is their polyanionic nature. Polyanions present in the tubular fluid can coat the crystals and prevent their adhesion to tubular cells. Additionally, separate signaling mechanisms regulate the cellular response to crystals that do bind, indicating a distinct set of signals involved in crystal-cell interactions. (Aggarwal et al. 2013)

III. Material and Methods

Collection of plant and Preparation of Crude Extract

Spermacoce neohispida Goaverts (seeds) commonly known as Nathai choori was collected and got authenticated from Siddha Central Research Institute, Arumbakkam, Chennai. The Sample code issued for the seed was S14112201N.

The Me.Sh seeds were thoroughly cleaned and then dried under shade at room temperature and were finely ground using a mechanical grinder to obtain a powder. A Soxhlet extractor was employed to extract the constituents of the dried powder using methanol as the solvent. The methanolic extract was then subjected to drying using a rotary evaporator to remove the solvent completely. The resulting product was stored in a desiccator at room temperature.

Physicochemical analysis

S.hispida goaverts seeds were subjected to physicochemical analysis in accordance with accepted test methods for the quanitative identification of total ash content, Acid insoluble ash, Loss on drying, pH, Alcohol soluble Ash, Water soluble ash. (Ajeesh Krishna et al. 2014)

Phytochemical analysis

S.hispida goaverts seeds were subjected to phytochemical screening in accordance with accepted test methods for the qualitative identification of secondary metabolites, such as alkaloids, glycosides, flavonoids, phenolic compounds, tannins, saponins, anthraquinones, coumarins, and saponins.(“Nirali Publication Pharmacognosy 55th Edition By C.K Kokate, A.P Purohit, S.B. Gokhale (English Medium)” n.d.)

Determination of Antioxidant potential

The DPPH assay is a widely used method in natural product antioxidant studies due to its simplicity and sensitivity. It is based on the principle that antioxidants act as hydrogen donors and can scavenge free radicals. The assay utilizes DPPH, a stable organic nitrogen radical, which changes color from purple to yellow upon accepting hydrogen from an antioxidant. Prepared a methanol solution containing 0.1 mM DPPH. 300 ml of the NC-methanol sample solution was taken in various concentrations of 500, 250, 100, 50, and 10 g/ml. Each sample solution should receive 100 µl of the prepared DPPH solution and should be thoroughly shaken before standing at room temperature for 30 minutes. Using ascorbic acid as the reference substance, determine the

absorbance of the reaction mixtures at 517 nm using a UV-VIS spectrophotometer. Lower absorbance values imply that samples are more effective at scavenging free radicals. Following formula was used to determine the DPPH scavenging effect. (Baliyan et al. 2022)

DPPH scavenging effect (%) = [(absorbance of control - absorbance of reaction mixture) / absorbance of control] x100.

Hydrogen peroxide possesses weak oxidizing properties and can directly deactivate certain enzymes by oxidizing essential (-SH) groups. The method described by Ruch et al. was used to determine the hydrogen peroxide-scavenging capacity of plant extracts with some slight modifications. In phosphate buffer, a solution of hydrogen peroxide (43 mM) was created (1 M pH 7.4). Various sample NC concentrations (500, 250, 100, 50, and 10 g/ml) were added to a solution of hydrogen peroxide (0.6 ml, 43 mM). After 10 minutes, the absorbance of hydrogen peroxide at 230 nm was measured in comparison to a blank solution made of phosphate buffer without hydrogen peroxide. Ascorbic acid served as the reference. The percent inhibition was used to calculate the free radical scavenging activity. **% inhibition = [(Control- Test)/control] ×100.**

Evaluation of the In Vitro Crystallization Assay

Aggregation assay. The method employed to determine experimental kidney stones made of calcium oxalate (CaOx) crystals was based on the approach developed by Mazni Abu Zarin et al., with a minor modification. To prepare the CaOx monohydrate crystals, equal concentrations of calcium chloride (50 mmol/L) and sodium oxalate (50 mmol/L) were mixed and allowed to equilibrate in a water bath at 60 °C for 1 hour. Subsequently, the crystals were cooled to 37 °C prior to evaporation. The final concentration of CaOx monohydrate crystals was 0.8 mg/mL in a Tris buffer (Tris 0.05 mol/L and NaCl 0.15 mol/L) at pH 6.5. Me.Sh plant extracts at different concentrations (500, 250, 100, 50, and 10 µg/ml) were added to the CaOx monohydrate crystals solution and incubated at 37 °C for 24 hours. Cystone, a known medication, was used as a positive control at a concentration of 10 mg/ml. The aggregation activity was assessed by measuring the turbidity using a microplate reader at 620 nm in the presence of the plant extract compared to the control. The experiment was performed in triplicate. The percentage inhibition of aggregation caused by the test sample was calculated as follows: **% inhibition = (OD control - OD test) / OD control x 100.**(Abu Zarin et al. 2020)

Animal Model of Urolithiasis

Male Wistar rats weighing between 200-250g were obtained from the central animal house of Madras Medical College, Chennai. The rats were housed under controlled conditions with a temperature of 22±3°C, relative humidity ranging from 30% to 70%, and a 12-hour light/dark cycle. Throughout the study, the animals had unrestricted access to water and a standard pelleted laboratory animal diet. To ensure acclimatization, the rats were kept in the laboratory environment for one week before the commencement of the experiment. All experimental procedures were conducted in accordance with the ethical guidelines set by the Institutional Animal Ethical Committee, which follows the regulations outlined by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India.(Venkateswarlu, Preethi, and Chandrasekhar 2016)

Acute Toxicity Study

Studies on acute toxicity were carried out in accordance with the OECD Guideline No. 423 (short term toxicity). Wistar rats of either sex (n = 3) were chosen using a random sample procedure and housed in their cages for a minimum of five days before the dose to allow them to acclimate to the laboratory environment. When 2/3 or 3/3 of the animals died, the dose given to them was regarded as toxic. If 1/3 of animals died, the same dose was then administered once again to confirm the toxic effect.

Urolithiasis induction

0.2% of Ammonium Chloride (AC) and 0.75% of Ethylene Glycol (EG) were thoroughly combined and dissolved in the necessary amount of purified water. Taking into account the quantity and weight of the animals, this solution was produced daily and administered orally as feed. Each animal's body weight was noted every day.

Assessment of Antiurolithiatic activity

A urolithiasis model was induced in Wistar rats using EG and AC. The rats were divided into five groups, each containing 6 animals, and housed in metabolic cages. Throughout the 28-day experimental period, all animals, including those in the normal group, had unrestricted access to a regular diet and drinking water. In the control, standard, and treated groups (TGI & TGII), the animals were supplemented with a solution containing 0.75% EG and 2% AC in distilled water, which they had ad libitum for 15 days. In the case of the standard group, animals were additionally administered CST (5mL/kg BW) from the 15th day until the 28th day. The TGI and TGII groups received Methanolic extract of *S.hispida Goaverts* seed at doses of 200mg/kg BW and 400mg/kg BW, respectively, from the 15th day until the 28th day.

Urine analysis

On the 28th day (24 hours), urine samples were collected, and before being stored, a drop of strong HCl was added. Urine volume, pH, calcium, urea, creatinine, and uric acid concentration were calculated.

Serum analysis

Under anesthesia, blood samples were collected from the tail vein. The collected blood was then subjected to centrifugation at 10,000 rpm for 10 minutes to separate the serum. The serum was further analyzed to determine the levels of creatinine, uric acid, urea, and calcium contents.

Histopathology

After making an incision in the abdomen, either kidney from each animal was collected/removed. The isolated kidneys were carefully cleaned of any extraneous tissue and preserved in a 10% neutral formalin solution. One of the isolated kidneys was then embedded in paraffin using conventional methods. Thin sections of approximately 5µm were obtained from the embedded kidney and stained with H&E dye. Finally, the stained sections were mounted in diphenyl xylene and observed under a microscope to examine any changes in the histopathology of the kidney architecture. The weight of each kidney was measured.

Statistical analysis

All the data was expressed as mean (n=6) ± SEM. To determine the statistical significance between different groups, a two-way analysis of variance (ANOVA) was performed. Subsequently, Dunnett's multiple comparison test was applied using a computer-based fitting program, specifically GraphPad Prism. The results were considered statistically significant, if $p<0.005$, $P<0.001$, $P<0.0001$.

IV. Results

The Me.Sh seed extract possesses an aromatic odor, exhibits a dark brown color with a slightly bitter taste, and appears opaque.

Physicochemical analysis. The physicochemical analysis of Me.Sh(*S.hispida goaverts*) seed extract was observed.

Table 2: Physicochemical analysis of Me.Sh seeds

Total ash content	9.64% w/w
Acid insoluble ash	0.08 w/w
Loss on drying	9.6% w/w
pH	7.1
Alcohol soluble Ash	6.95% w/v
Water soluble ash	7.04% w/v

Phytochemical Analysis. Phytochemical screening of *S.hispida Goaverts* seed extract, determined the presence of phenols, alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, and terpenes.

Antioxidant Activity

DPPH assay: The Me.Sh and ascorbic acid exhibited a dose-dependent increase in antioxidant activity, reaching maximum inhibition levels of 65.83 % and 86.88 %, respectively, at a concentration of 500 µg/ml. The free radical scavenging activity of the Me.Sh was measured to have an IC50 value of 113 µg/ml, while the IC50 value of ascorbic acid was found to be 93.65 µg/ml. Me.Sh exhibited comparable antioxidant activity to the standard ascorbic acid.

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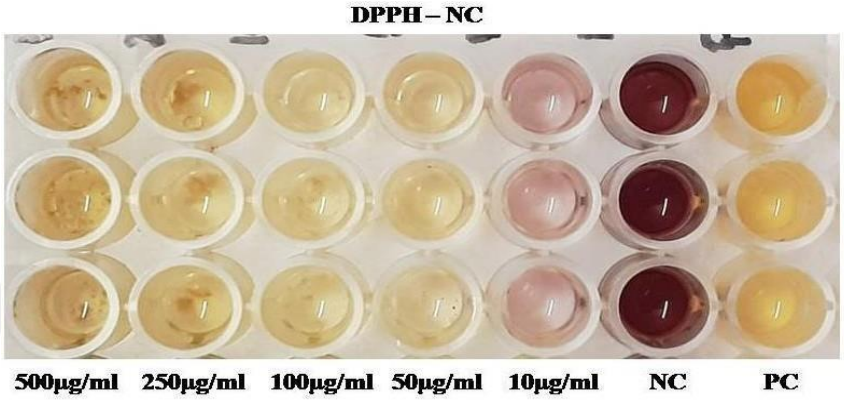

Figure 1: Inhibition of 2,2-Diphenyl-1-picrylhydrazyl in dose dependent manner.

H_2O_2 assay: The Me.Sh and ascorbic acid exhibited a dose-dependent increase in antioxidant activity, reaching maximum inhibition levels of 79.55 % and 85.39%, respectively, at a concentration of 500 µg/ml. The free radical scavenging activity of the Me.Sh was measured to have an IC50 value of 72.02 µg/ml, while the IC50 value of ascorbic acid was found to be 68.32 µg/ml. Me.Sh exhibited comparable antioxidant activity to the standard ascorbic acid.

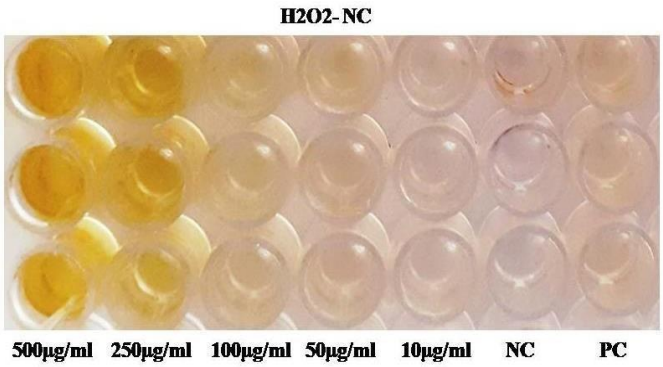

Figure 2: Inhibition of Hydrogen peroxide radical in dose dependent manner.

Evaluation of the in Vitro Aggregation Assay

The aggregation assay revealed that Me.Sh exhibited a percent inhibition of 87.17%, while cystone displayed a percent inhibition of 93.15% at a concentration of 500 µg/ml.

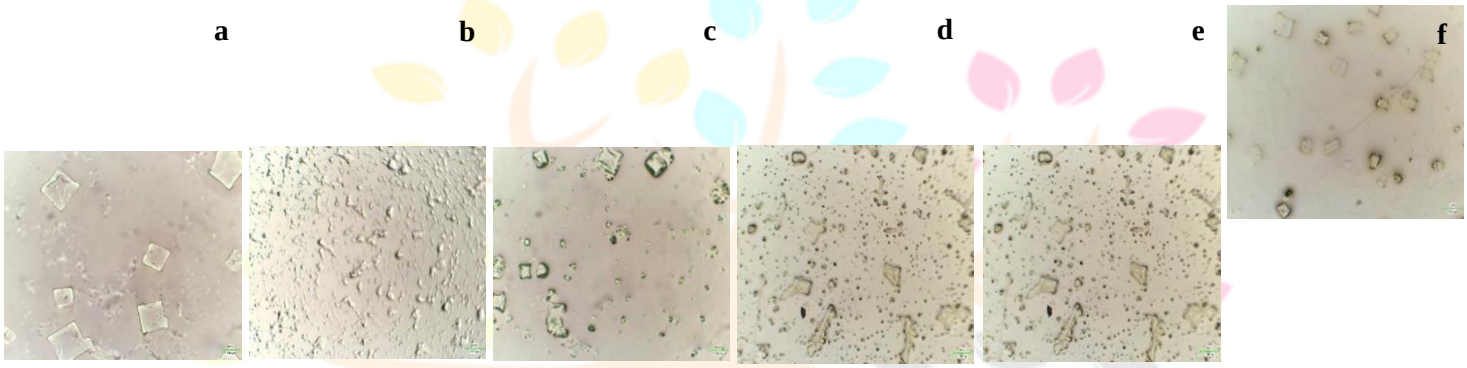

Figure 3: Microscopic view of in vitro model of crystallization a) Cystone and Me.Sh b) 500 µg c) 250 µg d) 100 µg e) 50 µg f) 10 µg

Models of Urolithiasis

Acute toxicity Study. Me.Sh was found to be safe up to the dose of 2000 mg/kg as there were no signs of toxicity observed till 14th day.

Urine analysis

The effects of Me.Sh and Cystone on urine parameters was assessed and shown in (Table 3). Following the induction of urolithiasis, experimental animals exhibited increase in parameter such as calcium, oxalate and decrease in the levels of magnesium, urine Ph and urine volume. On treatment with Me.Sh at the dose of 400 mg/kg BW,200 mg/kg BW and Cystone significantly reduced the levels of calcium , oxalate and increased the levels of magnesium the urine pH and urine volume compared to disease control.

Table 3: Effect of Me.Sh on urine parameters in urolithiatic rats

Groups	Urine parameters				
	Calcium (mg/dL)	Oxalate (mg/dL)	Magnesium (mg/dL)	pH	Volume (ml)
Normal group	8.34 ±0.45	6.34±0.69	0.80±0.012	6.89±0.13	14.53±0.35
Disease Control	19.43±0.37$^{\#\#\#\#}$	16.49±0.57$^{\#\#\#\#}$	0.41±0.014$^{\#\#\#\#}$	5.59±0.55	9.24±0.15
Standard group	10.34±0.62****	7.21±0.39****	0.71±0.016****	7.47±0.43*	13.10±0.43*
TGI	16.67±0.12***	11.40±0.32****	0.54±0.022****	6.31±0.51	10.63±0.45
TGII	11.36±0.39****	8.58±0.36****	0.68±0.010****	7.14±0.36	11.91±0.34

Note: All the values of mean ± SD; n=6; Significant from control *p<0.05, ***p<0.001, ****p<0.0001;Significant from normal #### = p<0.0001.

Serum analysis

In the urolithic group, there was a significant increase (P < .0001***) in serum creatinine, blood urea nitrogen (BUN) levels, uric acid, and calcium compared to the normal group. However, treatment with Me.Sh at doses of 200 mg/kg and 400 mg/kg, as well as cystone at a dose of 750 mg/kg, effectively normalized creatinine, BUN levels, uric acid, and calcium in a dose-dependent manner.

Table 4: Effect of Me.Sh on blood parameters in urolithiatic rats

Groups	Blood parameters			
	Creatinine (mg/dL)	Calcium (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)
Normal group	2.38±0.12	4.73±0.46	2.74±0.23	22.11±0.41
Control group	6.67±0.19####	9.51±0.56####	6.11±0.73####	48.09±0.37####
Standard group	2.46±0.12****	5.16±0.43****	2.59±0.03****	26.38±0.36****
TGI	2.75±0.06****	7.41±0.09**	4.09 ±0.51**	39.33 ±0.51****
TGII	2.51±0.06****	6.74±0.05***	2.75±0.06****	30.70±0.50****

Note: All the values of mean ± SD; n=6; Significant from control **p < 0.01, ***p < 0.001, ****p<0.0001;Significant from normal #### = p < 0.0001.

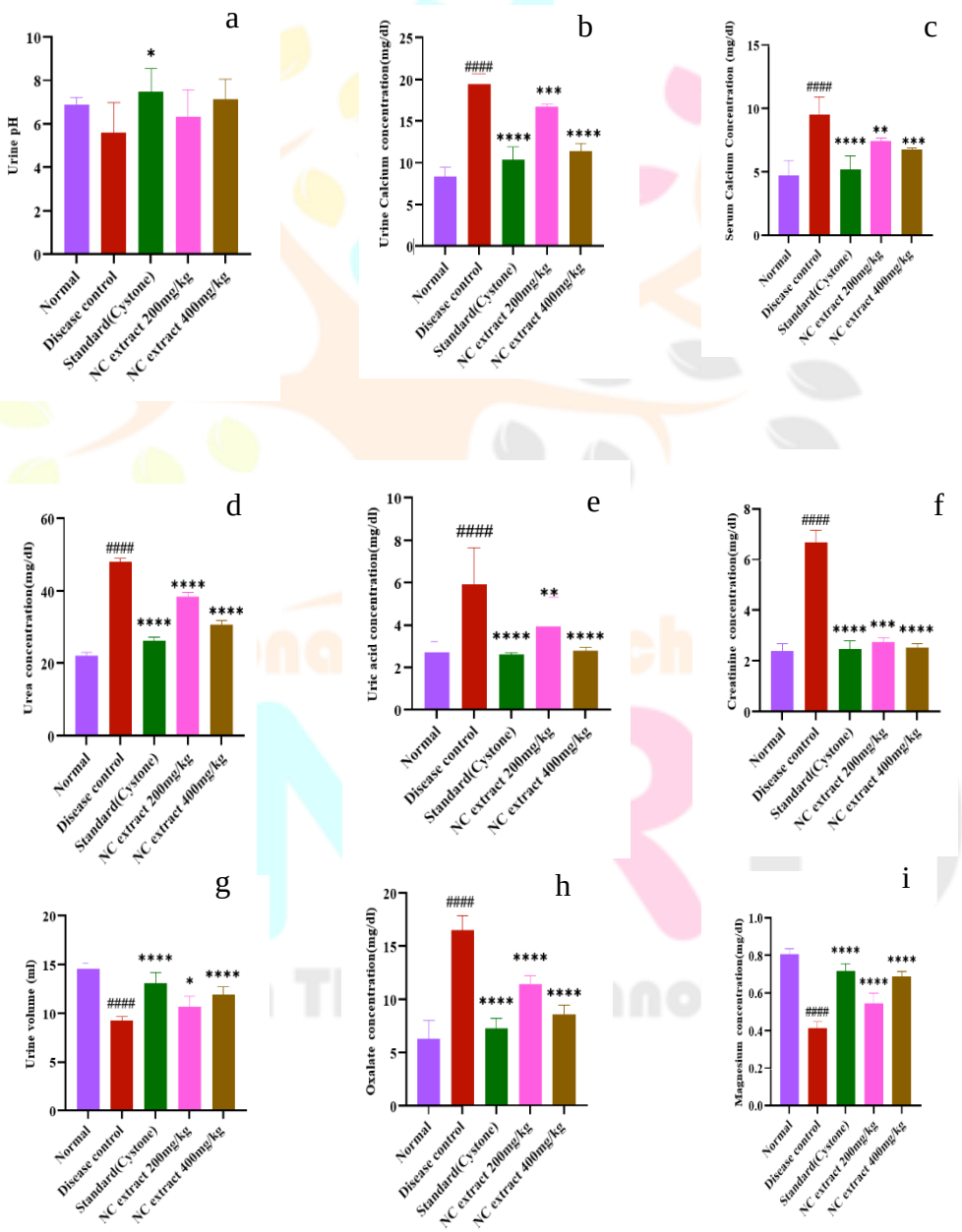

Figure 4: The bar diagrams showing the effects of the Methanolic extract of *S.hispida goaverts* on a) Urine pH b) Urine calcium c) Serum calcium d) Serum urea e) Serum uric acid f) Serum Creatinine g) Urine volume h) Urine oxalate i) Urine magnesium.

Histopathological study

Histological examination of kidney sections under a microscope revealed pronounced dilation of renal tubules, structural disorganization, inflammation, and enlarged interstitial spaces throughout the kidney in the untreated group compared to the control group. However, treatment with Me.Sh at doses of 200 mg/kg and 400 mg/kg, as well as Cystone at a dose of 750 mg/kg, resulted in reduced inflammation, improved integrity of the renal epithelial membrane, and normalization of interstitial spaces between the cells. Me.Sh at a dose of 400 mg/kg demonstrated high effectiveness in mitigating CaOx oxalate-induced epithelial injury.

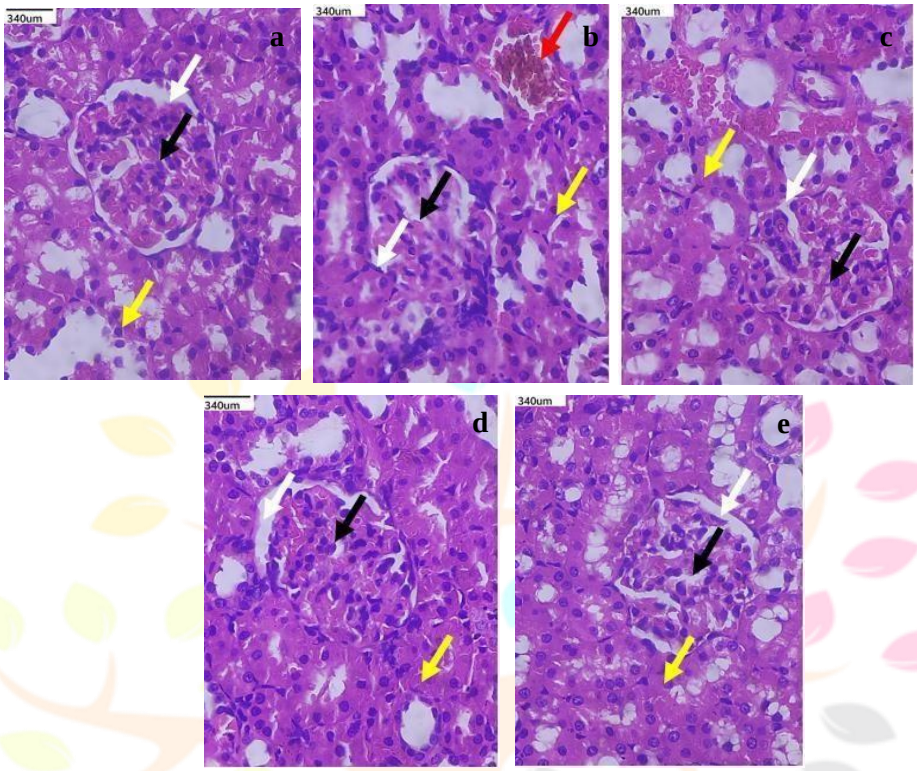

Figure 5: Histopathological slides of kidney sections observed under polarized light microscope (100 X) using Hematoxylin & Eosin staining a) normal b) disease control c) cystone and Me.Sh ; d) 200mg/kg e) 400 mg/kg

V. DISCUSSION

Phytochemical analysis of the Me.Sh seeds revealed the existence of several bioactive compounds. These include flavonoids, alkaloids, proteins, phenols, and terpenoids. This comprehensive profile of phytochemical constituents underscores the potential therapeutic value. The Anti-Urolithiatic activity was may be due to the presence of flavonoids and saponins.

The in-vitro antioxidant study such as DPPH radical scavenging activity and Hydrogen peroxide scavenging assay of Me.Sh seeds was evaluated with standard reference compound, ascorbic acid. The results highlight the ability of Me.Sh to exhibit robust antioxidant activity, suggesting its efficacy in combating oxidative stress and promoting overall cellular health.

In the investigation of the potential medicinal application of Me.Sh seeds in urolithiasis, an in-vitro calcium oxalate dissolution study was conducted, comparing it to the standard drug Cystone. The results revealed that Me.Sh seeds exhibited anti-urolithiatic activity, with a significant maximum dissolution on calcium oxalate crystals. The dissolution ability of Me.Sh seeds increased in a dose dependent manner. Calcification, a multifactorial phenomenon triggered by supersaturation, involves a cascade of events including crystal nucleation, growth, aggregation, and retention. Crystal inhibitors, such as thiazide, decrease the saturation of calcium oxalate and inhibit crystal nucleation, growth, and aggregation. They have also been shown to reduce crystallization in the urine of stone-forming rats. Me.Sh seeds may exert their anti- urolithiatic activity by interfering with crystal growth and aggregation. The methanolic extract of Me.Sh seeds demonstrated promising in-vitro efficacy against urolithiasis.

In the acute toxicity study, a dose of 2000mg/kg was found to be non-toxic for Me.Sh seeds. Therefore, for therapeutic purposes, doses of 200mg/kg/b.w and 400mg/kg/b.w were selected. Repeated administration of ethylene glycol (0.75%V/V) and ammonium chloride (2%) leads to the generation of kidney stones, primarily composed of calcium oxalate. The presence of hyperoxaluria, which increased the urinary concentration of oxalate, was considered a major factor in stone formation. The glomerular filtration rate (GFR), a crucial parameter for renal function, decreased in urolithiasis due to obstruction caused by urinary stone, resulting in reduced urine volume. Animals with induced urolithiasis showed a decrease in urine volume (Group II), while the treated groups (III, IV, V) exhibited an increase in urine volume compared to Group II.

In rats induced with ethylene glycol and ammonium chloride, urine pH was significantly reduced compared to normal animals, treatment with Me.Sh seeds demonstrated a significant increase in urine pH in a dose dependent manner. Chronic administration of 0.75%V/V ethylene glycol and 2% W/V ammonium chloride solution for 28 days resulted in hypercalciuria in rats. Oxalate and calcium excretion were significantly increased ($p<0.0001$), while magnesium levels decreased in the urine of ethylene glycol and

ammonium chloride treated animals (Group II) compared to Group I. Treatment with Me.Sh seeds at doses of 200mg/kg and 400mg/kg body weight, as well as cystone at a dose of 750mg/kg, significantly ($P<0.0001$) reduced the levels of oxalate and calcium in the urine compared to Group II animals. Magnesium levels in the standard and test groups returned to normal levels.

Obstruction in the kidneys due to stone formation leads to the accumulation of waste products in the blood, resulting in increased levels of Blood Urea Nitrogen (BUN), uric acid, and creatinine. Calculi-induced rats (Group II) exhibited renal damage indicated by elevated serum levels of creatinine, uric acid, BUN and calcium. Treatment with Me.Sh seeds (Group IV and V) and cystone (Group III) significantly ($P<0.0001$) reduced the elevated serum uric acid levels compared to Group II.

The anti-urolithiatic effect of Me.Sh seeds was further substantiated through kidney histopathological studies. Kidney sections of untreated rats exhibited abundant crystal depositions, along with evident tubular dilatation and damage manifested by large spaces in the tissue. In contrast, treated rats showed reduced crystal depositions compared to untreated animals, and the extent of necrosis and tubule dilatation was minimal. Renal stone deposition can lead to damage in renal tissue and deterioration of renal function. Renal epithelial injury contributes to crystal retention, and the presence of flavonoid constituents in Me.Sh seeds may play a role in restoring renal antioxidant enzymes and preventing renal cell injury. The lithogenic treatment resulted in impaired renal function in the untreated rats, as indicated by markers of glomerular and tubular damage such as urea, uric acid, and creatinine. However, animals receiving Me.Sh seeds extract showed lowered levels of these markers, indicating the preservation of renal function.

VI. CONCLUSION

In conclusion, the anti-urolithiatic activity of Me.Sh extract demonstrates promising potential in the prevention and treatment of urinary stone formation. Throughout this study, the extract had shown significant beneficial effects that contribute to its anti- urolithiatic properties. Me.Sh extract, derived from a specific plant or herb, contains bioactive compounds that exert diuretic, anti-lithogenic, and anti-inflammatory actions. These properties were crucial in inhibiting the formation and growth of urinary stones. The diuretic effect of Me.Sh increased urine output, facilitating the elimination of stone-forming substances and preventing their crystallization. This promotes the overall health of the urinary system and reduced the risk of stone formation. Additionally, the anti-lithogenic activity of the extract helps in breaking down existing stones, making them easier to pass through the urinary tract. This action can alleviate pain and discomfort associated with urolithiasis and facilitate the expulsion of stones from the body. The antioxidant properties of Me.Sh seeds reduced the stress induced damage in renal epithelial tissue.

Furthermore, the anti-inflammatory properties of Me.Sh play a crucial role in reducing inflammation in the urinary tract. Inflammatory processes can contribute to stone formation and aggravate the symptoms of urolithiasis. By suppressing inflammation, the extract helps in preventing stone recurrence and promoting healing. However, it is important to note that while the anti-urolithiatic activity of Me.Sh is promising, further research is needed to fully understand its mechanism of action, optimal dosage, and potential side effects.

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Declaration of Conflicting Interests

The authors declare no conflict of interest with respect to the authorship, research and publication of this article.

REFERENCES

1. Abu Zarin, Mazni, Joo Shun Tan, Paramasivam Murugan, and Rosma Ahmad. 2020. “Investigation of Potential Anti-Urolithiatic Activity from Different Types of Musa Pseudo-Stem Extracts in Inhibition of Calcium Oxalate Crystallization.” *BMC Complementary Medicine and Therapies* 20 (1): 1–12. https://doi.org/10.1186/S12906-020-03113-0/TABLES/4.
2. Aggarwal, Kanu Priya, Shifa Narula, Monica Kakkar, and Chanderdeep Tandon. 2013. “Nephrolithiasis: Molecular Mechanism of Renal Stone Formation and the Critical Role Played by Modulators.” *BioMed Research International* 2013. https://doi.org/10.1155/2013/292953.
3. Ajeesh Krishna, TP, TP Adarsh Krishna, R Kumuthakallavalli, VN Sanyo Raj, Sanis Juliet, T Shobha Rani, U Darsana, Suresh N Nair, and Reghu Ravindran. 2014. “Physico-Chemical Evaluation and Biochemical Quantification of Crude Drug Powder (Stem) of Chassalia Curviflora (Wall. Ex Kurz.) Thwaites; A Folk Medicinal Plant.” *~ 121 ~ Journal of Pharmacognosy and Phytochemistry* 3 (4): 121–24.
4. Alelign, Tilahun, and Beyene Petros. 2018. “Kidney Stone Disease: An Update on Current Concepts.” *Advances in Urology* 2018. https://doi.org/10.1155/2018/3068365.
5. Baatiah, Nada, Raghad Alhazmi, Fatmah Albathi, Esraa Albogami, Abdullah Mohammedkhalil, and Basim Alsaywid. 2020. “Urolithiasis: Prevalence, Risk Factors, and Public Awareness Regarding Dietary and Lifestyle Habits in Jeddah, Saudi Arabia in 2017.” *Urology Annals* 12 (1): 57. https://doi.org/10.4103/UA.UA_13_19.
6. Baliyan, Siddartha, Riya Mukherjee, Anjali Priyadarshini, Arpana Vibhuti, Archana Gupta, Ramendra Pati Pandey, and Chung Ming Chang. 2022. “Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus Religiosa.” *Molecules (Basel, Switzerland)* 27 (4). https://doi.org/10.3390/MOLECULES27041326.
7. Geraghty, R., K. Wood, and J. A. Sayer. 2020. “Calcium Oxalate Crystal Deposition in the Kidney: Identification, Causes and Consequences.” *Urolithiasis* 48 (5): 377. https://doi.org/10.1007/S00240-020-01202-W.
8. López, Michelle, and Bernd Hoppe. 2010. “History, Epidemiology and Regional Diversities of Urolithiasis.” *Pediatric Nephrology (Berlin, Germany)* 25 (1): 49. https://doi.org/10.1007/S00467-008-0960-5.

9. "Nirali Publication Pharmacognosy 55th Edition By C.K Kokate, A.P Purohit, S.B. Gokhale (English Medium)." n.d. Accessed June 30, 2023. https://shop.exam360.in/2022/01/nirali-publication-pharmacognosy-55th.html.
10. "Pharmacological Evaluation of Mentha Piperita Against Urolithiasis: An In Vitro and In Vivo Study - PMC." n.d. Accessed June 30, 2023. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8777360/.
11. Venkateswarlu, Kambham, Jami Komala Preethi, and K. B. Chandrasekhar. 2016. "ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF TAXILLUS TOMENTOSUS PLANT ON ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED UROLITHIASIS IN WISTAR RATS." *Indonesian Journal of Pharmacy* 27 (2): 66. https://doi.org/10.14499/INDONESIANJPHARM27ISS2PP66.