ANTIUROLITHIC ACTIVITY OF AQUEOUS EXTRACTS OF LEAVES OF *CAPPARIS MOONII IN* ETHYLENE GLYCOL-INDUCED UROLITHIASIS MODEL USING RATS

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IN
PHARMACOLOGY

Submitted by

Mr. SANTHANAM A

Reg. No. 261825211

Under the Guidance of

Prof. DR. R. SHANMUGA SUNDARAM., M.Pharm., Ph,D.,

Vice Principal & HOD

DEPARTMENT OF PHARMACOLOGY

J.K.K.NATTRAJA COLLEGE OF PHARMACY

KOMARAPALAYAM - 638183

TAMILNADU

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CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"ANTIUROLITHIC ACTIVITY OF AQUEOUS EXTRACTS OF LEAVES OF *CAPPARIS MOONII* IN ETHYLENE GLYCOL-INDUCED UROLITHIASIS MODEL USING RATS''** submitted by the student bearing Reg.No: **261825211** to "**The Tamilnadu Dr. M.G.R.Medical University**, **Chennai**", in partial fulfilment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on …….

Internal Examiner **External Examiner**

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Prof. DR. R. SHANMUGA SUNDARAM., M.Pharm., Ph,D.,
Vice Pricncipal & HOD
Department of Pharmacology
J.K.K.Nattraja College of Pharmacy
Kumarapalayam - 638 183

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Prof. Dr. R. Sambathkumar, M.Pharm.,PhD.,
Principal
J.K.K.Nattraja College of Pharmacy
Kumarapalayam - 638 183

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Prof. Dr. R. Shanmuga Sundaram, M.Pharm.,PhD.,
Vice Principal & HOD
Dept. of Pharmacology
J.K.K.Nattraja College of Pharmacy
Kumarapalayam - 638 183

Prof. Dr. R. Sambathkumar, M.Pharm.,PhD.,
Principal
J.K.K.Nattraja College of Pharmacy
Kumarapalayam - 638 183

DECLARATON

I hereby declare that the dissertation **“ANTIUROLITHIC ACTIVITY OF AQUEOUS EXTRACTS OF LEAVES OF CAPPARIS MOONII IN ETHYLENE GLYCOL-INDUCED UROLITHIASIS MODEL USING RATS’’** submitted to “**The Tamil Nadu Dr. M.G.R Medical University - Chennai”,** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work which has been carried out by me during the academic year 2019-2020, under the guidance and supervision of **Prof.Dr.R. Shanmuga Sundaram, M.Pharm.,Ph,D.,** Vice Principal, Head of the Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place: Kumarapalayam **Mr. SANTHANAM A**

Date: **Reg. No.261825211**

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Mr. SANTHANAM A **Reg.No.261825211**

CONTENTS

INTRODUCTION

Urolithiasis is the formation of stones in the urinary tract. The urinary system is the organ system that produces, stores, and eliminates urine. The urinary tract consists of two kidneys, two ureters, one urinary bladder, and one urethra. Kidney stones are common in industrialized nations with an annual incidence of 0.5 to 1.9%. Areas with higher incidence of kidney stones are Scandinavian countries, Mediterranean countries, British Isles, Northern Australia, Central Europe, portions of the Malayan Peninsula, China, Pakistan and northern India whereas the incidence of kidney stone formation is lower in areas like Central and South America and some parts of Africa. In Asia, stone-forming belt has been reported to stretch across Sudan, Saudi Arabia, the United Arab Emirates, the Islamic Republic of Iran, Pakistan, India, Myanmar, Thailand, Indonesia and Philippines. India has higher incidence of urinary calculi especially in Gujarat, Rajasthan, Punjab and Madhya Pradesh. Countries in tropical and subtropical areas have also reported a high incidence of urolithiasis (Rizvi *et al*., 2002). Furthermore, urolithiasis is largely a recurrent disease with a relapse rate of 50% in 5–10 years and 75% in 20 years (Trinchieri, 2008). Thus, urolithiasis imposes substantial economic consequences and a great public health importance.

PATHOPHYSIOLOGY OF RENAL STONE FORMATION

Kidney stone formation is a complex process that results from succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the renal tubules.

Supersaturation and Nucleation

The supersaturation of urine is the driving force behind crystal formation in the kidney. Supersaturation actually refers to a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. These results in nucleation which is defined as the formation of solid crystal phase in a solution. The process of nucleation in a pure solution i.e., formation of initial crystal phase, is known as homogenous nucleation.

In secondary nucleation, the newly formed crystals deposit on pre-existing crystal surfaces of similar type which then results in the mass production of the crystals. However, in another process that is “epit axy”, material of one crystal type is precipitated upon the surface of another type whose lattice dimensions are almost identical. Both these processes referred as “heterogenous nucleation”.

Since urine is not a pure solution, nucleation in urine often occurs over an existing surface. The most heterogenous frequent nucleation sites in urine are epithelial cells, cell debris, urinary casts, other crystals and bacteria. The renal tubular cell injury can promote crystallization of calcium oxalate crystals by providing substances which are the sources for heterogenous nucleation. The renal tubular cell injury followed by the cellular degradation produces numerous membrane vesicles, which have been shown to be potent nucleators of calcium crystals. The crystals observed in the renal tubules of hyperoxaluric rats are reported to be associated with cellular degradation products.

2. Crystal growth

The growth of the crystal phase is determined by the molecular size and shape of the molecules, the physical properties of the material, urinary supersaturation level, pH and defects that may form in the crystal structure. The crystal growth is the driving force for the particle formation and thus for stone formation.

Crystal aggregation

In this process, crystals in solution stick to each other to form larger particles.

The smaller the inter-particle distance, the larger will be the attractive forces and this favors particle aggregation. Crystal aggregation is promoted by viscous binding i.e., foreign crystalline compounds with multiple binding sites, such as abnormally self aggregated macromolecules attach to the crystal surfaces. Thus the macromolecules secreted by the brush border of proximal tubular cells in the urine results in crystallization on the interaction between tubular cells and crystals.

Experimental studies have shown that the injury of renal epithelial cells due to free radicals result in sloughed membrane fragments in the tubular lumen which provides a suitable surface for nucleation of calcium phosphate and oxalate (Khan *et al*., 1999). It is now widely accepted that the process of calcium stone formation in supersaturated urine initiates as a precipitation of calcium phosphate in the loop of Henle or the distal part of the distal tubule (Kok, 1997). Whereas in normal physiochemical conditions, repulsion occurs between the calcium phosphate crystals and tubular cells and thus result in elimination of small calcium phosphate crystals by dissolution in spontaneous urine.

This is then followed by formation of masses of crystals by growth and aggregation leading to the adherence of calcium phosphate crystal aggregates to the tubular surface.

Crystal retention

Crystal retention is the actual association of crystals with the epithelial cells lying in the renal tubules. The initial formation of the crystals in the urine depends on the composition of the tubular fluid, whereas the retention of crystals depends on the composition of the renal tubular epithelial cell surface (Schepers *et al*., 2002). Crystal retention can be caused by the association of crystals with the epithelial cells lining the renal tubules. The process of attachment of endocytosis of crystals to renal tubular cells is generally accomplished by crystal – cell i nteraction. A non-adherent surface of the distal tubules, collecting ducts, ureters, bladder, and the urethra may provide a natural defence mechanism against crystal retention, and may become defective when the anti-adherence properties are compromised.

MANAGEMENT OF STONE DISEASE

The management of stone disease depends on the size and location of the stones. The stones which are smaller than 5 mm have a higher probability of spontaneous passage. In contrast, stones larger than 5 mm, stones in patients with a higher risk of developing renal insufficiency (patients with a single kidney), or stones that fail to pass through should be treated by some interventional procedures including extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL) as well as other therapeutic treatments.

Extracorporeal shock wave lithotripsy (ESWL)

The extracorporeal shock wave lithotripsy is a non - invasive procedure which uses high-intensity acoustic pulse shock waves to fragment calculi. This technique is the most widely used method for managing renal and ureteral stones. However, treatment success rates depend on stone composition, size, properties and location of the stone as well as the instrumentation type and shock frequency. It also needs to be considered that the same forces that are directed at the stones have deleterious effects on surrounding tissues. The damage to almost every abdominal organ system has been, but by far the most common injury is acute renal hemorrhage although its true incidence is unclear and poorly defined. The most often renal hemorrhage can be managed conservatively; however, in rare instances the complications are fatal. The reports of post - ESWL perirenal hematoma range from less than 1% to greater than 30% . Furthermore, ESWL has also been associated with long-term medical effects such as diabetes mellitus and hypertension.

Ureteroscopy (URS)

In addition to ESWL, other procedures such as ureteroscopy have also been developed for removal of ureteral stones. Ureteroscopy is usually performed with an endoscope that is "uteroscopes" which is passed thr ough the urethra, bladder, and then directly into the ureter. The new generations of uteroscopes are flexible, smaller in diameter, stiffer and more durable, and have an improved tip deflection. However, there are many drawbacks also associated with this technique. The major drawback of URS is that it is more invasive than ESWL and the rate of ureteric perforation and stricture formation remains around 2 to 4%. In contrast, the major advantage of URS is that it is cheaper and results in higher and faster stone free rates.

Percutaneous nephrolithotomy (PCNL)

Percutaneous nephrolithotomy is a surgical procedure to remove stones from the kidney by a small puncture wound through the skin. It is most suitable to remove stones of more than 2 cm in size and which are present near the pelvic region. However, this also involves several complications like parenchymal bleeding, septicaemia and colonic or pleural injury.

THERAPEUTIC TREATMENTS

Along with the other interventional procedures as described earlier, therapeutic agents are also used routinely. The most effective hypocalciuric agents are thiazide diuretics which hypocalciuric action enhances calcium reabsorption in the distal renal tubules. However, long-term use in up to 50% of patients is limited because of side-effects including fatigue, dizziness, impotence, musculoskeletal symptoms, or gastrointestinal complaints . Another reported complication is thiazide - induced potassium depletion, which causes intracellular acidosis and can lead to hypokalemia and hypocitraturia (Moe, 2006). Potassium citrate is effective in the treatment of patients who have calcium stones and normal urinary calcium. By providing an alkali load, potassium citrate increases urinary pH and citrate, therefore mediating the inhibitory effects of macromolecular modulators of calcium oxalate crystallization. The main limitation for a more widespread use of alkali citrate preparations is the relatively low tolerability of available alkali citrate preparations. Adverse effects that reduce treatment compliance have been noted mainly in the gastrointestinal tract and include eructation, bloating, and diarrhea (Mattle and Hess, 2005). In conclusion, none of the listed treatment modalities is without any side-effects. Thus, the focus should be on the development of novel strategies for the prevention and treatment of kidney stone disease. Herbal medicines could close a gap in this regard.

SCREENING MODELS FOR INDUCING UROLITHIASIS

Several animal models have been developed to investigate hyperoxaluria and its consequences.Calcium oxalate type of kidney stones are produced in rats by the induction of acute or chronic hyperoxaluria (Khan and Hackett, 1987; Khan, 1991) using a variety of agents such as sodium oxalate, ammonium oxalate, hydroxy-L-proline, ethylene glycol, and glycolic acid. These lithogenic agents are generally administered either orally in food or water or by gavage but have also been injected intraperitoneally.

The acute hyperoxaluria can be induced by intraperitoneal administration of sodium oxalate (30, 70, or 100 mg/kg body weight of rat) resulted in increased urinary excretion of oxalate and an almost instant appearance of calcium oxalate crystals in lumina of the renal proximal tubules. The crystals were later seen in collecting ducts of the cortex and papilla. The amount and duration of urinary excretion of excess oxalate and the size, number, and location of crystals within the kidneys depended on the amount of sodium oxalate given. However, the largest amount of oxalate was excreted within the first 6 h of the challenge. At the lower dose of sodium oxalate, crystals were restricted to the tubular lumens and cleared the kidneys within a few days. At higher doses, crystals were initially located in tubular lumina and then they were later seen in the interstitium. Apparently, some crystals and crystal aggregates remained small and therefore, did not adhere to the renal epithelium and moved with the urine and finally were flushed out. The larger crystals and their aggregates also moved but at a slower rate.

Renal CaOx deposition induced by ethylene glycol is most appropriate model which is frequently used to mimic the urinary stone formation in human beings. In the most common model, ethylene glycol, a precursor of oxalate, is given to rats in their drinking water, with or without additional ammonium chloride or vitamin D. Today it is used as an experimental model in many *in vivo* systems. It is without doubt, the most intensively studied lithogen with dose ranges from 0.5% to 1.5%.

Ethylene glycol is a colourless, odourless, sweet-tasting and relatively non-volatile liquid. It has a low vapour pressure and is completely miscible in water. Ethylene glycol is used in the manufacture of polyethylene terephthalate, in natural gas processing, and as an antifreeze agent.

The step-wise metabolism of ethylene glycol in liver, proceeds in a nicotinamide adenine dinucleotide (NAD) - dependent fashion. The first step is oxidation of ethylene glycol to glycoaldehyde by alcohol dehydrogenase. Subsequently, glycoaldehyde is oxidised to glycolic

acid, to glyoxylic acid and finally to oxalic acid. Because the conversion of glycolic to glyoxylic acid is the rate-limiting step in this process, the accumulation of glycolic acid is largely responsible for the metabolic acidosis seen in this. Approximately 80% of an absorbed dose of ethylene glycol is hepatically metabolized, with the remainder excreted unchanged by the renal system.

Renal CaOx deposition induced by ethylene glycol is associated with proximal tubule cell necrosis leading to production of several metabolites glycolaldehyde, glycolate, glyoxylate and oxalate, in order) and accumulation of large CaOx crystals in tubular lumen have reported the induction of severe calcium oxalate crystalluria, both biochemically and histopathologically in rats treated with ethylene glycol in drinking water. In rats fed diets containing ethylene glycol for 16 weeks, the development of nephropathy correlated highly with the kidney oxalate crystal accumulation in response to chronic treatment. The chronic hyperoxaluria can be induced by the administration of 0.75% ethylene glycol alone in drinking water which shows persistent crystalluria. Initially, small dipyramidal crystals were seen in the urine. Later, most of the urinary crystals were develop into large aggregates of dumbbell-shaped CaOx monohydrate crystals and twinned CaOx dihydrate crystals. The crystals were shown to be located in both the cortex and the medulla. During chronic hyperoxaluria, crystals were initially distributed randomly in the renal medulla. Eventually, collecting ducts at the renal papillary tip and papillary base were the preferred sites of crystal deposition. Most crystals were found to be intraluminal aggregates. After 4-6 weeks of ethylene glycol administration, crystals were seen to be developing between the tubular epithelial cells as well as inside the epithelial cells and the interstitium. These stones contained both CaOx mono- and dihydrate crystals and reached a size of over 1000 µm, occupying and c alcifying the entire papillary tip. The light microscopy and scanning and transmission electron microscopic studies of the papillary stones revealed that they originated in the lumina of collecting ducts near the renal papillary surface.

The acute and chronic hyperoxaluria induced by ethylene glycol resulted in increased crystal deposition which was also associated with increased urinary excretion of the membrane marker enzymes alkaline phosphatase, and gamma- glutamyl transpeptidase. The renal tubular epithelium of nephrolithic kidneys was damaged with the crystals.

The other degenerative changes in epithelial cells included an increase in the number of lysosomes, swelling of mitochondria, dilatation of endoplasmic reticulum, cytoplasmic edema,

and vacuolization. Some cells appeared burst open and release their contents into the tubular lumen, whereas others sheared away from the basal lamina. The renal papillary surfaces were shown to be badly damaged. The intercellular spaces between the intact epithelial cells also reported to be enlarged. In addition, the injury caused by the exposure to elevated levels of oxalate and CaOx crystals may reduce the crystallization-inhibitory activity of the urine. The CaOx crystallization was also seen in all the rats administered with 0.5% ethylene glycol, but to a lower extent.

Oxidative stress imposed by reactive oxygen species (ROS) plays a crucial role in the pathophysiology associated with kidney stone disease. The ethylene glycol-induced hyperoxaluria and crystal deposition was reported to be associated with renal cell damage along with lipid peroxidation suggesting that cell injury due to the production of free radicals is augmented by crystal deposition in the renal tubules. A study conducted in Sprague Dawley rats, has reported that ethylene glycol-induced hyperoxaluria is accompanied by enzymuria, which is suggestive of renal tubular damage. Furthermore, levels of antioxidative enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione transferase (GST) in the renal cortex were significantly decreased with the concomitant increase in renal cortical content of lipid hydroperoxide (Green *et al*., 2005).

A previous study performed to ascertain the effect on oxidative injury in response to treatment with ethylene glycol for 28 days have indicated that ethylene glycol caused a variety of changes like alterations in the activities/levels of renal tissue enzymic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase and glucose-6-phosphate dehydrogenase) and non-enzymic (reduced glutathione, ascorbate and α-tocopherol) antioxidants, along with high malondialdehyde levels in the male hyperoxaluric rats (Coothan *et al*., 2007).

INTRODUCTION TO HERBAL DRUGS

Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. Interest in natural products research is strong and can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques to detect biologically active natural

products, improved techniques to isolate, purify and structurally characterize these active constituents and advances in solving the demand for supply of complex natural products. The use of herbal remedies for prevention and cure of ailments is of increasing interest due to the superiority and efficiency of activity provided by phytoconstituents in herbs and undesirable effects of modern medicine. Modern medicines are proved to target only one aspect of urolithiatic pathophysiology whereas herbal remedies have been shown to exert effectiveness at different stages of stone pathophysiology.

Herbal remedies produce multiple mechanism of action such as diuretic activity (beneficial in increasing the urinary volume that allows the easy passage of small calculi out of the body in urine), crystallization inhibition activity (helps to inhibit the different stages of stone formation by maintaining the balance between inhibitors and promoters of stone formation), lithotriptic activity (avoid binding mucin of calculi to prevent crystal aggregation to form a large stone) and antioxidant activity (prevent renal tissue injury). The traditional herbs also improve the renal function and regulate oxalate metabolism which help in reducing the re-occurrence of renal calculi.

The vast Ayurvedic literature claims a number of plants to be useful in urinary stones. It is now widely accepted that most herbs exhibit their effects by a variety of chemical constituents present therein and the idea of synergy within and between them is also gaining acceptance. Renal stone was well known in Ayurveda. Aqueous extract of *Costus spiralis* reduced the growth of calcium oxalate calculi in the urinary bladder of rats. Fourteen patients with renal calculi and sixteen patients with ureteric calculi have been treated with the herbomineral combination containing *Bergenia ligulata* and *Tribulus terrestris*; 28.57% of patients with renal calculi and 75% patients with ureteric calculi passed their calculi completely and in other patients there was a marked or partial expulsion of calculi along with changes in the shapes and sizes of calculi. *Crataeva nurvala* was reported to be effective in the prophylaxis of oxalate antiurolithiasis induced by simultaneous administration of sodium oxalate and methionine in guinea pigs. Lupeol, a triterpene compound has been isolated from *Crataeva nurvala* and was shown to have dose related prophylactic and curative activities in albino rats when studied by foreign body insertion method using glass beads.

Tamarindus indicus intake at the dose of 10 gm per patient showed significant beneficial effect in inhibiting spontaneous crystallization in both normal subjects and in stone formers. The

ethanolic extract of *Ammania baccifera* was found to be effective as prophylactic and curative against phosphate type of stones. This has given rise to stimulation in the search for investigating natural resources showing antiurolithiatic activity. It is necessary to explore extensively the potential usage of medicinal plants with traditional claims to be having activity against urolithiasis and subject them to systematic phytochemical and pharmacological study. It is under this context lies the relevance of the present study which intends to study the antiurolithiatic properties of two indigenous and widely distributed medicinal plants namely: *Bergenia ciliata* and *Dolichos biflorus*

D) PHYTOCHEMICAL CONSTITUENTS

Analysis of seeds showed moisture 11.8%, crude protein 22.0%, fat 0.5%, mineral matter 3.1%, fibre 5.3%, carbohydrate 57.35%, calcium 0.28% and phosphorous 0.39%; iron 7.6mg, nicotinic acid 1.5 mg, carotene 119 (international vitamin unit A unit) per 100 gm and rich in various enzymes. Other chemical constituents present are quercetin, streptogenin, beta-sitosterol, a phyto-haemagglutinin, beta-N-acetylglucosaminidase, alpha and beta galactosidases, alpha mannosides and beta glucosides.

SCOPE OF THE STUDY

Urolithiasis is a major healthcare problem worldwide because it appears to be increasing substantially in incidence and its incidence is very high in Asian countries. Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutics, natural products still remain as one of the best reservoirs of new structural types. Many plants have been in use since the ancient times for the treatment of various urolithiatic disorders. This presumptive evidence of efficacy renewed the interest in plant based medicines, especially in the treatment of urolithiasis. Instead of screening randomly selected synthesized chemicals against available targets, screening of traditionally claimed plants is more logical. Hence, there is a need to generate systematic scientific evidence for the activity and study the phytochemical aspects of the potential plants.

The central event in urolithiasis is hyperoxaluria and hypercalciuria with accumulation of large calcium oxalate monohydrate crystals. Ethylene glycol, is a potent lithogen, is known to cause hyperoxaluria and is normally used to induce urolithiasis in animal models. Therefore the initial phase of the present study was focused on determination of the best dose of ethylene glycol for induction of urolithiasis in rat model.

As medicinal plants, indeed, are gaining universal agreement as potential drugs, *B. ciliata* and *D. biflorus* was selected for the study that has several biological effects. The present study was designed to investigate antiurolithiatic property of *B. ciliata* and *D. biflorus in vivo* by biochemical and histopathological analysis.

Then the involvement of reactive oxygen species in pathophysiology of calcium oxalate stones was determined and the effect of medicinal plants on modulation of antioxidant markers was also evaluated.

Calcium oxalate is the primary constituent of the majority of stones formed in the urinary system of patients with urolithiasis. Therefore, *in vitro* anticrystallisation property of the selected plants for calcium oxalate was determined.

The protective effects of natural medicinal herbs are due to the presence of active components in them. Therefore, phytochemical studies of both the plants were also conducted and HPLC analysis was used to identify and quantify major active components present in the plant extracts.

Kidney Stones

The incidence of kidney stones has been increasing in western societies in the last five decades, in association with economic development. Most calculi in the urinal system arise from a common component of urine, e.g. calciumoxalate (CaOx), representing upto 80% of analyzed stones. Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of Extracorporeal ShockWaveLithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones.

However, in addition to the traumatic effects of shock waves, persistent residual stone fragments and the possibility of infection, suggest that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence. .Urolithiasis is still a mysterious disease even after extensive research in urology.Sophisticated instruments, investigation etc., have failed to trace out the exact mechanism of urolithiasis, but they are manifesting this condition.The treatment in modern medicine is not only expensive but also not easily affordable to the needy poor. Actually there is no satisfactory drug in modern medicine which can dissolve the stone and the physician remains to be depending on alternative systems of medicine for better relief.

Herbal medicines are efficacious and have lesser side effect compared to modern medicines and also reduce the recurrence rate of renal stone Although the complete mechanism of action of these remedies are lacking but, plant based phytotherapeutic agents represent the chiefity used in medicine for urolithiasis. Unlike allopathic medicines which targets only one aspectof urolithiatic pathophysiology, most of the plant based therapy have been depictn to be effective at different stages of stone pathophysiology. The plant based drugs exerts their antilithogenic property by altering the ionic composition of urine i.e. decreasing the calcium ion concentration or increasing the magnesium and citrate excretion. These remedies also express diuretic effect or lithotripticactivity. Drug with multiple mechanisms of protective action may be one way forward in minimizing tissue injury in human. Herbal medicines have several phytoconstituents and exert their beneficial effects in urolithiasis by multiple mechanisms.

1. Helps in spontaneous passage of calculi by increasing urine volume, PH and anti-calcifying activity.
2. Balance the inhibitors and promoter of the crystalisation in urine and effects the crystal nucleation, aggregation and growth(crystallisation inhibition activity)
3. Relieves the binding mucine of calculi (lithotriptic activity)
4. Improve renal functions.
5. Regulate oxalate metabolism.
6. Regulate the crystalloid colloid imbalance and improve renal function, thus prevents recurrence of urinal calculi
7. Improve renal tissue antioxidant status ans cell membranenintegrity and prevent reoccurrence (Antioxidant activity)
8. Exerts noteworthy anti-infective action in aligned with the chief causative organisms (antimicrobial activity).
9. Reveals marked improvement in symptoms of urinal calculi like pain, burning micturation and haematuria (Analgesic and anti-inflammatory activity)

In Herbal treatment of kidney stones, drugs used to dissolve the stone or aid their passing to guard aligned with further retention. Diuretic action is also needed to increasing the amount of fluid going through the kidneys and flush out the deposits (Arafat, OM, 2008). Lithotripsy means breaking and disintegrating or dissolution of the preformed stones. Some of the drugs increasing the urine volume decreasing the saturation of the salts and prevents the precipitation of the

crystals at physiological pH.Some of the herbal drugs disaggregatemucoproteins, which actually binds the crystal to the renal cells. Stones occur when urinal chemistry consequences in increase concentrations of stone salts (Oxalates, Calcium, Phosphates) that leads to super-saturation (SS) and exceeds the limit of metastability for that salt in solution.Increased urine volume decreases the saturation of the salts and prevents the precipitation of the crystal at physiological pH. All herbal medicines used for the treatment of the urolithiasis also have diuretic action and some are known to alkalize the urine.

Inhibitors are defined as molecules that increase the Super Saturation (SS) required to initiate nucleation, decrease crystal growth rate and aggregation, and inhibit secondary nucleation. In contrast promoters reduce the formation product of the supersaturated solution. Some of the common promoters are oxalate, calcium, cystine, uric acid and inhibitors are citrate and magnesium. An imbalance between urinal-promoting and inhibiting factors has been suggested as more important in urinal stone formation than a disturbance of any single substance. An assortment of physiological inhibitors of urolithiasis found in urine including inorganic (e.g., magnesium) and organic (e.g., Citrate, Urinal prothrombin fragment, Glycosaminoglycans (GAGs) and other macromolecule) substances are known to inhibit stone formation. Organic inhibitory compounds adsorb to the surface of the crystal, thereby inhibiting crystal nucleation, growth and aggregation. Interference with crystal growth and aggregation therefore seems a possible therapeutic strategy for the prevention of recurrent stone disease. The medicinal plants contain chemical compounds like Glycosaminoglycans (GAGs) which themselves possess an inhibitor effect in the crystallization of calcium oxalate. Macromolecule of higher molecular weight of plant extract excerpts their action similar to natural urinal inhibitors and inhibits crystal.

In urine there are a number of crystalloids of different types (oxalate, uric acid, calcium, cystine) which are kept in solution by the presence of colloids (mucin and sulphuric acid) in the urine by the process of absorption. When there is imbalance in the crystalloid-colloid ratio, i.e., increase in crystalloid and fall in colloid level leading to formation of renal stones or when the colloid lose the solvent action or adhesive property, urinal stones are formed.In this condition the Glomerular Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinal system. Due to this, the waste products, particularly nitrogenous substances such

as urea, creatinine and uric acid get accumulated in blood. Herbal therapy improves the renal function by increasing the excretion of urea and creatinine. Most of the phytothereupatic agent exerts their antiurolithiatic effect through this mechanism given.

Hyperoxaluria is a most noteworthy risk factor in the pathogenesis of renal stone. It has been perceived and documented that oxalate play an important role in stone formation and has about 15-fold greater effect than urinal calcium. Increased oxalate concentration is responsible for precipitation and deposition of CaOx crystals. Herbal extract interfere with the metabolism of oxalate in male rats fed sodium glycolate. Glycolate feeding consequenceed in hyperoxaluria as well as increased activities of oxalate synthesizing enzymes of the liver i.e., glycolate oxidase (GAO), glycolate dehydrogenase (GAD) and lactate dehydrogenase (LDH), and lessened kidney LDH activity. Increased excretion of phosphorus has been perceived and documented in stone formers. Increased urinal phosphorus excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which induces calcium oxalate deposition. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of GAGs. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Supersaturation of these urinal colloids consequences in precipitation as crystal initiation particle which when trapped acts as a nidus leading to subsequent crystal growth and Cystone (polyherbal formulation) maintain crystalloid-colloid balance by decreasing excretion of urinal calcium, oxalate, uric acid, phosphorus and protein in urolithiasis.

In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of aqueous and alcoholic extract of *Capparis moonii* using ethylene glycol induced hyperoxaluria model in rats and some relevant symptoms like anti-analgesic, anti-inflammatory, antioxidant and antimicrobial activity studies also validated.

Calcium oxalate is the primary constituent of the majority of stones formed in the urinary system of patients with urolithiasis. Therefore, *in vitro* anticrystallisation property of the selected plants for calcium oxalate was determined.

The protective effects of natural medicinal herbs are due to the presence of active components in them. Therefore, phytochemical studies of both the plants were also conducted and HPLC analysis was used to identify and quantify major active components present in the plant extracts.

Figure No 1. An assortment of parts of Kidney

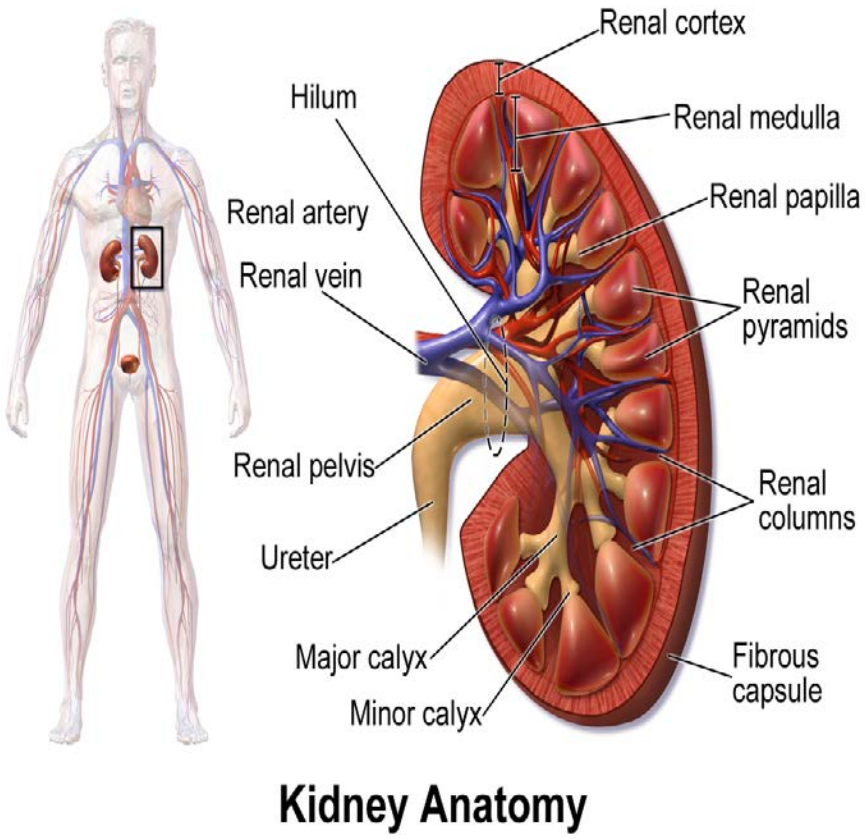
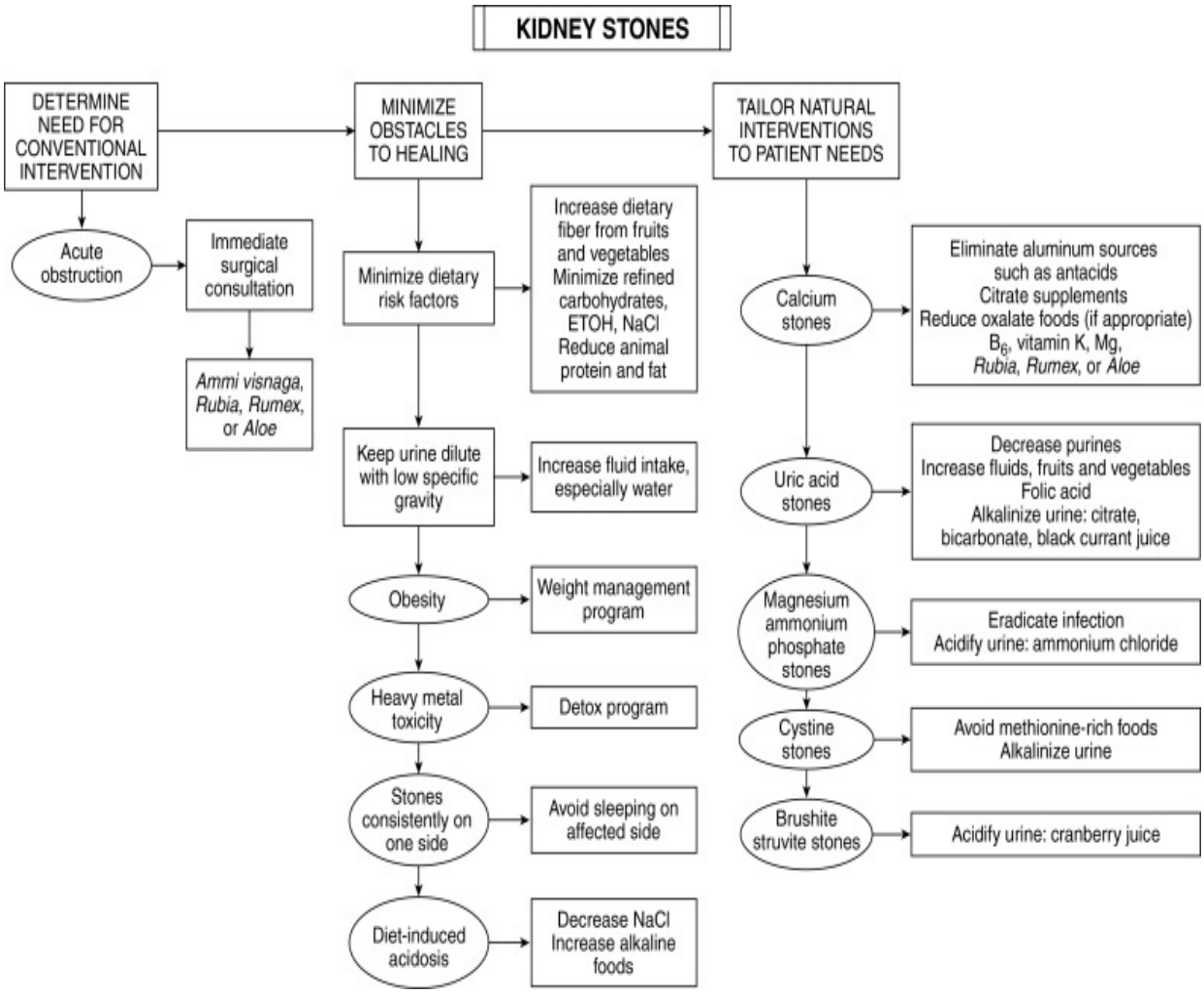


Figure No 2. Mechanism of Kidney Stone Formation



1.2. Classification of renal stones

Kidney stones may contain an assortment of combination of chemicals. The four most common types of kidney stones contain.

- Calcium
- Struvite
- Uric acid
- Cystine

1.3. Etiology of stone formation

An etiology of the urinal calculi is by no means clear, but the following possible factors may be considered.

- Stones can be classified into those caused by infection, or non-infectious causes, genetic defects or adverse drug effects (drug stones)
- **Non-infection stones**
  - Calcium oxalate
  - Calcium phosphate (including brushite and carbonate apatite)
  - Uric acid
- **Infection stones**
  - Magnesium ammonium phosphate
  - Carbonate apatite
  - Ammonium urate
- **Genetic causes**
  - Cystine
  - Xanthine

  2, 8-dihydroxyadenine
- **Drug stones**
- **Dehydration**
- **p^H of the urine**
- **Concentration of urinal salts**
- **Vitamin a deficiency**
- **Parathyroid hormone**
- **Prolonged immunity**
- **Nephrocalcinosis**

1.4. Stone composition

Metabolic aspects are important in stone formation, and metabolic evaluation is required to rule out anydisorders. Analysis in relation to metabolic disorders is the basis for further diagnostic and managementdecisions. Stones are often formed from a mixture of substances.

Most renal calculi contain calcium, usually in the form of calcium oxalate (CaC_2O_4) and often mixed with calcium phosphate ($CaPO_4$). In most instances no specific cause can be identified, although most patients have idiopathic hypercalcuria without hypercalcaemia.

Brushite is a unique form of calcium phosphate stones that tends to recur quickly if patients are not treated aggressively with stone prevention measures and are resistant to treatment with shock wave lithotripsy.

Interestingly hyperuricosuria is also associated with increased calcium containing stone formation, and is thought to be related to the uric acid crystals on which calcium oxalate and calcium phosphate can precipitate.

Rarely the underlying cause is primary oxaluria a liver enzyme deficiency leading to massive medullary nephrocalcinosis and renal failure.

Small asymptomatic stones in the kidney can be safely ignored, and if patients maintain good states of hydration, the risk of recurrent symptoms can be dramatically abridged. In all settings a search for a possible underlying cause of hyperoxaluria/hypercalcuria should be sought and if present corrected when possible.

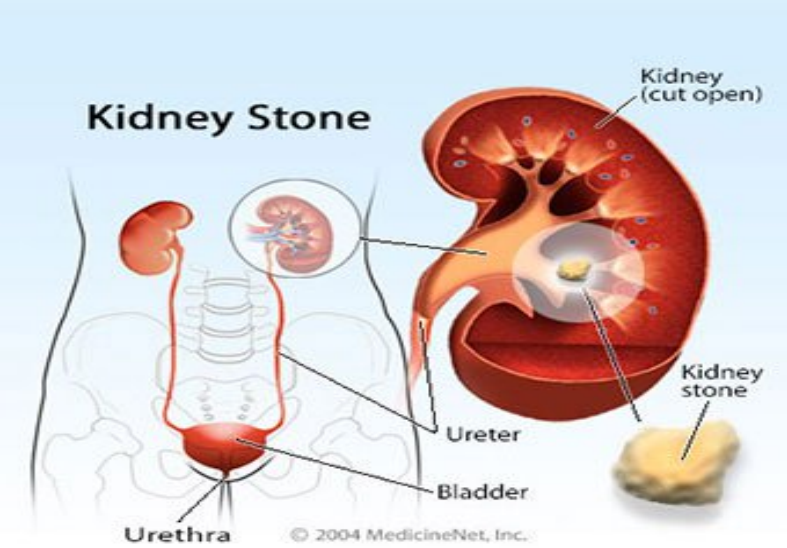
1.5. Struvite stones

Struvite (magnesium ammonium phosphate) stones are usually seen in the setting of infection with urease producing bacteria (e.g. *Proteus*, *Klebsiella*, *Pseudomonas* and *Enterobacter*), consequenceing in hydrolysis of urea into ammonium and increase in the urinal pH. They can grow very large and form a cast of the renal pelvis and calices consequenceing in so-calledstaghorn calculi. The struvite accounts for approximately 70% of these calculi, and is usually mixed with calcium phosphate thus rendering them opaque. Uric acid and cystine are also found as minor components. Struvite stones are usually large (staghorn calculi) and consequence from infection. These stones need to be treated surgically and the entire stone removed, including small fragments, as otherwise these residual fragments act as a reservoir for infection and recurrent stone formation.

1.6. Uric acid

Hyperuricosuria is not always associated with hyperuricoaemia, and is seen in a variety of settings, although in most instances uric acid stones occur in patients with no identifiable underlying aetiology. Uric acid crystals form and remain insoluble at acidic urinal pH below 5.

Figure No 3. Location of Kidney Stone



1.7. Cystine stones:

Cystine stones are also formed in acidic urine, and are seen in patients with congenital cystinuria.Cystine stones may be difficult to treat and are difficult to shatter with ESWL. Hydration and alkalinisation are usually first line therapy.

1.8. Ephedrine Calculi:-Ephedrine and its metabolites (norephedrine, pseudoephedrine, and norpseudoephedrine) are sympathomimetic agents that have been used for the treatment of enuresis, myasthenia gravis, narcolepsy, and rhinorrhea. In addition to numerous side effects, ephedrine and its derivatives have been associated with the production of urinal stones (Blau, 1998). The diagnosis of these calculi is similar to that of other radiolucent calculi. Twenty-four hour urine metabolic analyses can aid in identifying ephedrine or its respective metabolites.

1.9. Guaifenesin Calculi:-Guaifenesin is a widely used expectorant that has been recently associated with nephrolithiasis. Guaifenesin calculi are radiolucent and present in patients who

ingest this medication in excess. Twenty-four hour urine metabolic analysis can aid in the identification of guaifenesin or b-2-methoxyphenoxy-lactic acid.

1.10. Indinavir Calculi:-Indinavirsulfate (Crixivan) is currently one of the most frequently used protease inhibitors used aligned with human immunodeficiency virus, the virus that causes AIDS. The incidence of calculi in patients taking indinavir ranges from 3% to 20%. Indinavir calculi are radiolucent when they are pure, and are radiopaque when they contain calcium.

1.11. Xanthine Calculi:-These stones occur due to a rare hereditary condition with xanthine oxidase deficiency.The deficiency in these enzyme consequences in lessened levels of serum and urinal uric acid. Acidic urine causes crystal precipitation, consequenceing in stone formation. These stones are also seen in patients treated with iatrogenic inhibition of xanthine oxidase with xanthine oxidase inhibitors for hyperuricosuria such as allopurinol.

1.12. Causes of kidney stones

- Age
- Gender
- Diet
- Family history
- Urinal infections and blockage of the urinal tract
- Kidney diseases, such as cystic kidney disease
- Medicinal condition like gout
- Excess vitamin d intake
- Metabolic disorders, such as hyperparathyroidism.
- Certain medications such as diuretics,calcium based antacids
- Inherited disease such as cystinuria, hyperoxaluria, hypercalciuria or hyperuricosuria.

1.13. Kidney **stones diagnosis**

In order to diagnose a patient with kidney stones, doctors will typically:

- Gather a complete medical history
- Ask about the patient’s occupation
- Ask about the patient’s food habits
- Order laboratory tests, which include urine and blood tests

1.14. Laboratory tests:

- X-rays
- Ultrasound (sonogram)
- CT (computed tomography) scan
- Intravenous pyelogram IVP)

1.15. Treatments for kidneystone

- Most of the kidney stones pass through the urinal system with plenty of water.
- Extracorporeal shock wave lithotripsy
- Ureteroscopy

Larger stones may be treated with:

- Extra corporeal Shock Wave lithotripsy(ESWL)
- Percutaneous nephrostomynt
- Tunnel surgery

Medical Management:

Effective kidney stone prevention is dependent on the stone type and identification of risk factors for stone formation. An individualized treatment plan incorporating dietary changes, supplements, and medications can be developed to help prevent the formation of new stones. Certain conservative recommendations should be made for all patients regardless of the underlying etiology of their stone disease. Patients should be instructed to increase their fluid intake in order to maintain a urine output of atleast 2,000 ml/day. Patients should also limit their dietary oxalate and sodium intake, thereby decreasing the urinal excretion of oxalate and calcium. A restriction of animal proteins is encouraged for patients with "purine gluttony" and hyperuricosuria.

1.16. Chemical composition of stones:

There are several types of renal stones that differ in composition and pathogenesis. The most common type of kidney stone is composed of calcium oxalate and is caused by metabolic disorders that are often treatable.

1.17. Calcium stones

Most stones contain calcium combined with oxalate and phosphate or occasionally uric acid. All calcium stones are radio-opaque, and calcium oxalate and calcium phosphate stones are black, grey, or white and small dense and sharply circumscribed on radiographs

1.18. Hypercalciuria

Hypercalciuria or hypercalcinuria is the condition of elevated calcium in the urine. Chronic hypercalcinuria may lead to impairment of renal function nephrocalcinosis, and renal insufficiency.

1.19. Hypocitrauria

It's also associated with renal litho genesis. Citrate acts in the tubular lumen combining with calcium to form a non-dissociable but soluble complex. Hypocitraturia could consequence from a cause of intracellular acidosis such as nephritic failure potassium deficiency, distal renal tubular acidosis, chronic diarrhea state, and drugs such as acetazolamide.

Some studies suggest people who take supplemental calcium have a higher risk of developing kidney stones, and these findings have been used as the basis for setting the recommended daily intake for calcium in adults. In the Womens Health Initiative, postmenopausal women who consumed 1000 mg of auxiliary calcium and 400 units of vitamin D per day in seven years had a 17% higher risk of developing kidney stones than subjects taking a placebo.

1.20. Uric acid stone

Uric acid stones are smooth, round, yellow orange and nearly radio graphically transparent unless mixed with calcium crystals or struvite. Diets high in purines, especially those containing meats and fish, consequence in hyperuricosuria, and in combination with low urine volume and low urinal p^H can exacerbate uric acid stone formation.

1.21. Struvite or phosphate stones

Struvite is a crystalline substance composed of magnesium ammonium phosphate. Radiographs depict Struvite stones as large, gnarled, and laminated. They are associated with substantial morbidity infection. Signs of Struvite stones include urinal p^H greater than 7, stag horn calculi, and urease that grow bacteria on culture.

1.23. Cystine stone

Formation of cystine stone is the only clinical expression of cystinuria an autosomal recessive intestinal and renal tubular disorders of four amino acids.

Cystine, Arginine, Lysine, Ornithine.

People who are homozygous for Cystinuria excrete more than 600mg per day of insoluble cystine. The stones are greenish –yellow flecked with shiny crystals and are moderately radio – opaque rounded appearance.

1.24. Protease –related stone

This the newest type of stone described. The increasing incidences of HIV-positive patients have led to widespread use of the protease inhibitor Indinavir sulphate. Although the drug is generally well tolerated, it can be associated with urolithiasis 4-12% of patients. It thus may coexist or from a nidus for indinavir stones vice versa.

1.25. Prevention of stone

The first step in preventing kidney stones is to understand what is causing the stones to form. The health care provider may ask the person to try to catch the kidney stone as it passes, so it can be sent to a lab for analysis. Stones that are retrieved surgically can also be sent to a lab for analysis.Kidney stones may be prevented through changes in eating, diet, and nutrition and medications.

1.26. Diet and Nutrition

People can help prevent kidney stones by making changes in their fluid intake. Depending on the type of kidney stone a person has, changes in the amounts of sodium, animal protein, calcium, and oxalate consumed can also help. Drinking enough fluids each day is the best way to help prevent most types of kidney stones. Health care providers recommend that a person drink 2 to 3 litters of fluid a day. People with cystine stones may need to drink even more. Though water is best, other fluids may also help prevent kidney stones, such as citrus drinks.

The following recommendations based on the specific type of kidney stones,

Calcium Oxalate Stones

- Reduction of sodium intake
- Reduction of animal protein, such as meat, eggs, and fish
- Getting enough calcium from food or taking calcium supplements with food

- Avoiding foods high in oxalate, such as spinach, rhubarb, nuts, and wheat bran

Calcium Phosphate Stones

- Reduction of sodium intake
- Reduction of animal protein
- Getting enough calcium from food or taking calcium supplements with food

Uric Acid Stones

- Limiting animal protein

Medications

The health care provider may prescribe certain medications to help prevent kidney stones based upon the type of stone formed or conditions that make a person more prone to form stones:

Hyperuricosuria	: Allopurinol (Zyloprim), which decreases uricacid in the blood and urine
Hypercalciuria	: Diuretics, such as hydrochlorothiazide
Hyperoxaluria	: Potassium citrate to raise the citrate and pH of urine
Uric acid stones	: Allopurinol and potassium citrate
Cystine stones	: Mercaptopropionyl glycine, which decreases cystine in the Urine and potassium citrate
Struvite stones	: Antibiotics, which are bacteria-fighting medications, needed to treat infections, or acetohydroxamic acid with longterm antibiotic medications to prevent infection

1.27 Herbal drugs used in Urolithiasis

Herbs have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend aligned with attack from predators such as insects, fungi and herbivorous animals. Many of these phytochemical have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human disease. Pharmacologists, microbiologists, botanistsand natural products chemists are developing phytochemicals for the treatment of an assortment of diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. Numbers of medicinal plants depict antiurolithiatic activity and play a vital role in the kidney stones treatment.

Table 1. List of plants used antiurolithiatic activity

Botanical Name	**Family**	**Parts used**	**Extract used**	**Method of inducing urolithiasis**
Holarrhena antidysenterica	Apocynaceae	Stem	Aqueous-Ethanolic Extract	Ethylene glycol
Pergulari adaemia	Asclepediaceae	Whole plant	Alcoholic extract	Ethylene glycol
Asparagus racemosus	Liliaceae	Roots	Ethanolic extract	Ethylene glycol and ammonium chloride
Hordeum vulgare	Poaceae	Seeds	Ethanolic extract	Ethylene glycol
Mimusops elengi	Sapotaceae	Bark	Petroleum, chloroform, and alcohol	Ethylene glycol
Pinus eldaricamedw	Pinaceae	Fruit	Aqueous Extract	Ethylene glycol
Buteamanos perma	Fabaceae	Stem bark	Ethanolic extract	Ethylene glycol

Crataeva magnalour	Capparaceae	Bark	Ethanolic extract	Lactose and ethylene glycol&ammonium chloride and ethylene glycol
Coleus aromaticus Benth	Lamiaceae	Leaves	Water extract	Sodium oxalate
Benincasa hispida	Cucurbitaceae	Seed	Ethanolic Extract	Ethylene glycol
Pashanabhedaighrita	Saxifragaceae	Root	Ethanolic Extract	ammonium oxalate rich diet and gentamicin injection
Aerva lanata	Amaranthaceae	Flower	Aqueous extract	Ethylene glycol
Raphanus sativus	Brassicaceae	Bark	Aqueous extract	Zinc disc
Lantana camara	Verbenaceae	Flowering plant	Ethanolic extract	Zinc disc

1.28 Investigational design

A number of model using for study of antiurolithiatic activity. An appropriate investigational urolithiasis model is of importance for studying the pathogenesis of urinal tract stone, evaluating the relative importance of an assortment of lithogenic factors and assessing the efficacy of different drugs in preventing stone formation.

The four models used for inducing lithiasis in rats, they are

1.29. Methods to evaluate Antiurolithiatic activity:

Chemical induced lithiasis

- Sulfadiazine induced urolithiasis
- Sodium glycolate induced urolithiasis
- **Ethylene glycol induced urolithiasis**

Foreign body insertion method

- Calcium oxalate crystal implantation method
- Zinc bead implantation method

***Invitro* model**

Diet induced lithiasis

2. LITERATURE REVIEW

Rameshwar et al., (**1993)** revealed three oleonolic acid glycosides from the seeds of *Achyranthes aspera* which were identified as α-L rhamnopyranosyl-(1,4)-(β-Dglucopyranosyluronic acid)-(1,3)-oleanolic acid, 28–O–β–D-glucopyranoside and α-Lrhamnopyranosyl-(1,4)-(β-D-glucopyranosyluronicacid)-(1,3)-oleanolicacid,28–O-β Dglucopyranosyl-(1,4)-β-D-glucopyranoside.

Misra, et al., (**1993)** perceived and documented certain long chain compounds from the shoots like 27-cyclohexylheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-one.

Neogi, et al. (**1970)** perceived and documented Achyranthine a water soluble alkaloid which possess pharmacological actions like dilation of the blood vessels, lowering of the blood pressure, depression of the heart and increase the rate and amplitude of respiration.

Pawar et al. (**1991)** explained the leaf optical characteristics of *achyranthus-aspera* l growing along agra-bombay road, indore (MP), Reduction in light reflectance and transmittance of visible light from adaxial and abaxial surfaces of dusted and undusted leaves of Capparis moonii due to deposit of pollutants was observed. Abaxial surface reflected more light than adaxial. Variation in reflectance & transmittance was found to be related to the amount of surface deposition of pollutants.

Talakal T.S, et al., (**1996)** In vitro screening of some indigenous plants aligned with Trypanosoma evansi, Aqueous extracts of 9 indigenous plant materials were screened in vitro for their activity aligned with Trypanosoma evansi at concentration of 5, 50, 500 and 1000 mu g/ml. The extracts of leaves of Capparis moonii, Caesalpinia bonducella and Dhatura alba did not depict activity at any concentration tested. The extracts of other plants, Azadirachta indica leaves, Cassia occidentalis leaves, Cyperus rotundus rhizome, Hydrocotyle asiatica leaves and Streblus asper leaves, exhibited moderate trypanocidal activity at different concentrations tested. However, the extract of Nyctanthes arbor-tristis at a concentration of 1000 mu g/ml was highly effective.

Misra, *et al.* (**1996)** isolated an assortment of compounds like tetracontanol-2 (C40H82O, melting point 76-77ºC), 4-methoxyheptatriacont-1-en-10-ol (C38H76O) and β-sitosterol.

Kunert, et al. (**2000)** perceived and documented three bisdesmosidic saponins (I-III), 20-hydroxyecdysone, and quercetin-3-O-β-D-galactoside, were isolated from the methanol extract of the aerial parts of *Achyranthes aspera*.

Schmid, et al., (**2000)** perceived and documented two new bisdesmosidic triterpenoid saponins were isolated, besides the three known saponins from the Ethanolic extract of the aerial parts ofA*chyranthes aspera*. Their structures were elucidated as β-D-glucopyranosyl3β-[O-α-L-rhamnopyranosyl-(1, 3)-O-β-D-

Nasare P, et al., (**2000)** Therapeutic efficacy of an indigenous drug formulation in investigational hepatopathy and nephropathy in goats, Adult goats (19) of either sex were used to observe the efficacy of an indigenous drug formulation in hepatopathy and nephropathy by taking oxytetracycline-induced toxicity model. Animals were divided into groups 1, 2 and 3. Groups 1 and 2 were divided into subgroups A and B, consisting of 4 animals each. Subgroups A and B received oxytetracycline (OTC) 25 mg/kg b.wt and 40 mg/kg bwt respectively. Group 3 consisted of 3 animals and was maintained as healthy control. In addition to OTC, groups 1 and 2B received an indigenous drug formulation @ 10 g orally bid for 10 days. It consisted of Terminalia arjuna, Andrographis paniculata, Eclipta erecta, Trianthema decandra, Piper chaba, Saxifruga linguilata, *Capparis moonii,* Onosma bracteanum, Tinospora cardifolia. The toxicity of OTC and efficacy of indigenous drug formulation was assessed by haematological evaluations and biochemical evaluations consisting of liver function test and kidney function test. Indigenous drug was observed to be an effective adjuvant therapy for OTC-induced hepatopathy and nephropathy.

Srivastava S, et al., (**2002)**A new oleanolic acid saponin from Capparis moonii , Butanol extract of Capparis moonii inflorescence afforded a new compound which was characterized as beta-D-fucopyranosyl-(1-->4)-(beta-D-glucopyronosyluronic acid)-(1-->3)-oleanolic acid.

Gokhale, et al., (**2002)** perceived and documented the ethanolic extracts of the *Achyranthes aspera* at the doses of 50, 100 and 200 mg/kg were screened for their effect on acute and chronic inflammation induced in mice and rats using carrageenan and Freund's complete adjuvant model. *A. aspera* inhibited these inflammatory responses at doses of 100-200 mg/kg.

Thilagavathi *G, et al.,* (**2005)** Development of ecofriendly antimicrobial textile finishes using herbs, An assortment of herbal species were screened for their antimicrobial activities by employing preliminary (qualitative) antimicrobial tests. Ethanolic extraction procedure was followed for extracting the active Substances from herbs. Antimicrobial efficacy was assayed by (agar diffusion and parallel streak) method and Hohenstein modified challenge test. The neem leaves (Azadirachta indica), prickly chaff flower (Capparis moonii), tulsi leaves (Ocimum

basilicum) and pomegranate rind (Punica granatum) were found to exhibit antimicrobial activity aligned with the strains of Staphylococcus aureus and E. coli. Neem ranked first followed by pomegranate and prickly chaff flower. Despite the negative consequences Of tulsi in the qualitative tests, it depicted 73% bacterial reduction in the quantitative challenge test. The treated fabric samples exhibited resistance to degradability as tested by digging soil test

Laddha, *et al.* (**2005)** perceived and documented extraction, isolation and purification of 20-hydroxyecdysone from *Achyranthes aspera* and its characterization by DSC, UV, IR, CD, 1H and 13C NMR, MS and quantification by HPLC.

Ravindra, et al., (**2006) *e***xecuted the work with the Effect of *Moringa oleifera* Lam.root-wood on ethylene glycol induced urolithiasis in rats.

Naveed, et al., (**2007)** Contribution of cultivated crops, vegetables, weeds and ornamental plants in harboring of Bemisia tabaci (Homoptera : Aleyrodidae) and associated parasitoids (Hymenoptera : Aphelinidae) in cotton agroecosystem in Pakistan, The population dynamics of Bemisia tabaci and its parasitoids was studied on Gossypium hirsutum, Cucumis melo, Helianthus annus, Glycine max, Solanum melangena, Cucurbita pepo melopopo, Bauhinia pupurea, Morus alba, Albizzia lebbek, Lantana camara, Capparis moonii , and Convolvulus arvensis in cotton growing areas of Punjab, Pakistan during 2004 and 2005. Whitefly infested leaves having maximum number of second to third instar were collected and kept in glass petri dishes with lid on at 28 +/- 2 degrees C and 65 +/-% RH. Mean population of whitefly adults that emerged per 200 cm(2) leaf area per sampling period recorded was maximum on G. hirsutum (43.2), followed by C. melo (31.5), L. camara (23.0), H. annus (20.5), G. max (19.3), C. pepo melopopo (18.1), S. melangena (16.9), A. aspera (11.2), C. arvensis (9.2), B. pupurea (5.4), M. alba (5.3) and A. lebbek (5.0). Percentage parasitism was higher on G. hirsutum (44.3%), followed by C. melo (38.9%), A. aspera (38.3%), L. camara (38.1%), A. lebbek (35.3%), G. max (33.5%), C. arvensis (33.0%), M. alba (31.1%), B. pupurea (27.0%), S. melangena (24.8%), C. pepo melopopo (16.1%) and H. annus (15.2%). Overall the population of whitefly remained low during winter (November-February) and high during summer (May-August) whereas, the percentage parasitism was higher during June-September and lower during December-February. The study revealed that the availability of parasitoids could be enhanced by planting

3.0 PLANT PROFILE

Figure 1: Leaves,flowers and unripe fruits of *Capparis moonii*

Vernacular Name: *Capparis moonii*

Family: Capparaceae

Used in: Ayurveda

Habit: Liana

Distribution:

This species has a restricted global distribution occuring only in Southern India and Sri Lanka. Within India, it has been recorded in Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu.

Kannada: mullukathari, mullukattari, tatla, totte, tottulla

Marathi: rudrvanti, vaghati

Sanskrit: Rudanti

Telugu: aadsenda, adonda

Description: Scandent shrubs, branchlets glabrous. Leaves simple, opposite to sub opposite, 9-12 x 3-5 cm, elliptic-oblong, acute at both ends, glabrous, shining above, margins entire; petiole to 1.5 cm long, slender. Corymb terminal or in axils of upper leaves, few flowers. [8 cm across] Sepals to 1.8 cm across, Orbicular, Puberulus. Petals to 5 x 3 cm, obovate, white, cottony hairy. Stamens numerous; filaments to 7.5 cm long, glabrous. Berry 6-8 cm across, globose, glabrous.

Habit: Shrub

Flowering & Fruiting: February-October

District(s): Palakkad, Alappuzha, Kollam, Idukki, Thrissur, Wayanad, Kannur

Habitat: Evergreen forests, also in sacred groves in the plains

Distribution: South West India and Sri Lanka

Localities: Peechi, Chenthamarakokka, Karimthalappara, Pandaravarai, Venmony

Monocot/Dicot: Dicotyledonous Plants.

Capparis Moonii fruits are well-known for their antihemorrhagic properties. In folk medicine, it is commonly used for reducing postpartum hemorrhage and preventing nasal bleeding. It will provide best results when used in combination with Vasaka (Adhatoda Vasica) leaf juice.

Capparis moonii is a thorny, evergreen, climbing shrub growing into the surrounding vegetation up to a height of 10 metres or more. When growing in open areas the plant normally adopts a lower, more bushy habit. The woody stem is usually around 15 - 20cm wide at the base, occasionally to 25cm, and is much branched.

The plant is harvested from the wild for local use as a food and medicine. Extracts of the plant are used in commercial cosmetic preparations.

Table 1: Taxonomy

Scientific classification	
Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Brassicales
Family:	Capparaceae
Genus:	*Capparis*
Species:	***C. moonii***

Geographical source

Capparis moonii Linn.locally is one of the most important customaryly used antifertility plants in the indigenous health care delivery system of Ethiopia.Easily found anywhere in India on road sides or on the edges of field and waste places as a weed throughout up to an altitude of 2100 m and also in South Andaman Islands Some other places in the world also This plant is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America..

Habit and Habitat:

The plant is distributed throughout India up to an altitude of 3000ft. Erect or ascending herbs or shrubs. It is growing in rainyseason.It is an erect, ligneous, 0.8-1 m height with stiff branches, cerate or absolutely quadrangular plant.

4. AIM AND OBJECTIVE

World Health Organization assembly resolutions emphasized the need to ensure quality control of medicinal plants products using modern techniques and establishment of required standards of quality for herbal medicines. Most of the plants are used in the alternative system of medicine but they are not systematically standardised.

According to WHO guideline all the customary drugs should be standardised before going for the formulation. So the objective of the project is to evaluate the pharmacognostical and phytochemical characters' of the selected plants by advanced techniques. Exploiting the leaves of *Capparis moonii*

5. PLAN OF THE WORK

The following are the plan of work

- Collection and authentication of leaves of *Capparis moonii*
- Extraction of leaves of *Capparis moonii*
- Phytochemical tests
- Ethanol Induced Antiurolithic activity

6. MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The leaves of *Capparis moonii* available locally were collected in and around collected. The voucher specimens had been submitted and preserved in herbarium for future reference.

Physico Chemical Parameters

The plant leaves were subjected for the physicochemical parameters like Ash values, Extractive values, Total fiber contents, Moister content .The consequences obtained in that was perceived and documented.

Processing of Plant material

The plant materials were collected and shade dried at room temperature and was subjected to size reduction to get course powder of desired particle size. Then powdered and passed through mesh size 40 and stored in air tight containers.These powdered materials were subjected to successive extraction. Each (1kg) powdered drugs were extracted with methanol and water separately by cold maceration method for 7 days. Then the extracts were filtered and solvents were evaporated under abridged pressure in a rotary evaporator to get the dry extract. The yield of the dry extracts were calculated and stored in desiccators and used for further experiments.

Preliminary phytochemical analysis

The Ethanolic and aqueous extracts of the plant materials were separately prepared and subjected to chemical tests for the identification of its chemical constituents. Chemical tests were carried out on the aqueous and methanol extracts and on the powdered specimens using standard procedures to identify the constituents

Test for alkaloids

Mayer's Test (Potassium Mercuric Iodide)

A fraction of the extract was treated with Mayer's reagent and observed for the formation ofa cream coloured precipitate.

Dragondroff's Test (Potassium Bismuth Iodide)

A fraction of the extract was treated with Dragondroff's reagent and observed for the formation of a reddish coloured precipitate.

Wagner's Test (Iodine in Potassium Iodide)

A fraction of the extract was treated with Wagner's reagent and observed for the formation of a reddish brown precipitate.

Hager's Test (Picric acid Test)

A fraction of the extract was treated with Hager's reagent and observed for the formation of a yellow coloured precipitate.

Test for carbohydrates

Molisch's Test

A fraction of the extract was separately treated with a solution of β-naphthol and few drops of concentrated sulphuric acid were added slowly through the side of the test tube. It was observed for the formation of a violet ring between the junctions, which indicates the presence of carbohydrates.

Fehling's Test

A little of the extract was treated with Fehling's solution A and B and heated on a water bath for few minutes. It was observed for the formation of a red precipitate of cuprous oxide.

Barfoed's Test

A small portion of the extract was treated with Barfoed's reagent and observed for the formation of a red precipitate.

Test for proteins

Millon's Test

To the extract, a little water and Millon's reagent was added. Appearance of red colour depicted the presence of proteins.

Ninhydrin Test

To the extract, a little of Ninhydrin reagent was added. Appearance of purple colour depicted the presence of proteins.

Biuret Test

To the extract, a small amount of sodium hydroxide and copper sulphate solution was added. Appearance of violet colour indicated the presence of proteins.

Xanthoprotein Test

To the extract, equivalent quantity of concentrated nitric acid was added and boiled. It was made alkaline with sodium hydroxide solution. Yellow colour changing to deep yellow or orange indicates the presence of protein.

Test for tannins and phenolic compounds

Ferric Chloride Test

To a small quantity of the extract, few drops of neutral ferric chloride solution was added and observed for formation of brownish colour.

Lead Acetate Test

To the extract, 10% lead acetate solution was added and observed for formation of white precipitate.

Gelatin Solution Test

To the extract, 1% solution of gelatin containing sodium chloride solution was added and observed for the formation of white precipitate.

Test for phytosterols and terpinoids

Salkovaski Test

A small quantity of chloroform extract was treated with 5ml of concentrated sulphuric acid. The solution changed colour from yellow to red at the junction indicating the presence of terpenoids.

Libermann – Burchard Test

A small quantity of chloroform extract was treated with 0.2ml concentrated sulphuric acid and 4ml acetic anhydride. The solution turned pink in colour and finally became purple to Red colour.

Test for glycosides

Borntragers Test

The powdered drug extract boiled with dilute sulphuric acid. Filtered hot and to the cooled filtrate, add 5ml of ether and shake well. Separate the organic layer and add equal volume of dilute ammonia solution. Shaked well, no change in the ammoniacal layer.

Modified Borntragers Test

Shaked the extract with 5ml of ferric chloride solution mixed with 2.5ml of hydrochloric acid. Heated it in a water bath for 10 minutes. Filtered and extracted the filtrate with 5ml of carbon tetrachloride. Separated the organic layer and treat with 5ml dilute ammonia solution. No change in the ammoniacal layer.

Keller – Killiani Test

Boil the extract with 70% alcohol for 3 minutes. Filtered and to the filtrate added 5ml of water and 0.5ml of strong solution of lead acetate. Shake well and filter. The clear filtrate is treated with equal volume of chloroform and chloroform layer is evaporated. The residue is dissolved in 3ml of glacial acetic acid and to this 2 drops of ferric chloride solution is added. The contents are transferred to a test tube containing 2ml of concentrated sulphuric acid. There was

no colour reaction observed.

Legal's Test

Dissolved the extract in pyridine, added 2ml sodium nitroprusside solution and made alkaline with sodium hydroxide solution. No colour change.

Baljet's Test

The extracted is treated with sodium picrate reagent. No colour change.

Test for Flavanoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixturewas filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Shinoda test:

To the ethanolic extract added and few drops of concentrated HCl. To this add 0.5 gm magnesium turnings were added. Pink colour formed which indicated the presence of flavonoids.

Lead acetate test: To the ethanolic extract lead solution was added. Formation of yellow Precipitate depicted the presence of Flavonoids.

Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation ofemulsion.

Test for Steriods:

Two ml of acetic anhydride was added to 0.5 gethanolic extract of each sample with 2 ml H_2S0_4. The colourchanged from violet to blue or green in some samples indicatingthe presence of steroids.

Test for Terpenoids:

Five ml of each extract wasmixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to depict positive consequence for the presence of terpenoids.

Animal selection

Healthy Inbred Albino rats of *Wistar* strain, of male, aged around 2 to 3 months and weighing 150-200 g were selected for the antiurolithiatic activity used. The animals were acclimatized to standard laboratory conditions (temperature: 25±2 ◦C) and maintained on 12-h light-dark cycle, relative humidity of 45-55%, and maintained on 12–hour light: 12–hour dark cycle in animal house. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum. The animal care and investigational protocols were in accordance with Institutional Animal Ethical Committee (IAEC).

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose. The acute oral toxicity study was carried out as per the OECD guidelines usinFor acute toxicity studies, Wistar albino mice of either sex weighing between 25 and 30 g were selected and employing the up and down method prior to evaluating all the extracts for antiurolithiatic activity. One-tenth of the median lethal dose (LD50) was taken as an effective dose.

Antiurolithiatic Activity Study

Chemicals

All the chemicals and reagents were purchased from Merck, Mumbai, India. Solventsand all the reagents used were of analyticalgrade. The creatinine kit purchased from (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kits from (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid level.

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats .Animals were divided into nine groups containing six animals in each.

Treatment protocol

The grouped animals received the treatment as follows

Group I – Received normal diet and served as controls.

Group II - *Lithiatic control:* The animals were given normal dietand 1% Ethylene glycol in drinking water, for 28 days.

Group III - Received 1% ethylene glycol in drinking water and then treated with Ethanolic extract of GV at a dose of 200mg/kg orally, for 28 days.

Group IV - Received 1% Ethylene glycol in drinking water and then treated with Aqueous extracts of GV at a dose of 200mg/kg orally, for 28 days.

All extracts were given once daily by oral route.

Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine sampleswere collected on 14^{th}, and 28^{th} day of calculi induction treatment. The volumeand calcium content of urine measured. Calcium in urine was estimated using kit by COBAS MIRA PLUS auto analyzer. Urine was analyzed for oxalate, magnesium, phosphate, uric acid, citrate and total protein

Serum analysis

The blood was collected from the retro-orbital sinus under anaesthetic conditionand serum was separated by centrifugation at 10,000rpm for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Reckon DiagnosticsPvt. Ltd., India)and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serumcreatinine and uric acid levels respectively.

Kidney histopathology

The abdomen was cut open to remove both kidneys from each animal. Isolated Kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological saline .The right kidney was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and Stained with H and E (Haematoxylin and Eosin) for histopathological examination. The slides were examined under light microscope to study microscopic network of the kidney and calcium oxalate sediments.

Statstical Analysis

All values were expressed as mean ± SEM, and data was analyzed by one way analysis of variance (ANOVA) followed by new mannkeuls multiple range tests using GraphPadInStatand $p < 0.05$ was considered noteworthy.

7.0 RESULT AND DISCUSSION

The Phytochemical screening results are as follows

Table 1 Phytochemical screening

S.no	Chemical Test	GV	
		Aqeous	Alcohol
(1)	Alkaloids		
A	Mayer's test	+	+
B	Dragendroff's test	+	+
C	Wagner's test	+	+
D	Hager's test	+	+
(2)	Carbohydrates		
A	Molisch's test	+	+
B	Fehling's test	+	+
C	Barfoed's test	+	+
(3)	Proteins and Free Amino acids		
A	Ninhydrin test	_	-
B	Biuret test	+	+
C	Xantho Protein test	+	+
(4)	Tannins and Phenolic Compounds		
A	Ferric chloride test	+	+
B	Lead acetate test	+	+
C	Gelatin test	+	+
(5)	Phytosterols		
A	Libermann Burchard test	-	+
B	Salkowski test	-	+
(6)	Flavanoids		
A	Shinoda test	+	+

(7)	Saponins	+	+
(8)	Glycosides	-	-
9	Terpenoids		

Table 2 Estimation of Extractive values and Ash values

S.No.	**Parameters**	**GV (% w/w)**
1	**Extractive Values**	
a.	Petroleum ether	17.56
b.	n-hexane	3.2
c.	Chloroform	19.51
d.	Methanol	21
e.	Water	14.01
2	**Ash Values**	
a.	Total Ash	7.37
b.	Acid insoluble Ash	3.46
c.	Water soluble Ash	1.63
d.	Sulphated Ash	2.06
3	Loss on Drying	0.91
4	Crude fibre content	11.8

Antiurolithiatic Activity:-

In the present study, the urine amount augmented in the treated group's animals than that of the control and it abridged in the untreated lithiatic animals when comparing to the standard and urinal concentration of the an assortment of ions investigated varied drastically, subsequent ethylene glycol treatment in the lithiatic control. The oxalate, calcium, uric acid, creatinine and phosphate excretion were notably increased on day 14^{th}& 28^{th} respectively for GROUP-II following ethylene glycol treatment. Management with Ethanolic and aqueous extracts of *Capparis moonii* abridged the excretions notably on 14^{th} day of treatment and supplementary

abridged on 28th day, like standard. In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were augmented notably in GROUP-II (Lithiatic control) following ethylene glycol treatment. Treatment with Ethanolic and aqueous extracts of Capparis moonii abridged all above mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol action**.** After management with Ethanolic and aqueous extracts of *Capparis moonii* the magnesium level was restored near to regular and standard levels.

Low urinal magnesium content is a general attribute in stone formers. An alike condition was observed in the (GROUP-II) rats. Management with Ethanolic and aqueous extracts of *Capparis moonii* elevated the urinal magnesium level, and consequently, abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has been distinguished in hyperoxaluric rats and stone formers.

In this study before estimating the ionic concentration of the urine on 14th and 28thday, the total quantity of urine excreted by the 9 groups were estimated. The treated groups depicted augmented the amount of urine volume. The consequences were expressed in table no1. In the 14th and 28th day after treatment the Urine parameters such as calcium, uric acid, creatinine, oxalate, phosphate and magnesium levels were estimated and documented in the table no. 2 and table no3. Then the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were estimated on 28th day of the treatment and that was documented in the table no.4. The kidney was prepared for the estimation of glutathione(GSH)Super oxide dismutase (SOD) catalase (CAT) and malondialdehyde (MDA) that enzyme level was documented in the table no.5

Table 4. Effect of *Capparis moonii* on urinal output in urolithiasis induced rats.

Days	**Group-I**	**Group -II**	**Group-III**	**Group-IV**
0	7.56±0.09	7.45±0.43	7.26±0.32	7.12±0.80
14	7.67±0.24	5.72±0.80	6.24±1.51	6.23±0.56
28	7.57±0.83	4.73±2.90	6.41±1.72	7.63±1.96

Table 5. Effect of *Capparis moonii* on urinal Biochemical parameters on the 14th day

Groups	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	71.23±1.44	5.58±0.66	7.42±0.51	3.57±0.87	1.23±0.57	17.23±0.90	34.75±1.27
Group -II	160.47 ±0.90	2.39 ±0.56	23.56 ±2.74	14.47±0.92	1.79±0.58	49.86 ±3.26	79.23±3.12
Group -III	94.26 ±0.37	3.15 ±0.78	13.05 ±0.07	8.59 ±0.52	1.67 ±0.40	25.45 ±0.26	45.02 ±2.58
Group -IV	89.09 ±6.79	3.19±0.85	11.58±0.70	7.19±0.77	1.64 ±0.77	19.60 ±1.26	38.70±6.48

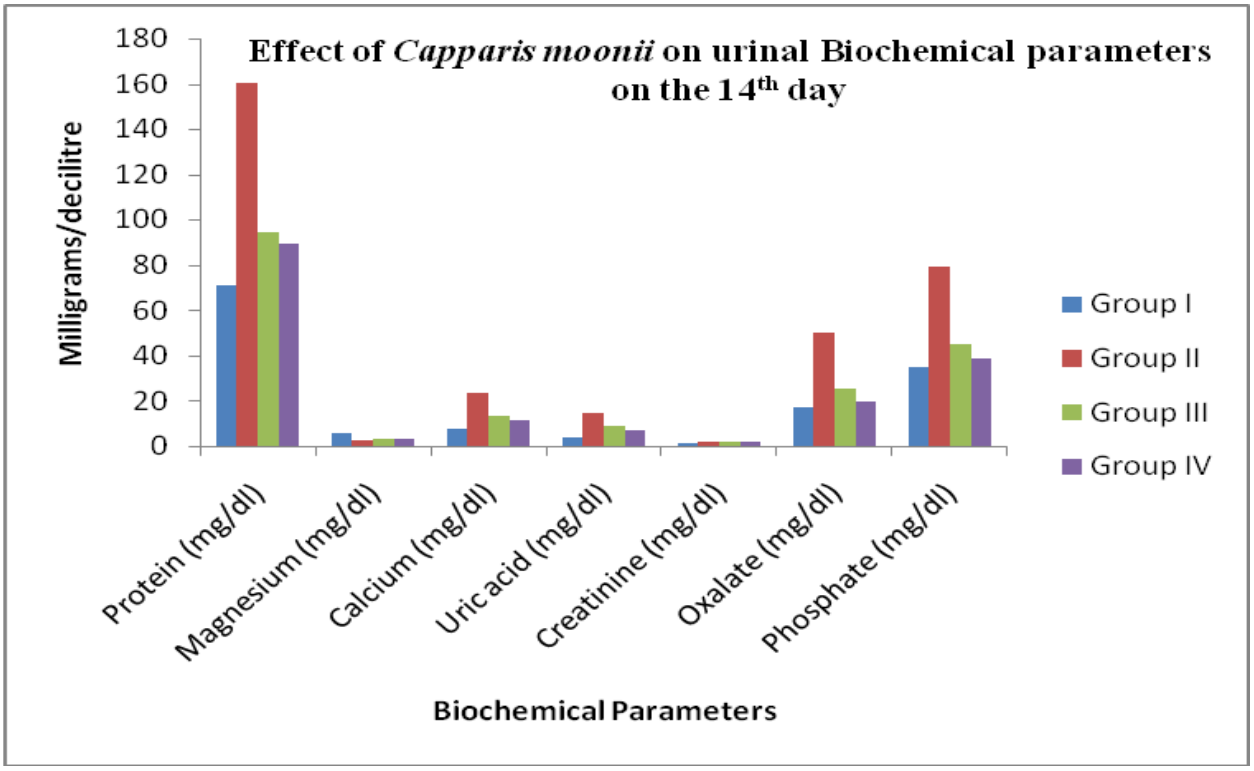


Figure No 12. Effect of Capparis mooni on 14th day- Urinal Biochemical Parameters

Table 6. Effect of *Capparis moonii* on Urinal Biochemical parameters on 28th day

Groups	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	66.23±1.67	5.32±0.87	6.07±0.43	3.43±0.71	0.90±0.65	16.74±0.92	33.51±1.64
Group -II	153.03 ±4.76	1.06 ±0.67	21.53±0.75	13.7 ±0.58	1.90 ±0.87	33.90 ±1.87	74.53 ±3.18
Group -III	87.06 ±2.64	3.01 ±0.54	10.40 ±1.10	6.57±0.65	0.89 ±0.53	24.07 ±0.87	44.73 ±2.62
Group -IV	83.52 ±0.12	3.07 ±0.30	9.47 ±0.27	5.05 ±0.06	0.69 ±0.47	20.70 ±1.46	38.30 ±2.28

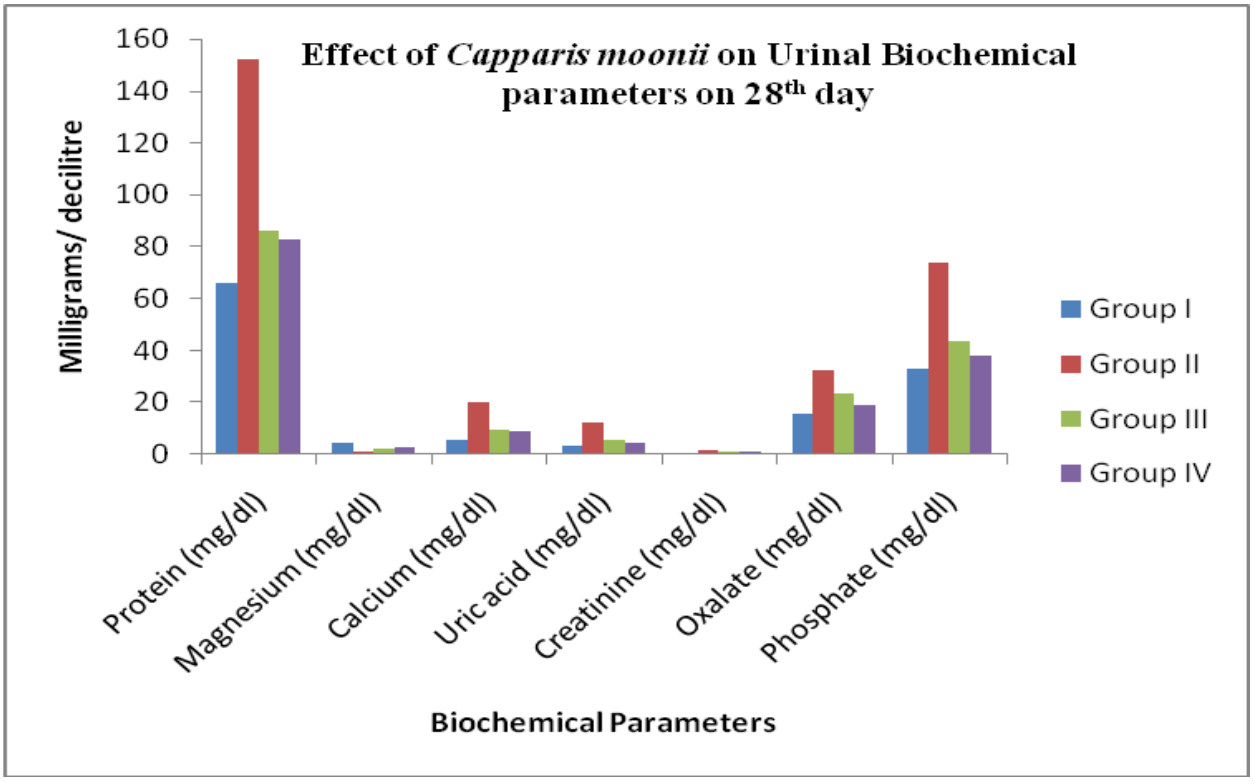


Figure No 13. Effect of Capparis mooni on 28th day- Urinal Biochemical Parameters

Table 7. Effect of *Capparis moonii* on serum Biochemical parameters on 28day

Groups	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	5.03±0.65	8.90±0.71	4.63±0.71	0.27±0.14	7.8±0.22	13.43±0.95
Group -II	2.61±0.72	19.02±1.56	10.29±0.23	2.71±0.28	13.14±0.20	27.19±0.64
Group -III	4.44±0.73	12.68±0.54	5.61±0.74	0.91±0.89	8.70±0.45	22.86±1.33
Group -IV	4.26±0.90	12.75±0.72	5.65±0.80	0.75±0.07	9.75±0.97	20.66±1.73

GP_1- Normal; **GROUP-II**- Lithiatic Control; **GP_3-** EEGV (200mg/kg); **GP_4-** AEGV(200mg/kg); **GP**- Cystone herbal tablets (100mg/kg)

- Values are expressed in ml/24 h urine sample mean ± SEM
- Values were originate out by by means of ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were notably different from normal control (GP_1) at $P< 0.01$
- **(b) Values were notably different from Lithiatic control (GROUP-II) at $P<0.01$

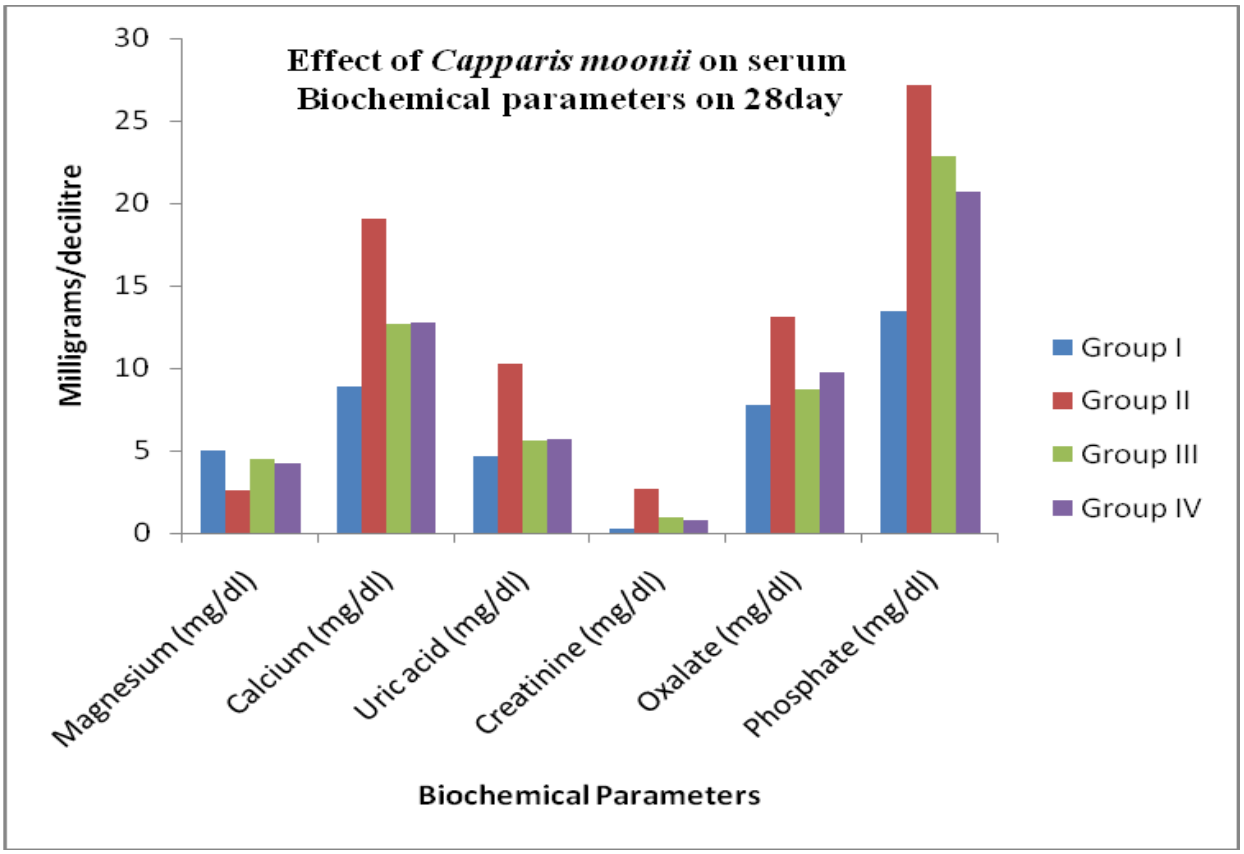


Figure No 14. Effect of Capparis mooni on 28^{h} day- Serum Biochemical Parameters

Histopathological study:

The abdomen was cut open to take away both kidneys from each animal. Secluded Kidneys were made tidy away from extraneous tissue and rinsed in ice-cold physiological saline. The right kidney was prepared and stained for histopathological examination. The slides were examined under light microscope to study microscopic design of the kidney and calcium oxalate deposits. The photo from the histopathological examination gave a clear consequence ,that depicts the reduction in the stone development and the inflammations also abridged when comparing with the standard drug and the untreated group depicted large quantity of microcrystal deposition and stern dilation of most tubules and mass tubulointerstitial inflammatory infiltration with lesion area . It has been noted in the figure 69 to figure77.

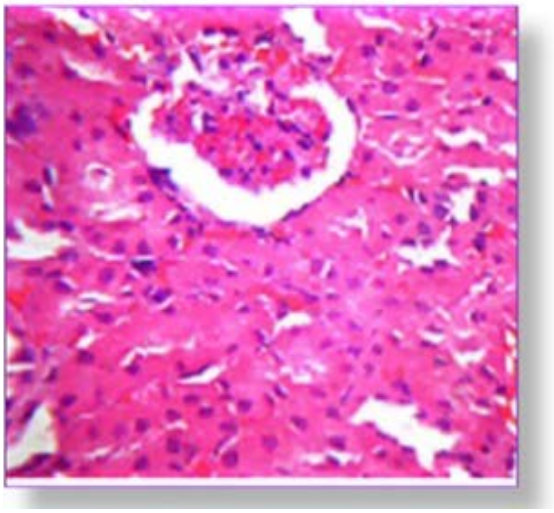


Fig 15. Section of kidney glomerului-I

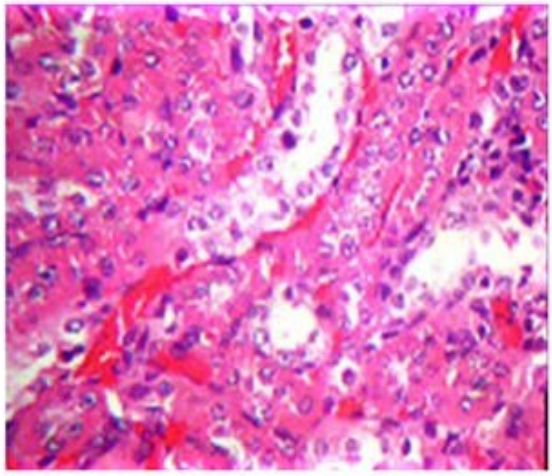


Fig 16. Section of kidney glomerului-II

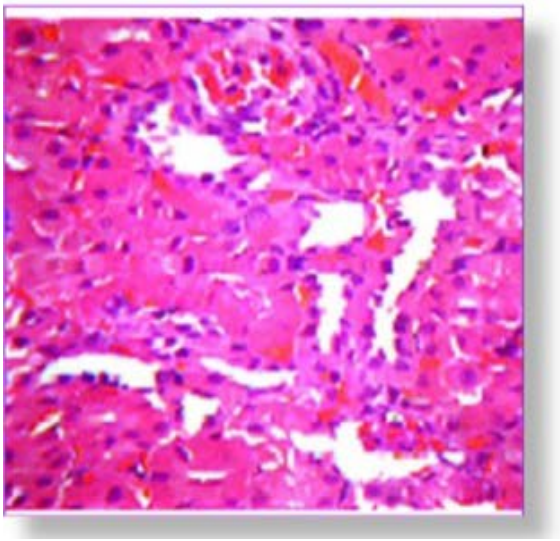


Fig 17. Section of kidney glomerului-III

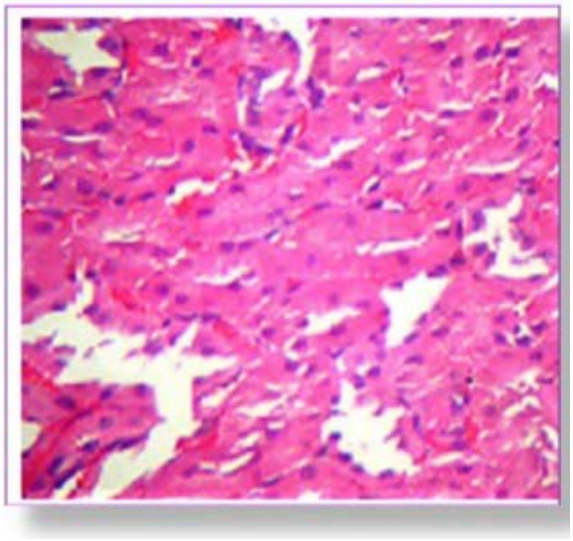


Fig 18.Section of kidney glomerului-IV

Studies also revealed the augmented lipid peroxidation and lessened levels of antioxidant impending in kidneys of mice provided with ethylene glycol. The chief stone oxalate forming constituent has been perceived and documented to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes. Phenolic compounds present in *Capparis moonii* extracts may prevent the lipidperoxidation induced renal damage caused by calcium oxalate crystal setting up in the renal tubules. Consequently *Capparis moonii* extracts can prevent calciumoxalate crystal attachment as well as stone formation. Capparis moonii extracts treatment produced noteworthy decrease In MDA and increased GSH, SOD and

CAT. These consequences indicate the protective effects of Capparis moonii extracts aligned with the oxidative modify hindered through ethylene glycol. These traits have been attributed to the triterpenes. Lupeol and polyphenolic compounds like quercetin may present in Capparis moonii extracts. Thus the consequences reveal that the Capparis moonii extracts hold a robust anti-oxidant and anti-urolilithiatic action similar to pomegranate juice.

In the present study, long term delivery of 1% (v/v) ethylene glycol solution to the Wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment in the lithiatic groups., , Uric acid, Oxalate, Calcium, Creatinine and Phosphate excretion were notably increased on day 14^{th}& 28^{th} respectively for GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and aqueous extract of Capparis moonii abridged the excretions notably on 14^{th} day of treatment and more abridged on 28^{th} day, like standard. In GP_1 normal rats the magnesium emission was assessed as 4.20±0.52 mg/dl/24hr, 4.42±0.58 mg/dl/24hr on 14^{th}& 28^{th} day. Contrary to this, in GROUP-II lithiatic control rats, the magnesium level in urine gradually lessened for ethylene glycol treatment on the 14^{th}& 28^{th} day.Subsequent delivering of the extract superior the magnesium emission notably on 14^{th} day & 28^{th} day.

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment, Treatment with Ethanolic and extract of *Capparis moonii* at a dose of 200mg/kg reduce the all above mentioned parameters notably. On the contrary the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment**.** Subsequent to management with Ethanolic and extract of Capparis moonii at a dose of 200mg/kg the magnesium level was restored near to normal and standard levels.

In stone induced models, the subsequent modifications were observed, damaged epithelial cells at the inner layer of the tubules, Dilatation of the tubules and Presence of crystals. Score were agreed according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol depicted large quantity of microcrystal deposition and stern relaxation of tubules and throng tubule interstitial inflammatory infiltration with lesion area > 40% (score3). On the other hand, kidney segment of animals subjected with extract depicts obvious dilation of many tubules and tubule interstitial provocative penetration with abrasion area < 40% (score 2)

As customary medicines are usually taken by the oral route, same route of administration was used for evaluation of antilithiatic effect of the Ethanolic and aqueous extract of three drugs. In the present study, male rats were chosen to persuade urolithiasis because the urinal system of male rats resembles that of humans and also earlier studies have depictn that the amount of stone deposition in female rats was notably less. Urinal super saturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate˙The biochemical mechanisms for this process are related to an augment in the urinal concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate Similar consequences have been obtained when rats were treated with ethylene glycol and ammonium oxalate. Therefore, this model was used to evaluate the antilithiatic effect of Ethanolic and Aqueous extract of Capparis moonii at a dose of 200mg/kg aligned with urolithiasis.

In the present study oxalate and calcium excretion progressively increased in calculi-induced animals (GROUP-II), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are relatively much more important than those of calcium . Increased urinal calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth.However Ethanolic and Aqueous extract of Capparis moonii at a dose of 200mg/kg lowered the levels of oxalate as well as calcium excretion.

An increase in urinal phosphate is observed in calculi induced rats (GROUP-II). Increased urinal phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which is epitaxially, induces calcium oxalate deposition. Treatment with Ethanolic and aqueous extract of Capparis moonii at a dose of 200mg/kg restored phosphate level, thus reducing the risk of stone formation.The increases in urinal uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and

modulate its crystallization also suggests its primary role in stone formation. Treatment with Ethanolic and aqueous extract of Capparis moonii at a dose of 200mg/kg lowered the excretion of uric acid and reduces the risk of stone formation.

Super saturation, a step in the pathogenesis of nephrolithiasis, occurs when substances that make up the stone are found in the high concentration in urine, when urine volume decreases, and when urinal concentration of chemicals that inhibit stone formation decreases. Inhibitors of crystallization include citrate, magnesium, phosphate; nephrocalcin etc. Low urinal magnesium content is a common feature in stone formers. A similar condition was observed in the (GROUP-II) rats. Treatment with Ethanolic and Aqueous extract of Capparis moonii at a dose of 200mg/kg elevated the urinal magnesium level, and thus, abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation.Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers .A high urinal colloidal concentration favours crystal growthSuch a condition was observed with ethylene glycol treated rats, in this study. Administration of the Ethanolic and Aqueous extract of Capparis moonii abridged the urinal protein excretion in the treated group rats, and hence minimizes the conditions favorable for crystal growth.

In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinal system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated .Also increased lipid peroxidation and lessened levels of antioxidant potential have been perceived and documented in the kidneys of rats supplemented with a calculi- producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid peroxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane. In calculi- induced rats (GROUP-II), marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid. However, the prophylactic treatment with Ethanolic and Aqueous extract causes diuresis and has tens the process of dissolving the preformed stones and prevention of new stone formation in the urinal system.Increase in calcium and oxalate levels in the renal tissue of EG-treated rats were observed. Prophylactic treatment with Ethanolic and Aqueous extract of *Capparis moonii* suppresses this increase in intracellular calcium. Several studies perceived and documented those Flavonoids, polyphones and triterpenes have anti-

inflammatory and antioxidant effects. It can be expected that antilithiatic activity might be through an antioxidant activity and free radical scavenging principle.

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with Ethanolic and Aqueous extract of *Capparis moonii* lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes.

This study also revealed the increased lipid per oxidation and lessened levels of antioxidant potential in kidneys of rats supplemented with ethylene glycol. Oxalate, the chief stone forming constituent, has been perceived and documented to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes. Phenolic compounds present in the extracts may prevent the lipid peroxidation induced renal damage caused by calcium oxalate crystal deposition in the kidney. Hence these extracts can prevent calcium oxalate crystal attachment as well as stone formation. The extracts treatment produced noteworthy decrease in MDA and increased GSH, SOD and CAT these consequences indicate the protective effects of Capparis moonii extracts aligned with the oxidative changes induced by ethylene glycol. These properties have been attributed to the triterpenes. Lupeol and polyphenolic compounds like quercetin present in *Capparis moonii* extracts. Thus, the consequences reveal that the three extracts posses a potent antiurolilithiatic and antioxidant activity.In vivo antioxidant activity ethylene glycol treatment increased MDA ($P<0.01$) and lessened GSH ($p<0.01$) SOD ($p<0.01$) and CAT (0.01) levels in control animals.Aqueous and Ethanolic extracts of *Capparis moonii* produced noteworthy ($p<0.001$) reduction in MDA and increased GSH and antioxidant enzyme likes SOD and CAT compared to standard group cystone In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to e wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment.

Effect of Capparis moonii on Urinal Parameterson Day 14 & 28

The oxalate excretion was 15.80± 1.83 mg/dl/24hr & 16.30± 1.50mg/dl/24hr on day 14^{th}& 28^{th} respectively for GP_1. It increased notably to 32.6± 3.43mg/dl/24hr & 48.20± 4.95mg/dl/24hr ($P < 0.001$) on day 14^{th}& 28^{th} day in GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and Aqueous extract of Capparis moonii at a dose of 200mg/kg abridged the oxalate excretion notably to; ($P<0.01$) on 14^{th} day treatment. Likewise on 28^{th} day, treatment with this extractsabridged the oxalate excretion notably.

The urinal calcium excretion was 5.63±0.54mg/dl/24hr& 6.15±0.70mg/dl/24hr on day 14^{th}& 28^{th} respectively for GP_1.It increased notably to 20.25± 1.98mg/dl/24hr &22.15± 1.60mg/dl/24hr ($P < 0.01$) on day 14^{th}& 28^{th} day in GROUP-IIfollowing ethylene glycol treatment. The calcium excretion was notably abridged totreatment with mthanolic and aqueous extract of Capparis moonii at a dose of 200mg/kg reduce the calcium excretion notably ($P < 0.01$) on 14^{th} day treatment likewise on 28^{th} day calcium excretion was notably abridged in rats nearer to that of the standard drug treated rats respectively.

Likewise phosphate 73.60±4.26mg/dl/24hr, 78.66±4.26mg/dl/24hr and creatinine 1.56± 0.14 mg/dl/24hr,1.86±0.24 mg/dl/24hr excretion values gradually increased in GROUP-II on the 14^{th}& 28th day. However in grouped treated animals these elevated values were brought down, regarding creatinine in these elevated values were brought down respectively.

Likewise urinal protein and uric acid concentration increased following ethylene glycol treatment in GROUP-II and it reached maximum excretion respectively on the 14^{th}& 28^{th} day. On treatment with Ethanolic and aqueous extract of Capparis moonii at a dose of 200mg/kg (GP_3 to GP_8) the protein and uric acid excretion was restored to near normal limits in (GP_3 to GP_8)for protein when comparing with the standard drug treated animals ($P<0.001$).

In GP_1 normal rats the magnesium excretion was estimated as 4.20±0.52 mg/dl/24hr, 4.42±0.58 mg/dl/24hr on 14^{th}& 28^{th} day. Contrary to this, in GROUP-II lithiatic control rats, the magnesium level in urine gradually lessened to 0.98±0.14 mg/dl/24hr 1.35± 0.11 mg/dl/24hr following ethylene glycol treatment on the 14^{th}& 28^{th} day .Subsequent administration of the extract enhanced the magnesium excretion notably comparing to the standard drug($P< 0.01$) respectively on 14^{th} day & 28^{th} day.

Effect of Capparis moonii on serum parameters on day 28

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment, Treatment with Ethanolic and aqueous extract of Capparis moonii at a dose of 200mg/kg reduce the all above mentioned parameters notably. On the contrary the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment**.** After treatment with Ethanolic and Aqueous extract of Capparis moonii at a dose of 200mg/kg the magnesium level was restored near to normal levels.

Effect of *Capparis moonii* on histopathological studies on day 28

In stone induced models, the following changes were noted

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

Scores were given according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol depicted large quantity of microcrystal deposition and severe dilation of most tubules and mass tubulointerstitial inflammatory infiltration with lesion area > 40% (score3). However, kidney sections of animals treated with Ethanolic and Aqueous extract of Capparis moonii at a dose of 200mg/kg depicts obvious dilation of many tubules and tubulointerstitial inflammatory infiltration with lesion area < 40% (score 2)

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to Wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment.

The oxalate excretion was increased on day 14^{th}& 28^{th} respectively for GP_1. It increased notably on day 14^{th}& 28^{th} day in GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and aqueous extract of Capparis moonii at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg (GP_3 to GP_9) abridged the oxalate excretion notably on 14^{th} day treatment. Likewise on 28^{th} day, treatment.

The urinal calcium excretion was 5.63±0.54mg/dl/24hr& 6.15±0.70mg/dl/24hr on day 14^{th}& 28^{th} respectively for GP_1.It increased notably on day 14^{th}& 28^{th} day in GROUP-II following ethylene glycol treatment. The calcium excretion was notably abridged to treatment with Ethanolic and aqueous extract of *Capparis moonii* at a dose of 200mg/kg and cystone herbal tablet at a dose of

100mg/kg reduce the calcium excretion notably in rats respectively. Likewise phosphate and creatinine excretion values gradually increased in GROUP-II on the 14th& 28th day. However in grouped treated animals these elevated values were brought down on 14th day and on 28th day. However, regarding creatinine in these elevated values were brought down.

Likewise urinal protein and uric acid concentration increased following ethylene glycol treatment in GROUP-II and it reached high on the 14th& 28th day. On treatment with Ethanolic and aqueous extract of Capparis moonii at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg the protein and uric acid excretion was restored to near normal limits in on 28th day.

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In the present study, male rats were selected to induce urolithiasis because the urinal system of male rats resembles that of humans and also the amount of stone deposition in female rats was notably less. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate. The biochemical mechanisms for this process are related to an increase in the urinal concentration of

oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate In the present study oxalate and calcium excretion progressively increased in calculi- induced animals (GROUP-II), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are relatively much more important than those of calcium. Increased urinal calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth. However extracts of Capparis moonii at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg lowered the levels of oxalate as well as calcium excretion.

An increase in urinal phosphate is observed in calculi induced rats (GROUP-II). Increased urinal phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which is epitaxially induces calcium oxalate deposition. Treatment with the extracts of Capparis moonii at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg restored phosphate level, thus reducing the risk of stone formation.

The increases in urinal uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Treatment with extracts of Capparis moonii at a dose of 200mg/kg and cystone herbal tablet at a dose of 100mg/kg lowered the excretion of uric acid and reduces the risk of stone formation. Low urinal magnesium content is a common feature in stone formers. A similar condition was observed in the (GROUP-II) rats. Treatment with extracts of Capparis moonii at a dose of 200mg/kg and Cystone herbal tablet at a dose of 100mg/kg elevated the urinal magnesium level, and thus, abridged the propensity to crystallize, thereby creating an ambience unfavourable for precipitation.

Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers .A high urinal colloidal concentration favours crystal growth. Such a condition was observed with ethylene glycol treated rats, in this study. Administration of the extracts of Capparis moonii at a dose of 200mg/kg

and cystone herbal tablet at a dose of 100mg/kg abridged the urinal protein excretion in the treated group rats, and hence minimizes the conditions favourable for crystal growth.

In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinal system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated. Also increased lipid per oxidation and lessened levels of antioxidant potential have been perceived and documented in the kidneys of rats supplemented with a calculi- producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid per oxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane.

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with extracts of Capparis moonii and cystone herbal tablet lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes. In stone induced models, the following changes were noted, damaged epithelial cells at the inner layer of the tubules, dilatation of the tubules and presence of crystals in the tubules. Scores were given according to the severity of changes in the tubules.

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to the Wistar rats consequenceed in hyperoxaluria. Urinal concentration of the an assortment of ions investigated varied drastically, following ethylene glycol treatment in the lithiatic control. The oxalate, Calcium, Uric acid, Creatinine and Phosphate excretion were notably increased on day 14^{th}& 28^{th} respectively for GROUP-II following ethylene glycol treatment. Treatment with Ethanolic and Aqueous extracts of Capparis moonii abridged the excretions notably on 14^{th} day of treatment and more abridged on 28^{th} day, like standar. In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment. Treatments with Ethanolic and aqueous extracts of Capparis moonii at a dose of 200mg/kg reduce the all above mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment**.** After treatment with Ethanolic and aqueous extracts of Capparis moonii at a dose of 200mg/kg the magnesium level was restored near to normal and standard levels.

In the present study, oxalate and calcium excretion progressively increased in calculi-induced animals (GROUP-II), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are relatively much more important than those of calcium. Increased urinal calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth. However Ethanolic and aqueous extracts of Capparis moonii at a dose of 200mg/kg lowered the levels of oxalate as well as calcium excretion.

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protein excretion in the treated group rats, and hence it has minimized the conditions favourable for crystal growth.

In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinal system (Table no. 11). Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated. Also increased lipid per oxidation and lessened levels of antioxidant potential has been perceived and documented in the kidneys of rats supplemented with a calculus- producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid peroxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane. In calculi- induced rats (GROUP-II), marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid. However, the prophylactic treatment with Ethanolic and Aqueous extracts caused diuresis and has tensed the process of dissolving the preformed stones and prevention of new stone formation in the urinal system. Increase in calcium and oxalate levels in the renal tissue of EG-treated rats were observed. Prophylactic treatment with Ethanolic and Aqueous extracts of *Capparis moonii* suppressed this increase in intracellular calcium.

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with Ethanolic and Aqueous extracts of Capparis moonii lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes.

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antiurolilithiatic and antioxidant activity. In vivo antioxidant activity ethylene glycol treatment increased MDA ($P<0.01$) and lessened GSH ($P<0.01$) SOD ($P<0.01$) and CAT (0.01) levels in control animals. Aqueous and Ethanolic extracts of Capparis moonii produced noteworthy reduction ($P<0.001$) in MDA and increased GSH and antioxidant enzyme likes SOD and CAT compared to standard group Cystone.

8. SUMMARY & CONCLUSION

This present study evaluated *Capparis moonii* for urolithiasis and related problems in human. As conventional medicines are typically taken by the oral route, same course of administration was used for assessment of antilithiatic consequence of the extracts of *Capparis moonii* at a dose of 200mg/kg and Cystone herbal tablet at a dose of 100mg/kg aligned with ethylene glycol induced urolithiasis in rats. In the present study, male rats were selected to persuade urolithiasis because the urinal system of male rats resembles that of humans and also the quantity of stone deposition in female rats was drastically fewer. Substantiation in preceding studies indicated that in response to 14 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi encompassed mainly of calcium oxalate. The biochemical mechanisms for this progression are related to a raised in the urinal concentration of oxalate. Stone development in ethylene glycol fed animals is caused by hyperoxaluria, which causes augmented renal retention and emission of oxalate. In the present study oxalate and calcium excretion progressively augmented in calculi-induced animals, since it is customary that hyperoxaluria, is a far more risk reason in the pathogenesis of renal stones than hypercalciuria, and the changes in urinal oxalate levels are comparatively much more important than those of calcium. Augmented urinal calcium is a feature favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and consequent crystal growth. Conversely, extracts of *Capparis moonii* treated animals lowered the levels of oxalate as well as calcium excretion.

In the present study, *Capparis moonii* are having good antioxidant and antiurolithiatic activity, it was proved obviously in this juncture. Meticulous percevings of the patho-physiology of illness and method of action of these herbal medicines have great importance in improvement of effective and safe antiurolithiatic agent. The antioxidant action was calculated as free radical scavenging activity technique, Nitric oxide scavenging, DPPH method, Reducing control determination technique, Hydrogen peroxide method. All the methods depict good response due to thepresence of phenolic compounds and flavonoids in three species. The herbal drugs exert their urolithiatic consequence by varying the ionic content of urine lessening the ca+ and oxalate ion strength or escalating magnesium and citrate excretion and also with diuretic activity. In this respect this information provides a fruitful area for scientific research by willing investigators.An attempt may be made to develop new herbal formulation to treat Kidney stone by *Capparis moonii* plants. From

5this present study we can conclude by using this *Capparis moonii*, we can go for herbal formulation development to treat Kidney stone.

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CERTIFICATE

Name of the student : **Santhanam . A**

This is to certify that the project **"Evaluation of Antiurolithic Activity of Hydro alcoholic extracts of leaves of *Capparis moonii* in Ethylene Glycol (EG) Induced Urolithiasis"** has been approved by the IAEC, meeting held on 10-04-2019.

Proposal number	: **JKKN/IAEC/M.Pharm/13/ 2019**
Approval date	: **10-04-2019**
No of animals sanctioned	: **06 Wistar rats**

10/4/19

Dr. R.Sambath kumar

IAEC Chairperson

Dr. R.SAMBATH KUMAR
Chair Person
Institutional Animal Ethical Committee
JKK Nattraja College of Pharmacy
Komarapalayam - 638 183

10/04/19

Dr.P.Srinivsan

CPCSEA link nominee

CPCSEA LINK NOMINEE
Institutional Animal Ethical Committee
JKK Nattraja College of Pharmacy
Komarapalayam - 638 183