ANTI UROLITHIATIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *APIUM GRAVEOLENS* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS

A Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University

Chennai – 600 032

In Partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

MAHESH KUMAR S

Registration No: 261725251

Under the guidance of

M.GOMATHI M.Pharm.,

Assistant Professor

DEPARTMENT OF PHARMACOLOGY,

ULTRA COLLEGE OF PHARMACY,

4/235, COLLEGE ROAD, THASILDAR NAGAR,

MADURAI- 625020.

NOVEMBER 2019

DECLARATION

I hereby declare that the Dissertation work entitled **"ANTI UROLITHIATIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *APIUM GRAVEOLENS* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS"** submitted by me in partial fulfilment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, work carried out at Department of Pharmacology, Ultra College of Pharmacy, Madurai during the academic year 2017-2018 under the valuable and efficient guidance of **Mrs. M.GOMATHI M.Pharm.,** Assistant Professor, Ultra College of Pharmacy, Madurai, I also declare that the matter embodied in it is a genuine work and the same has not found formed the basis for the award of any degree, diploma, associate ship, fellowship of any other university or institution.

Place: Madurai Reg. No: 261725251

Date: **MAHESHKUMAR.S**

ULTRA COLLEGE OF PHARMACY
4/235, COLLEGE ROAD,
THASILDAR NAGAR,
MADURAI – 625020.

CERTIFICATE

This is to certify that the Dissertation work entitled "**ANTI UROLITHIATIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *APIUM GRAVEOLENS* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS"** submitted in partial fulfilment of the requirements for the award of degree of Master of Pharmacy in Pharmacology, of the Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide work carried out by **MAHESH KUMAR.S (Reg. No: 261725251)** guided and supervised by Mrs. **M.GOMATHI M.Pharm.,** during the academic year 2018-2019.

Place: Madurai
Date:

Mrs. M.GOMATHI M.Pharm.,
Assistant Professor,
Department of pharmacology.
Ultra College of Pharmacy,
4/235, College Road, Thasildar Nagar,
Madurai-625020

ULTRA COLLEGE OF PHARMACY
4/235, COLLEGE ROAD,
THASILDAR NAGAR,
MADURAI – 625020.

CERTIFICATE

This is to certify that the Dissertation work entitled "**ANTI UROLITHIATIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *APIUM GRAVEOLENS* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS"** submitted in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology, of the Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide work carried out by **MAHESH KUMAR S (Reg. No: 261725251)** guided and supervised by **Mrs. M.GOMATHI M.Pharm.,** during the academic year 2018-2019.

Dr. P.S.S. RAMKUMAR,
M. Pharm., Ph.D.,
Vice-Principal
Ultra College of Pharmacy,
4/235, College Road, Thasildar Nagar,
Madurai- 625020

Dr. A. BABU THANDAPANI,
M. Pharm. Ph.D.,
Principal
Ultra College of Pharmacy,
4/235, College Road, Thasildar Nagar,
Madurai- 625020

ULTRA COLLEGE OF PHARMACY
4/235, COLLEGE ROAD,
THASILDAR NAGAR,
MADURAI – 625020.

CERTIFICATE

This is to certify that the Dissertation work entitled "**ANTI UROLITHIATIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *APIUM GRAVEOLENS* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS"** submitted in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology, of the Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide work carried out by **MAHESH KUMAR S (Reg. No: 261725251)** guided and supervised by **Mrs. M.GOMATHI, M.Pharm.,** during the academic year 2018-2019 was evaluated by us.

Place: Madurai.

Date:

Examiners:

1.

2.

WE DEDICATE THIS PROJECT TO ALMIGHTY GOD, OUR ENLIGHTING STAFFS, LOVABLE PARENTS AND OUR MOTIVATING FRIENDS.

ACKNOWLEDGMENT

I humbly thank the Almighty who has given me the health and ability to pass through all the difficulties in the compliance and presentation of my project.

With pride and pleasure, I wish to express my thanks to **Prof. K.R. Arumugam, M.Pharm,** Chairman, **Ultra** College of Pharmacy, Madurai, for his encouragement and source of inspiration my project work.

I deem it my privilege in expressing fidelity Principal **Dr. A. Babu Dhandapani, M. Pharm, Ph.D.,** Ultra College of Pharmacy, Madurai, for his unswerving support and open handed help in allowing us to exploit all the facilities available in the department

I am indebted to **Dr. P.S.S. Ramkumar. M. Pharm, M.B.A., Ph.D.,** vice principal Ultra College of Pharmacy, Madurai for his support and encouragement during my project work

I take privilege and honour to extent deepest gratitude and cordial thanks to my guide **M.GOMATHI, M.Pharm.,** Asst .Professor, Department of Pharmacology, Ultra College of Pharmacy, Madurai for her supervision and guidance during the whole project, together with her enormous support, perpetual encouragement, abundant help and constructive criticism were the real driving force as well as the keen interest in my project encouraged me a lot, madam without your help this work not in this form. Thank you so much madam

I wish to offer my respectable thanks to the teaching staff **Mr.Balu, Mr. Sampath Kumar, Mr. Pratheesh, Mr. K. Senthil Kumar and Mr. S.K. Satheesh Kumar** for their valuable help and suggestions throughout my thesis work. I would like to thank **Miss. Vinothani** madam. I must record my special thanks to **Mrs. B. Masila,** B. Com, Lab technician (P.G), Lab technician (U.G), Department of Pharmacology, for her continuous assistance in carrying out the project work and special thanks to the Librarian **Mr.Thirunavukkarasu, M.A., M.Literauture** (U.G) and asst Librarian madam.

My endless thanks to Dr.Chithambaranathan Ph.D., Department of Pharmacology, KMCP, for their timely support abundant help and everlasting encouragement throughout the project.

I offer flowers of gratitude to **Mrs.V. Esther Pandiammal** (mother), ROSE (sister) and my family members; whose inspiration, motivation, blessings and moral support continue to contribute a great deal to my academic endeavours.

I would whole heartedly thank my senior vinciya T, and my friends fayas, Ibrahim, for their timely support, abundant help and everlasting encouragement throughout my project.

Completing task is never a one man effort. It is often result of individual contribution of a number of individual in a direct or indirect manner.

Last but not the least, I express my gratitude and apologize to everybody whose contributions, I could not mention in this page.....

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

IH	Idiopathic Hypercalciuria
CUD	Cardio vascular diseases
Caox	Calcium oxalate
OPN	Osteopontin
IaI	Inter-a-Inhibitor
HC	Heavy chain
UPTF	Urinary prothrombin fragment
THP	Tamm-Horstall protein
TAL	Thick Ascending Limb
GAGs	Glycosamino Glycans
CaCo3	Calcium carbonate
RL	Renal Lithostathine
KUB	Kidney Ureters Bladders flim
CT	Computer Tomography
GFR	Glomeruli Filtration
TPA	Terephthalic Acid
HE	Hematoxylin Eosin
r.p.m	Rotation per minutes
ESWL	Extra Corporal Shock Wave Lithotripsy
CPD	Calculi Producing Diet
EG	Ethylene Glycol
ANOVA	Analysis of Variance

SEM	Standard Error of Mean
CPCSEA	Committee for the Purpose of Control and Supervision of Experimental on Animal.
OECD	Organisation for Economic Co-operation and Development.
HCL	Hydrochloric acid
NAOH	sodium hydroxide
g	gram
kg	kilogram
nm	nano meter
mmol/l	millimoles per liter
v/v	volume /volume

CERTIFICATE

This is certify that the project title **"ANTI UROLITHIATIC AND ANTI OXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *APIUM GRAVEOLENS* AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN WISTAR ALBINO RATS"** has been approved by the IAEC. Proposal No: **IAEC/S.MAHESHKUMAR/M.PHARM/TNMGRMU/ 261725251/KMCP/67/2019.**

Name of Chairman/
Member Secretary IAEC:

Dr. N. CHIDAMBARANATHAN

Name of CPCSEA nominee:

Dr. P. THIRUPATHY KUMARESAN

Signature with date

30/5/19

Chairman/ Member Secretary of IAEC:

I. A. E. C. CHAIRMAN
INSTITUTIONAL ANIMAL ETHICAL COMMITTEE
K. M. COLLEGE OF PHARMACY
MADURAI-625 107.

30/5/19

CPCSEA nominee:

CPCSEA NOMINEE
INSTITUTIONAL ANIMAL ETHICS COMMITTEE
K M. COLLEGE OF PHARMACY
MADURAI-625 107

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Dr. D. STEPHEN
ASSISTANT PROFESSOR
DEPARTMENT OF BOTANY

THE AMERICAN COLLEGE
MADURAI 645002
Ph. 9944792299, 0452-2642289

CERTIFICATE

This is to certify that the plant specimen brought to me by **Mr. S. Mahesh kumar (Reg. No. 261725251)** II M.Pharm., **Department of Pharmacology, Ultra College of Pharmacy, Madurai** has been identified as ***Apium graveolens* L.** belongs to the family **Apiaceae**.

(Dr. D. Stephen)

Dr. D. STEPHEN, Ph.D.,
LECTURER
DEPARTMENT OF BOTANY
THE AMERICAN COLLEGE
MADURAI-625 002
TAMILNADU - INDIA

Introduction

Review of Literature

Plant Profile

Aim & Objectives

Phytochemical Analysis

Pharmacological Evaluation

Results and Analysis

Discussion

Conclusion

Bibliography

1. INTRODUCTION[1,2]

Kidney stones is also major disorders all over the world. About 75% of kidney stones are composed of calcium oxalate crystals. Gall stone problem is mainly affected in global countries. More than half a million people are affected annually in United States and more than 50,000 people in Canada. Canada endures surgical treatment to remove their gall bladder because of gall stone. About 80 % of the all gall stones be evidence for no symptoms and may continue for years.

A large number of people are suffering from urinary stone problem all over the globe. Not only the humans but animals and birds also suffer from the urinary stone problem. The occurrence in some areas is so alarming that they are known as Stone Belts. Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 7081% in males, and 4760% in females. Approximately 50% of patients with previous urinary calculi have a recurrence within 10 years. Stone disease is 2-3 times more common in males than in females. Most urinary calculi occur in patients aged 2049 years. (1)

This higher rate of occurrence in men than in women can also be due to enhancing capacity of testosterone and inhibiting capacity of oestrogen in stone formation. Also, the increase daily breakdown of the tissues in men could result in increased metabolic waste and a predisposition to stone formation. The other more significant cause may be because of the male urinary tract being more complicated than the female urinary tract. Estrogen may also help to prevent the formation of calcium stones by keeping urine alkaline and raising protective citrate levels.

An unbalanced diet or particular sensitivity to various foods in stone formers can lead to urinary alterations such as hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitrauria and excessive acid urinary pH. Over the course of time, these conditions contribute to the formation or recurrence of kidney stones, due to the effect they exert on the lithogenous salt profile. The fundamental aspects of the nutritional approach to the treatment of idiopathic nephrolithiasis

are body weight, diet and water intake Stone disease is a multifactorial disease; the cause of calcium oxalate stones is heterogeneous and might involve both genetic and environmental factors. Although extensive genetic studies were carried out, no chromosomal mapping has been conducted in patients with stones and idiopathic hypercalciuria (IH). The only conclusive evidence through genetic studies is that urolithiasis is a polygenic defect and partly penetrative (2).

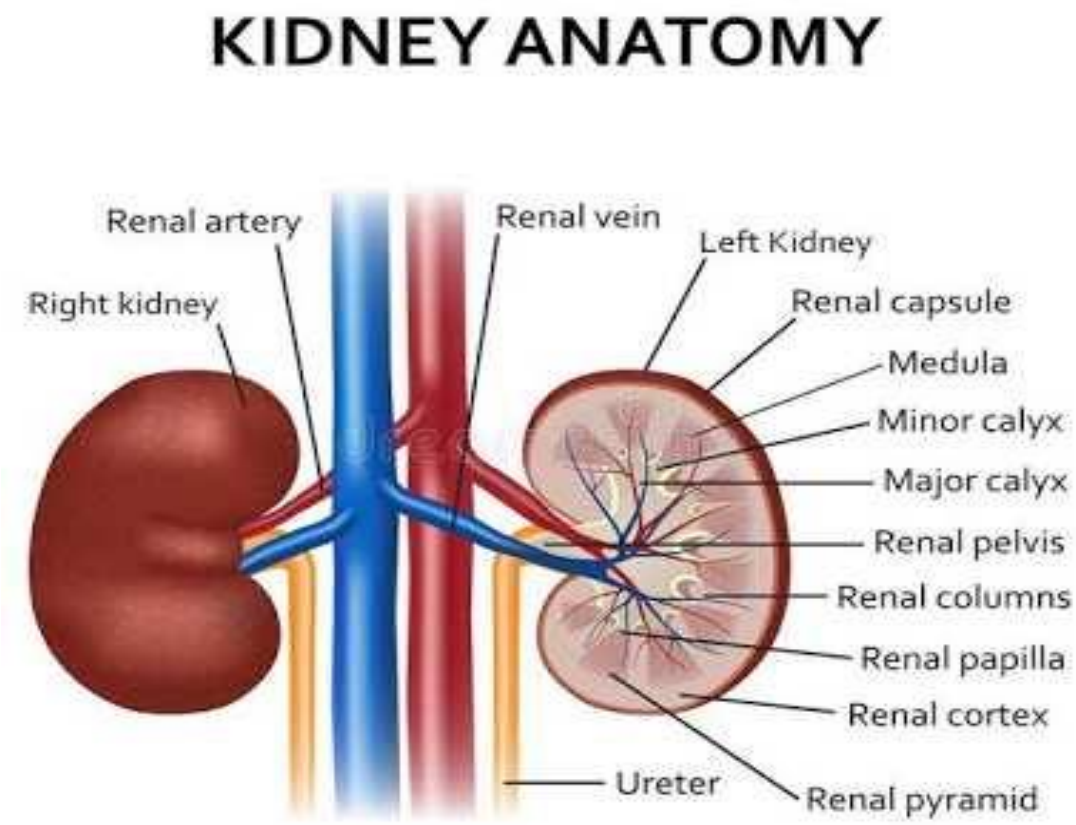


FIGURE 1 : ANATOMY OF KIDNEY

KIDNEY

The urinary system is the main excretory system and consists of the following structures, 2 kidneys, 2 ureters, urinary bladder and urethra.

Definition

Kidneys are a pair of excretory organs situated on the posterior abdominal wall, one on each side of the vertebral column, behind the peritoneum. They remove waste products of metabolism and excess of water and salt from the blood and maintain its PH.

Location:

The kidneys occupy the epigastric, hypochondriac, lumbar and umbilical regions. Vertically they extend from the upper border of twelfth thoracic vertebra to the centre of thebody of third lumbar vertebra. The right kidney is slightly lower than the left, and the left kidney is little nearer to the median plane than the right.

The transpyloric plane passes through the upper part of the hilus of the right kidney and through the lower part of the hilus of the left kidney.

Shape, Size, Weight and Orientation:

Each kidney is bean shaped. Each kidney is about 11cm long, 6cm broad, and 3cm thick. The left kidney is a little longer and narrower than the right kidney. On an average the kidney weighs 150 g in males and 135g in females. The kidneys are reddish brown in colour.

The kidneys perform many crucial functions, including:

- maintaining overall fluid balance
- regulating and filtering minerals from blood
- filtering waste materials from food, medications, and toxic substances
- creating hormones that help produce red blood cells, promote bone health, and regulate blood pressure

Nephrons

Nephrons are the most important part of each kidney. They take in blood, metabolize nutrients, and help pass out waste products from filtered blood. Each kidney has about 1 million nephrons. Each has its own internal set of structures.

Renal corpuscle

After blood enters a nephron, it goes into the renal corpuscle, also called a Malpighian body. The renal corpuscle contains two additional

- **The glomerulus.** This is a cluster of capillaries that absorb protein from blood traveling through the renal corpuscle.
- **The Bowman capsule.** The remaining fluid, called capsular urine, passes through the Bowman capsule into the renal tubule

Renal tubules

The renal tubules are a series of tubes that begin after the Bowman capsule and end at collecting ducts.

Each tubule has several parts:

- **Proximal convoluted tubule.** This section absorbs water, sodium, and glucose back into the blood.
- **Loop of Henle.** This section further absorbs potassium, chloride, and sodium into the blood.
- **Distal convoluted tubule.** This section absorbs more sodium into the blood and takes in potassium and acid.

By the time fluid reaches the end of the tubule, it's diluted and filled with urea. Urea is by product of protein metabolism that's released in urine.

Renal cortex

The renal cortex is the outer part of the kidney. It contains the glomerulus and convoluted tubules.

The renal cortex is surrounded on its outer edges by the renal capsule, a layer of fatty tissue. Together, the renal cortex and capsule house and protect the inner structures of the kidney.

Renal medulla

The renal medulla is the smooth, inner tissue of the kidney. It contains the loop of Henle as well as renal pyramids.

Renal pyramids

Renal pyramids are small structures that contain strings of nephrons and tubules. These tubules transport fluid into the kidney. This fluid then moves away from the nephrons toward the inner structures that collect and transport urine out of the kidney.

Collecting ducts

There's a collecting duct at the end of each nephron in the renal medulla. This is where filtered fluids exit the nephrons.

Once in the collecting duct, the fluid moves on to its final stops in the renal pelvis.

Renal pelvis

The renal pelvis is a funnel-shaped space in the innermost part of the kidney. It functions as a pathway for fluid on its way to the bladder

Calyces

The first part of the renal pelvis contains the calyces. These are small cup-shaped spaces that collect fluid before it moves into the bladder. This is also where extra fluid and waste become urine.

Hilum

The hilum is a small opening located on the inner edge of the kidney, where it curves inward to create its distinct beanlike shape. The renal pelvis passes through it, as well as the:

- **Renal artery.** This brings oxygenated blood from the heart to the kidney for filtration.
- **Renal vein.** This carries filtered blood from the kidneys back to the heart.

Ureter

The ureter is a tube of muscle that pushes urine into the bladder, where it collects and exits the body.

FUNCTONS OF KIDNEY [3]

- Regulation of extracellular fluid volume. The kidneys work to ensure an adequate quantity of plasma to keep blood flowing to vital organs.
- Regulation of osmolarity.
- Regulation of ion concentrations.
- Regulation of pH. .
- Excretion of wastes and toxins.
- Production of hormones.
- Maintaining overall fluid balance
- Regulating and filtering minerals from blood.
- Filtering waste materials from food, medications, and toxic substances.
- Creating hormones that help produce red blood cells, promote bone health, and regulate blood pressure.
- Maintaining overall fluid balance.
- Regulating and filtering minerals from blood.
- Filtering waste materials from food, medications, and toxic substances.
- Creating hormones that help produce red blood cells, promote bone health, and regulate blood pressure

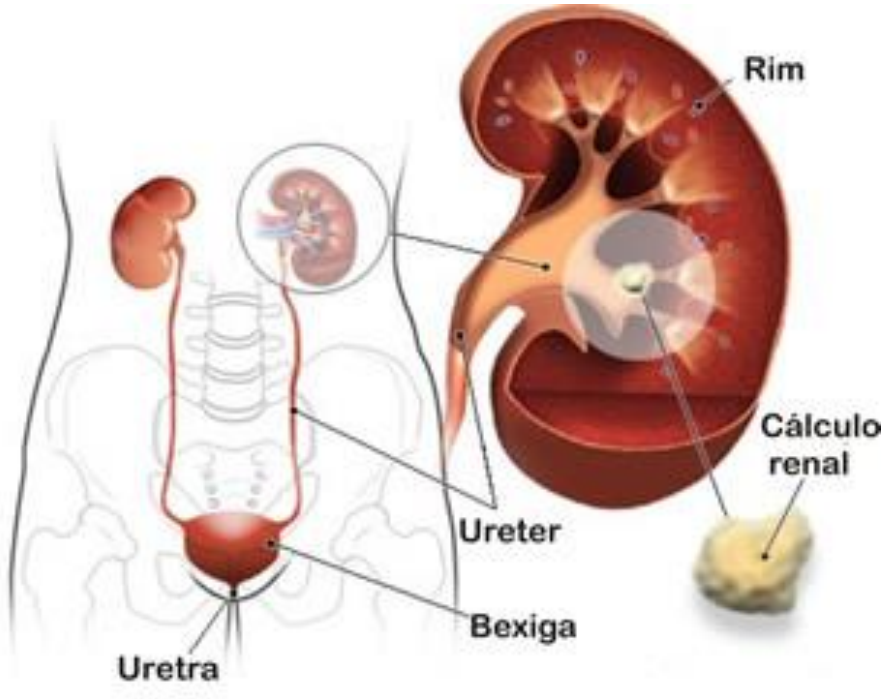


FIGURE 2: KIDNEY STONE FORMATION

UROLITHIASIS:[4-7]

The development of stones is related to decreased urine volume or increased excretion of stone forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate. The stones form in the urine collecting area (the pelvis) of the kidney and may range in size from tiny to staghorn stones the size of the renal pelvis itself.

The pain with kidney stones is usually of sudden onset, very severe and colicky (intermittent), not improved by changes in position, radiating from the back, down the flank, and into the groin. Nausea and vomiting are common.

Factors predisposing to kidney stones include recent reduction in fluid intake, increased exercise with dehydration, medications that cause hyperuricemia (high uric acid) and a history of gout.

Treatment includes relief of pain, hydration and, if there is concurrent urinary infection, antibiotics.

The majority of stones pass spontaneously within 48 hours. However, some stones may not. There are several factors which influence the ability to pass a stone. These include the size of the person, prior stone passage, prostate enlargement, pregnancy, and the size of the stone. A 4 mm stone has an 80%chance of passage while a 5 mm stone has a 20% chance. If a stone does not pass, certain procedures (usually by a urology specialist doctor) may be needed.

The process of stone formation, urolithiasis, is also called nephrolithiasis. "Nephrolithiasis" is derived from the Greek nephros-(kidney) lithos (stone) = kidney stone

"Urolithiasis" is from the French word "urine" which, in turn, stems from the Latin "urina" and the Greek "ouron" meaning urine= urine stone. The stones themselves are also called renal calculi. The word "calculus" (plural: calculi) is the Latin word for pebble.

Etiology:

Several etiological factors contribute to the pathogenesis of stone formulation.

- **Geography**

Kidney stone incidence varies in different parts of the world, thus projecting the significance of the stone belt areas. The effect of geography on the incidence of stone formation may be direct, through its effect on temperature, high temperatures increase perspiration, which may result in concentrated urine, which in turn promotes increased urinary crystallization.

- **Age and Sex**

The disease affected all age groups from less than 1 year old to more than 70, with a male to female ratio of 2:1.

- **Nutritional aspects**

An unbalanced diet or particular sensitivity to various foods in stone formers can lead to urinary alterations such as hypercalciuria, hyperoxaluria, hyperuricosauria, hypocitrauria and excessive acid urinary pH.

- **Diet**

Some reports have described that vegetarians are at lower risk for stone formation in contrast to non-vegetarians.

- **Water intake**

Supersaturation of the urinary environment with stone -forming constituents is a prerequisite for calculus formation and increased fluid consumption results in excretion of higher volume of urine, which is less supersaturated with stone -forming constituents.

- **Body weight**

Overweight condition and obesity was found in 59.2% of the men and 43.9% of the women and both these conditions were strongly associated with an elevated risk of stone formation in both genders due to increased urinary excretion of promoters but not inhibitors of calcium oxalate stone formation and further concluded that overweight and obese men are more prone to stone formation than overweight women.

- **Kidney stone and other diseases**

It has been proposed that essential hypertension, cardiovascular diseases (CVD), diabetes, and other medical conditions predispose to stone disease.

- **Recurrence**

The recurrent nature of stone disease is a well-recognized Clinical problem. Urinary metabolic abnormalities such as low urine volume, hypercalciuria, hyperoxaluria, hyperuricosuria and hypocitraturia predispose a patient to early recurrence. Male gender, multiple stones, stone location, residual fragments and

some anatomic or functional urinary tract abnormalities are known to be major risk factors for recurrence.

- **Occupation**

The role of occupation in stone formation is highly debated. Kidney-related complications are on the increase because of geographic factors: residence in the "stone belt, occupation related lifestyle changes - in case of indoor occupation - sedentary habits, stress, unhealthy dietary plan in terms of healthy or over healthy food intake, irregular food habits and fluid intake (intake of juices and beverages instead of water) or the other spectrum of physical manual labour - involving working outside exposed to heat and sun, low socioeconomic status, malnutrition and reduced fluid intake. Some experts speculated that this increased risk might be due to a hormone called vasopressin, which is released during stress, which increases the concentration of urine.

- **Molecular Aspects**

Stone disease is a multifactorial disease; the cause of calcium oxalate stones is heterogeneous and might involve both genetic and environmental factors. Although extensive genetic studies were carried out, no chromosomal mapping has been conducted in patients with stones and idiopathic hypercalciuria (IH). The only conclusive evidence through genetic studies is that urolithiasis is a polygenic defect and partly penetrative.

EPIDEMIOLOGY OF UROLITHIASIS:[8-11]

The epidemiology of urolithiasis varies according to geographical area in term of prevalence and age, incidence, and sex distribution, stone composition and stone location. Such differences have been explained in terms of race, diet and climate factors.

Socioeconomic conditions have generated difference in the prevalence, incidence and distribution for age, sex and type of lithiasis in terms of both the site and the chemical-physical composition of the calculi

Epidemiological surveys proves that the prevalence rate ranged between 4% and 20% for economically developed countries. Urolithiasis, urinary stone development, is the third most common problem of the urinary tract, with lifetime incidence of 12% and 7% in men and women respectively in U.S.A. and 34% and 6-9% in women and men respectively in the other western countries, In India its reversion rate is about 50% in 5-10 years and 75% in 20 years9,10. The disease lead to loss of about $5 billion per annum in the USA . About 12% of India population are suffering from the problem of urinary stones, 50% of which may result in kidney and renal injury. About 80% of these calculi are made up of calcium oxalate (CaOx). In the 20th century, occurrence and frequency of upper urinary tract stones were still increasing in Western countries probably resulting from developments in clinical-diagnostic procedures and differences in nutritional and environmental factors. Endemic infantile bladder stone disease, with features similar to those previously defined in Europe in the 19th century, was fairly prevalent in huge areas of Turkey, Iran, India, China, Indochina and Indonesia with stones composed of calcium oxalate and ammonium urate due to malnutrition

kidney stones:[12,13]

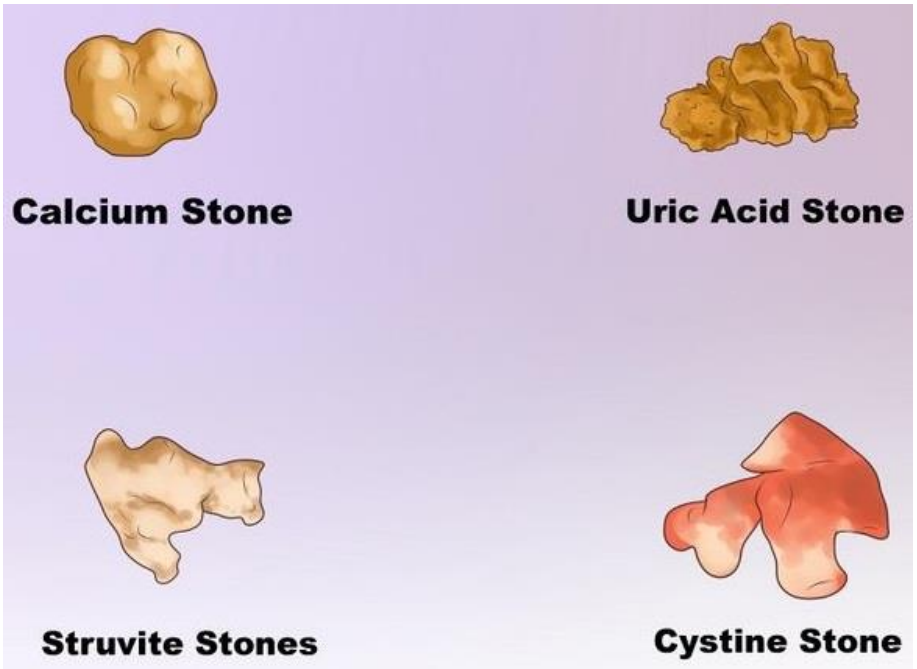


FIGURE 3 TYPES OF KIDNEY STONES

Types of urinary calculi

There are 4 main types of urinary calculi-calcium containing, mixed (struvite), uric acid and cystine stones, and a few rare types.

1. CALCIUM STONES

Calcium stones are the most common comprising about 75% of all urinary calculi. They may be pure stones of calcium oxalate (50%) or calcium phosphate (5%), or mixture of calcium oxalate and calcium phosphate (45%).

Etiology

Etiology of calcium stones is variable

About 50% of patients with calcium stones have idiopathic hypercalciuria without hypercalcaemia. Approximately 10% cases are associated with hypercalcaemia and hypercalciuria, most commonly due to hyper parathyroidism, or a defect in the bowel (i.e. absorptive hypercalciuria), or in the kidney (i.e. renal hypercalciuria).

About 15% of patients with calcium stones have hyper uricosuria with a normal blood uric acid level and without any abnormality of calcium metabolism.

In about 25% of patients with calcium stones, the cause is unknown as there is no abnormality in urinary excretion of calcium, uric acid or oxalate and is referred to as "idiopathic calcium stone disease"

Pathogenesis

The mechanism of calcium stone formation is explained on the basis of imbalance between the degree of supersaturation of the ions forming the stone and the concentration of inhibitors in the urine. Most likely site where the crystals of calcium oxalate and or calcium phosphate are precipitated is the tubular lining or around some fragment of debris in the tubule acting as nidus of the stone. The stone grows, as more and more crystals are deposited around the nidus. Other

factors contributing to formation of calcium stones are alkaline urinary pH, decreased urinary volume, and increased excretion of oxalate and uric acid.

Morphology

Caicium stones are usually small (less than a centimetre), ovoid, hard, with granular surface. They are dark brown due to old blood pigments deposited in them as a result of repeated trauma caused to the urinary tract by these sharp edged stones.

MIXED (STRUVITE) STONES

About 15% of urinary calculi are made of magnesium-ammonium-calcium-phosphate, often called struvite; hence mixed stones are also called as 'struvite stones' or triple phosphate stones.

Etiology

Struvite stone are formed as a result of infection of the urinary tract with urea splitting organisms that produce urease such as by species of proteus and occasionally klebsiella, pseudomonas and enterobacter. These are, therefore, also known as infection –induced stones. However, E.coli does not form urease.

Morphology

Struvite stones are yellow white or grey. They tend to be soft and friable and irregular in shape. Staghorn stone which is a large solitary stone that take the shape of the renal pelvis where it is often formed is an example of struvite stone.

URIC ACID STONE

Approximately 6% of urinary calculi are made up of uric acid. Uric acid calculi are radiolucent unlike radio opaque calcium stones.

Etiology

Uric acid stones are frequently formed in cases with hyper uricaemia and hyper uricosuria such as due to primary gout or secondary gout due to

myeloproliferative disorders especially those on chemotherapy and administration of uricosuric drugs (egsalicyates, probenecid). Other factors contributing to their formation are acidic urinary pH (below 6) and low urinary volume .

Pathogenesis

The solubility of uric acid at pH of 7 is 200 mg/dl while at pH of 5 is 15 mg /dl . Thus as the urine become more acidic ,the solubility of uric acid in urine decreases and precipitation of uric acid increases favouring the formation of uric acid stones while hyper uricaemia is found in half the cases .

Morphology

Uric acid stones are smooth ,yellowish brown hard and often multiple .On cut section they show laminated structure .

CYSTINE STONES

Cystine stones comprises less than 2% of urinary calculi. Formation of cystine stones is the only clinical expression of cystinuria, an autosomal recessive disorder. People who are homozygous for cystinuria excrete more than 600 mg per day of insoluble cystine. The stones are greenish-yellow, flecked with shiny crystallites, and are moderately radio-opaque in appearance. These stones represent only a small percentage of kidney stones

Etiology

Cystine stones are associated with cystinuria due to genetically determined defects in the transport of cystine and other amino acids across the cell memebrane of the renal tubules and the small intestine mucosa.

Pathogenesis

The resultant excessive excretion of cystine which is least soluble of the naturally occurring aminoacids leads to the formation of crystals and eventually cystine calculi.

Morphology

Cystine stones are small, rounded, smooth often multiple. They are yellowish and waxy.

OTHER CALCULI

Less than 2% of urinary calculi consist of other rare types such as due to inherited abnormality of enzyme metabolism. E.g. hereditary xanthinuria developing xanthine stones.

Symptoms of kidney stones : (14)

- A kidney stone may not cause symptoms until it moves around within your kidney or passes into your ureter — the tube connecting the kidney and bladder. At that point, you may experience these signs and symptoms.
- Severe pain in the side and back, below the ribs
- Pain that radiates to the lower abdomen and groin
- Pain that comes in waves and fluctuates in intensity
- Pain on urination
- Pink, red or brown urine
- Cloudy or foul-smelling urine
- Nausea and vomiting
- Persistent need to urinate
- Urinating more often than usual
- Fever and chills if an infection is present
- Urinating small amounts

Pain caused by a kidney stone may change — for instance, shifting to a different location or increasing in intensity — as the stone moves through your urinary tract.(15,16).

FACTOR'S INDUCING LITH'S FORMATION:**[13,17,18]**

High levels of calcium

Too much calcium in the urine -- hypercalciuria -- can be a risk factor for kidneystones and is frequently genetically determined. Certain medications such as calcium containing antacids, loop diuretics and glucocorticoids can increase calcium secretion into theurine. Too much vitamin D can also lead to increased calcium.

Hyperparathyroidism occurswhen too much parathyroid hormone is produced by the body, causing calcium to be pulledfrom the bones into the blood and subsequently into the urineThis helps to explain the association between kidney stones and low bone density. Kidney disease, too, can cause high calcium levels in the urine when calcium is not properly absorbed back into the bloodstream. High blood pressure and obesity have also been associated with hypercalciuria.

High levels of oxalates

Some people are born with a genetic tendency to secrete excess oxalate into the urine. This condition, hyperoxaluria, is rare; most cases of hyperoxaluria arise from other causes. For one, diets rich in oxalate may place someone at risk for kidney stones. Oxalate-rich foods include beets, chocolate, nuts, rhubarb, spinach, strawberries, tea and wheat bran. Excessive amounts of vitamin C can also increase oxalate levels, as can inflammatory bowel disease

High levels of Protein

High amounts of dietary protein can lead to increases in both calcium and oxalate levels in the urine. The elevated protein results in lower urine pH -- an acidic environment that makes it easier for calcium oxalate kidney stones to form. It also decreases citrate levels in the urine that help prevent kidney stones from forming. The risks of kidney stone formation can often be minimized by paying close attention to diet and good hydration. If you are concerned about kidney

stones, speak with a health-care provider who can evaluate the type of stones you might have and what dietary changes would be most helpful for you.

Uric acid stones

Uric acid stones are the most common cause of radiolucent kidney stones in children. Several products of purine metabolism are relatively insoluble and can precipitate when urinary pH is low. These include 2- or 8-dihydroxyadenine, adenine, xanthine, and uric acid. The crystals of uric acid may initiate calcium oxalate precipitation in metastable urine concentrates. The terms gouty nephropathy, urate nephropathy, and uric acid nephropathy are used to describe renal insufficiency due to uric acid precipitation within the renal tubules. Uric acid urolithiasis or uric acid kidney stones refer to development of a stone or calculuscomposed of significant amounts of urate in the renal pelvis, ureter, or bladder.

FLOW CHART NO 1: PATHOPHYSIOLOGY OF LITH'S FORMATION

Mechanism of stone formation[19]

AGE	PROFESSIOON	NUTRITION	CLIMATE	INHERITANCE
SEX	MENTALITY	CONSTITUITIONS	RACE	

ABNORMAL RENAL MORPHOLOGY DISTURBED URINE FLOW	URINARY TRACT INFECTIONS	METABOLIC ABNORMALITIES	GENETIC FACTORS

INCREASED EXCREATION STONE FORMING CONSISTUENTS	DECREASED EXCREATION OF INHIBITORS OF CRYSTALLIZATIONS

PHYSICO CHEMICAL CHANGEIN THE STATE OF SUPERSATURATION

ABNORMAL CRYSTALLURIA

CRYSTALS AGGREGATION

CRYSTAL GROWTH

FORMATION OF STONE

MODES OF STONE GROWTH[20-26]

Nucleation

Nucleation is the process by which free ions in solution associate into microscopic particles. Crystallization can occur in solution micro-environments, such as may be present in certain points in the nephron, as well as on surfaces, such as those of cells and on extracellular matrix. There is considerable dispute about the importance of free solution crystallization versus crystallization at other sites, in renal tubules or on bladder walls, on normal or damaged cells, on areas denuded of cells by certain forms of injury, or at interstitial sites.

Aggregation

Aggregation is a process by which there is agglomeration of crystals that form in free solution into larger multicomponent particles. It may also encompass the phenomenon of secondary nucleation of new crystals on the surface of those already formed. The structure of stones suggests that one or other of these processes must occur for the stone to grow to a clinically significant size. Kidney stones can be thought of as being similar to concrete, a mixture of a binding agent (cement), and particulates such as sand, pebbles, or glass. Stones are an aggregation of crystals and an organic matrix, the latter serving as the binding agent. The organic matrix contains proteins, lipids, polysaccharides, and other cell-derived material.

Crystal growth

Growth of microscopic crystals is accomplished by movement of ions out of solution onto the growing crystal. While some growth of nuclear crystals must occur by movement of ions from solution, this is clearly a limited process, as giant single crystals of stone constituents are not generally observed. It is more likely that stone growth is accomplished through aggregation of preformed crystals or secondary nucleation of crystal on the matrix coated surface of another It has been proposed that the growth of these microscopic crystals to the extent that they can

be retained in the kidney on the basis of size alone cannot occur without aggregation or attachment to specific intrarenal structures.

INHIBITORS OF CRYSTALLIZATION

Inhibitors of calcium stone formation prevent crystal growth and aggregation by coating the surface of growing calcium crystals or by complexing with calcium and oxalate.

Citrate

Citric acid is a tricarboxylic acid that circulates in blood complexed to calcium, magnesium and sodium at physiological pH of 7.4. Most of the circulating citrate is derived from endogenous oxidative metabolism. It is filtered freely through the glomerulus. Approximately 75% of the filtered citrate is reabsorbed in the proximal convoluted tubule. Apart from idiopathic causes, other aetiological factors of hypocitraturia are – use of drugs like acetazolamide and thiazides, renal tubular acidosis, urinary tract infection, hypokalemia, hypomagnesemia and inflammatory bowel disease.

Thiazide diuretics may induce hypocitraturia owing to hypokalemia with resultant intracellular acidosis. Hypocitraturia is a common disorder occurring in >50% of patients with nephrolithiasis. Citrate has been widely studied for its stone inhibiting action in urine and it has been found to be particularly effective against the calcium oxalate and phosphate stones. Citrate appears to alter both calcium oxalate monohydrate and calcium phosphate crystallisation.

Pyrophosphates

At low concentrations, 16 mM, pyrophosphate inhibits calcium oxalate monohydrate crystal growth by 50%. The urinary pyrophosphate level is in the range of 20–40 mM and therefore, theoretically levels are high enough to inhibit Calcium oxalate and Calcium phosphate crystallisation. Pyrophosphate and diphosphate have shown to inhibit the precipitation of Calcium phosphate, where asdiphosphates also inhibits the growth of apatite crystals. Pyrophosphate will

reduce the absorption of calcium in the intestine and this action probably mediated by formation of 1.25 (OH)2 – vitamin D. Sharma et al reported low 24-hour urinary excretion of pyrophosphate in stone formers (50.672.16 mmol/24 h) as compared to normal subjects (71.465.46 mmol/ 24 h) ($p < 0.01$) . Oral administration of orthophosphate has shown little benefit in prevention of stone recurrence. Conversely, patients treated in a randomised, placebo-controlled study recorded increased stone formation in the orthophosphate treated group over placebo treated subjects over a 3-year period. There is a lack of scientific evidence to support preventive role of orthophosphate.

Magnesium

Magnesium is the fourth most abundant mineral in the body and is largely found in bones. Dietary magnesium is absorbed in the small intestines and excreted through the kidney. Only 1% of total body magnesium circulates in blood. In a supersaturated Calcium oxalate solution 2 mmol/L magnesium reduced particle number by 50%. Magnesium can form complexes with oxalate and decreases Super Saturation. Oral intake of magnesium will decrease the oxalate absorption and urinary excretion, in a manner similar to calcium by binding to oxalate in the gut. Magnesium supplementation in subjects with magnesium deficiency increases the excretion of citrate in urine. However, there is little evidence to recommend magnesium therapy in patients with urolithiasis.

Inter-alpha-trypsin inhibitor family of proteins

Inter-a-inhibitor (IaI) belongs to the Kunitz-type protein superfamily, a group of proteins possessing a common structural element(kunin) and the ability to inhibit serine proteases .I aI is a glycoprotein composed of 2 heavy chains (HC1 and HC2) and one light chain, also known as bikunin .

Bikunin circulates free in plasma and is excreted in urine where it degrades further to fragments HI14 and HI8. Bikunin, a Kunitz type protease inhibitor found in human amniotic fluid and urine, exhibits anti-inflammatory and anti metastatic functions in animals and humans . It is expressed mainly in the

proximal tubules and the thin descending segment near the loop of Henle. It may contribute to the regulation of crystal adhesion and retention within tubules during kidney stone formation. Furthermore, the potent inhibition of Calcium oxalate crystal growth by these proteins, coupled with the known presence of bikunin and its fragments in urine, suggested the possible existence of a relationship between IaI and Calcium oxalate stone formation .

Osteopontin (Uropontin)

Osteopontin (OPN) is a negatively-charged aspartic acid rich protein that inhibits growth of Calcium oxalate crystals in a supersaturated solution. OPN is intimately involved in the regulation of both physiological and pathological mineralization. OPN is a phosphorylated protein of wide tissue distribution that is found in association with dystrophic calcification including in the organic matrix of kidney stones. OPN is synthesised within the kidney and present in the human urine at levels in excess of 100 nM.

Urinary prothrombin fragment 1

The blood clotting factor prothrombin is degraded into three fragments – thrombin, fragment 1 and fragment 2. Fragment 1 is excreted in urine and is named Urinary prothrombin fragment (UPTF1) and is a potent inhibitor of Calcium oxalate stone formation inviter.

The organic matrix of Calcium oxalate crystals contains UPTF1, providing evidence that links the role of blood coagulation proteins with urolithiasis. UPTF1 is an important inhibitor of Calcium oxalate crystal aggregation and adherence of crystals to renal cells. In South Africa the incidence of urolithiasis in blacks is significantly less compared to whites. UPTF1 from the black population has a superior inhibitory activity over UPTF1 from the white population. Further studies indicate that saliyatedglycoforms of UPTF1 afford protection against Calcium oxalate stone formation, possibly by coating the surface of Calcium oxalate crystals.

Tamm-Horsfall protein

Tammand Horsfall isolated a mucoprotein from the human urine nearly 50 years ago, and showed that the protein was able to interact and inhibit viral haemagglutination. Tamm-Horsfall protein (THP), also known as uromucoid, is an 80-kDa glycoprotein synthesized exclusively in the thick ascending limb of the loop of Henle'sloop (TAL) with exception the of the macula densa. THP is the most abundant protein in the urine of normal mammals. THP production ranges from 30 to 60 mg/24 h in humans.

THP may be involved in the pathogenesis of cast nephropathy, urolithiasis, and tubule interstitial nephritis. There is good evidence that the excessive intake of animal protein predisposes to stone disease.

Much controversy exists about whether THP is a promoter or an inhibitor of crystal aggregation. Most authors believe that it is an effective inhibitor of calcium oxalate monohydrate crystal aggregation in solutions with high pH, low ionic strength and low concentration of divalent ions and THP. In contrast, with low pH, high concentrations of calcium, sodium, and hydrogen ions as well as low THP, inhibitory activity is lost and it may even become a promoter of aggregation.

Glycosaminnoglycans

Glycosaminnoglycans (GAGs) have been identified as one of the macromolecules present in the stone matrix. chondroitin sulphate, heparin sulphate and hyaluronic acid are excreted in the urine. Recently, the main GAGs found in stone matrix were identified as heparan sulphate and hyaluronic acid. They are thought to play an important role in Calcium oxalate crystallization. GAGs concentration in the urine is too low to decrease calcium Super Saturation. In vitro, GAGs have shown to act as inhibitors of Calcium oxalate crystal growth and crystal aggregation. However, investigators have failed demonstrate any qualitative and/ or quantitative significant difference in total excretion of GAGs between stone formers and controls.

Renal lithostathine

Lithostathine is a protein of pancreatic secretion inhibiting calcium carbonate crystal growth. A protein immunologically related to lithostathine is actually present in urine of healthy subjects and in renal stones, renal lithostathine (RL). Immunocytochemistry of kidney sections localized the protein to cells of the proximal tubules and thick ascending limbs of the loop of Henle. Because of its structural and functional similarities with pancreatic lithostathine, it was called renal lithostathine. RL seems to control growth of calcium carbonate crystals. Several reports showing the presence of calcium carbonate (CaCO3) in renal stones suggested that crystals of CaCO3 might be present in the early steps of stone formation. Such crystals might therefore promote Calcium oxalate crystallization from supersaturated urine by providing an appropriate substrate for heterogeneous nucleation.

PROMOTERS

On the cell surfaces of the kidney, cell debris, protein aggregates and other crystals may provide analogous site for nucleation. These nucleation sites may lower the Super Saturation required to initiate crystallisation and therefore promote Calcium oxalate crystallisation. Strong geometric similarities between the crystals of uric acid dehydrate and calcium oxalate monohydrate may promote over growth of one on the other, a process similar to the relationship between apatite and calcium oxalate monohydrate. Evidence suggests that uric acid and Calcium phosphate may promote heterogeneous nucleation. Another factor that may promote the formation and growth of intrarenal crystals is ionic calcium. Hypercalciuria can decrease inhibitor function and lead to factors that modulate these crystal-cell interactions could stimulate the initiation of an intrarenal stone. [27]

DIAGNOSIS

Diagnosis of kidney stones is made on the basis of information obtained from the history, physical examination, urinalysis, and radiographic studies.

Clinical diagnosis is usually made on the basis of the location and severity of the pain, which is typically colicky in nature (comes and goes in spasmodic waves). Pain in the back occurs when calculi produce an obstruction in the kidney. Physical examination may reveal fever and tenderness at the costovertebral angle on the affected side.

Imaging studies

In people with a history of stones, those who are less than 50 years of age and are presenting with the symptoms of stones without any concerning signs do not require helical CT scan imaging. A CT scan is also not typically recommended in children.

Otherwise a non-contrast helical CT scan with 5 millimeters (0.2 in) sections is the diagnostic modality of choice in the radiographic evaluation of suspected nephrolithiasis. All stones are detectable on CT scans except very rare stones composed of certain drug residues in the urine, such as from indinavir. Calcium-containing stones are relatively radiodense, and they can often be detected by a traditional radiograph of the abdomen that includes the kidneys, ureters, and bladder (KUB film). Some 60% of all renal stones are radiopaque. In general, calcium phosphate stones have the greatest density, followed by calcium oxalate and magnesium ammonium phosphate stones. Cystine calculi are only faintly radiodense, while uric acid stones are usually entirely radiolucent.

Where a CT scan is unavailable, an intravenous pyelogram may be performed to help confirm the diagnosis of urolithiasis. This involves intravenous injection of a contrast agent followed by a KUB film. Uroliths present in the kidneys, ureters, or bladder may be better defined by the use of this contrast agent. Stones can also be detected by a retrograde pyelogram, where a similar contrast agent is injected directly into the distal ostium of the ureter (where the ureter terminates as it enters the bladder).

Renal ultrasonography can sometimes be useful, because it gives details about the presence of hydronephrosis, suggesting that the stone is blocking the

outflow of urine. Radiolucent stones, which do not appear on KUB, may show up on ultrasound imaging studies. Other advantages of renal ultrasonography include its low cost and absence of radiation exposure. Ultrasound imaging is useful for detecting stones in situations where X-rays or CT scans are discouraged, such as in children or pregnant women. Despite these advantages, renal ultrasonography in 2009 was not considered a substitute for non-contrast helical CT scan in the initial diagnostic evaluation of urolithiasis. The main reason for this is that, compared with CT, renal ultrasonography more often fails to detect small stones (especially ureteral stones) and other serious disorders that could be causing the symptoms.

Laboratory examination

Laboratory investigations typically carried out include:

- Microscopic examination of the urine, which may show red blood cells, bacteria, leukocytes, urinary casts, and crystals;
- Urine culture to identify any infecting organisms present in the urinary tract and sensitivity to determine the susceptibility of these organisms to specific antibiotics;
- Complete blood count, looking for neutrophilia (increased neutrophil granulocyte count) suggestive of bacterial infection, as seen in the setting of struvites stones.
- Renal function tests to look for abnormally high blood calcium blood levels (hypercalcemia);
- 24 hour urine collection to measure total daily urinary volume, magnesium, sodium, uric acid, calcium, citrate, oxalate, and phosphate;
- Collection of stones (by urinating through a Stone Screen kidney stone collection cup or a simple tea strainer) is useful. Chemical analysis of collected stones can establish their composition, which in turn can help to guide future preventive and therapeutic management.[28]

Current management and treatment of Urolithiasis is aimed in the prevention and treatment of recurrent urolithiasis is to increase the daily fluid intake to at least 2.5 L to 3 L per day along with pain controlling drugs and medications to monitor salts that may increase or reduce formation of stones. On the contrary, most stones with a diameter >8 mm will ultimately necessitate intervention. Many allopathic agents like thiazide diuretics (hydrochlorothiaide), alkali (potassium citrate), allopurinol, sodium cellulose phosphate (SCP), penicillamine (cuprimine), analgesic (diclophenac sodium), bisphosphonates, potassium phosphate and probiotics (Oxalobacterformigenes) are used in treating stones. Thiazide diuretics (e.g., hydrochlorothiazide, chlorthalidone and indapamide) produce an increase in tubular reabsorption of calcium, which diminishes calciuria, and hence are effective in reducing calciuria and stone recurrence. However, most of these standard pharmaceutical drugs used to prevent and cure urolithiasis are not effective in all cases, costly, quite common reoccurrences, risks of long term fertility, potential side effects and no guarantee. [29]

A large number of Indian medicinal plants have been used in the treatment of urolithiasis and they have been reported to be effective with fewer side effects.

Before the advent of lithotripsy and ureteroscopy, most patients with symptomatic upper tract calculi underwent open surgical lithotomy. However, lithotripsy and ureteroscopic extraction have dramatically reduced the role of open stone surgery. Despite these advancements, techniques such as extracorporeal shock wave lithotripsy and percutaneous nephrostolithotomy do not assure the prevention of recurrence of the stone. They cause side effect such as haemorrhage, hypertension, tubular necrosis, and subsequent fibrosis of the kidney leading to cell injury, and ultimately recurrence of renal stone formation. Also these methods are costly, non-affordable by the poor section and the re-occurrence rate is also high (50-80%). Thus, even with the improved understanding of the mechanisms of stone formation and treatment, the worldwide incidence of urolithiasis is quite high and there is no truly satisfactory drug for treatment of renal calculi. [30]

The recurrence of urolithiasis represents a serious problem, as patients who have formed a stone are more likely to form another, and thus stone prevention is highly recommended. Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of 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. Furthermore, although some drugs used to prevent the disease have some positive effects, they are not effective in all patients and often have adverse effects that compromise their use in long-term medical treatment. Alternative treatment using phytotherapy has been sought; indeed, in recent years there has been a resurgence of interest in medicinal plants that are effective, safe and culturally acceptable. [31]

The worldwide incidence of urolithiasis is quite high and there is no truly satisfactory drug for treatment of renal calculi. A large number of Indian medicinal plants have been used in the treatment of urolithiasis and they have been reported to be effective with fewer side effects.

Many remedies have been employed during ages to treat renal stones. Most of remedies were taken from plants and proved to be useful, though the rational behind their use is not well established except for a few plants and some proprietary composite herbal drugs and they are reported to be effective with no side effects. The present day medical management of nephrolithiasis is either costly or not without side effects. Hence the search for antilithiatic drugs from natural sources has assumed greater importance. [33]

2. LITERATURE REVIEW

Hall PM, (2009) identified the mechanism of stone formation and outlined treatment for prevention of kidney stone recurrences. He also found factors that promote stone formation. Those factors include low daily volumes, saturation of the urine with calcium, oxalate, calcium phosphate, uric acid or cystine, acidic urine and bacterial infection. Most of stones are composed of calcium oxalate or calcium phosphate. Alkalinization of urine may help in the prevention of uric acid stones and cystine stones.

Basavaraj DR *et al*., (2007) explained the role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. Calcium, sodium, oxalate, urate, cystine, low urinary pH, low urinary flow are promoting factors that promote urinary stones. Inhibiting factors include inorganic (citrate, magnesium, pyrophosphate) and organic (Tamm-Horsfall protein, urinary prothrombin fragment 1, inter α inhibitor, glycosaminoglycans, osteopontin, renal lithostathine, bikunin, calgranulin, high urine flow) that inhibit urinary stones.

Sharma AP *et al*., (2010) explained the epidemiology of paediatric urolithiasis. Calcium oxalate is the most common stone worldwide and accounts for 60-90% of paediatric urolithiasis. Calcium phosphate stones accounts for 10-20%. Struvite constituents 1-18% of the stones in developed countries. Uric acid constituents 5-10%, cystine 1-5% and mixed or miscellaneous 4% of the paediatric stones. Some metabolic risk factors such as hypercalciuria, hyperoxaluria and hypocitraturia increase the risk of stone recurrence. Idiopathic stone disease has been reported to be more frequent in white Caucasians than in Africa.

Ruchi Roper *etal*(2017)Celery, botanically known as *Apiumgraveolens* is belongs to the family of apiaceae, an annual or bionomical herbaceous plant that is native of Mediterranean regions like Asia, Africa and Europe. *Apiumgraveolens* is an important plant with great Ayurvedic medicinal properties. The medicinal properties of celery includes antioxidant, anti-inflammatory, antispasmodic, antibacterial, antifungal, anticancer, diuretic and sedative activities. Celery is used

as a salt and in salads for its good culinary tastes. Celery requires comparatively high humidity, but does not needhigh temperature. Therefore its best product comes in cool weather and temperate regions. The present paper reviews the geographical distribution, history, cultivation, uses, side effects, synonyms, botanical description, taxonomical classification, phytochemical constituents and pharmacological activities.[34]

Syed Sufiyan Fazal *etal* (2011) Pharmacological properties of medicinal plants and various natural products of plant origin lie in the chemical constituents they contain. Thus, in most cases, the principal aim of phytochemical analysis of plants and natural products is to detect, isolate, characterize and identify these chemical substances. *Apiumgraveolens*(Celery plant) is an indigenous plant belongs to family Apiaceae. According to ayurveda, the plant is having a broad spectrum of use as an aphrodisiac, anthelmintic, antispasmodic, carminative, diuretic, emmenagogue, laxative, sedative, stimulant, and toxic. Celery is known as mild diuretic and urinary antiseptic and has been in the relief of flatulence and griping pains. Literature data revealed that *Apiumgraveolens* have many pharmacological properties as antifungal, anti-hypertensive and hypolipidemic, diuretic, anticancer and many more. Currently review article tried to critically cover all the necessary aspects related of *Apiumgraveolens*.[35]

Hiba Khaleel Ibrahim *et al*(2016) Natural metabolites especially those extracted from plants are useful for curing human cancer through their cytotoxicity and antioxidant activities as well as for the control of bacterial infection. Celery *Apiumgraveolens* is a medicinal herb used as a food, and also in traditional medicine. The extract of *Apiumgraveolens* seeds was prepared using methanol. The antioxidant activity of *Apiumgraveolens* seeds alcoholic extract (0.5, 1, 1.5, 2 and 2.5 mg/ml) were tested using DPPH method. The antimicrobial activity was assessed using agar diffusion method against Staphylococcus mutans, Streptococcus pyogenes, Klebsiella pneumonia, Pseudomonas aeruginosa, and Escherichia coli. Results indicated that the extract showed a scavenging activity of the DPPH in which the IC50 value for alcoholic extract was 2.31 mg/ml which is

comparatively higher than the IC50 (1.10 mg/ml) of ascorbic acid. The extract exhibited antimicrobial activity on almost microorganisms tested, in which an inhibition zone recorded by using 2.5 mg/ml on Pseudomonas aeruginosa and Streptococcus pyogenes, however no inhibition zone showed using the same concentration on Klebsiella pneumonia, Staphylococcus mutans and Escherichia coli. Increasing the concentration of the extract, resulted in increasing the inhibitory activity against the sensitive microbial isolates and started to affect Klebsiella pneumonia and Staphylococcus mutans, though still no inhibitory activity against E. coli at 5 mg/ml. The antimicrobial effect reached its higher activity by using 10 mg/ml from the alcoholic extract, in which all the microbial isolates were affected.

WesamKooti*et al*(2017) Plants are an important source of natural active products that are different, based on mechanism and biological properties. Celery *Apiumgraveolens*L) is a plant from the apiaceae family and phenolic and antioxidant compounds of this plant have been studied by several scientists. The aim of this study was to review systematically the antioxidant activity of celery. Required articles were searched from databases such as Science Direct, PubMed, Scopus, and Springer. Keywords used in this study were *Apiumgraveolens* L, celery, antioxidant, free radical, leaf, and seed. Out of 980 collected articles (published in the period 1997-2015), 9 studies finally met the inclusion criteria and were considered. Celery, because of compounds such as caffeic acid, p-coumaric acid, ferulic acid, apigenin, luteolin, tannin, saponin, and kaempferol, has powerful antioxidant characteristics, to remove free radicals. It is clear that celery, with different compounds and diverse concentration can have varied healing effects. It is suggested that the next studies concentrate on other therapeutic and industrial attributes of celery.[36]

M. C. Powanda*etal*(2010) An extract of the seed from celery (*Apiumgraviolens*) (CSE), and fractions thereof, have been found to possess anti-inflammatory activity, gastro-protective activity, and anti-Helicobacter pylori activity. In view of the potential for employing these extracts for therapeutic use, toxicological

investigations were undertaken with an alcoholic extract (A-CSE) which has previously been shown to have the above pharmacological activities. Methods A 28-day toxicity study was performed in rats according to Good Laboratory Practice (GLP) conditions. Eighteen adult male and 18 adult female rats were randomly assigned to 3 treatment groups of 6 rats/sex/group and were dosed orally with A-CSE of 0, 150 or 5,000 mg/kg per day. Daily observations of vital signs and body weights were recorded and ophthalmological investigations were performed. At autopsy, the principal organs were weighed and sections collected for histological analysis. Serum and urine samples were collected at termination for routine clinical chemistry. Under non-GLP conditions alpha-2-l-globulin immunohistochemistry was performed on kidney tissues and hepatic cytochrome P450 protein was determined, as well as, the enzymatic activities of the principal isoforms.[37]

Rhema Niteen Dongre *et al* (2018) The present study aimed at the *invitro*evaluation of Xanthine Oxidase Inhibitory Activity of *Apiumgraveolens* dulce and *Bryophyllumpinnatum*kurz. The activity was performed usingethanolic extract of leaves and stalk of *Apiumgraveolens* dulce and fresh juice of leaves of Bryophyllumpinnatumkurz at various concentrations (100µg, 200µg, 300µg, 400µg, 500µg, 600µg, 700 µg, 800µg). In this study Allopurinol was used as a standard drug. The results were noted in terms of highest percentage inhibition of Xanthine oxidase. The combination of ethanolic extract of leaves and stalk of *Apiumgraveolens* dulce and fresh juiceof leaves of Bryophyllumpinnatumkurz shows significant effect at highest concentration such as 800µg in combination group.[7]

Choubey Ankur *et al*(2010) Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of various ailments. Today large number of population suffers from kidney stone, gall stone and urinary calculi. Stone disease has gained increasing significance due to changes in living conditions i.e. industrialization and malnutrition. Changes in prevalence and incidence, the occurrence of stone types

and stone location, and the manner of stone removal are explained. Medicinal plants are used from centuries due to its safety, efficacy, cultural acceptability and lesser side effects as compared to synthetic drugs. The present article deals with measures to be adopted for the potential of medicinal plants in stone dissolving activity.

Faruk H. Al Jawad *et al*(2010) Nephrocalcinosis (NC) is a state characterised by deposition of calcium phosphate or oxalate in the renal parenchyma due to different clinical conditions. *Apiumgraveolens* (Celery) is a popular vegetable added to salads and many cooked dishes, used in Chinese medicine to reduce high blood pressure and in Arabic medicine to relieve renal pains. To evaluate the effect of *Apiumgraveolens* in reducing calcium deposits from renal parenchyma in rabbit models with induced NC by a large dose of oxalic acid. *Apiumgraveolens* produced a significant reduction of blood urea nitrogen (5.7±0.05 vs 7.3±0.2) mmol/l, serum creatinine (87.2±0.63 vs 97.3±0.5) mmol/l and serum Na+ levels (136.8±0.2 vs 142.16±0.7) mmol/l with non-significant reduction in serum K+ (3.3±0.8 vs 3.8±0.03). There is a significant reduction in calcium deposition in renal parenchyma in comparison to the control group after ten days of treatment. *Apiumgraveolens* showed a significant diuretic effect that accentuates the excretion of urinary calcium.

John D. Everard *et al*(1994) Both mannitol and sucrose (Suc) are primary photosynthetic products in celery (*Apiumgraveolens*). In other biological systems mannitol has been shown to serve as a compatible solute or osmoprotectant involved in stress tolerance. Although mannitol, like SUC, is translocated and serves as a reserve carbohydrate in celery, its role in stress tolerance has yet to be resolved. Mature celery plants exposed to low (25 mM NaCI), intermediate (100 mM NaCI), and high (300 mM NaCI) salinities displayed substantial salt tolerance. Shoot fresh weight was increased at low NaCl concen- trations when compared with controls, and growth continued, although at slower rates, even after prolonged exposure to high salinities. Gas-exchange analyses showed that low NaCl levels had little or no effect on photosynthetic carbon assimilation (A), but at

intermediate levels decreases in stomatal conductance limited A, and at the highest NaCl levels carboxylation capacity (as measured by analyses of the CO, assimilation response to changing internal CO, partial pressures) and electron transport (as indicated by fluorescence measurements) were the apparent prevailing limits to A. Increasingsalinities up to 300 mM, however, increased mannitol accumulation and decreased SUC and starch pools in leaf tissues, e.g. the ratio of mannitol to SUC increased almost 10-fold. These changes were due in part to shifts in photosynthetic carbon partitioning (as measured by "C labeling) from SUC into mannitol. Salt treatments increased the activity of mannose-6-phosphate reductase (MCPR), a key enzyme in mannitol biosynthesis, 6-fold in young leaves and 2-fold in fully expanded, mature leaves, but increases in MCPR protein were not apparent in the older leaves. Mannitol biosynthetic capacity (as measured by labeling rates) was maintained despite salt treatment, and relative partitioning into mannitol consequently increased despite decreased photosynthetic capacity. The results support a suggested role for mannitol accumulation in adaptation to and tolerance of salinity stress.

Sara Ali-Akbari *et al*(2014) Medicinal plants are used in traditional medicine to treat many diseases. Celery (*Apiumgraveolens*) is a native medicinal plant to Europe. This plant has a very wide range of usage and cultivation. The wild type was found in countries such as Algeria, the Caucasus, Iran, India and America. However, due to increasing value and the special place of the plant in the new pharmaceutical industry, it is necessary to recognize the potential in the field of manufacturing and processing. This article presents morphological characteristics, vegetation compounds and evaluation of the therapeutic properties of this valuable medicinal plant. Methods: The information of this review article have been gathered from accessible journals in databases such as Web of Science, PubMed, Scopus, Embase, SID and Iran Medex. The search terms were "Celery" and "*Apiumgraveolens*" that searched in Persian and English books on medicinal plants and traditional medicine, as well as reputable sites mentioned. Results: Various studies have shown that celery plays a role in prevention of cardiovascular disease, lowering blood glucose and serum lipid, decrease blood pressure and

strengthener the heart. This herb has anti- bacterial, anti-fungal and anti-inflammatory effects. Also, a powerful antioxidant property has been attributed to compounds such as apigenin, apiein, vitamins A and C. Conclusion: Celery widely used in pharmaceutical, food and ornamental industries, that causes its significant commercial value. Various combinations and numerous medicinal properties of seeds, leaves and stems, cause the need further and more research about the other useful and unknown properties of celery

M.S.Seraga*et al* (2013) The present study aims to determine the relationship between soil chemical characteristics and the yield, qualitative and quantitative composition of the essential oils, as well as, phenolic and flavonoid content of the *Apiumgraveolens* L. (Apiaceae) aerial parts and fruits. Field study indicated that *Apiumgraveolens* L. is widely distributed in the Nile Delta coast namely, sandy fertile cultivated lands, banks of irrigation canals, Orchards, reclaimed lands and waste land. Soil analysis indicated that *A. graveolens* L. grow in a wide range of soil variables such as conductivity, calcium carbonates, organic carbon, chlorides and potassium, sodium, calcium and magnesium cations. The volatile constituents were analyzed by GC/MS. The detected compounds were identified by their retention times and mass spectral data, as well as comparison with published data or with reference compounds and mass spectrometric libraries of REPLIB and MAINLIB. Significant differences in the proportion of volatile constituents from oils of different habitat were detected. Besides, the phenolic and flavonoid content of the aerial parts and fruits varied widely according to habitat type. Hence, great attention must be paid to the type of soil and cropping strategies, to obtain satisfactory yields of high quality products, respecting their safety and medicinal value.(39)

Anwar Ali Shad *et al*(2011) The present study investigates the nutritive and pharmacological potential of *Apiumgraveolens* commonly available as wild plant in Peshawar and suburbs of Khyber Pakhtunkhwa region of Pakistan. For this purpose, plants of *Apiumgraveolens* L. was collected from its natural habitat at mature stage (seedling stage) from Palusi, near KPK Agricultural University,

Peshawar Pakistan. Similarly, celery seeds were obtained from 3 different ecological zone markets of Khyber Pakhtunkhwa including Peshawar, Swat and Dera Ismail Khan Regions of KPK Pakistan. Analysis of the data revealed that all the examined plants contained appreciable amount of moisture, ash, fat, protein and fiber. The wild celery had good level of vitamin C and β-carotene. The phytochemical screening of methanolic extracts of celery showed the presence of various groups of compounds including tannins, steroids, phenolic acids, terpenoids, flavonoids, volatile oils and saponins, whereas cardiac glycosides was absent in all the samples. Among the antioxidant parameters, a significant amount of total phenols and tannins were observed. Likewise elements analyzed were in the order of Na> P> K> Ca> Mg> Fe> Cu> Mn> Zn> Ni> Pb> Pt> Cd> Se> Cr. The results further revealed that Mg, Ca, P, K and Na were present in fairly high amount. The methanolic extract of all the examined celery showed positive antibacterial activity against different strains tested. Similarly, antifungal potential of the celery was determined against Trichphytonlongifuss, Candida albicans, Aspergillus flavus, Microsporumcanis, Fusarium solani and Candida glabrata in concentration 200 µg ml-1 of dimethyl sulfoxide (DMSO). The results revealed that all the organic solvents (hexane, chloroform, methanol) and water extracts showed no brine shrimp lethality and leishmanicidalactivity.(40)

Ali Esmail Al-Snafi*et al*(2018) Anti-urolithiatic drugs are the drugs which dissolve or prevent the formation of urinary calculi, while diuretics are drugs which increase the volume of urine excreted. Several medicinal plants can inhibit urolithiasis by many mechanisms: maintains crystalloid-colloid balance by decreasing excretion of urinary calcium, oxalate, uric acid, phosphorus and protein in urolithiasis, improves the renal function by increasing the excretion of urea and creatinine, diuretic and ACE inhibition activity. On the other hand many drugs can produce diuretic effect via their effects on renal water channels, on renal carriers, on nitric oxide-cGMP pathway, on prostaglandin-cAMP pathway, on the renin-angiotensin-aldosterone system (RAAS), on the kinin–kallikrein system (KKS), s on carbonic anhydrase and osmotic effects on kidneys.

The current review will discuss the medicinal plants with anti-urolithiatic and diuretic effects and their potential in the treatment of urinary stone.(41)

Sandhya *et al*(2010) The purpose of the present review is to provide an update about the most common risk factors or medical conditions associated with renal stone formation as the incidence of kidney stone disease is increasing in tropical developing countries .The potent risk factors identified include the "classic" risk factors in the urine (low urine volume, hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitrauria, and hypomanesuria) and epidemiological factors include climate, race, ethnicity, age, sex and body weight. We have found that sedentary lifestyle habits, an unhealthy dietary plan, and overweight problems may be important promoters. We suggest that there is a need for further studies to be carried out in larger sample sizes with emphasis on above risk factors for rational, efficient and specific management.

Basavaraj DR *et al* (2007) explained the role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. Calcium, sodium, oxalate, urate, cystine, low urinary pH, low urinary flow are promoting factors that promote urinary stones. Inhibiting factors include inorganic (citrate, magnesium, pyrophosphate) and organic (Tamm-Horsfall protein, urinary prothrombin fragment 1, inter α inhibitor, glycosaminoglycans, osteopontin, renal lithostathine, bikunin, calgranulin, high urine flow) that inhibit urinary stones.

Pak CYC, *et al* (1998) found that kidney stones developed by various metabolic and environmental-nutritional factors including hypercalciuria, hyperoxaluria, hyperuricosuria, hypercitraturia, under urinary acidity, cystinuria and low urine volume. For the treatment of urolithiasis, There are different drugs are used such as thiazide diuretics, potassium citrate, low calcium diet for hypercalciuria, allopurinol for hyperuricosuria, magnesium citrate for hyperoxaluria, chelating agents for cystinuria and antibiotics for infection stones.

Kumkum Agarwal *et al* (2014) evaluated the *Ocimumgratissimum L*. has been used to treat various diseases including urinary stone diseases, since ancient time in India. The inhibition of *in-vitro* calcium-oxalate crystal formation by

Ocimumgratissimum L. extract was investigated by different methods i.e. nucleation assay and synthetic urine assay. In nucleation assay, the aim was to evaluate the effectiveness of different concentrations of the extract (100-1000 mg/ml) on calcium oxalate crystallization in-vitro while in synthetic urine method the percentage inhibition and growth of the calcium oxalate monohydrate crystals from synthetic urine at different % concentrations of extract (25-100%) was investigated. In both the assay % inhibition for calcium oxalate crystal formation was found directly proportional to the increase in concentration of the plant extract with maximum inhibition of 66.08% at 1000 mg/ml, while in synthetic urine assay maximum inhibition was 62.07 % at 100% concentration of extract. Thus*Ocimumgratissimum L*. was found to be a potent and promising anti-urolithiatic agent, which is in accordance with its use in traditional medicine.(42)

Chandra Shekhar Tailor *et al* (2015) studied the, ethanolic & aqueous extracts of roots and rhizomes of *Hedychium coronarium J. Koenig* plant were evaluated for their potential to dissolve experimentally prepared kidney stones like calcium oxalate by titrimetric method with an invitro model and Antioxidant activity performed by DPPH scavenging assay method. Phytoconstituents were also isolated by chromatographic techniques of this plant species. For performing *invitro* antilithiatic activity titrimetric method was adopted and for antioxidant activity 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay method was adopted. Phytoconstituents were isolated by column and thin layer chromatographic techniques. Results indicated the ethanolic roots & rhizomes extract of this plant produced highest dissolution of stones when compare to standard drug cystone and at10mg concentration. Also this study showed alcoholic extract of roots & rhizomes of *Hedychium coronarium J. Koenig* plant in higher concentration possess best antioxidant potential as compare to standard ascorbic acid with IC50 value 9.0 and 18.9 µg/ml. for ascorbic acid and alcoholic extract respectively. Isolated phytoconstituent from alcoholic extract of this plant was 8a, hydroxy hedychilactone and its structure was confirmed by IR, NMR and Mass spectroscopic datas.[43].

Wongtawatchai, *etal* (2016) *Apiumgraveolens*is a potent antioxidant and effective agent on neurological disease. However, the neuroprotective effect against stress still unclear. The present study investigates the anxiolytic effects of a methanol exact of *A. graveolens* in immobilization stressed rats. The five groups of rats used were control, vehicle, positive controls (diazepam 2 mg/kg) and treated groups with 125 and 250 mg/kg of *A. graveolens* for 21 days. On day 15, the rats were placed in the restrainer (12 h daily for 7 days). Restraint stress-induced anxiety-like behavior were assessed using the open field, hole-board and elevate plus maze test. For biochemical parameters, corticosterone, monoamine oxidase enzyme-A (MAO-A) activity, malondialdehyde (MDA), percentage of inhibition of superoxide anion (O-2) and glutathione peroxidase (GPx) were measured. Administration of *A. graveolens* showed a significant increase in the frequency of head dips in the hole board, line crossings and rearing in the open field, time spent in open arm in elevate plus maze, the discrimination index in object recognition and an decrease in escape latency time in the Morris water maze test compared with vehicle. Moreover, results of the biochemical parameter were represented by treated versus vehicle group. Corticosterone level (250 mg/kg) was 9.76 ± 1.87 versus 27.75 ± 5.90 ng/mL. In cortex and striatum, MAO-A and MDA were significantly decreased while GPx and % inhibition of O-2 were significantly increased. These results indicated that *A. graveolens*has anxiolytic potential to prevent stress without cognitive deficit, whereas diazepam can cause cognitive deficit.

Sandhya Abbagani*et al*(2010) The purpose of the present review is to provide an update aboutthe most common risk factors or medical conditions associated with renal stone formation as the incidence of kidney stone disease is increasing in tropical developing countries .The potent risk factors identified include the "classic" risk factors in the urine (low urine volume, hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitrauria, and hypomanesuria) and epidemiological factors include climate, race, ethnicity, age, sex and body weight. We have found that sedentary lifestyle habits, an unhealthy dietary plan, and overweight problems may be important promoters. We suggest that there is a need for further studies to be carried out in larger sample sizes with emphasis on above risk factors for rational, efficient and specific management.[21]

3. PLANT PROFILE

FIG.NO:*4 Apium graveolens*

Table no :1 Taxnomical Classification of *Apium graveolens* [34]

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Apiales
Family	Apiaceae
Genus	Apium
Species	Graveolens

Table no :2 International Synonyms of *Apium graveolens* [34]

Name	**language**	**Country/ Region**
Celeri	French	France
Zeller	Hungarian	Hungary
Apio	Spanish	Spain
Seller	Swedish	Sweden
Soilire	Irish	Ireland
Seller	Danish	Denmark, Greenland
SeledriI	Indonesian	Indonesia

Table no:3 Indian Synonyms of *Apium graveolens* [34]

Name	Language	State
Siviri keerai	Tamil	Tamilnadu
Omam	Malayalam	Kerala
Oma, Ajavana, Omakki	Kannada	Karnataka
Banajuani	Oriya	Eastern India
Ajmod	Urdu	North India
Bonjamani, Yamani,	Ajowan	Assam

Geographical Distribution [44]

Celery is native to Mediterranean regions. Celery was first cultivated in Italy and France. From here, the plant spreads to Central and Northern Europe.

World Scenario

Apium graveolens is cultivated in Italy, France, Sweden, Algeria, Egypt, Ethiopia, Kingdom of Saudi Arabia and India.

Indian Scenario

Apium graveolens is cultivated in north –western Himalayas, Punjab, Haryana and western utter Pradesh.

Cultivation

The range of cultivation and consumption of celery is very extensive. Celery requires comparatively high humidity, but does not need high temperature. Therefore its best products comes in cool weather and temperate regions. Celery is a shade oriented vegetable and high light intensity decreased its quality and growth.

Climate

Celery can be grown in all but extreme climates, although it prefers cool temperature weather, of between 580F (150C-260C).

Soil

It grows best in average to moist soil that is light and sandy with a neutral pH of 6.6 to 7.5, and hardy to zone 6, though they can be damaged by hard frosts. It can adapt to a variety of soil types and conditions, but has waterlogging intolerance. *Apium graveolens* flowers are hermaphrodite (have both male and female organs) and are pollinated

Propagation

The easy and cost effective method of propagation of celery is by sowing seeds. Seed – sow spring or autumn in situ. If seed is in short supply it can be sown in a cold frame in spring. The seed can harbor certain diseases of celery, it is usually treated by seed companies before being sold but if you save your own seed you should make sure that only seed from healthy plants is used. Soaking of seed in water for 24 hours improves germination

Botanical Description of *Apium graveolens* [34]

Leaves -Shiny pinnate, toothed leaflets

Flowers -White

Petals -Small and entire

Fruits -Small and slightly compressed

Stems -Branched, angular, green

Seeds -Small

Flowering- period June to august

Root -Succulent, well developed

Phytochemical Constituents [45-51]

The constituents of the celery include glycosides, steroids, and different types of phenolic including furanocoumarins, flavones, and trace elements (sodium, potassium, calcium and iron). There is variability of the constituents in the different parts of the plants. The main chemical constituents present in each part of the plant are as follows:

Roots

The roots contains falcarinol, falcarindiol, panaxidol, and polyacetylene 8-O-methylfalcarindiol.

Stems

The stem contains pectic polysaccharide (apiuman) containing d-galacturonic acid, 1-rhamnose, 1-arabinose, and d-galactose.

Leaves

Twenty-eight components are obtained from gas chromatography-mass spectrometry study of the volatile oil obtained from the leaf. The important compounds are 1-dodecanol, 9-octadecen-12-ynoic acid, methyl ester, and tetradecence-1-ol acetate.

Fruits/seed

Caffeic acid, chlorogenic acid, apiin, apigenin, rutaretin, ocimene, bergapten, and isopimpinellin are reported to be found in celery seed. The other substances such as seslin, isoimperatorin, osthenol, and gravebioside A and B were also found in the seeds Literature also showed that seslin, isoimperatorin, osthenol, gravebioside A and B, umbelliferone are present in the seeds of the plants.

The seed oil is composed of palmitic acid, stearic acid, oleic acid, linoleic acid, petroselinic acid, d-limonene, selinene, terpineol, and santolol. The aroma of the oil is due to the presence of sedanonic anhydride and sedanolide in the seed oil. The maximum concentration of the oil was found in 5-week-old fruits.

Medicinal Uses [52,53,54,55-67]

Hepatoprotective

The methanolic extract of celery seed was found to be effective paracetamol-induced and carbon tetra chloride-induced liver damage. It was noticed that celery has the protective activity against thioacetamide drugs.

Anti-diabetic

The aqueous extract of the celery stimulate pancreas to secrete insulin to reduce blood glucose levels, so that it can used to reduce or treat diabetes complications.

Pain and Inflammation

Some of the compounds in its seed show antiinflammatory and analgesic effects. The juice extracted from the petioles can be used for ede ma, rheumatic tendencies, gout, flatulence, chronic pulmonary catarrh, tendencies toward overweight and lack of appetite.

Diuretic

It is a strong diuretic, and is used as a urinary antiseptic, mainly because of the volatile oil apiol. It was shown that essential oil obtained from celery is reported to have a calming effect on the central nervous system. Moreover, some of its constituents showed antispasmodic, sedative and anticonvulsant actions

Anti-hysteric

Wild celery is said to be useful in cases of hysteria, promoting restfulness and sleep, and diffusing through the system a mild sustaining influence.

Anti-cancer

These have anti-cancer properties due to anti-cancer chemicals like polyacetylens and phthalide. These can detoxify carcinogens present in cigarette.

In animal studies, it was shown that perillyl alcohol in celery causes regression of tumors of the pancreas, liver, and breast. Celery has a protective effect on the gastric mucosa and it Isanti - gastric ulcer, also a diuretic plant with antioxidant properties. It is also used to treat stomach pain. This plant increases the secretion of breast milk. It also reduces jaundice.

Anti-microbial Activity

The ethanolic extract of celery was found to be exhibit antibacterial activity against Escherichia coli.

Culinary Uses

Celery recipes are famous for their aromatic flavor. Celery leaves are typically used as a salad ingredient. Its seeds are used as a seasoning or dry spice in many celery dishes. Even a spice called 'celery salt' is also prepared by mixing salt and grounded celery seeds.

This spice is widely used as a flavoring in savory dishes as well as cocktails, namely 'Bloody Mary', Along with onion and carrot; celery makes some of the most flavorful celery recipes. Celery stalk and leaves are also considered a base ingredient in many soups and stew dishes. An additional use of celery is to extract 'volatile oil' from its seeds.

Contraindications

Due to the irritating effect of the volatile oils, is contraindicated in acute kidney conditions. The volatile oils have an empirical emmenogogue and possible abortifacient effect and should be avoided during pregnancy. Empirical evidence also suggests increased photosensitivity due to the furanocoumarins.

Side-Effects

The plant is generally safe for the common use. Excessive use of celery may cause kidney inflammation. High dose of celery seeds can stimulate the uterus in pregnant women. High intake of celery during the last four weeks of pregnancy may increase the complications.

4. AIM & OBJECTIVE

Need of the Present Study

In spite of advances in the understanding of urolithogenesis there is no satisfactory drug treatment of the 'idiopathic' oxalocalcic stone formers (nor hyperoxaluria nor hypercalciuria).This, in part, is due to many causes that provoke the disease in a non-uniform group of patients. Thus the genesis of the calculus must be attributed to a deficit of crystallization inhibitors (nucleation inhibitors) and /or an increase of promoters (heterogeneous nucleation). A deficit of citrate is an alteration related to oxalocalcic stone formation as a consequence of activity inhibiting calcium salt nucleation. Persistent acid urinary pH values (<5.5) is another risk factor due to the formation of colloidal uric acid. Persistent urinary pH values >6.5 is also an important risk factor due to the formation of colloidal calcium phosphates. It is evident that depending on the cause, different therapies are appropriate. It is curious to observe how throughout the world a variety of single and compound herbal preparation appears to be used with success in urolithiasis therapy.(68)

Many remedies have been employed during the ages to treated urinary stones. The recent medical treatment procedure like surgical removal, percutaneous technique and extra corporal shock wave lithotripsy (ESWL) are prohibitively costly, for common man and with those procedures reoccurrence is quiet common and patient have to follow up for number of years.(69) In modern medicine no cheap and effective therapy is available to dissolve or to prevent recurrence of urinary stones. Many tribal people in cold desert Ladakh,(70) Muzaffarnagar (Utter Pradesh, India) ,(71)Arunachal Pradesh and other parts of India follow Ayurvedic treatment for various ailments. Knowingly or unknowingly, most of the diseases cured by having edible plant products, which we use in our day today food consumption.(72) Traditional indigenous systems like Ayurveda and Siddha claim to be successful in the treatment of urinary calculi. The traditional system of Indian medicine "Ayurveda" recommends several plants for the treatment of urolithiasis.

Scientific Bases

Ayurvedic therapies for urolithiasis are effective in either alone or as an adjunct with other herbs those also used to treat urolithiasis is very effective. Ayurveda search has revealed more than 20 herbs have been shown to have antilithiatic activity. Some of these herbs have been frequently in Ayurvedic formulas.

Table 4 : Some Important Ayurvedic Formulation Available in the Market.

Drug Name	Company	Ingredients
Cystone	Himalaya	Shilapuspha (*Didymocarpus pedicellata*), Pasanabheda (*Saxifraga ligulata* Syn.*Bergenia ligulata / ciliata*), Indian madder / Manjishtha (*Rubia cordifolia*), Umbrella's edge / Nagarmusta (*Cyperus scariosus*), Prickly chaff flower / Apamarga (*Achyranthes aspera*), Sedge / Gojiha (*Onosma bracteatum*), Purple fleabane / Sahadevi (*Vernonia Cinerea*), Lime silicate calx / Hajrul yahood Bhasma / Badrashma bhasma, Shilajit
Distone	Ind-Swift	*Crataeva nurvala, Dolicus biflaous, Raphanus Sativus, Achranthes Aspra(Apamark Kashaar), Boerhavia Diffusa(Punarnava Kashaar), Tribulus terristris*
Nilstone	Domagk Smith Labs Pvt. Ltd.	*Crataeva*, *Bergenia ligulata*, Processed Alum compound, *Cucumis Sativus, Picrorrhiza* Kurroa, *jawakshar*, *elettaria Cardamomum*, Meeta Soda, *Boerhavia Diffusa*
Neeri	Aimil Pharmaceuticals Ltd.	*Bergenia Ligulata, Butea monosperma, Boerhavia Diffusa, Crataeva nurvala, vemonia cinerea, Achyranthes aspera, Tribulus terristris*, Purified Asphaltum, *Mimosa pudica*, L. ash of *Hordeum vulgare*

Traditional medicine has attributed some beneficial effects for a variety of kidney disorders to ***Apium graveolens***. Therefore they are selected to evaluate their effectiveness in preventing the formation of oxalate calculi.(73,74,75)

So in the present work, an attempt is made to find out the extent of ***anti-lithiatic activity*** of the above mentioned plant.

Aim of Work

To identify a plant with potential antiurolithiatic activity and to compare the effect of three traditional herbs with ethylene glycol induced urolithiasis in rats.

Plan of Work

Antilithiatic activity of ***Apium graveolens***, animal model are not yet evaluated till date. Therefore this study was proposed to perform with the following objectives.

- Phytochemical investigation of the hydro alcoholic extract of whole plant of ***Apium graveolens***.
- Evaluation of antilithiatic activity of ***Apium graveolens*** with ethylene glycol induced lithiasis model.

5. PHYTOCHEMICAL ANALYSIS

Preliminary Phytochemical Studies

Standardization of traditional plants is a critical and essential issue to be considered in assuring the therapeutic efficacy, safety and to rationalize their use in the health care. However information on such profiles is not available for all the plants and the scientific literature concerning the quality, safety and efficacy of *Apium graveolens* is not available. Therefore, the present investigations were undertaken to identify the phytochemical constituents.

The first step in the standardization of plant material is correct identification of the species concerned. This study reports physicochemical values and identifications of secondary metabolites in hydroalcoholic extracts. To facilitate this process, guidelines for assessment of the quality, safety and efficacy of herbal medicines have been developed.(76)

Plant Material

The collection of the plant materials of *Apium graveolens* were done in the month of April-2019 at Alagar hills, Madurai- Tamilnadu. Early April will be the ideal time for the collection of medicinal plants since the plants will be enriched with phytoconstituents during that time. The identification and authentication of the plant was carried out by Dr. D.Stephan, Ph.D., Department of Botany, American College, and Madurai-Tamilnadu.

Drying

The whole plant of *Apium graveolens* were washed and dried under shade for 15 days on fresh cotton cloth. After 15 days the dry weight is taken.

Grinding of the plant for extraction

Cleaned and mixer was used for grinding the plant materials.After proper grinding, the weight of the powder was obtained. These powders were used for hot extraction.

Solvents order and temperature

Petroleum ether	:	60º-80º C
Chloroform	:	60º-62º C
Ethyl acetate	:	74º-78º C
Hydro alcohol	:	100º C

Preparation of extracts of *Apium graveolens* by hot continuous percolation method

The flask with the given solvent is heated to a particular temperature. The vapour produced passes through the siphon tube into the thimble kept above where it is condensed and tickles down into the flask again through the thimble dissolving the active constituents in it. The method is described as the continuous extraction. The process is continued until all the soluble constituents get separated. The extract at the bottom was collected and dried under reduced temperature and pressure. Each time, before the extraction with other solvents, the powdered substance is air dried.

About 200 gm of dried powder was properly packed in Whatmann filter paper (grade no.1) and kept in thimble and the soxhlet apparatus was set up. The extraction of powder was done with different solvents with solvents of increasing polarities like petroleum ether (60-80º C), chloroform, and hydro alcohol. Here temperature maintenance is based on the solvents used for extraction. The solvents were removed under reduced pressure using rotary evaporator and stored in desiccators (77).The consistency of the extract is semi solid. (This method is repeated until getting desired extract).

FLOW CHART NO 2: Extraction of *Apium graveolens*

Apium graveolens

↓

Dried

↓

Powdered

↓

Extracted with Petroleum ether (40hrs)

↓

Extracted with chloroform (30hrs)

↓

Marc

↓

Soxhlet extraction with Hydro alcohol (70:30) (72hrs)

↓

Marc

↓

Freeze

↓

For Anti-lithiatic activity

Fig no : Flow chart of extraction of *Apium graveolens*

Table 5: Percentage Yield of Various Extracts of *Apium graveolens*

S. No.	Solvent used for Extraction	Time required for complete extraction (Hrs.)	Colour of extract	Percentage of yield (w/w)
1.	Petroleum ether	40	Dark green	7.20%
2.	Chloroform	40	Dark greenish brown	9.80%
3.	Hydro alcohol (70:30%)	72	Dark brown	18.30%

The crude extract of *Apium graveolens* were subjected to qualitative tests for identification of various plant constituents.

Qualitative Phytochemical Analysis

The hydro alcoholic extracts of *Apium graveolens* were subjected to various tests for identification of constituents.

1) Detection of Carbohydrates

Small quantities of hydro alcoholic extracts of *Apium graveolens* were dissolved in distilled water separately and filtered. The filtrates were taken for the various tests to detect the presence of carbohydrates.

A. Molisch's Test

The filtrates were treated with 2-3 drops of 1% alcoholic α- napthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. A brown ring was not formed in *Apium graveolens*.

B. Fehling's Test

Small portion of the filtrates were treated with equal volume of Fehling's solution A and B and then heated. A brick red precipitate not formed in *Apium graveolens.*

C. Benedict's Test

Small portion of the filtrates were treated with equal volume of Benedict's reagent. A yellow precipitate was not formed in *Apium graveolens.*

D. Barfoed's Test

Small portion of the plant extracts were treated with Barfoed's reagent. Red precipitate was not formed in *Apium graveolens.*

E. Test for Starch

A small amount of the plant extracts were treated with dilute iodine solution. No bluish black colour was observed in the *Apium graveolens* extracts showing the absence of starch**.(78)**

2) Tests for Gums and Mucilage's

Alcoholic Precipitation and Molisch's Test.

The plant extracts were treated with absolute alcohol, stirred and filtered. The filtrate was dried and examined for its swelling properties. *Apium graveolens* extracts were not answered for the absence of gums and mucilage.

3) Test for Proteins and Amino Acids

Small quantities of hydro alcoholic extract was dissolved in few ml of distilled water and subjected to Ninhydrin test, Xanthoprotein test, test with tannic acid and heavy metals.

A. Ninhydrin Test

Hydro alcoholic extracts of the *Apium graveolens* plants were treated with ninhydrin reagent (0.1% solution) and boiled. Purple colour was not observed indicating the absence of protein in *Apium graveolens* extract.

B. Biuret Test

To a portion of the above prepared extracts, equal volumes of 5% w/v sodium hydroxide and 4 drops of 1% w/v copper sulphate solution were added. Violet colour was not formed, indicating the absence of protein in *Apium graveolens.*

C. Millon's Test of Cole's Mercuric Nitrite Test.

To the above-prepared extracts, Millon's reagent was added. White precipitate was not formed, showing the absence of protein in the extracts of *Apium graveolens.*

D. Xanthoprotein Test

To 3 ml of the above-prepared extracts, 1 ml of the concentrated nitric acid was added, boiled for one minute, cooled and concentrated ammonia was added till alkaline. An orange colour was not formed, showing the absence of protein in *Apium graveolens.*

4) Test for Fixed Oils and Fats

A. Spot Test

A small quantity of plant extracts was pressed between two filter papers. Oil stains were observed with the extracts indicating the presence of fixed oils and fats.

B. Saponification Test

Few drops of 0.5 N alcoholic potassium hydroxide was added *Apium graveolens* extracts along with of few drops of phenolphthalein. The mixture was heated on a water bath for one hour. Soap was formed with the extracts indicating the presence of fixed oils and fats.

5) Test for Alkaloids

Small amount of the solvent free hydro alcoholic extracts were separately stirred with a few ml of dilute HCl and filtered. The filtrates were tested with various alcoholic reagents.

A. Mayer's Test

To the small quantities of the extracts, Mayer's reagent was added. Presence of cream-colored precipitate indicates the presence of alkaloids in *Apium graveolens* plant extracts.

B. Dragendorff's Test

To small quantity of extracts, Dragendorff's reagent was added. Presence of orange brown precipitate indicates the presence of alkaloids in *Apium graveolens* plant extracts.

C. Wagner's Test

To small quantity of the extracts, Wagner's reagent was added. Presence of reddish brown precipitate, indicate the presence of alkaloids in *Apium graveolens* plant extracts.

D. Hager's Test

To small quantity of the extracts, Hager's reagent was added. Presence of yellow precipitate, indicate the presence of alkaloids in *Apium graveolens* plant extracts.(79)

6) Tests for Glycosides

A small amount of the extracts of *Apium graveolens* plants were dissolved separately in 5 ml of distilled water and filtered. Another portion of the extracts were hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolyzed was subjected to Legal's, Baljet's, Borntrager's, Keller-Killani's tests and for the presence of cyanogenetic glycosides.

A. Legal's Test

To the hydrolyzed, 1 ml of pyridine and a few drops of sodium nitroprusside solution was added and then made alkaline with sodium hydroxide solution. Pink colour was observed in *Apium graveolens* extracts.

B. Baljet's Test

To a section of plant extract, sodium picrate solution was added. Yellowish orange colour was observed in *Apium graveolens* extract.

C. Borntrager's Test

Hydrolyzed was treated with chloroform and the chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added. Pink colour was observed in the ammonical layer of chloroform in *Apium graveolens* extract showed the presence of glycosides.

D. Test for Deoxy Sugar (Keller-Killani Test)

To the *Apium graveolens* extracts 10 ml of 70% alcohol were added, boiled on a water bath, filtered. The filtrates were diluted with 1 ml of distilled water; 1ml of strong lead acetate solution was added and filtered. The filtrates were extracted with an equal volume of chloroform. The chloroform layer was pipette out and evaporated to dryness. The residue obtained was dissolved in 3 ml of 3.5% of ferric chloride in glacial acetic acid, left for one minute and then transferred to a test tube. To the side of the test tube, 1.5 ml of sulphuric acid was added carefully, which formed a separate layer at the bottom and kept for few minutes.

Blue colour at the interface and pale green colour in the upper layer was observed in *Apium graveolens* extract showed the presence of glycosides.

7) Test for Phytosterols

Small quantities of the *Apium graveolens* extract were dissolved in the 5 ml of chloroform separately. Then these chloroform solutions were subjected to Libermann's test, Libermann-Burchard's test, Salkowski's test.

A. Libermann-Burchard's Test

The residue was dissolved in chloroform. To this Libermann-Burchard's reagent was added. Green colour was produced. Green colour was produced in three extracts indicating the presence of phytosterols.

B. Salkowski's test

A few drops of concentrated sulphuric acid were added to chloroform solution. The lower layer of the solution turned brownish red colour with the three extracts indicating the presence of phytosterols.

8) Test for Flavanoids

The *Apium graveolens* extracts were separately dissolved in ethanol and then subjected to the following tests.

A. Ferric chloride Tests

To a small quantity of the hydro alcoholic extract, few drops of neutral ferric chloride were added. Blackish red colour was observed in three plant extracts indicating the presence of Flavonoids.

B. Shinoda's test

A small quantity of the extract was dissolved in alcohol and to this magnesium metal followed by concentrated hydrochloric acid, was added drop wise and heated. A magenta colour was produced in *Apium graveolens* plant extracts indicating the presence of Flavonoids.

C. Flavones

1. With sodium hydroxide solution, the extract gave yellow colour.

2. Extract gave orange colour with concentrated sulphuric acid.

D. Reaction with alkali and acid

When alcoholic solution was treated with alkali and acid, yellowish colour indicating the presence of Flavonoids.

9) Test for Tannins

The extracts were dissolved in water and filtered. The filtrates were treated with various reagents.

A. Ferric chloride test

Few ml of the filtrates were treated with 5% ferric chloride solution. A bluish black colour was not observed indicating the absence of tannins in *Apium graveolens* extract.

B. Reaction with lead acetate

Few ml of the filtrates were treated with lead acetate solution. White precipitates were not produced in *Apium graveolens* extract indicating the absence of tannins.

C. Gelatin Test

The extracts were dissolved separately in minimum amount of water and filtered. To the filtrate, add 1 ml of 1% solution of gelatin. The *Apium graveolens* extracts did not produce any white precipitate.

10) Test for Saponins

The extracts were diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes. One-centimeter layer of foam was formed in *Apium graveolens* plant extracts indicating the presence of saponins.(80,81,82).

Table No 6: Qualitative Chemical Analysis of hydro alcoholic Extract of *Apium graveolens*

S.No	Test for Plant Constituents	Hydro alcoholic Extracts
		Apium graveolens
1	***Test for Carbohydrates***	
	a. Molisch's test	–
	b. Fehling's test	–
	c. Benedict's test	–
	d. Barfoed's test	–
	e. Test for Starch	–
2	***Test for Gums and Mucilage***	
	Alcoholic precipitation and Molisch's test	–
3	***Test for Proteins and amino acids***	
	a. Ninhydrin Test	–
	b. Biuret Test	–
	c. Millon's test or Cole's Mercuric Nitrate test	–
	d. Xanthoprotein test	–
4	***Test for fixed oils and fats***	
	a. Spot test	+
	b. Saponification Test	+
5	***Test for alkaloids***	
	a. Mayer's Test	+
	b. Dragendorff's Test	+
	c. Wagner's Test	+
	d. Hager's Test	+
6	***Test for Glycosides***	
	a. Legal's Test	+
	b. Baljet's Test	+

	c. Borntrager's Test	+
	d. Keller-Killani's Test	+
7	***Test of Phytosterols***	
	a. Liebermann-Burchard's Test	+
	b. Salkowski's Test	+
8	***Test for Flavanoids***	
	a. Ferric chloride Test	+
	b. Shinoda's Test	+
	c. Fluorescence Test	+
	d. Reaction with alkali and acid	+
9	***Test for Tannins and Phenolic Compounds***	
	a. 5% Ferric chloride solution test	–
	b. Reaction with lead acetate	–
	c. Gelatin test	–
10	***Test for Saponins***	
	a. Frothing Test	+

6. PHARMACOLOGICAL EVALUATION

Approval of Experimental Work from IAEC/CPCSEA

The protocol of the animal experiments involved in this research work has been approved by IAEC/CPCSEA constituted for this purposes.

Proposal No:

IAEC/S.MAHESHKUMAR/M.PHARM/TNMGRU/261725251/KMCP/67/2019

Acute toxicity study

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hrs. Acute toxic class method (OECD guidelines, (2000) (83) was followed to arrive at the maximum safety dose of the drug extracts. Three wistar strain female albino rats (8-12 weeks old, 180-200g body weight) were used in each group. Single dose (2g/Kg) of the extracts were orally administered to overnight fasted (food but not water withheld) animals while control animals received the vehicle (0.3%w/v CMC).Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 h, with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. Body weights of the animals were recorded. The other observations include changes in skin, fur, eyes and mucous membranes, respiratory, circulatory, and autonomic and central nervous system and somatomotor activity and behavior pattern. At the end of 14 days, all animals were subjected to gross necropsy.

Evaluation of Anti-Lithiatic activity

Experimental Models

For the study of urolithiasis an animal model was added that would satisfy the following conditions.

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

Selection of Laboratory Model

Animals such as sheep, cats, dogs, rats, rabbits and guinea pigs have been used in experimental study of urolithiasis. Rats are the most common model used in the study of Nephrolithiasis. In the present study rats have been used because, "the rats' urine resembles human urine in characteristics believed to contribute to stone formation." The urinary system of rat is similar to that of human being.(84)

Induction of Lithiasis

Lithiasis can be experimentally induced by different methods.

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

Dietary Factor:

A number of dietary items contribute to renal stone production.

- ➢ A high oxalate intake may contribute to ca -oxalate stone production.
- ➢ Excessive purine intake may contribute to the production of stones containing uric acid and uric acid plus calcium components.

- A diet high in protein from animal sources increases urinary calcium that may contribute the stone formation.
- Increased dietary magnesium intake causes formation of magnesium – ammonium phosphate calculi in rats.
- Excessive intake of vitamin A and D can contribute to calcium urolithiasis
- Vitamin B_6 deficient diet produces hyperoxaluria and oxalate nephrocalcinosis in rats.(85)

Chemically Induced

- Literature shows that 0.75% ethylene glycol for 28 days or by combination of 0.5% ethylene glycol and 0.5 mg 1 alpha (OH) D_3 in drinking water, if taken for 4 weeks, produce calcium oxalate urolithiasis.
- It is also reported that calcium oxalate urolithiasis can be induced by administration of 1% or 0.75% ethylene glycol in drinking water.(86)
- Feeding rats with 3% glycolic acid with feed for 45 days produces stone formation.(87)
- Uninephrectomy enhances urolithiasis in ethylene glycol treated male Sprague – Dawley rats which produces calcium oxalate stones.
- Nephrolithiasis has been experimentally induced by administering both gentamicin (40 mg/kg body weight, i.p) ammonium oxalate (2%, p.o).(88)
- A stone-forming animal model was developed involving renal tubular injury using a cyclooxygenase- 2 selective inhibitor in which male Sprague-Dawley rats were fed chow containing 3% sodium oxalate with or without 100 mg/kg celecoxib.(89)
- Exposure of 3-5% terephthalic acid (TPA) or 1-3% dimethyl terephthalate in the diet for less than two weeks induced calculi in the urinary tract of weaning Fischer - 344 rats.(90)

Foreign Body Insertion Technique

Sterile zinc disc of 4mm diameter is inserted into the bladder through a small incision. A silk suture is made to close the incision of the bladder. After seven days, stone deposition occurs and its weight is compared with the control animals. Calcium oxalate crystals can also implanted in the urinary bladder of adults for the stone formation.

Infection Induced Stone Formation

- Evidence shows that these stones are formed in the presence of infection by urea splitting organisms. The chief urea- splitting organisms are *Staphylococcus, Streptococcus Proteus, Klebsiella, serratia* and *Mycoplasma create* alkaline urine and thus enhance the production of magnesium ammonium phosphate (struvite) stones.
- Nanobacteria have been isolated from kidney stones and it has been suggested that they may act as a nucleus for the initiation of the renal stones.
- Bladder stones were induced in rats by using human urinary stones. D-Penicillamine increases the hepatic oxalate production and urine oxalate excretion.(91)

In Vivo Method

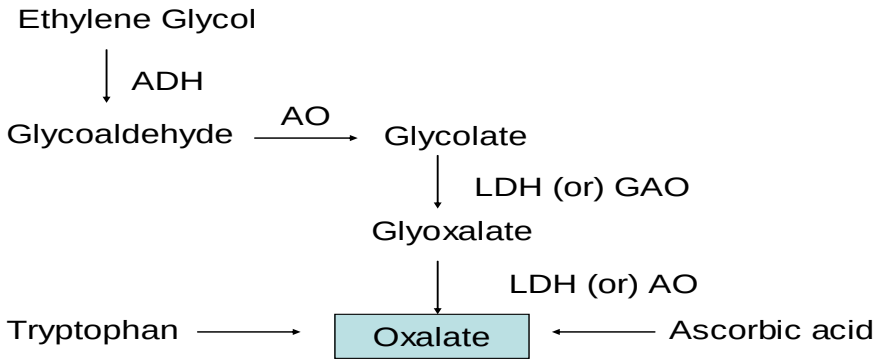
Hydro alcoholic extracts of the *Apium graveolens* were screened for their antilithiatic activity as follows:

Principle

The ethylene glycol 1% will result in hyperoxaluria, which is due to the conversion of ethylene glycol to glycoaldehyde, glyoxalic acid, glyoxal and oxalate by the enzyme alcohol dehydrogenase.

Flow chart 3

Pathways for metabolism of ethylene glycol showing mechanism of production of Oxalate



ADH	-	Alcohol Dehydrogenase
AO	-	Alcohol Oxidase
LDH	-	Lactate Dehydrogenase
GAO	-	Glycolic Acid Oxidase

Selection & Acclimatization of Animals

Adult male albino rats of the wistar strain weighing between 180-200gms were selected and were fed with standard pellet diet and water *ad Libitum*. They were housed in well-ventilated cages (3 to 4 per cage), maintained at room temperature under 12h light – dark cycle. Animals were acclimatized for a week to the laboratory condition.

Induction of Lithiasis by Ethylene Glycol (1%)

(1%) Ethylene glycol was given orally with drinking water for 28days to all groups of animals except normal control.(92)

Materials and Methods:

Animals : Male albino Wistar rats

Drugs : Hydro alcoholic Extract of *Apium graveolens*

Chemical : 1% Ethylene glycol

After the acclimatization of animals they were divided in to 10 groups of six each, designed as per the treatment protocol.

Treatment Protocol

The grouped animal's received the treatment as follows,

Prophylactic Study:

Group I Received normal diet and serves as control.

Group II Lithiatic control: The animals were given normal diet and 1% ethylene Glycol in drinking water, for 28 days.

Group III Received 1% ethylene glycol in drinking water and then treated with Hydro alcoholic extract of *Apium graveolens* at a dose of 200mg/kg.

Group IV Received 1% ethylene glycol in drinking water and then treated with Hydro alcoholic extract of *Apium graveolens* at a dose of 400mg/kg.

Group V Received 1% ethylene glycol in drinking water and then treated with Cystone at a dose of 750 mg/kg.

Curative Study:

Group I Received normal diet and served as curative normal Controls.

Group II Lithiatic control: The animals were given normal diet and 1% ethylene Glycol in drinking water, for 28 days and ordinary water for 15 days.

Group III Received 1% Ethylene glycol in drinking water for 28 days and then Treated with Hydro alcoholic extract of *Apium graveolens* at a dose of 200mg/kg for next 15days.

Group IV Received 1% Ethylene glycol in drinking water for 28 days and then Treated with Hydro alcoholic extract of *Apium graveolens* at a dose of 400mg/kg for next 15days.

Group V Received 1% ethylene glycol in drinking water for 28 days and then Treated with cystone at a dose of 750 mg/kg for next 15days..

Preparation of drugs

Hydro alcoholic extracts of *Apium graveolens* was dissolved in sterile water administered orally at a dose of 200 and 400 mg/kg rat.

The 24 hour urine samples were collected from rats, housed in metabolic cages, on the 14th, 28th and 43rd days and the volume noted. Urinary parameters like calcium, phosphate, magnesium, oxalate, protein, uric acid and creatinine concentration were estimated using standard methods.

Biochemical Analysis

Preparation of Samples for Biochemical Studies

Urine Sampling

On the final day of the experiment, 24hours urine was collected by housing the rats of the respective group in different metabolic cages. The urine samples were kept at 4°C and analyzed for calcium, magnesium phosphate, oxalate, uric acid, creatinine and protein.

Blood Sampling

All rats were anaesthetized with diethyl ether; blood was collected from retro orbital plexus by using capillary tube, and then centrifuged for 10 minutes at 3000 r. p. m. to separate the serum. The serum of each animal of all the groups

was estimated for calcium, magnesium, oxalate, phosphate, creatinine and uric acid using their respective kits in the laboratory.

Histopathological Study

To confirm the incidence of lithiasis, the animals were scarified and their kidneys were subjected to histopathological studies. The kidneys were carefully removed, washed in ice cold, 0.15 M Kcl. The kidneys were fixed in formaldehyde (10%) for H.E (Hematoxylin Eosin) stain. The crystal deposit was visually examined under light microscope.

Assay Method

Estimation of Serum Calcium

Principle

Serum is treated with ammonium oxalate to precipitate calcium as calcium oxalate. The precipitate is washed with ammonia to remove excess oxalate and treated with sulphuric acid to convert calcium oxalate into oxalic acid. The later is titrated with standard potassium permanganate.

Procedure

Measure 2 ml of serum, 2 ml of water and 1 ml of 4% ammonium oxalate in a centrifuge tube. Mix thoroughly and allow standing for at least half an hour. Mix again and centrifuge at 1500 r. p. m. for 15 minutes. Pour off the supernatant fluid and drain the tube by keeping it inverted on a filter paper for a few minutes. Wipe the mouth of the tube dry with a filter paper. Add 3 ml of 2% ammonia, shake, centrifuge and drain as before. Add 3 ml of 1N sulphuric acid and shake vigorously, keep the tube in a boiling water bath, shaking intermittently, until the precipitate is completely dissolved. While the tube is still hot, titrate with 0.01N potassium permanganate exactly as before. Let the volume of potassium permanganate used be y ml.

Serum calcium (mg/100ml) = (x – y) X 0.2 X 100/2

= (x – y) X 10

Estimation of Urine Calcium

The urine is diluted 1 in 10 with water. The calcium in diluted urine is estimated as described above and the result is multiplied by 10(93)

Estimation of Serum Creatinine

Principle

Tungstic acid filtrate of serum is treated with alkaline picrate. The resulting colour is measured by colorimetry.

Procedure

Measure 7 ml of water in a test tube. Add 1 ml of serum and 1 ml of 10% sodium tungstate. Mix and add 1 ml of 2/3N sulphuric acid with constant shaking. Let it stand for a few minutes and filter. Transfer 5 ml of the filtrate into a tube labeled unknown. Measure 5 ml of the working standard creatinine solution in a tube labeled Standard and 5 ml of water in a tube labeled Blank. Add 2.5 ml of alkaline picrate solution to each tube and mix. Let the tubes stand for 10 minutes. Read unknown and standard against blank at 520 nm or using a green filter.

Serum Creatinine (mg/100ml) = u/s X 0.01/0.5 X 100

= u/s X 2

Estimation of Urine Creatinine

Urine creatinine may be estimated in the same way as serum creatinine after diluting the urine. The urine is diluted according to the urinary output. The urine is diluted according to the urinary output. The urine should be diluted 1 in 100 if the urine volume is 1-2 litres/ day. The dilution should be grater if the urine volume is more than 2 liters/day. The result should be multiplied by the number of times the urine was diluted.(94)

Estimation of Serum Uric acid-

Principle

Serum, deproteinized by Tungstic acid, is allowed to react with phosphotungstic acid in an alkaline medium provided by sodium carbonate. Uric acid reduces the colorless phospho Tungstic acid to blue colored phosphotungstic acid (tungsten blue). The intensity of the colour is measured calorimetrically.

Procedure

Measure 7 ml of water in a test tube. Add 1 ml of serum and 1 ml of 10% sodium tungstate. Mix add 1 ml of 2/3 N sulphuric acid slowly with constant shaking. Let it stand for a few minutes and filter. Transfer 5 ml of the filtrate into a tube labeled Unknown. Measure 5 ml of the working standard uric acid solution in a tube labeled Standard and 5 ml of water in a test labeled Blank. To each tube, add 1 ml of 10% sodium carbonate and 1 ml of dilute phosphotungstic acid. Mix and keep the tubes in a water-bath at 25^0C for 30 minutes. Read unknown and standard against blank at 700nm or using a red filter.

Serum uric acid (mg/100ml) = Au/As X0.025/0.5 X 100

= Au/As X 5

Estimation of Urine Uric acid

The urine is diluted 1 in 10 with water. Uric acid in diluted urine is estimated by the above procedure and the result multiplied 1 in 100 with water and 5 ml of the diluted urine taken instead of protein-free filtrate.(95)

Estimation of Serum Phosphate

Principle

Serum is deproteinized with trichloracetic acid. Protein free filtrate is treated with acid molybdate which combines with phosphate to form phosphomolybdic acid. This is reduced by 1, 2, 4-aminonaphthosulphonic acid to

blue colored phosphomolybdous acid (molybdenum blue). The intensity of the colour is measured calorimetrically.

Procedure

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

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

= Au/As X 4

Estimation of Urine Phosphate

The urine is diluted 1 in 10 with water. Phosphate in diluted urine is estimated by the above procedure and the result multiplied by 10. If the urine does not contain proteins, it may be diluted 1 in 100 with water and 5 ml of the diluted urine taken instead of protein-free filtrate.(96)

Estimation of Urine Protein

Principle

Urine proteins are precipitated by sulphosalicylic acid, which gives a white precipitate, and the degree of the precipitate is proportional to the protein level.

Procedure

To 2ml of urine taken in a 13 x 100mm glass tube, add 2 ml of 3g% sulphosalicylic acid. Mix gently. Leave for 5 minutes at room temperature. Compare the degree of the precipitate with 4ml of sulphosalicylic acid taken in a similar test tube(97)

Estimation of Urine Oxalate

Principle

Oxalic acid is co-precipitated with calcium sulphate, reduced to glycolic acid by boiling with dilute sulphuric acid and a zinc pellet and estimated calorimetrically with chromo tropic acid.

Procedure

Acidify the urine sample by adding conc. HCl to ensure the solution of any crystals of calcium oxalate which may be present. Measure 0.5 ml of urine into a 25 ml graduated stoppered centrifuge tube, followed by 1.5 ml of water and one drop of 0.04% bromo-thymol blue indicator solution. Adjust the pH 7.0 by addition of dilute NaOH or dilute acetic acid. Add 2 ml of a saturated aqueous solution of calcium sulphate, followed by 14 ml of ethanol, mix gently and allow the solution to stand at room temperature for at least 3 h. Centrifuge at 2000r.p.m. for 10 min, carefully decant the super decant fluid and allow the tube to drain for a few minutes on a filter paper. Wipe the mouth of the tube with clean tissue and dissolve the precipitate in 2 ml of 2N sulphuric acid solution. Add a piece of freshly cleaned zinc and heat in a boiling water bath for 30 min; remove the zinc with a bent glass rod. Wash the zinc with 0.5 ml of 1% chromo tropic acid solution, adding the washing to the tube. Add 5 ml of conc. Sulphuric acid slowly, with mixing, and heat in a boiling water bath for 30 min. Cool, dilute to 20 ml with 10N Sulphuric acid. Read unknown and standard against blank at 570 nm.

The concentration of oxalic acid in the original sample of urine is given by the equation:

Concentration (mg of anhydrous oxalic= Reading from calibration curve (μg) X100/0.5 X 1/1000

Estimation of Serum Oxalate

Serum oxalate may be estimated in the same way as urine oxalate after diluting the urine. The serum is diluted according to the serum output.(98)

Estimation of Urine Magnesium

Principle

A method for the quantitative determination of magnesium in a single specimen of urine and serum determination of their respective phosphate.

Procedure

1. To a 15 ml conical centrifuge tube add 10 ml of well shaken urine, 2 drops of methyl orange, Conc. HCl drop wise, to a red colour, and 1 ml of 5% ammonium phosphate slowly with shaking.
2. Add 2 ml of ammonium hydroxide (28%), mix and let stand for at least 1 hour.
3. Centrifuge, decant and wash the precipitate three times with 5 ml of alcohol wash.
4. Dissolve the precipitate with 0.5 ml of 1:4 HCl and wash quantitatively into a 10 ml. volumetric flask, bring to volume with distilled water and mix.
5. 1 or 2 ml of this are then used for total phosphate determination.
6. 5 ml of the aliquot from item (4) are taken and 1 ml of 2.5 % oxalic acid is added, as well as a drop of methyl orange. Sodium acetate solution is then added slowly until the pH is approximately 4; i.e., until the indicator just turns from red to orange. The mixture is the allowed to stand for 4 hours or more for the complete calcium precipitation of the calcium oxalate. This assures complete calcium precipitation and magnesium solution.
7. The precipitate of calcium oxalate is then centrifuged and washed twice with 3 ml of 2% ammonium hydroxide. The supernatant and the washings are saved for the magnesium determination.
8. To the supernatant and the washing from the above, 0.5 ml of ammonium phosphate solution and 2 ml of strong ammonia are added and allowed to stand for 1 hour. The precipitate (Magnesium ammonium phosphate) is centrifuged and washed twice with 5 ml of alcohol wash and then redissolved in 1:4 HCl. Phosphate analysis is then performed upon the

magnesium ammonium phosphate. Calorimetery is performed with any standard photoelectric colorimeter, having a green a filter with an approximate spectral range of 500 to 570 mμ.

Estimation of Serum Magnesium

Serum magnesium may be estimated in the same way as urine magnesium after diluting the urine. The serum is diluted according to the serum output.(99)

Statistics

The results are expressed as Mean ± SEM. Data was evaluated using ONE WAY ANOVA followed by Newman – Keuls multiple range test. Probability values less than ($p < 0.01$) were considered significant.

7. RESULTS AND ANALYSIS

Pharmacological Evaluation

Acute toxicity studies

No normality was observed in the acute oral toxicity testing in rats with the single dose of 2g/kg of the individual extracts. Therefore the herbal extracts are categorized under acute toxicity class 5-> 2000mg- 5000mg/kg (OECD Guidelines, 2000).These findings provide information on the doses to be given in the subsequent pharmacological studies.

The extracts neither caused any of the toxic signs noted below, less than 14 days observation in the tested animals.

Behavioural toxic signs: Sedation, restlessness, drooping head, severe depression, excessive preening, gnawing paw, panting, irritability, aggressive and defensive hostility, fear, confusion.

Respiratory toxic signs: Hypopnea, dyspnea, gasping apnea.

Ocular toxic signs: Mydriasis, miosis, lacrimation, ptosis, nystagmus, cycloplegia, papillary light reflex.

No changes in body weight, feed intake and water intake of animals were observed. These observations have proved the safety of the extracts in large doses.

Pharmacological Evaluation

Antilithiatic Activity (Prophylactic and curative)

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to male wistar rats resulted in hyperoxaluria. Urinary concentration of the various ions investigated varied drastically, following ethylene glycol treatment.

Effect of HAEAG on Urinary Parameters on Day 14 & 28

In prophylactic study the urinary parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased significantly in G2 (Lithiatic control) following ethylene glycol treatment, treatment with hydro alcoholic extract of *Apium graveolens* at a dose of 200 and 400 mg/kg and cystone treatment reduce the all above mentioned parameters significantly. On the contrary the magnesium levels were decreased significantly in G2 (Lithiatic control) following ethylene glycol treatment**.** After treatment with hydro alcoholic extract of *Apium graveolens* at a dose of 200 and 400 mg/kg and cystone treatment the magnesium level was restored near to normal levels.

Effect of HAEAG on serum parameters on day 28

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased significantly in G2 (Lithiatic control) following ethylene glycol treatment, treatment with hydro alcoholic extract of *Apium graveolens* at a dose of 200 and 400 mg/kg and cystone treatment reduce the all above mentioned parameters significantly. On the contrary the magnesium levels were decreased significantly in G2 (Lithiatic control) following ethylene glycol treatment**.** After treatment with hydro alcoholic extract of *Apium graveolens* at a dose of 200 and 400 mg/kg and cystone treatment the magnesium level was restored near to normal levels.

Effect of HAEAG on urinary & serum parameters in curative study.

The curative study was designed to evaluate the effect of these extracts in lithiasis, after it has been induced as treatment is normally instituted in humans only after the incidence of renal stones. So in this study treatment was instituted from 29th day after treatment with ethylene glycol for 28 days. After treatment with extracts for 2 weeks starting from 28th day the above mentioned parameters were studied (table no: 4,5). The values show that all parameters like protein, calcium, creatinine, oxalate, uric acid and phosphate were increased significantly in ethylene glycol treated group (G2), whereas magnesium levels were decreased

significantly. However after treatment with hydro alcoholic extract of *Apium graveolens* at a dose of 200 and 400 mg/kg and cystone treatment,all above mentioned parameters reduced significantly and magnesium levels restore to near normal limits.

Histopathological Studies

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 (GP-2 in Fig 2) showed 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 hydro alcoholic extract of *Apium graveolens* at a dose of 200 and 400 mg/kg and cystone treatment shows obvious dilation of many tubules and tubulointerstitial inflammatory infiltration with lesion area < 40% (score 2) in Fig 3,4,5 showed improvement in the above symptoms and reduced crystal deposition.(score 1)

TABLE 7 : EFFECT OF HAEAG ON URINARY BIOCHEMICAL PARAMETERS ON THE DAY 14

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	64.80± 3.55	4.10± 0.46	5.60± 0.54	3.14± 0.60	0.78± 0.06	14.76± 1.70	32.80± 2.20
G2	150.20 ± 8.35**(a)	0.95 ± 0.20**(a)	19.30 ± 1.92**(a)	12.45 ± 1.50**(a)	1.50 ± 0.16**(a)	32.45 ± 3.40**(a)	72.60 ± 4.10**(a)
G3	85.30 ± 6.24 **(b)	2.45 ± 0.30**(b)	9.35 ± 1.05**(b)	5.50 ± 0.90**(b)	0.90 ± 0.12**(b)	22.20 ± 2.60**(b)	42.40 ± 3.40**(b)
G4	80.65 ± 6.05**(b)	2.60 ± 0.34**(b)	8.75 ± 0.90**(b)	5.25 ± 0.80**(b)	0.88 ± 0.10**(b)	20.15 ± 2.30**(b)	37.80 ± 3.25**(b)
G5	78.30± 5.80**(b)	2.95 ± 0.50**(b)	7.70 ± 0.60**(b)	4.90 ± 0.65**(b)	0.82 ± 0.08**(b)	18.10 ± 1.85**(b)	34.30 ± 2.50**(b)

G1- Normal control; **G2**- Lithiatic Control;

G3- HAEAG 200MG/KG

G4- HAEAG 400MG/KG

G5- CYSTONE 750MG/KG

Values are expressed as Mean± SEM

- Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test
- **(a)values were significantly different from normal control G1 at P< 0.01
- **(b) values were significantly different from Lithiatic control G2 at P<0.01

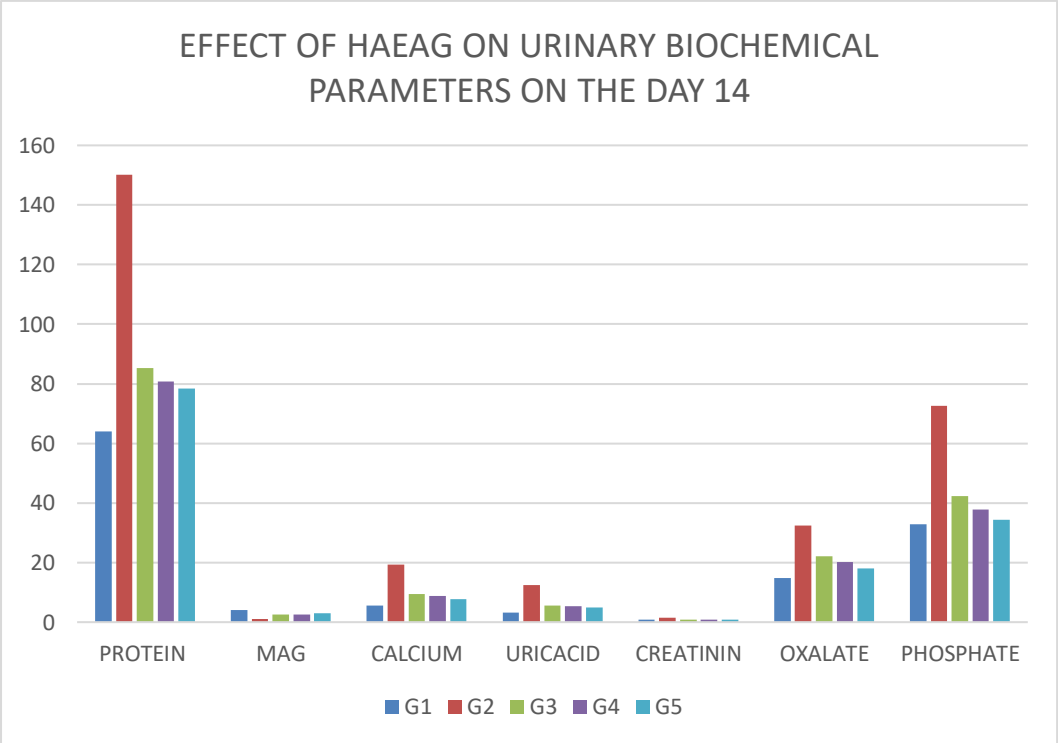


Figure no :5 EFFECT OF HAEAG ON URINARY BIOCHEMICAL PARAMETERS ON THE DAY 14

TABLE 8: EFFECT OF HAEAG ON URINARY BIOCHEMICAL PARAMETERS ON THE 28TH DAY.

GROUP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	70.85 ±3.30	4.40 ±0.70	6.20 ±0.70	3.30 ±0.58	0.85 ±0.06	15.30 ±1.50	32.50 ±2.20
G2	158.40 7.30**(a)	1.35 ±0.25**(a)	20.10 1.60**(a)	13.60 ±1.35**(a)	1.78 ±0.20**(a)	47.20 ±4.50**(a)	75.60 ±4.10**(a)
G3	93.10 5.80**(b)	2.90 ±0.35**(b)	10.60 0.80**(b)	7.80 ±0.75**(b)	1.10 ±0.10**(b)	24.40 ±2.70**(b)	43.75 ±3.60**(b)
G4	89.15 4.90**(b)	3.15 ±0.45**(b)	10.15 ±0.55**(b)	7.20 ±0.60**(b)	1.02 ±0.08**(b)	22.20 2.40**(b)	41.50 ±3.10**(b)
G5	85.60 ±3.70**(b)	3.60 ±0.55**(b)	9.20 ±0.50**(b)	6.60 0.45**(b)	0.88 ±0.04**(b)	19.10 ±2.15**(b)	38.25 ±2.75**(b)

G1- Normal control; **G2**- Lithiatic Control;

G3- HAEAG 200MG/KG

G4- HAEAG 400MG/KG

G5- CYSTONE 750MG/KG

Values are expressed as Mean± SEM

- Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test
- **(a)values were significantly different from normal control G1 at P< 0.01

  **(b) values were significantly different from Lithiatic control G2 at P<0.01

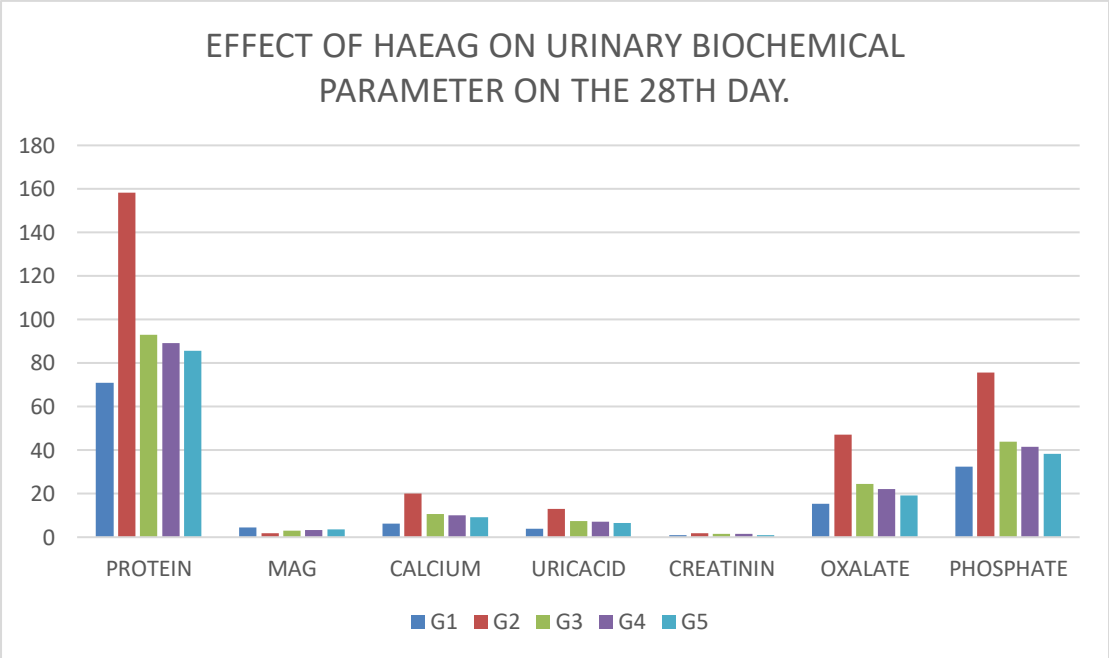


Figure no : 6 EFFECT OF HAEAG ON URINARY BIOCHEMICAL PARAMETERS ON THE 28TH DAY.

TABLE 9: EFFECT OF HAEAG ON SERUM PARAMETERS IN PROPHYLACTIC TREATMENT OF ANIMALS

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	4.75 ±0.70	9.40 ±1.25	3.50 ±0.30	0.60 ±0.06	6.60 ±0.60	12.10 ±1.45
G2	1.30 ±0.35**(a)	18.10 ±2.40**(a)	9.70 ±1.10**(a)	1.05 ±0.13**(a)	12.65 ±1.60**(a)	26.05 ±3.25**(a)
G3	3.60 ±0.58**(b)	11.70 ±1.35**(b)	4.40 ±0.55**(b)	0.90 ±0.09**(b)	8.45 ±0.85**(b)	20.10 ±2.65**(b)
G4	3.85 ±0.45**(b)	11.30 ±1.25*(b)	4.15 ±0.40**(b)	0.85 ±0.07**(b)	8.10 ±0.75**(b)	19.80 ±2.05**(b)
G5	4.10 ±0.55**(b)	10.45 ±1.40**(b)	3.90 ±0.35**(b)	0.80 ±0.06**(b)	7.60 ±0.65**(b)	16.20 ±2.20**(b)

G1- Normal control; **G2**- Lithiatic Control;

G3- *HAEAG* 200MG/KG

G4- HAEAG 400MG/KG

G5- CYSTONE 750MG/KG

Values are expressed as Mean± SEM

- Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test
- **(a)values were significantly different from normal control G1 at P< 0.01

  **(b) values were significantly different from Lithiatic control G2 at P<0.01

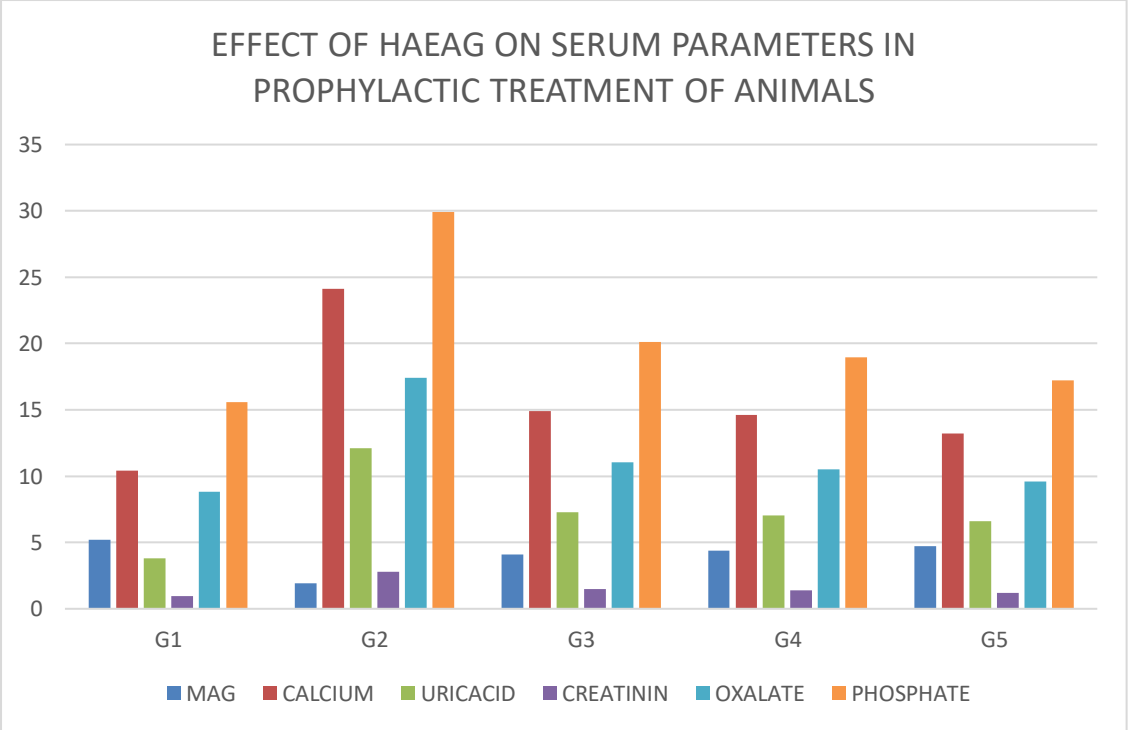


FIGURE NO :7 EFFECT OF HAEAG ON SERUM PARAMETERS IN PROPHYLACTIC TREATMENT OF ANIMALS

TABLE 10 : EFFECT OF HAEAG ON URINARY BIOCHEMICAL PARAMETERS IN CURATIVE TREATMENT OF ANIMALS

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	73.50 ±3.90	4.70 ±0.70	6.45 ±0.65	3.80 ±0.80	14.95 ±2.15	19.20 ±1.75	35.10 ±2.90
G2	170.85 ±7.95**(a)	1.50 ±0.20**(a)	17.90 ±2.10**(a)	15.00 ±2.50**(a)	55.90 ±4.15**(a)	49.90 ±3.45**(a)	80.30 ±4.75**(a)
G3	112.85 5.05**(b)	3.20 ±0.40**(b)	11.75 ±1.20**(b)	9.50 ±1.30**(b)	36.90 ±3.20**(b)	26.85 ±2.45**(b)	43.30 ±3.10**(b)
G4	96.35 4.70**(b)	3.80 ±0.45**(b)	10.95 ±1.15**(b)	9.00 ±1.20**(b)	34.60 ±3.05**(b)	24.30 ±2.15**(b)	41.70 ±2.90**(b)
G5	90.65 ±3.80**(b)	4.10 ±0.50**(b)	10.30 ±1.05**(b)	8.30 ±1.05**(b)	32.60 ±2.90**(b)	21.10 ±1.95**(b)	38.35 ±2.60**(b)

G1- Normal control; **G2**- Lithiatic Control;

G3- *HAEAG* 200MG/KG

G4- HAEAG 400MG/KG

G5- CYSTONE 750MG/KG

Values are expressed as Mean± SEM

- Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test
- **(a)values were significantly different from normal control G1 at P< 0.01

  **(b) values were significantly different from Lithiatic control G2 at P<0.01

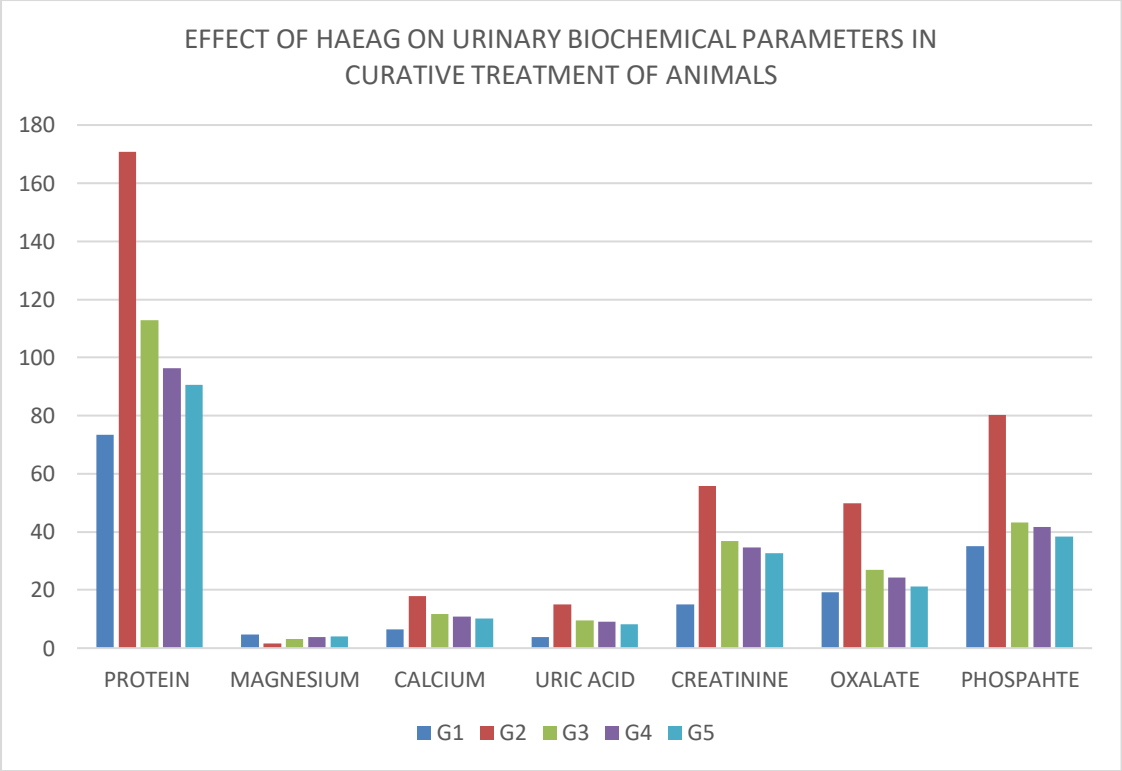


Figure no :8 EFFECT OF HAEAG ON URINARY BIOCHEMICAL PARAMETERS IN CURATIVE TREATMENT OF ANIMALS

TABLE 11 : EFFECT OF HAEAG ON SERUM PARAMETERS IN CURATIVE TREATMENT OF ANIMALS

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	5.20 ±0.90	10.40 ±1.30	4.20 ±0.90	0.95 ±0.20	8.8 ±0.75	15.60 ±2.00
G2	1.90 ±0.50**(a)	24.10 ±2.40**(a)	12.10 ±2.40**(a)	2.80 ±0.60**(a)	17.40 ±1.20**(a)	29.90 ±3.05**(a)
G3	4.10 ±0.82**(b)	14.90 ±1.60**(b)	7.30 ±1.05**(b)	1.50 ±0.50**(b)	11.05 ±1.00**(b)	20.80 ±2.45**(b)
G4	4.40 ±0.90**(b)	14.60 ±1.25**(b)	7.05 ±0.95**(b)	1.40 ±0.35**(b)	10.50 ±0.85**(b)	18.95 ±2.22**(b)
G5	4.75 ±0.98**(b)	13.20 ±1.05**(b)	6.60 ±0.75**(b)	1.22 ±0.28**(b)	9.60 ±0.80**(b)	17.20 ±2.05**(b)

G1- Normal control; **G2**- Lithiatic Control;

G3- *HAEAG* 200MG/KG

G4- HAEAG 400MG/KG

G5- CYSTONE 750MG/KG

Values are expressed as Mean± SEM

- Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test
- **(a)values were significantly different from normal control G1 at P< 0.01

  **(b) values were significantly different from Lithiatic control G2 at P<0.01

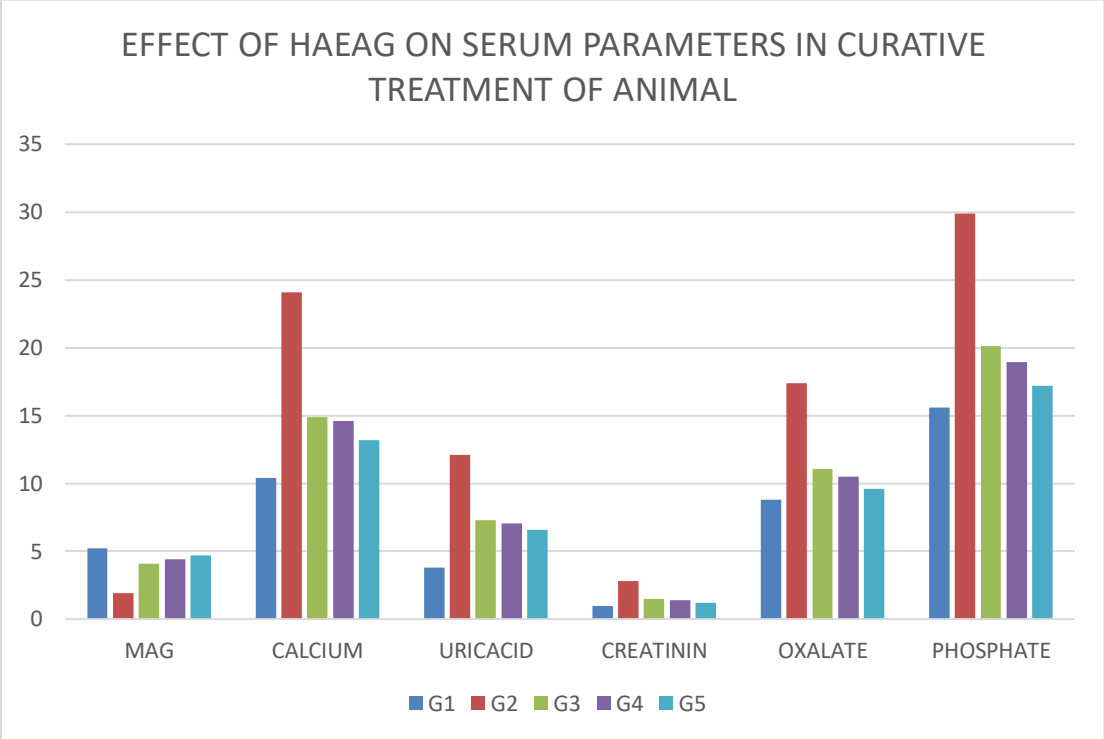


Figure no : 9 EFFECT OF HAEAG ON SERUM PARAMETERS IN CURATIVE TREATMENT OF ANIMALS

HISTOPATHOLOGY

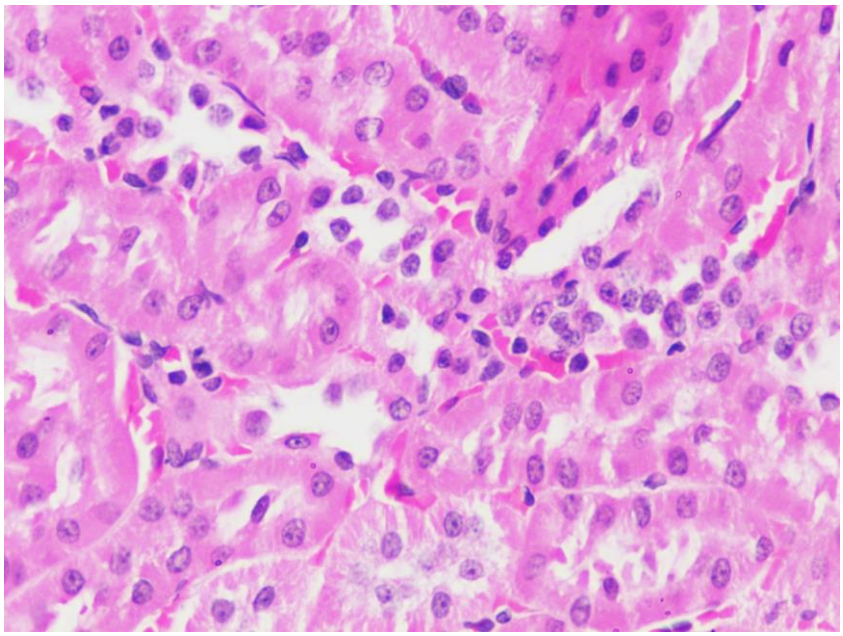


FIG.NO: 10 NORMAL CONTROL (CURATIVE STUDY)

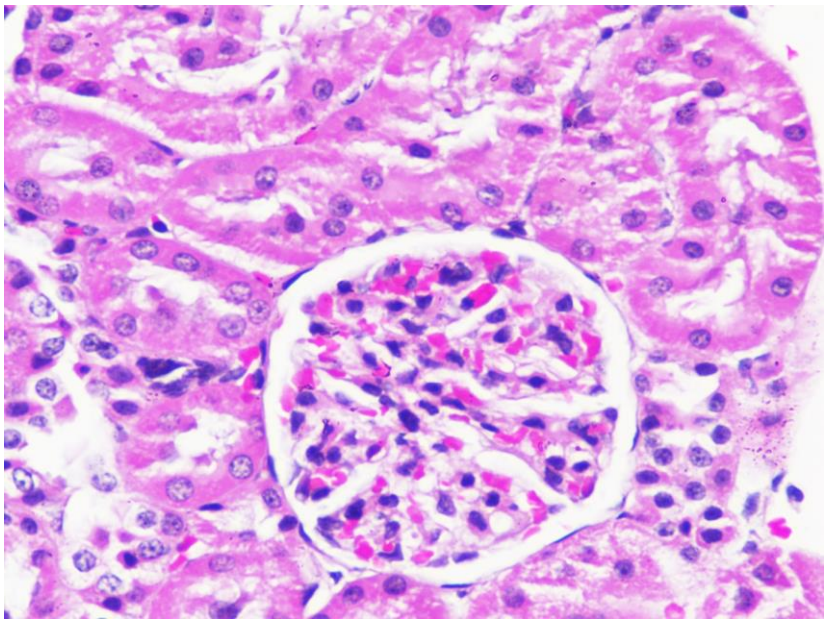


FIG NO :11 LITHIATIC CONTROL (CURATIVE CONTROL)

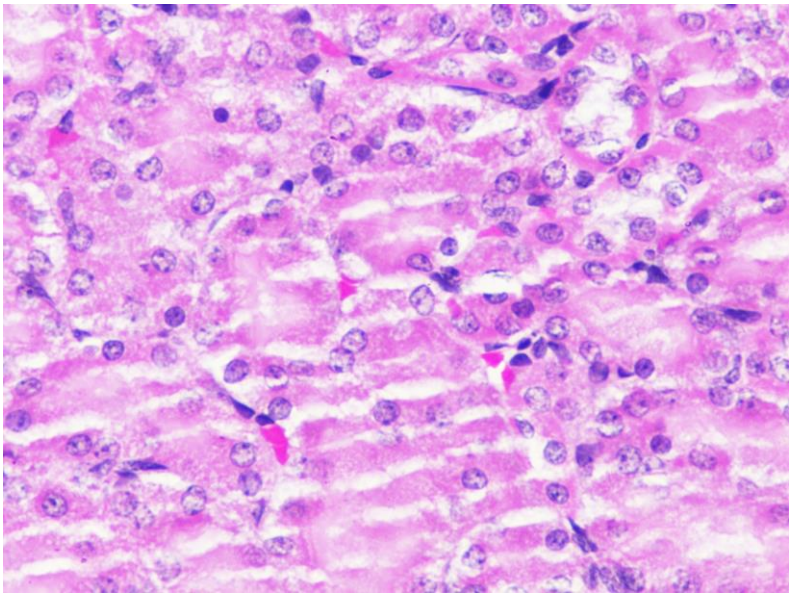


FIG NO :12 HAEAG 200MG/KG

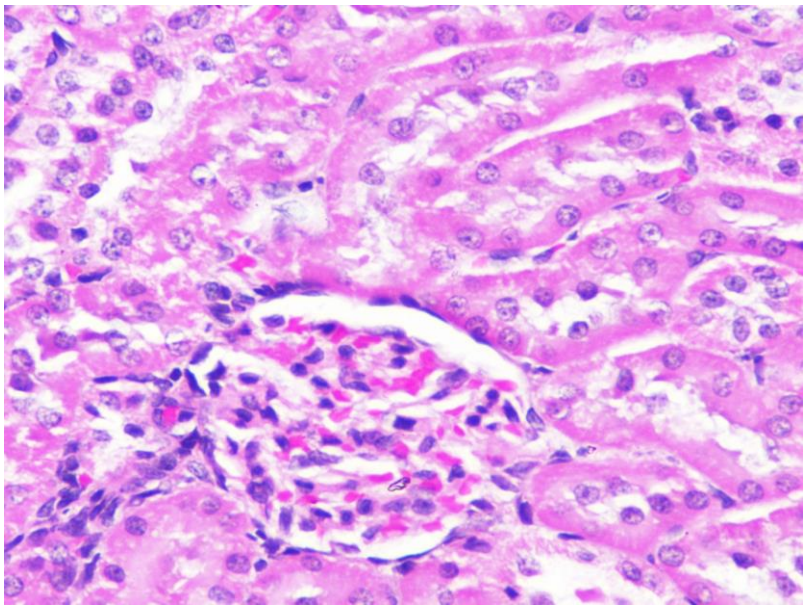


FIG NO : 13HAEAG 400MG/KG

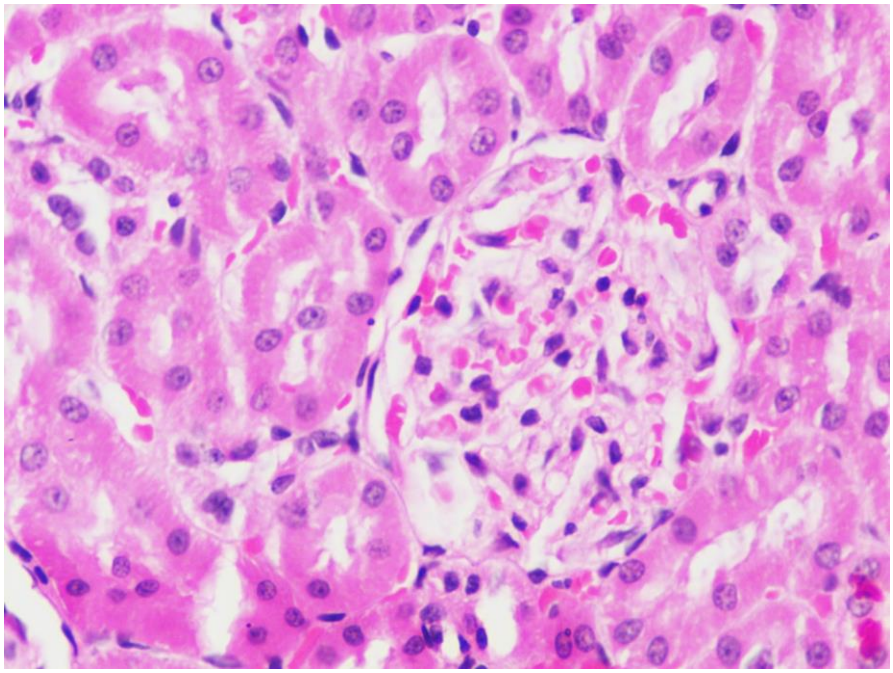


FIG NO: 14 CYSTONE 750MG/KG

8. DISCUSSION

As traditional medicines are usually taken by the oral route, same route of administration was used for evaluation of antilithiatic effect of the hydro alcoholic extract of *Apium graveolens* against ethylene glycol induced urolithiasis in rats.

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and also earlier studies have shown that the amount of stone deposition in female rats was significantly less.

Urinary supersaturation 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 increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate.(100) Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate. Therefore, this model was used to evaluate the antilithiatic effect of hydro alcoholic extract of *Apium graveolens* against urolithiasis.

In the present study oxalate and calcium excretion progressively increased in calculi- induced animals (G2), since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria (100), and the changes in urinary oxalate levels are relatively much more important than those of calcium(101). Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth.(102) However hydro alcoholic extract of *Apium graveolens* lowered the levels of oxalate as well as calcium excretion.

An increase in urinary phosphate is observed in calculi induced rats (G2). Increased urinary 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.(103) Treatment with hydro alcoholic extract of *Apium graveolens* restored phosphate level, thus reducing the risk of stone formation.

The increases in urinary uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been reported 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 hydro alcoholic extract of *Apium graveolens* lowered the excretion of uric acid and reduces the risk of stone formation.

Supersaturation, 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 urinary concentration of chemicals that inhibit stone formation decreases. Inhibitors of crystallization include citrate, magnesium, phosphate; nephrocalcin etc.(104) Low urinary magnesium content is a common feature in stone formers.(105)A similar condition was observed in the (G2) rats. Treatment with hydro alcoholic extract of *Apium graveolens* elevated the urinary magnesium level, and thus, reduced the propensity to crystallize, thereby creating an ambience unfavorable for precipitation.

Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers (106)A high urinary colloidal concentration favors crystal growth.(107) Such a condition was observed with ethylene glycol treated rats, in this study. Administration of the hydro alcoholic extract of *Apium graveolens* reduced the urinary 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 urinary system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated.(108)Also increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi- producing diet (CPD).(109,110)Elevated oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane. (111)In calculi- induced rats (G2), marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid. However, the curative and prophylactic treatment with hydro alcoholic extract of *Apium graveolens* causes diuresis and hastens the process of dissolving the preformed stones and prevention of new stone formation in the urinary system.

Increase in calcium and oxalate levels in the renal tissue of EG-treated rats were observed. Curative and prophylactic treatment with hydro alcoholic extract of *Apium graveolens* suppresses this increase in intracellular calcium. Several studies reported that Flavonoids, polyphones and triterpenes have anti-inflammatory and antioxidant effects.(112,113,114)It can be expected that antilithiatic activity might be through an antioxidant activity and free radical scavenging principle(s)(115,116).

Microscopic examination of kidney sections derived from ethylene glycol induced urolithiasis rats showed 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 the hydro alcoholic extract of *Apium graveolens* decreased the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes.

9. CONCLUSION

In conclusion, the presented data indicate that administration of the hydro alcoholic extract of *Apium graveolens* to rats in ethylene glycol induced lithiasis, reduced and prevented the growth of urinary stones, supporting the hill tribals' claim regarding antilithiatic activity of the plants.

Earlier studies reported that antilithiatic activity might be through an antioxidant activity and free radical scavenging principle(s). Hydro alcoholic extract of *Apium graveolens* are having Polyphenols- Tyrosol, Pyrogallol, saponins and Flavonoids. These components are responsible for antilithiatic activity.

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

10. BIBILIOGRAPHY

INTRODUCTION

1. Choubey Ankur, Parasar Amarchand, Choubey Aadarsh, Iyer Deepa, Pawar R S, Patil U K POTENTIAL OF MEDICINAL PLANTS IN KIDNEY, GALL AND URINARY STONES International Journal of Drug Development & Research | April-June 2010 | Vol. 2 | Issue 2 | ISSN 0975-9344.
2. K. D. Mwambete in vitro antimicrobial activity of fruit and leaf crude extracts of Momordica charantia: A Tanzania medicinal plant African Health Sciences 2009; 9(1):34-39.
3. http://www.kidneyfund.org/kidney-disease/kidney-problems/kidney-stones/
4. Definition of Urolithiasis - Medicine Net. https:// www.medicinenet.com/ main/ ar
5. Lewis's Medical –Surgical Nursing. Second edition; vol-2: page no: 1129-1133.
6. Kidney stones - Symptoms and Causes – mayoclinic .https:// www.mayoclinic.org/ diseases-conditions/ kidney-stones/ symptoms-causes/ syc-20355755 and h1=en-IN.
7. Maso T, Sujihata. International Journal of Urology. 2008; 15: 115-120.
8. Coe FL, Keck J, Norton ER. The natural history of calcium urolithiasis. Jama. 1977;238:1519–1523.
9. Pearle MS, Calhoun EA, Curhan GC, Urologic diseases in America project: Urolithiasis, J Urol 2005; 173: 848–57.
10. 9.Hughes P, Kidney stones epidemiology. Nephrol 2007; 12: 26–30. 10. Dawson PA, Russell CS, Lee S, McLeay SC, van Dongen JM, Cowley DM, Clarke LA, Markovich D. Urolithiasis and hepatotoxicity are linked to the anion transporter Sat1 in mice, J Clin Invest 2010; 120(3): 706–12.

11. Saigal CS, Joyce G, Timilsina AR, Direct and indirect costs of nephrolithiasis in an employed population: Opportunity for disease management? Kidney Int 2005; 68: 1808–14.
12. A. P. Evan, ―Physiopathology and etiology of stone formation in the kidney and the urinary tract,‖ Pediatric Nephrology, vol. 25, no. 5, pp. 831–841, 2010.
13. NKF (National Kidney Federation)
14. Harrison's Principles of internal medicine. 15th ed. 1998.
15. Maso T, Sujihata. International Journal of Urology. 2008; 15: 115-120.
16. Ureter Stones: Symptoms types causes treatment, diagnosis, prevention https:// www.epainassist.com/pelvic-pain /urethera / ureter -stones.
17. Worcester EM, Coe FL; Clinical practice. Calcium kidney stones. N Engl J Med. 2010 Sep 2 363(10):954-63.
18. Renal or ureteric colic - acute; NICE CKS, April 2015 (UK access only)
19. H. Ghelani,M. Chapala, andP. Jadav, ―Diuretic andantiurolithiatic activities of an ethanolic extract of Acorus calamus L.rhizome in experimental animal models,‖ Journal of Traditional and Complementary Medicine, vol. 6, no. 4, pp. 431–436, 2016.
20. Ryall RL, Harnett RM, Marshall VR. The effect of urine, pyrophosphate, citrate, magnesium and glycosaminoglycans on the growth and aggregation of calcium oxalate crystals in vitro. Clin Chim Acta. 1981;112:349–356.
21. I. Sharma, W. Khan, and S. Ahmad, ―In vitro and ex vivo approach for anti urolithiatic potential of bioactive fractions of gokhru with simultaneous HPLC analysis of six major metabolites and their exploration in rat plasma,‖ Pharmaceutical Biology, vol. 55, no. 1, pp. 701–711, 2017
22. D. B. V. S. R. Radha, ―Herbal plants used in the treatment of urolithiasis: a review,‖ International Journal of Pharmaceutical Research and Development, vol. 5, no. 974, pp. 66–70, 2013.
23. Ecobichon D J. The basis of Toxicity testing, 2nd Edition, CRC press, New York,1997;43

24. Department of Pharmacology, Toxicology & Neuroscience, Louisiana State University Health Sciences Center, Shreveport, L A,USA November 2009, Vol.47,No.9,Pages 859-869
25. Bashir and A. H. Gilani, ―Antiurolithic effect of Bergenia ligulata rhizome: an explanation of the underlying mechanisms,‖ Journal of Ethnopharmacology, vol. 122, no. 1, pp. 106–116, 2009.
26. Khan SR. Animal models of kidney stone formation: an analysis. World J Urol. 1997;15:236–243.
27. Doddametikurre Ramegowada, Chandra, Shekhar Biyani, Antony J B, Jon J, Cartledge. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. . International Journal of Urology. 2007; 5: 126-136.
28. Kidney stone disease – Wikipedia. https://en.m.wwikipedia.org/wiki/ kidney-stone-disease.
29. Khaling Mikawlrawng, Suresh Kumar, Vandana. Current scenario of Urolithiasis and the use of medicinal plants as antiurolithiasis and the use of medicinal plants as antiurolithiatic agents in Manipur (north east india): A Review International Journal of Herbal Medicine. 2014; 2(1): 1-12.
30. Christian Turk, Ales Petrik, Kemal Sarica, Christian Seitz, Andreas Skolarikos, Michael Straub, Thomas Knoll. EAU guidelines on diagnosis and conservative management of urolithiasis. European Urology. (2016); 69: 468-474.
31. Atmani F, Slimani Y, Mimouni M, and Hacht B. Prophylaxis of calcium oxalate stones by Herniaria hirsute on experimentally induced nephrolithiasis in rats. BJU International. 2003; 92: 137-140.
32. Syed Sufiyan Fazal, Rajeev K Singla Indo Global Journal of Pharmaceutical Sciences, 2012; 2(1)
33. Sree Lakshmi, Namburu Sujatha, Dodoala, Bharathi Koganti K, S.Prasad. Antiurolithiatic activity of phaseolus vulgaris seeds against ethylene glycol-induced renal calculi in wistar rats. International Journal of Green Pharmacy. Oct-Dec 2017; 11(4): 281.

LITERATURE REVIEW

34. Ruchi Roper1,*Dharam Pal Pathak2, Vikash Gupta3, Uma, Garima Kapoor2, Rubina Bhutani2, Ravi Kant2 Int. J. Pharm. Sci. Rev. Res., 46(1), September - October 2017; Article No. 18, Pages: 95-99
35. Syed Sufiyan Fazal, Rajeev K Singla Indo Global Journal of Pharmaceutical Sciences, 2012; 2(1)
36. Wesam Kooti1, Sara Ali-Akbari1, Majid Asadi-Samani2*, Hosna Ghadery1, Damoon Ashtary-Larky3A review on medicinal plant of Apium graveolens Advanced Herbal Medicine, 2014; 1 (1): 48-59.
37. M. C. Powanda • K. D. Rainsford A toxicological investigation of a celery seed extract having anti-inflammatory activity Inflammopharmacol DOI 10.1007/s10787-010-0049-1
38. Rhema Niteen Dongre*, Dr. S. B. Patil, Dr. N.S. Naikwade, Mohsin J Jamadar In Vitro Evaluation of Combined Dosage of Apium graveolens Dulce and Bryophyllum pinnatum Kurz for Antigout Activity Saudi Journal of Medical and Pharmaceutical Sciences ISSN 2413-4929 (Print) Scholars Middle East Publishers ISSN 2413-4910 (Online)
39. M.S.Seraga*, S.R.Gedarab, A.Z. Abo El- Nagaa, N.G. Amera Habitat Effect on the Essential Oils, Phenolics and Flavonoids of the Medicinal Weed Apium graveolens L. Scientific Journal for Damietta Faculty of Science 2 (2013) 33-42.
40. Anwar Ali Shad, Hamid Ullah Shah, Jehan Bakht Nutraceutical potential and bioassay of Apium graveolens Journal of Medicinal Plants Research Vol. 5(20), pp. 5160-5166, 30 September, 2011
41. Ali Esmail Al-Snafi Arabian medicinal plants with antiurolithiatic and diuretic effects - plant based review Volume 8, Issue 6 Version. II (June 2018), PP. 67-80.
42. Spice Pages. Celery. (n.d.). Retrieved from http://gernot-katzers-spice-pages.com/engl/Apiu_gra.html .
43. Brunner and Suddarth's. Text Book of Medical-Surgical Nursing. 13th edition; vol-2: page no: 1591-1595.

PLANT PROFILE

44. Migahid AM. Flora of Saudi Arabia. Riyadh: Riyadh University Publication; 1978.
45. Al-Asmari AK, Al-Elaiwi AM, Athar MT, et al. A review of hepatoprotective plants used in Saudi traditional medicine. Evid Based Complementary Alter n Med 2014, 2014, 1-23.
46. Tyagi S, Dhruv M, Ishita M, Gupta AK, Usman MRM, Nimbiwal B, *et al* . Medical benefits of *Apium graveolens* (celery herb). J Drug Discov Ther 2013;1:36-8
47. Hussain MT, Ahmed G, Jahan N, Adiba M. Unani description of Tukhme Karafs (seeds of *Apium graveolens* Linn) and its scientific reports. Int Res J Biol Sci 2013;2:88-93.
48. Zidorn C, Jöhrer K, Ganzera M, Schubert B, Sigmund EM, Mader J, *et al* . Polyacetylenes from the *Apiaceae* vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. J Agric Food Chem 2005;53:2518-23
49. Ovodova RG, Golovchenko VV, Popov SV, Popova GY, Paderin NM, Shashkov AS, *et al* . Chemical composition and anti-inflammatory activity of pectic polysaccharide isolated from celery stalks. Food Chem 2009;114:610-5.
50. Nagella P, Ahmad A, Kim SJ, Chung IM. Chemical composition, antioxidant activity and larvicidal effects of essential oil from leaves of *Apium graveolens*. Immunopharmacol Immunotoxicol 2012;34:205-9.
51. Sastri B. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials; 1956. p. 4.
52. Bhatnagar JK, Handa SS. Thin layer chromatographic studies of volatile oils from common umbelliferous fruits during growth. Res Bull Punjab Univ Sci 1968;4:331-4.

53. Singh A, Handa SS. Hepatoprotective activity of ApiumGraveolens and Hygrophilaauriculata against paracetamol and thioacetamideintoxication in rats. J Ethnopharmacol 49, 1995, 119-26.
54. Ahmed B, Alam T, Varshney M, Khan SA. Hepatoprotective activity of two plants belonging to the Apiaceae and the Euphorbiaceae family. J Ethnopharmacol 79, 2002, 313-316.
55. Hamza AA, Amin A. Apiumgraveolens modulates sodium valproate-induced reproductive toxicity in rats. J ExpZool A Ecol Genet Physiol. 307(4), 2007, 199-206.
56. Roghani M, Baluchnejadmojarad T, Amin A, Amirtouri R. The effect of administration of Apiumgraveolens aqueous extract on the serum levels of glucose and lipids of diabetic rats. Iran J Endocrinol M etab 9, 2007, 177-181
57. Thimn JC, Burritt DJ, Sims IM, et al. Celery parenchyma cell walls: cell walls with minimal xyloglucan. Physiol Plant. 116(2), 2002, 164-71.
58. Atta A. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. J Ethnopharmacol. 60(2),1998, 117-24 .
59. Tsi D, Tan BK. The mechanism underlying the hypocholesterolaemic activity of aqueous celery extract, its butanol and aqueous fractions in genetically hypercholesterolaemic RICO rats. Life Sci. 66(8), 2000, 55-67.
60. Belanger JT. Perillyl alcohol: applications in oncology. Altern Med Rev. 3(6), 1998, 448-57.
61. Zidorn C, Johrer K, ganzera M, Schubert B, Sigmund EM, Mader J, et al. Polyacetylenes from the apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. J Agric Food chem. 53(7), 2005, 2518-23.
62. Yarnell E. Botanical medicines for the urinary tract. World J Urol. 20(5), 2002, 285-93.
63. Fluke H. Herb. Trans Tavakoli-Saberi H. 6th ed. Tehran: Gulsh an publicathion, 2005, 201.

64. Al-Howiriny T, Alsheikh A, Alqasoumi S, et al. Gastric antiulcer, ant secretory and cytoprotective properties of celery I rats. Pharm Biol. 48(7), 2010, 786-93.
65. Naema NF, Dawood B, Hassan S. A study of some Iraqi medicinal plants for their spasmolytic and antibacterial activities. J Basrah Res (Sci) 36, 2010, 67-8.
66. Kolarovic J, Popovic M, Mikov M, Mitic R, Gvozdenovic L. Pro tective effects of celery juice in treatments with Doxorubicin. Molecules, 14(4), 2009, 1627-38.
67. Jones AF. Perforation of small intestine by a fragment of cele ry. Can Med Assoc J. 63(3), 1950, 291-2.

AIM &OBJECTIVE

68. Grases,F., and costa-Bauza.Potentiometric study of the nucleation of calcium oxalate in the presence of several additives. *Clinical chemistry and enzymology communication*., **1991a**, 3:319-328.
69. Grases,F., costa-Bauza, A.,March, J.G., and Masarova, L.Glycosaminoglycans, uric acid and calcium oxalate urolithiasis. *Urological research*., **1991b,19:375**-380.
70. Volak,J., Stodola,J., and severa, F.Plantas Medicinales, 1983 ,Artia Ed., Praque,Czechoslovakia.
71. Ageel,A.M.,Tarig,M.,Moss,J,S.,Al-yahya,M.A.,Al-said,M.S. Plants used in Saudi folk medicine. King Abdulaziz City for Science and Technology, King Saud university press,Riyadh.Saudi Arabia.
72. Prasad, K.V.S.R.G., Sujatha, D., Bharathi, K. Herbal drugs in Urolithiasis. *A. Review. Pharmacognosy Review.*, **2007,** 1:175-177.
73. Basant, B., Chaurasis, O.P., Zakwan, A., Shashi, B.S. Traditional medicinal plants of cold desert Ladakh- used against kidney and urinary disorders. *Journal of Ethnopharmacology.*, **2008,** 118:331-339.
74. Prachi., Chauhan, N., Kumar, D., Kasana, M.S. Medicinal plants of Muzaffarnagar district used in treatment of urinary tract and kidney stones. *Indian Journal of Traditional Knowledge.*, **2009,** 8(2):191-195.

75. Nima, D., Namsa., Hui, T., Mandal, M., Kalita, P., Das, A.K. An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh. *Indian Journal of Ethnopharmacology.*, **2009,** 125:234-245.

PHYTOCHEMICAL ANALYSIS

76. Shashi, A., Monika, S., Shruti, R., Alok, M. Herbal drugs in Antilithiasis-A Review. *International Journa. of Pharma Research and Development.*, **2010,** 1(11):01-07.
77. WHO, Research Guidelines for evaluating the safety and efficacy of herbal medicines, World Health Organizations, Geneva, 1998; 30-33.
78. Hui Tag., Nima, D., Namsa, A.K., Das, P., Kalita, S.C., Mandal. Evaluation of anti-inflammatory potential of *Chloranthus erectus* (Buch.-Ham.) Verd. leaf extract in rats. *Journal of Ethnopharmacology.*, **2009**, 126(2):371-374.
79. Khandelwal K.R, Practical Pharmacognosy, Ist ed, Nirali Prakashan, Delhi, 2002,pp.157
80. Kokate CK, Practical Pharmacognosy, Ist ed, Vallabh Prakashan, Pune, 1986,pp.111-115.
81. Singh, H., Kapoor, V.K. Practical Pharmaceutical Chemistry, Ist ed, Vallabh Prakashan, New Delhi, 1994, pp.167-177.
82. Finar, I.J., Organic Chemistry, 5th ed, The English language Book Society and Longmann Green Publishers, London, 1973,pp.342, 653,656-657.

PHARMACOLOGICAL EVALUATION

83. OECD/OCDE guideline for the testing of chemicals. Revised draft guideline 423: Acute oral toxicity-Acute toxic class method. Revised document, October 2000,1-14.
84. Khan SR, Animal models of kidney stone formation: An analysis. *World Journal of Urology*,, **1997**, 15:236-243.

85. Grases, F., Ramis, M., Costa, B.A., March J.G., Effect of *Herniaria hirsuta* & *Agropyron repens* on calcium oxalate urolithiasis risk in rats. *Journal of Ethnopharmacology.*, **1995**, 45:211-214.
86. Ying, H. L., Jeng, Y.T., Jong, K.H. Combined use of 30% lactose rich diet and 1% ethylene glycol: A new animal model for study of urolithiasis. *Journal of Urology*., **2000**,11(4):149-153.
87. Varalakshmi, Vidhya L. Control of urinary risk factors of stones by betulin and lupeol in experimental hyperoxaluria. *Journal of Ethnopharmacology*., **1999**, 28:313-321.
88. Varalakshmi, P., Rajeswari, A. Low molecular heparin protection against oxalate – induced oxidative renal insult. *Clinica Chimica Acta*., **2006**, 370:108-114.
89. Jeong, B.C., Park, M.Y., Kwak, C., Kim, B.S., Kim, J.I., Kim, H.H. An animal model of calcium oxalate urolithiasis based on a cyclooxygenase selective inhibitor. *Urological Research*., **2005**, 33:453-459.
90. Theodore, YC. Chemical Urolithiasis. Characteristics of bladder stone induction by terepthalic acid and dimethyl terepthalate in weanling Fischer – 344 rats. *Toxicology and Applied Pharmacology.*, **1981**,58:307-321.
91. Malini, MM., Lenin, M., Varalakshmi, P. Protective effect of triterpenes on calcium oxalate crystal- induced peroxidative changes in experimental urolithiasis. *Pharmacological Research*., **2000**, 41:413-418.
92. Yargas, R.S., Perez, R.M.G., Perez, S.G.,Zavala, M.A.S, Perez, C.G. Antiurolithiatic activity of *Raphnus sativus* aqueous extract on rats. *Journal of Ethnopharmacology*., **1999**, 68: 335-338.
93. Caden, S., Tania, A.V., Cristina, D.D. Evaluation of the antiurolithiatic activity of the extract of *Costus spiralis* Roscoe in rats. *Journal of Ethnopharmacology*., **1999,** *66:*193-198.
94. Halabe, A., Shor, R., Wong, N.L.M., Sutton, R.A.L. Effect of Vitamin D_3 on the conversion of ethylene glycol to glycolate and oxalate in ethylene glycol- fed rats. *Clinica Chimica Acta*., **2003**, 330: 135-139.

95. Clark, E.P., Collip, J.B. Estimation of serum calcium. *Journal of Biological Chemistry.*, **1925**,63:461.
96. Folin, O., Wu, H. Estimation of serum creatinine. *Journal of Biological Chemistry*., **1919**, 38:81.
97. Caraway, W.T. Estimation of serum uric acid. *American Journal of Clinical Pathology.*, **1955**,25:840.
98. Fiske, C.H., Subbarow, Y. Estimation of serum phosphate. *Journal of Biological Chemistry*., **1925**,66:375.
99. Doumas, B.T., Bayse, D., Borner, K., Carter, R.J, Peter, S.T., Schafter, R. Estimation of urine protein. *Clinica. Chemica*., **1981,**27:1642.

DISCUSSION

1. Robertson, W.G., Peacock, M. The course of idiopathic calcium disease: Hypercalciuria or Hyperoxaluria? *Nephron.*, **1980,** 27:386-391.
2. Lemann, J.J., Worcestor, E.M., Gray, R.W. Hypercalciuria and stones. *American Journal of Kidney. Diseases.*, **1991,** 26:105-110.
3. Roger, K., Low, M.D., Stoller, M.L. Uric acid nephrolithiasis. *Urologic Clinics of North America.*, **1997,** 24:135-148.
4. Ryall, R.L., Harnet, R.M., Marshall, V.R. The effect of urine pyrophosphate, citrate, magnesium and glycosaminoglycans on the growth and aggregation of calcium oxalate crystals invitro. *Clin. Chem. Acta.*, **1991,** 112:349-356.
5. Grases, F., Genestar, C., Conte, A., March, P., Costa, B.A. Inhibitory effect of pyrophosphate, citrate, magnesium and chondriotin sulfate in calcium oxalate urolithiasis. *British Journal of Urology.*, **1989,** 64:235-237.
6. Khan, S.R. Animal models of kidney stone formation: An analysis. *World Journal of Urology.*, **1989,** 64:236-243.
7. Groyer, P.K., Resnick, M. Evidence for the presence of abnormal proteins in the urine of recurrent stone formers. *Journal of Urology.*, **1995,** 153:1716-1721.

8. Finch, A.M., Kasidass, G.P., Rose, G.A. Urine composition in normal subjects after oral ingestion of oxalate rich foods. *Clinical Science.,* **1981,** 60:411-418.
9. Ghodkar, P.B. Chemical Tests in Kidney Disease. In, Text book of Medical Laboratory Technology, 1st ed, Bhalani Publishing House, Mumbai, 1994,pp. 118-132.
10. Sumathi, R., Jayanthi, S., Kalpana, D.V., Varalakshmi, P. Effect of DL–alpha- Lipoic acid on tissue lipid peroxidation and antioxidant systems in normal and glycolate treated rats. *Pharmacological Research.,* **1993,** 27:1-10.
11. Saravanan, N., Senthil, D., Varalakshmi, P. Effect of L-cysteine on lipid peroxidation in experimental urolithiatic rats. *Pharmacological Research.,* **1995,** 32:165-169.
12. Ernster, L., Nordenbrand, K. Oxidation and Phosphorylation. In, Methods in Enzymology, (Ronald, W.E., Maynard, E.P. ed.) Academic Press, New York, 1967, 10:574 – 580.
13. Karadi, R.V., Palkar, M.B., Gaviraj, E.N., Gadge, N.B., Mannur, V.S., Alagawadi, K.R. Antiurolithiatic property of *Moringa oleifera* root bark. *Pharmaceutical Biology.,* **2008,** 46(12):861-865.
14. Varatharrajan, S., Veena, C.K., Varalakshmi, P. Antiurolithiatic effect of lupeol linoleate in experimental hyperoxaluria. *Journal of Natural Products.,* **2008,** 71(9):1509-1512.
15. Soundararajan, P., Mahesh, R., Ramesh, T., Hazeena, V.B. Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats. *Indian Journal of Experimental Biology.* **2006,** 44:981-986.
16. Mousa-Al-Reza, H., Alireza, K., Zahra, H., Mohammadreza, P. Ethanolic extract of *Nigella sativa* L seeds on ethylene glycol- induced kidney calculi in rats. *Urology Journal.,* **2007,** 4(2):86-90
17. Arafat, O.M., Tham, S.Y., Sadikun, A., Zhari, I., Haughton, P.J., Asmawi, M.Z. Studies on diuretic and hypouricemic effects of orthosiphon stamineus methanol extracts in rats. *Journal of Ethnopharmacology.,* **2008,** 118:354-360.