

An In Vitro Study on the Antiurolithiatic Activity of *Tribulus terrestris* Linn

AIM AND SCOPE

Utilization of plants for medical purposes in India has been documented long back in ancient literature. Many medical plants have been used since ages to treat urinary stones though the rationale behind their use is not well established through systematic and pharmacological studies, except their use is not well established through systematic and pharmacological studies, except for some composite herbal drugs and not plant medicines are because of their wide biological and medicinal activities, higher safety margin and costs.

Urolithiasis is a complex process that occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Data from in-vitro, in- vivo and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. Medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. Various plant species of *Tribulus terrestris*, have been reported to posses antiurolithiatic property. In this study ethenol extracts of *Tribulus terrestris* Leaves. Linn and standard for dissolving kidney stones- calcium oxalate by an in-vitro model. To check their potential to dissolve experimentally prepared kidney stones- calcium oxalate by an in-vitro model for *Tribulus terrestris* and cystone as a standard compound collected from market.

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INTRODUCTION:

Most people know that a major function of the kidneys is to remove waste products and excess fluid from the body. These waste products and excess fluid are removed through the urine. The production of urine involves highly complex steps of excretion and re-absorption. This process is necessary to maintain a stable balance of body chemicals.

The critical regulation of the body's salt, potassium and acid content is performed by the kidneys. The kidneys also produce hormones that affect the function of other organs. For example, a hormone produced by the kidneys stimulates red blood cell production. Other hormones produced by the kidneys help regulate blood pressure and control calcium metabolism.

The kidneys are powerful chemical factories that perform the following functions:

- remove waste products from the body
- remove drugs from the body
- balance the body's fluids
- release hormones that regulate blood pressure
- produce an active form of vitamin D that promotes strong, healthy bones
- control the production of red blood cells

There are two kidneys, each about the size of a fist, located on either side of the spine at the lowest level of the rib cage. Each kidney contains up to a million functioning units called nephrons. A nephron consists of a filtering unit of tiny blood vessels called a glomerulus attached to a tubule. When blood enters the glomerulus, it is filtered and the remaining fluid then

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passes along the tubule. In the tubule, chemicals and water are either added to or removed from this filtered fluid according to the body's needs, the final product being the urine we excrete.

The kidneys perform their life-sustaining job of filtering and returning to the bloodstream about 200 quarts of fluid every 24 hours. About two quarts are removed from the body in the form of urine, and about 198 quarts are recovered. The urine we excrete has been stored in the bladder for anywhere from 1 to 8 hours.

STRUCTURE OF KIDNEY

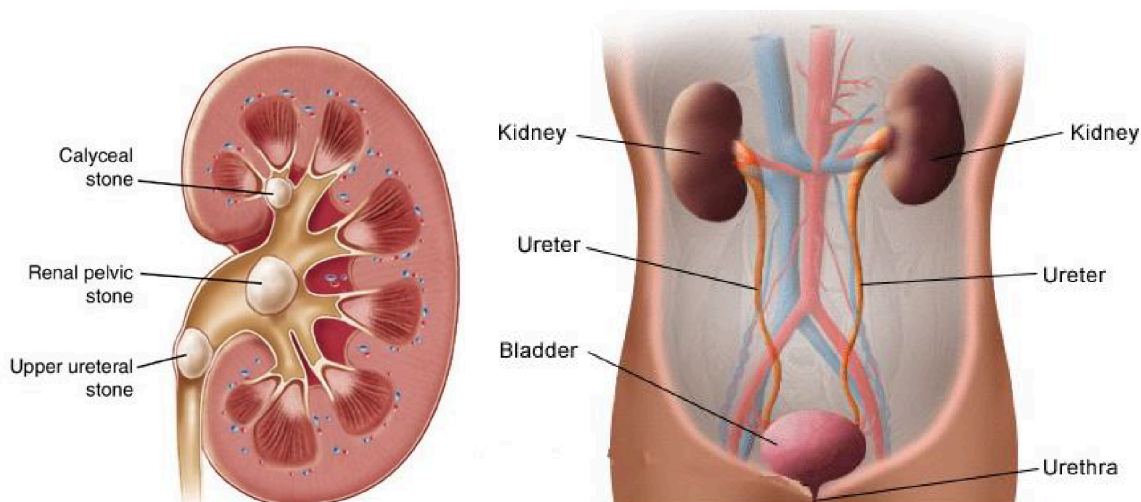
The kidneys are paired organs located in the posterior part of the abdomen on either side of the vertebral column. Underneath the capsule of fibrous tissue enclosing the kidney lies the cortex, which contains the glomeruli. The inner portion of the kidney, the medulla, contains the collecting ducts. The renal pelvis rapidly diminishes in caliber and merges into the ureter. Each ureter descends in the abdomen alongside the vertebral column to join the bladder. The bladder provides temporary storage for urine, which is eventually voided through the urethra to the exterior. (Kaplan and Pesce, 1996)

GROSS ANATOMY

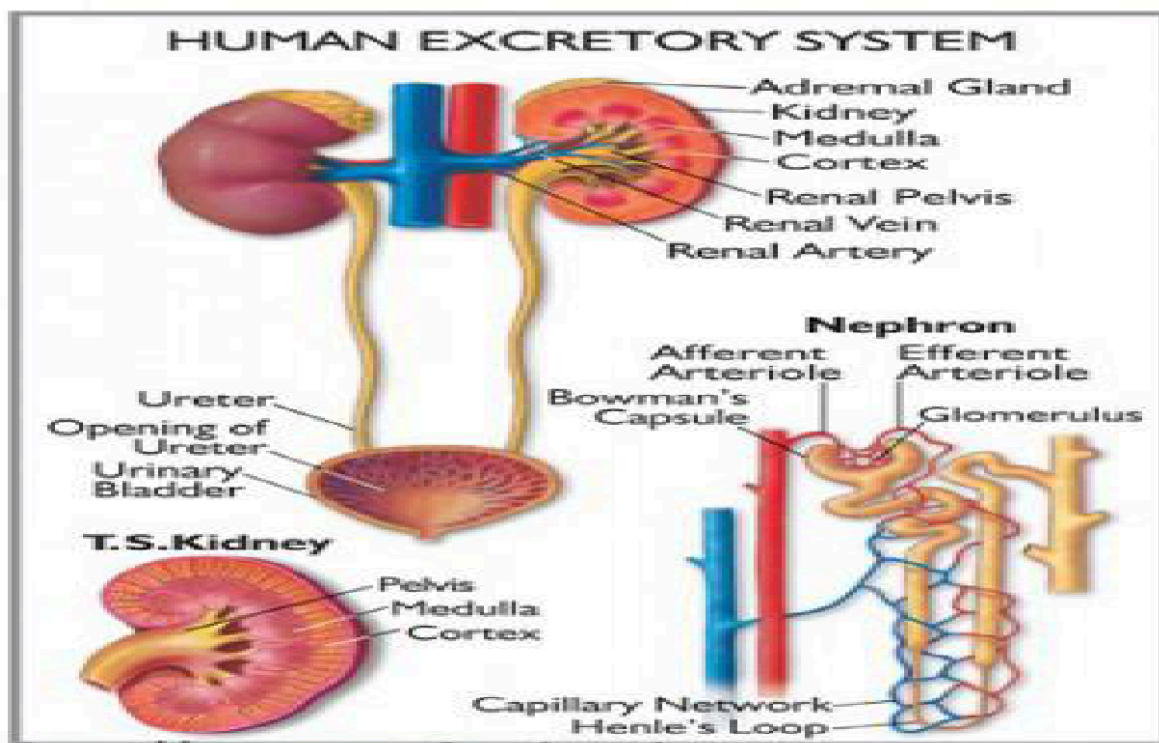
Kidneys are paired retroperitoneal organs situated in the posterior part of the abdomen on each side of the vertebral column. In the human, the upper pole of each kidney lies opposite the twelfth thoracic vertebra, and the lower pole lies opposite the third lumbar vertebra. The right kidney is usually slightly more caudal in position. The weight of each kidney ranges from 125 g to 170 g in the adult male and from 115 g to 155 g in the adult female. The human kidney is approximately 11 cm to 12 cm in length, 5.0 cm to 7.5 cm in width, and 2.5 cm to 3.0 cm in thickness

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Located on the medial or concave surface of each kidney is a slit, called the hilus, through which the renal pelvis, the renal artery and vein, the lymphatics, and a nerve plexus pass into the sinus of the kidney. The organ is surrounded by a tough fibrous capsule, which is smooth and easily removable under normal conditions. In the human, as in most mammals, each kidney is supplied normally by a single renal artery, although the presence of one or more accessory renal arteries is not uncommon. The renal artery enters the hilar region and usually divides to form an anterior and a posterior branch. Three segmental or lobar arteries arise from the anterior branch and supply the upper, middle, and lower thirds of the anterior Surface of the kidney. The posterior branch supplies more than half of the posterior surface and occasionally gives rise to a small apical segmental branch. However, the apical Segmental or lobar branch arises most commonly from the anterior division.



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Each kidney is made up of approximately 1 million nephrons. The nephron begins with the glomerulus, a unit of capillaries formed from the afferent (incoming) arteriole and drained by a smaller efferent (outgoing) arteriole. The glomerulus is surrounded by Bowman's capsule, formed by the blind dilated end of the renal tubule. The proximal convoluted tubule runs a tortuous course through the cortex, entering the medulla and forming first the descending end of the loop of henle and then ascending limb.

The ascending limb reenters the cortex, forming the distal convoluted tubule. The merging of distal tubules mark the beginning of a collecting duct. As the collecting duct descends through the cortex and medulla, it receives the effluent from a dozen or more distal tubules. The collecting ducts join and increase in size as they pass down the medulla. The ducts of each pyramid coalesce to form a central duct, which empties through the papilla into to a minor calyx, eventually draining into the renal pelvis (Kaplan and Pesce, 1996).

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RENAL PHYSIOLOGY:

The kidney is the chief regulator of all body fluids and is primarily responsible for maintaining homeostasis, or equilibrium of fluid and electrolytes in the body (Kaplan and Pesce, 1996).

The kidney has six main functions:

1. Urine formation
2. Regulation of fluid and electrolyte balance
3. Regulation of acid-base balance
4. Excretion of waste products and protein metabolism
5. Hormonal function
6. Protein conservation

URINE FORMATION:

The removal of potentially toxic waste products is a major function of the kidneys and is accomplished through the formation of urine. The basic processes involved in the urine formation are filtration, re-absorption and secretion. The kidneys filter large volumes of plasma, reabsorb most of what is filtered, and leave behind a concentrated solution of metabolic wastes called urine (Kaplan and Pesce, 1996).

GLOMERULAR FILTRATION:

The renal artery splits into numerous arterioles, each feeding a nephron. The arteriole splits into numerous capillaries, which form a knot called a glomerulus. The glomerulus is enclosed by the renal capsule (or Bowman's capsule)- the first part of the nephron. The arteriole leading into the glomerulus is wider than the one leading out, so there is high blood pressure in the capillaries of the glomerulus.

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This pressure forces plasma out of the blood by ultrafiltration. Both the capillary walls and the capsule walls are formed from a single layer of flattened cells with gaps between them, so that all molecules with a molecular mass of $< 68,000$ are squeezed out of the blood to form a filtrate in the renal capsule. Only blood cells and large proteins (e.g. antibodies and albumin) remain in the blood.

Each minute 1000 to 1500 ml of blood pass through the kidneys. The glomerulus has a semi-permeable basement membrane that allows free passage of water and electrolytes but is relatively impermeable to large molecules. In glomerular capillaries the hydrostatic pressure is approximately three times greater than in other capillaries; hence, substances are filtered through the membrane into Bowman's capsule at about 130 ml/min; this is the glomerular filtration rate (GFR). The glomerular filtrate is essentially plasma without the proteins. In an Average healthy person 187,000 ml of filtrate are formed per day. Normal urine output is around 1500 ml per day, which is only about 1% of the amount filtrate formed; hence the others 99% must be reabsorbed.

PROXIMAL TUBULE:

This structure is 15mm long and is composed of single layer of interdigitating epithelial cells united at their apices by tight junctions. About 80% of salt and water are reabsorbed from the glomerular filtrate in the proximal tubule. All the filtered glucose and most of the filtered amino acids are normally reabsorbed. Low-molecular-weight proteins, urea, uric acid, bicarbonate, phosphate, chloride, potassium, magnesium and calcium are reabsorbed to varying extents. Many organic acids and bases, as well as, hydrogen ion and ammonia, are secreted into the tubular fluid by tubular cells. Normally, no glucose is excreted in the urine; all that is filtered is reabsorbed. As the plasma concentration of glucose is increased above renal plasma threshold level, glucose appears in the urine. Tubular secretion, which transports substances into the tubular lumen, may also be an active or passive process.

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Substances that are transported from the blood to the tubules and excreted in the urine include potassium, hydrogen ions, ammonia, uric acid, and certain drugs, such as penicillin. The following table gives an idea of the magnitude and importance of these re-absorptive mechanisms. The luminal surfaces of these cells have microvillous brush borders which provides the large surfaces of these areas required for the absorptive function of the proximal tubule. The convoluted tubule drains into a short straight segment directed towards the outer medulla and continuous with the descending limb of the loop of Henle.

KIDNEY STONE:

Kidney stones are mainly lodged in the kidney(s). Mankind has been afflicted by urinary stones since centuries dating back to 4000 B.C., and it is the most common disease of the urinary tract. The prevention of renal stone recurrence remains to be a serious problem in human health. The prevention of stone recurrence requires better understanding of the mechanisms involved in stone formation. Kidney stones have been associated with an increased risk of chronic kidney diseases, end-stage renal failure, cardiovascular diseases, diabetes, and hypertension. It has been suggested that kidney stone may be a systemic disorder linked to the metabolic syndrome. Nephrolithiasis is responsible for 2 to 3% of end-stage renal cases if it is associated with nephrocalcinosis.

The symptoms of kidney stone are related to their location whether it is in the kidney, ureter, or urinary bladder. Initially, stone formation does not cause any symptom. Later, signs and symptoms of the stone disease consist of renal colic (intense cramping pain), flank pain (pain in the back side), hematuria (bloody urine), obstructive uropathy (urinary tract disease), urinary tract infections, blockage of urine flow, and hydronephrosis (dilation of the kidney). These conditions may result in nausea and vomiting with associated suffering from the stone event. Thus, the treatment and time lost from work involves substantial cost imposing an impact on the quality of life and nation's economy.

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Globally, kidney stone disease prevalence and recurrence rates are increasing , with limited options of effective drugs. Urolithiasis affects about 12% of the world population at some stage in their lifetime. It affects all ages, sexes, and races but occurs more frequently in men than in women within the age of 20–49 years. If patients do not apply metaphylaxis, the relapsing rate of secondary stone formations is estimated to be 10–23% per year, 50% in 5–10 years, and 75% in 20 years of the patient. However, lifetime recurrence rate is higher in males, although the incidence of nephrolithiasis is growing among females. Therefore, prophylactic management is of great importance to manage urolithiasis.

Recent studies have reported that the prevalence of urolithiasis has been increasing in the past decades in both developed and developing countries. This growing trend is believed to be associated with changes in lifestyle modifications such as lack of physical activity and dietary habits and global warming. In the United States, kidney stone affects 1 in 11 people, and it is estimated that 600,000 Americans suffer from urinary stones every year. In Indian population, about 12% of them are expected to have urinary stones and out of which 50% may end up with loss of kidney functions

CAUSES OF KIDNEY CHRONIC DISEASES

Chronic kidney disease is defined as having some type of kidney abnormality, or "marker", such as protein in the urine and having decreased kidney function for three months or longer. There are many causes of chronic kidney disease. The kidneys may be affected by diseases such as diabetes and high blood pressure. Some kidney conditions are inherited (run in families). Others are congenital; that is, individuals may be born with an abnormality that can affect their kidneys. The following are some of the most common types and causes of kidney damage.

Diabetes is a disease in which your body does not make enough insulin or cannot use normal amounts of insulin properly. This results in a high blood sugar level, which can cause problems in many parts of your body. Diabetes is the leading cause of kidney disease.

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High blood pressure (also known as hypertension) is another common cause of kidney disease and other complications such as heart attacks and strokes. High blood pressure occurs when the force of blood against your artery walls increases. When high blood pressure is controlled, the risk of complications such as chronic kidney disease is decreased.

Glomerulonephritis is a disease that causes inflammation of the kidney's tiny filtering units called the glomeruli. Glomerulonephritis may happen suddenly, for example, after a strep throat, and the individual may get well again. However, the disease may develop slowly over several years and it may cause progressive loss of kidney function.

Polycystic kidney disease is the most common inherited kidney disease. It is characterized by the formation of kidney cysts that enlarge over time and may cause serious kidney damage and even kidney failure. Other inherited diseases that affect the kidneys include Alport's Syndrome, primary hyperoxaluria and cystinuria.

Kidney stones are very common, and when they pass, they may cause severe pain in your back and side. There are many possible causes of kidney stones, including an inherited disorder that causes too much calcium to be absorbed from foods and urinary tract infections or obstructions. Sometimes, medications and diet can help to prevent recurrent stone formation. In cases where stones are too large to pass, treatments may be done to remove the stones or break them down into small pieces that can pass out of the body.

Urinary tract infections occur when germs enter the urinary tract and cause symptoms such as pain and/or burning during urination and more frequent need to urinate. These infections most often affect the bladder, but they sometimes spread to the kidneys, and they may cause fever and pain in your back.

Congenital diseases may also affect the kidneys. These usually involve some problem that occurs in the urinary tract when a baby is developing in its mother's womb. One of the most common occurs when a valve-like mechanism between the bladder and ureter (urine tube) fails

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to work properly and allows urine to back up (reflux) to the kidneys, causing infections and possible kidney damage.

Drugs and toxins can also cause kidney problems. Using large numbers of over-the-counter pain relievers for a long time may be harmful to the kidneys. Certain other medications, toxins, pesticides and "street" drugs such as heroin and crack can also cause kidney damage

TYPES OF KIDNEY STONES

The chemical composition of kidney stones depends on the abnormalities in urine composition of various chemicals. Stones differ in size, shape, and chemical compositions (mineralogy). Based on variations in mineral composition and pathogenesis, kidney stones are commonly classified into five types as follows

1. CALCIUM STONES: CALCIUM OXALATE AND CALCIUM PHOSPHATE

Calcium stones are predominant renal stones comprising about 80% of all urinary calculi. The proportion of calcium stones may account for pure calcium oxalate (CaOx) (50%), calcium phosphate (CaP, termed as apatite) (5%), and a mixture of both (45%) . The main constituent of calcium stones is brushite (calcium hydrogen phosphate) or hydroxyapatite. Calcium oxalate is found in the majority of kidney stones and exists in the form of CaOx monohydrate (COM, termed as mineral names: whewellite, $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$), and CaOx dihydrate (COD, weddellite, $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$), or as a combination of both which accounts for greater than 60%. COM is the most thermodynamically stable form of stone. COM is more frequently observed than COD in clinical stones.

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Many factors contribute to CaOx stone formation such as hypercalciuria (resorptive, renal leak, absorptive, and metabolic diseases), hyperuricosuria, hyperoxaluria, hypocitraturia, hypomagnesuria, and hypercystinuria. Mostly, urinary pH of 5.0 to 6.5 promotes CaOx stones, whereas calcium phosphate stones occur when pH is greater than 7.5. The recurrence of calcium stone is greater than other types of kidney stones.

2. STRUVITE OR MAGNESIUM AMMONIUM PHOSPHATE STONES

Struvite stones occur to the extent of 10–15% and have also been referred to as infection stones and triple phosphate stones. It occurs among patients with chronic urinary tract infections that produce urease, the most common being *Proteus mirabilis* and less common pathogens include *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Enterobacter*. Urease is necessary to split/cleave urea to ammonia and CO₂, making urine more alkaline which elevates pH (typically > 7). Phosphate is less soluble at alkaline versus acidic pH, so phosphate precipitates on to the insoluble ammonium products, yielding to a large staghorn stone formation. Women's are likely to develop this type of stone than the male. *Escherichia coli* is not capable of splitting urea and is not associated with struvite stones.

3. URIC ACID STONES OR URATE

This accounts approximately for 3–10% of all stone types. Diets high in purines especially those containing animal protein diet such as meat and fish, results in hyperuricosuria, low urine volume, and low urinary pH (pH < 5.05) exacerbates uric acid stone formation. Peoples with gouty arthritis may form stones in the kidney(s). The most prevalent cause of uric acid nephrolithiasis is idiopathic [38], and uric acid stones are more common in men than in women.

4. CYSTINE STONES

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These stones comprise less than 2% of all stone types. It is a genetic disorder of the transport of an amino acid and cystine. It results in an excess of cystinuria in urinary excretions, which is an autosomal recessive disorder caused by a defect in the rBAT gene on chromosome 2, resulting in impaired renal tubular absorption of cystine or leaking cystine into urine. It does not dissolve in urine and leads to cystine stone formation. People who are homozygous for cystinuria excrete more than 600 millimole insoluble cystine per day. The development of urinary cystine is the only clinical manifestation of this cystine stone disease.

5. DRUG-INDUCED STONES

This accounts for about 1% of all stone types. Drugs such as guaifenesin, triamterene, atazanavir, and sulfa drugs induce these stones. For instance, people who take the protease inhibitor indinavir sulphate, a drug used to treat HIV infection, are at risk of developing kidney stones. Such lithogenic drugs or its metabolites may deposit to form a nidus or on renal calculi already present. On the other hand, these drugs may induce the formation of calculi through its metabolic action by interfering with calcium oxalate or purine metabolisms.

KIDNEY STONE COMPOSITIONS

The chemical compositions of urinary stones include crystals and non-crystalline phases or the organic material (the matrix). The organic matrix of urinary stones consists of macromolecules such as glycosaminoglycans (GAG's), lipids, carbohydrates, and proteins. These molecules play a significant role by promoting or inhibiting the processes of kidney stone development (Table 1). The main components of the stone matrix are proteins (64%), nonamino sugars (9.6%), hexosamine as glucosamine (5%), water (10%), and inorganic ash (10.4%). The matrix acts as a template participating in the assembly of kidney stones. The matrix of all stones contains phospholipids (8.6%) of the total lipid, which in turn represents about 10.3% of stone matrix. Cell membrane phospholipids, as part of organic matrix, promote the formation of

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calcium oxalate and calcium phosphate stones. Albumin is the major component of the matrix of all stone types.

Serial Number	Name of protein	Role in crystallization			
		Nucleation	Growth	Aggregation	Cell adherence
1	Nephrocalcin (NC)	I	I	I	—
2	Tamm–Horsfall protein (THP)	P	—	I/P	—
3	Osteopontin/uropontin (OPN)	I	I	I	I/P
4	Albumin	P	—	I	—
5	Urinary prothrombin fragment-1 (UPTF1)	I	I	I	—
6	Alpha-1-microglobulin	—	—	I	—
7	S100A	—	I	I	—
8	Inter-alpha-inhibitor	I	I	I	I
9	Bikunin	I	I	I	I
10	Renal lithostathine	—	I	—	—

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11	Alpha defensin	—	P	P	—
12	Human phosphatocytidylyl transferase 1, choline, beta	—	I	—	—
13	Myeloperoxidase	—	P	P	—
14	Nucleolin	—	—	—	P
15	Histone-lysine N methyltransferase	—	I	I	—
16	Inward rectifier K channel	—	I	I	—
17	Protein Wnt-2	—	I	I	—
18	Alpha-2HS glycoprotein	P	I	—	—
19	Crystal adhesion inhibitor (CAI)	—	—	—	I
20	Hyaluronic acid (HA)	—	—	—	P
21	Chondroitin sulphate	—	I	I	—

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22	Heparin sulphate (HS)	—	I	—	—
23	Human urinary trefoil factor 1 (THF1)	—	I	—	—
24	Monocyte chemoattractant protein-1 (MCP 1)	—	—	—	P
25	Annexin II	—	—	—	P
26	CD44	—	—	—	P
27	Matrix Gla protein (MGP)	—	I	—	I
28	Histone H1B	—	P	—	—
29	Fibronectin	—	—	I	I
30	Collagen	P	—	—	—
31	Glycosaminoglycans	I	I	I	I
32	Citrate	—	I	—	—
33	Pyrophosphate	—	I	—	—
34	Magnesium	—	I	—	—

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Mechanisms of Renal Stone Formation

The pathogenesis of kidney stone or bio-mineralization is a complex biochemical process which remains incompletely understood. Renal stone formation is a biological process that involves physicochemical changes and super-saturation of urine. Supersaturated solution refers to a solution that contains more of dissolved material than could be dissolved by the solvent under normal circumstances. As a result of super-saturation, solutes precipitate in urine leads to nucleation and then crystal concretions are formed. That is, crystallization occurs when the concentration of two ions exceeds their saturation point in the solution. The transformation of a liquid to a solid phase is influenced by pH and specific concentrations of excess substances. The level of urinary saturation with respect to the stone-forming constituents like calcium, phosphorus, uric acid, oxalate, cystine, and low urine volume are risk factors for crystallization. Thus, crystallization process depends on the thermodynamics (that leads to nucleation) and kinetics (which comprises the rates of nucleation or crystal growth) of a supersaturated solution. Therefore, lithiasis can be prevented by avoiding super-saturation.

However, it should be noted that stone formation is usually dependent on the level of imbalance between urinary inhibitors and promoters of crystallization. All stones share similar events with respect to the mineral phase of stone formation. But, the sequence of events leading to stone formation differs depending on the type of stone and urine chemistry. For instance, crystallization of calcium-based stones (calcium oxalate or calcium phosphate) occurs in supersaturated urine if it is with low concentrations of inhibitors. Uric acid interferes the solubility of calcium oxalate and promotes CaOx stone formation. In healthy controls, crystallization process is opposed by inhibitory substances and gets safe. The sequence of events that trigger stone formation includes nucleation, growth, aggregation, and retention of crystals within the kidneys.

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Crystal Nucleation

The first step in the formation of kidney stone begins by the formation of nucleus (termed as nidus) from supersaturated urine retained inside the kidneys. In a super-saturated liquid, free atoms, ions, or molecules start forming microscopic clusters that precipitate when the bulk free energy of the cluster is less than that of the liquid. For example, charged soluble molecules such as calcium and oxalate combine to form calcium oxalate crystals and become insoluble. Nucleation may be formed in the kidney through free particle or fixed particle mechanism. In supersaturated solutions, if promoters exceed that of inhibitors, nucleation starts.

Once a nucleus is created (and/or if it is anchored), crystallization can occur at lower chemical pressure than required for the formation of the initial nucleus. Existing epithelial cells, urinary casts, RBCs, and other crystals in urine can act as nucleating centers in the process of nuclei formation termed as heterogeneous nucleation. The organic matrix, mucopolysaccharide acts as a binding agent by increasing heterogeneous nucleation and crystal aggregation. On the other hand, nanobacteria is claimed to form apatite structures serving as a crystallization center for stone formation. The whole process potentiates stone formation. The role of oxalate-degrading bacteria, such as *Oxalobacter formigenes*, in CaOx stone formation is a subject of current research. Thus, treatment which targets the process of nucleation intervention is one of the best approaches to control kidney stone.

Crystal Growth

Crystals in urine stick together to form a small hard mass of stone referred as crystal growth. Stone growth is accomplished through aggregation of preformed crystals or secondary nucleation of crystal on the matrix-coated surface. Once a nidus has achieved, the overall free

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energy is decreased by adding new crystal components to its surface. The total free energy of the cluster is increased by the surface energy. The process of stone growth is slow and requires longer time to obstruct the renal tubules. From organic matrix, mainly Tamm–Horsfall protein and osteopontin are promoters of CaOx stone formation. Under in vitro study, crystals induced in human urine demonstrated an intimate association between calcium-containing crystals and organic matrix (lipids and proteins). Lipids of cellular membranes are basically believed to involve in nucleation of crystals.

Crystal Aggregation

The process whereby a small hard mass of a crystal in solution sticks together to form a larger stone is called aggregation. All models of CaOx urolithiasis concede that crystal aggregation is probably involved in crystal retention within the kidneys. Crystal aggregation is considered to be the most critical step in stone formation.

Crystal-Cell Interaction

The attachment of grown crystals with the renal tubule lining of epithelial cells is termed as crystal retention or crystal-cell interaction. In individuals with hyperoxaluria, renal tubular epithelial cells were injured due to exposure to high oxalate concentrations or sharp calcium oxalate monohydrate (COM) crystals. Crystal-cell interaction results in the movement of crystals from basolateral side of cells to the basement membrane. Then, crystals could be taken into cells and anchored to the basement membrane of the kidneys. The interaction of COM crystals with the surface of renal epithelial cells could be a critical initiating event in nephrolithiasis. An increased retention force between the crystal and injured renal tubule epithelium cells promotes CaOx crystallization. Most of the crystals attached to epithelial cells are thought to be digested by macrophages and/or lysosomes inside cells and then discharged with urine.

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Following renal tubular cell injury, cellular degradation produces numerous membrane vesicles which are nucleators of calcium crystals as supported by in vitro and in vivo studies. Injured cells release substances like renal prothrombin fragment-1 or other anionic proteins which induce COM crystal agglomeration. Reactive oxygen species is thought to be one of the factors involved in renal cell injury. Thus, reduction of renal oxidative stress could be an effective treatment option.

Injured cells potentiate to invert its cell membrane which is anionic to the urinary environment and acts as site of crystal adherence. COM crystals have stronger affinity of attachment towards the inverted anionic membrane, than calcium oxalate dihydrate (COD) crystals. On the other hand, deposition of COM crystal was observed in Madin–Darby canine kidney epithelial cells (MDCK cells), than at proximal tubular epithelial cells derived from pig kidney (LLC-PK1 cells) study models. This preferential difference may be due to the presence of a binding molecule such as hyaluronan on Madin–Darby canine kidney epithelial cells for COM crystal attachment . Although the detailed mechanisms of crystal-cell interaction remain unexplored, one of the best ways to treat urolithiasis is to control crystal-cell retentions.

Endocytosis of CaOx Crystals

Endocytosis or engulfment of crystals by renal tubular cells is the earliest process in the formation of kidney stones. Studies on tissue culture crystal-cell interactions indicated that COM crystals rapidly adhere to microvilli on the cell surface and subsequently internalized. Polyanion molecules present in tubular fluid/urine such as glycosaminoglycans, glycoproteins, and citrate may coat crystals and inhibit the binding of COM crystals to cell membrane [41]. For example, Tamm–Horsfall glycoproteins (THP) have a dual biological role in stone formation. Lieske et al. reported that THP may promote renal stone formation by initiating the interaction of COM crystals with distal tubular cells of the nephron. Another study revealed that, upon lowering pH and raising ionic strength, THP's viscosity increases which exhibits high tendency of polymerization and fails to inhibit crystallization. Moreover, THP becomes a strong promoter of

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crystallization in the presence of additional calcium ions. In contrast, THP is thought to protect against COM stone formation by inhibiting COM aggregation when it is at high pH and low ionic strength as reported by Hess. COM aggregation assays revealed that desialylated THP promoted COM aggregation, while normal THP inhibited aggregation. Similar reports revealed that THP may inhibit calcium oxalate crystal aggregation, whereas uromodulin may promote aggregation. Inactivating the THP gene in mouse embryonic stem cells results in spontaneous formation of calcium crystals in adult kidneys. This is a convincing evidence that THP is a critical urinary inhibitor of human nephrolithiasis.

Various cellular and extracellular events are involved during stone formation. Modulators targeting the steps from supersaturation to crystal retention may be a potential means to block stone formation. Similarly, the blockage of crystal binding molecules (such as osteopontin, hyaluronic acid, sialic acid, and monocyte chemoattractant protein-1) expressed on epithelial cell membranes may be an alternative approach to prevent stone formation. Experimental findings demonstrated that stone calcification is triggered by reactive oxygen species (ROS) and the development of oxidative stress. In vitro and in vivo studies have demonstrated that CaOx crystals are toxic for renal epithelial cells that produce injury and renal cell death. Similarly, an exposure to hypercalciuria produces cellular injury and ROS-induced lipid peroxidation which stimulates calcium oxalate deposition. The pathophysiology of urinary stone formation is incompletely understood. A summary of the various steps involved in stone formation.

Cell Injury and Apoptosis

Exposure to high levels of oxalate or CaOx crystals induces epithelial cellular injury, which is a predisposing factor to subsequent stone formation. CaOx crystal depositions in the kidneys up regulate the expression and synthesis of macromolecules that can promote inflammation. Crystals may be endocytosed by cells or transported to the interstitium. It has been suggested that injured cells develop a nidus which promotes the retention of particles on the renal papillary surface. In individuals with severe primary hyperoxaluria, renal tubular cells are

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injured and crystals become attached to them . The addition of CaOx crystals onto Madin–Darby canine kidney (MDCK) cell lines showed an increase in the release of lysosomal enzymes, prostaglandin E₂, and cytosolic enzymes. A study on animal models also revealed that the administration of high concentrations of CaOx crystals or oxalate ions appears to be toxic causing renal tubular cell damage. It has been suggested that oxalate increases the availability of free radicals by inhibiting enzymes responsible for their degradation. For instance, reactive oxygen species can damage the mitochondrial membrane and reduce its transmembrane potential. These events are known features of early process in apoptotic pathways.

The activation of p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway regulates the expression of cellular proteins. The various extracellular stimuli or stresses like ultraviolet radiation and proinflammatory cytokines may activate p38 MAPK which results in phosphorylation and activation of transcription factors . The exposure of renal cells to oxalate increases an altered gene expression that induces apoptosis signaling cascades. A study revealed that the exposure of HK-2 cells to increased oxalate levels results in an increased transcriptional activation of IL-2R beta mRNA and consequently increases IL-2R beta protein levels which drive cellular changes like induction of inflammation. Oxalate-induced activation may trigger p38 MAPK signaling by acting on cell membranes, although the exact mechanisms have not been established.

Apoptosis at the level of renal tubular cells may lead to stone formation through cellular demise and postapoptotic necrosis which could promote calcium crystal aggregation and growth. This fact has been supported by in vitro study on MDCK cells being exposed to oxalate ions. However, it has to be noted that some cells did not respond to oxalate injury. This may be due to the fact that changes in gene expression could protect from apoptosis and then inhibit from lithiasis. These findings highlight the need for future studies clarifying novel biochemical targets of kidney stone formation and the utility of p38 MAPK inhibitors in preventing stone formation.

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Genetic Basis of Kidney Stone Formation

Environmental factors interacting with underlying genetic factors cause rare stone disease. The production of promoters and inhibitors of crystallization depends on proper functioning of the renal epithelial cells. Cellular dysfunction affects the super-saturation of urinary excretion by influencing ions such as calcium, oxalate, and citrate. Some genetic defects which lead to stone formation.

Randall's Plaques

Randall's plaques appear to be the precursor's origin of urinary stone development although it is unclear whether it involves in all stone types or not. Moreover, the pathogenesis of Randall's plaque itself is not clearly known. The majority of CaOx stones are found to be attached with renal papillae at the sites of Randall's plaque. It is located at the interstitial basement membrane in loop of Henle. Calcium phosphate, and purine crystal compositions were identified in plaques, whereas apatite is dominant. Initially, calcium phosphate crystals and organic matrix are deposited along the basement membranes of the thin loops of Henle and extend further into the interstitial space to the urothelium, constituting the so-called Randall plaques. Evidence suggests that a primary interstitial apatite crystal formation secondarily leads to CaOx stone formation. In supersaturated urine, crystals adhere to the urothelium which may enhance subsequent stone growth.

Due to renal cell injury, plaque is exposed to supersaturated urine. Renal epithelial cell damage (degradation) products promote heterogeneous nucleation and promotes crystal adherence in renal cells. Randall plaque calcification is triggered by oxidative stress. Cells may express molecules at distal and collecting tubules which act as crystal binding sites such as phosphatidylserine, CD44, osteopontin, and hyaluronan. Renal epithelial cells of the loop of

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Henle or collecting ducts produce membrane vesicles at the basal side which leads to plaque formation. Thus, apatite crystal deposits have been proposed to act as nidus for CaOx stone formation by attachment on further matrix molecules. However, the driving forces in plaque formation and the involved matrix molecules remain elusive.

Kidney stones are either attached to the renal papillae or found freely. According to the fixed particle pathway, the beginning of calcium phosphate (CaP) deposition in the interstitium establishes a nucleus for CaOx formation. CaP formed in the basement membrane of the loops of Henle, the inner medullary collecting ducts, and ducts of Bellini serves as an attachment site for stone development. Idiopathic stone formers develop CaOx attached to fixed sites of interstitial plaque. Stones of the distal tubular acidosis attach to plugs protruding from dilated ducts of Bellini, whereas cystinuria stones do not attach to the renal plaques (found freely). CaP, uric acid, or cystine crystals formed in the renal tubules plug at the terminal collecting ducts. When mineralization reaches the renal papillary surface, plaques rupture exposing CaP crystals to the pelvic urine. Then, urinary macromolecules deposit over the exposed CaP crystals and promote CaOx deposition on CaP.

Kidney Stone Inhibitors and Promoters

Inhibitors are substances which decrease the initiation of supersaturation, nucleation, crystal growth, rate of aggregation, or any other processes required to stone formation. Normally, urine contains chemicals that prevent crystal formation. Inhibitors in urine includes small organic anions such as citrate, small inorganic anions such as pyrophosphates, multivalent metallic cations such as magnesium, or macromolecules such as osteopontin, glycosaminoglycans, glycoproteins, urinary prothrombin fragment-1, and Tamm–Horsfall proteins. These inhibitors do not seem to work equally for everyone; therefore, some people form stones. But, if crystals formed remain tiny, usually it travels through the urinary tract and passes out from the body with

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urine splash without being noticed. Inhibitors may act either directly by interacting with crystal or indirectly by influencing the urinary environment. When inhibitory compounds adsorb onto the surface of the crystal, it inhibits nucleation, crystal growth, aggregation, or crystal-cell adherence

In contrast, promoters are substances which facilitate stone formation by various mechanisms. Some of the promoters include cell membrane lipids (phospholipids, cholesterol, and glycolipids), calcitriol hormone enhancement via parathyroid hormone stimulation, oxalate, calcium, sodium, cystine, and low urine volume. Among recurrent stone formers, urinary oxalate excretion was found to be higher, whereas citrate excretion was lower. Studies indicated that oxalate can increase chloride, sodium, and water reabsorption in the proximal tubule and activate multiple signaling pathways in renal epithelial cells. In general, an imbalance between urinary stone inhibitors and promoters has been suggested to be the cause for stone formation.

Preventive Options for Urolithiasis

Effective kidney stone prevention depends upon addressing the cause of stone formation. Generally, to prevent the first episodes of kidney stone formation or its secondary episodes, proper management of diet and the use of medications is required. Primary prevention of kidney stone disease via dietary intervention is low-cost public health initiative with massive societal implications. Thus, nutritional management is the best preventive strategy against urolithiasis.

Regardless of the underlying etiology and drug treatment of the stone disease, patients should be instructed to increase their water intake in order to maintain a urine output of at least 2 liter per day. A simple and most important lifestyle change to prevent stone disease is to drink more water/liquids. Enough fluid intake reduces urinary saturation and dilutes promoters of CaOx crystallization. Dietary recommendations should be adjusted based on individual metabolic abnormalities. For absorptive hyperoxaluria, low oxalate diet and increased dietary calcium intake are recommended.

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A high sodium intake boosts stone risk by reducing renal tubular calcium reabsorption and increasing urinary calcium. Restriction of animal proteins is also encouraged since animal proteins provide an increased acid load because of its high content of sulfur-containing amino acids. Thus, high protein intake reduces urine pH and the level of citrate and enhances urinary calcium excretion via bone reabsorption. Therefore, if you have very acidic urine, you may need to eat less meat, fish, and poultry and avoid food with vitamin D. Instead, an increase intake of fruits and vegetables rich in potassium is recommended.

People who form calcium stones used to be told to avoid dairy products and other foods with high calcium content. However, persons with a tendency of kidney stone formation should not be advised to restrict calcium intake unless it has been known that he/she has an excessive use of calcium. A reduced intake of calcium leads to an increased intestinal absorption of oxalate, which itself may account for an increased risk of stone formation. Calcium supplements may reduce oxalate absorption because the calcium binds dietary oxalate in the gut lumen. However, the benefit of taking calcium pills is controversial. Vitamin C has been implicated in stone formation because of in vivo conversion of ascorbic acid to oxalate. Therefore, a limitation of vitamin C supplementation is recommended.

For prevention of calcium oxalate, cystine, and uric acid stones, urine should be alkalized by eating a diet high in fruits and vegetables, taking supplemental or prescription citrate, or drinking alkaline mineral waters. For uric acid stone formers, gout needs to be controlled, and for cystine stone formers, sodium and protein intakes need to be restricted. For prevention of calcium phosphate and struvite stones, urine should be acidified. For struvite stones, acidifying the urine is the single most important step. Patients must receive careful follow-up to be sure that the infection has cleared. However, the current treatment modalities are not efficient to prevent urolithiasis, and further research is required.

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INTRODUCTION OF *Tribulus terrestris*:



Domain: *Eukaryota*

Kingdom: *Plantae*

Phylum: *Spermatophyta*

Sub-phylum: *Angiospermae*

Class: *Dicotyledonae*

Order: *Geraniales*

Family: *Zygophyllaceae*

Genus: *Tribulus*

Species: *Tribulus terrestris*

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T. terrestris is an annual (sometimes perennial in warm climates) herb with a long, slender, branched tap-root. The greenish-red stems are up to 2 m long, branched, radiating from a central axis and covered with fine hairs. Though usually prostrate, the stems become more erect in shade or when competing with other plants. Leaves, 3-7 cm long, are in opposite pairs with one of the pair slightly smaller than the other. Each leaf consists of three to eight pairs of opposite, oblong-lanceolate leaflets, each leaflet being 5 to 15 mm long and 3 to 5 mm wide. The upper surface of the leaflets is darker than the underside. Kranz anatomy is evident in cross sections of the leaves, indicating C4 metabolism. The joints as well as the axes of compound leaves can move in phototropic responses which, together with the C4 photosynthetic pathway, increase the efficiency of photosynthesis ([Yang and Yu, 1981](#)).

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The flowers are yellow, 5-petalled, 7 to 15 mm in diameter, solitary and borne on short stalks in the axils of the smaller of each pair of leaves; they open in the morning and close or shed their petals in the afternoon.

The fruit is a woody burr, approximately 1 cm in diameter, which splits into 4 or 5 wedge-shaped segments (carpels), each with 2 unequal pairs of spines and containing 1-4 seeds. Seeds are yellow, variable in shape but more or less ovoid and 2-5 mm long.

It is used as a folk medicine such as tonic, aphrodisiac, painkiller, astringent, to kill parasitic worms from gastro, antihypertensive, diuretic lithon-triptic and urinary anti-invectives

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(Abirami and Rajanderan 2011). In southern Europe, the plant parts of *T. terrestris* such as roots, stems and the leaves are used for the formulation of tonic. Besides its many advantages in treatment of dysfunction, the fruits are also used in cooling and diuretic purposes, which help in removing

harmful toxins and metabolic waste by increasing urination. Besides this, it is also used as a tonic in calculus affection and urinary disorders.

In China *T. terrestris* has been used for treatment of cutaneous pruritus, edema and inflammation (Abirami and Rajanderan 2011). The extract of *T. terrestris* is used for formulation of cream with antibacterial, anti-inflammatory, antiviral activities (Alexis 2001, Alexis 2005). The combinations of *T. terrestris* extracts with metals are found in several Asian patented literatures. The plant extracts with a combination of metals for preparation of antiviral pharmaceutical compositions have been already worked out (Alexiev 2003a, 2003b). Other patents describe the uses of the extract for skin disorders (Li 2010) and successfully help in dilating the skin permeability and stimulating the generation of melanophore (Zhang 2011) and numerous cosmetic applications (Jing 2009, Ke 2010).

OTHER ACTIVITIES OF THE *Tribulus terrestris*

Initial literature search retrieved numerous articles stating *Tribulus terrestris* is an important curable plant famous for its use in Indian and Chinese systems of medicine for several diseases. In India, these are classified under mishrak varga as ‘Dashmoola’ in Ayurveda and in chemotaxonomy as Saponin glycoside (Vyawahare et al. 2014). It contains various phytochemical such as alkaloids, resins, tannins, sugars, terols, flavonoids and saponins that are potential candidates for pharmaceutical industries. This plant has been used in dietary supplements, dermatological, cosmetic, hygiene, cough, kidney failure and even as an anticancer agent.

The extracts of *T. terrestris* have characteristic diuretic, astringent, analgesic, aphrodisiac, antiurolithic, immunomodulatory, antidiabetic, cardiotonic, central nervous system,

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anti-inflammatory, analgesic, antitumour, antibacterial, anthelmintic, larvicidal and anticariogenic activities. A preliminary analytical technique such as TLC is discussed for identification and separation of drug valued compounds.

In this discusses the pharmacologic aspects of *Tribulus terrestris* and describes its habitat, botany, minor and major bioactive constituents with their pharmacologically important products. All this valuable information will assist scientists, lecturers, pharmacologists, research students of under-graduates and post-graduates of botany and pharmacy for drafting diverse research work.

Biological activities of extracts and saponins of *T. terrestris*:

T. terrestris has been in use for medicinal purposes since time immemorial. In India and China, the medicinal use of this herb is traced 5,000 year back (Balch, 1990; Bartram, 1995). In Ayurveda this herb is known for its diuretic, aphrodisiac and anti-urolithiatic properties (Deepak et al., 2002). In the traditional Chinese medicine, its fruits have been used for treatment of eye trouble, abdominal distention, emission, edema, morbid leucorrhea, sexual dysfunction and veiling (Xu et al., 2000; Cai et al., 2001). In South Africa it is recommended in the form of a herbal tonic for diarrhea and diseases of the throat and the eyes (Drewes et al., 2003). In the Bulgarian folk medicine, *T. terrestris* is used for blood purification and in haemorrhoids (Stoyanov, 1973).

Sexual disorders:

Since long *T. terrestris* is known for its aphrodisiac properties and as a traditional medicine for treating male infertility (Gauthaman et al., 2002). A formulation of the saponins from *T. terrestris* was developed for veterinary application and production in Bulgaria (Tomova et al., 1966; Tomova & Gjulemetova, 1978b). This formulation was effective in stimulating the sexual system (spermatogenesis, libido sexualis). Using *T. terrestris* extracts, an increase in sexual function in rats was demonstrated and attributed to an increase in testosterone, dihydrotestosterone, and dehydroepiandrosterone (Gauthaman et al., 2002; Gauthaman & Ganesan. 2008). A Bulgarian formulation with the name of Tribestan was optimized and widely

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used for treatment of infertility and libido disorders in men and women (Tomova et al., 1978a; Protich et al., 1981; Viktorov et al., 1982). The furastanol saponins (protodioscin, protogracilin) from *T. terrestris* were reported to have stimulating effect on spermatogenesis via Luteinizing Hormone (LH) stimulating the secretion of male hormone Testosterone. Testosterone in turn significantly improved the quality and quantity of sperms (Tomova et al., 1981; Brown et al., 2002). Libilov is another formulation of the saponin fraction of *T. terrestris* and is reported for similar activities. Protodioscin, main component of Libilov, proved to be effective in treatment of male infertility and it increased the level of dehydroepiandrosterone (DHEA) in infertile men. Protodioscin in *T. terrestris* was suggested to be DHEA precursor in patients with low serum level of this hormone (Adimoelja & Adaikan, 1997; Adimoelja, 2000). Different formulations containing *T. terrestris* extracts are marketed in USA and Europe as food supplements for stimulation, vigor (De Combarieu et al., 2003; Mulinacci et al., 2003) and for other multiple ailments.

Cardiac diseases:

Research studies revealed that saponins of *T. terrestris* can play a role in dilating coronary artery and improving coronary circulation. The results of a clinical trial on 406 patients, when treated with saponin of *T. terrestris* showed that the total efficacious rate of remission angina pectoris was 82.3% and the total effective rate of ECG improvement was 52.7% higher (Wang et al., 1990). Therefore, *T. terrestris* was recommended for treatment of angina pectoris (Wang et al., 1990). Many research studies revealed that aqueous extract of *T. terrestris* fruits have some antihypertensive effect (Yang et al., 1991, Chui et al., 1992 and Lu et al., 1994). These beneficial effects have partly been attributed to its ability to increase nitric oxide (NO) release from the endothelium and nitrergic nerve endings (Adaikan et al., 2000). Antihypertensive mechanism of *T. terrestris* was studied in 2K1C hypertensive rats by measurement of circulatory and local ACE activity in aorta, heart, kidney and lung. The systolic blood pressure and ACE of *T. terrestris* fed hypertensive rats was significantly decreased compared to hypertensive rats indicating a negative correlation between consumption of *T. terrestris* and ACE activity in serum and different tissues

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in 2K1C rats (Sharifi et al., 2003). An another research study indicated that dietary intake of *T. terrestris* can significantly lower serum lipid profiles, decrease endothelial cellular surface damage and rupture and may partially repair the endothelial dysfunction resulting from hyperlipidemia in New Zealand rabbits fed a cholesterol-rich diet (Altug Tuncer et al., 2009).

Antimicrobial activity:

Antimicrobial activities of *T. terrestris* are reported to vary with the origin of the plant and the part of the plant used. The ethanolic extracts of Yemeni *T. terrestris* did not show antibacterial activity against bacteria tested whereas the methanolic/ethananolic extracts of different parts (fruits, roots and stems with leaves) of Iranian, Indian or Turkish *T. terrestris* inhibited the growth of different microorganisms tested (Bedir & Khan, 2000; Ali et al., 2001; Kianbakht & Jahaniani, 2003). Among the seven different saponins tested from *T. terrestris*, only the spirostanol saponins showed antifungal activity against *C. albicans* and *Cryptococcus neoformans*, while none of the furostanol derivatives exhibited inhibitory activity. Further, these compounds were not effective against *S. aureus*, *Aspergillus fumigatus*, *P. aeruginosa* and *Mycobacterium intracellulare* (Bedir et al., 2002). Antimicrobial activity of organic and aqueous extracts from fruits, leaves and roots of *T. terrestris* from Iraq was examined against 11 species of pathogenic and non-pathogenic microorganisms. All the extracts from the different parts of the plant showed antimicrobial activity against most tested microorganisms. The most active extract was ethanol extract from the fruits with a minimal inhibitory concentration of 0.15 mg/ml against different bacteria and fungi (Al-Bayati & Al-Mola, 2008).

Anti-cancerous activity:

Among the different saponins analyzed from different parts (stem & fruit) of *T. terrestris* collected from different regions (Bulgaria, China & India), only spiro compounds exhibited remarkable activity (Kostova & Dinchev, 2005). The most active spirostanol glycoside (Hecogenin) exhibited a broad range of anticancer activity against cell lines SKMEL, KB, BT-549 and SKOV- 3 (Bedir & Khan, 2000; Bedir et al., 2002). Dioscin & also prosapogenin A

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of dioscin showed anti-cancerous activity against the cancer cell line K562 in vitro (Hu et al., 1996). Protodioscin proved to be cytotoxic against cell lines from leukemia and solid tumors and particularly against one leukemia line (MOLT-4), one NSCLC line (A549/ATCC), two colon cancer lines (HCT-116 and SW-620), one CNS cancer line (SNB-75), one melanoma line (LOX IMVI), and one renal cancer line (Hu & Yao, 2002). Based on computer analysis of methylprotodioscin as a seed compound, a potential novel mechanism was suggested for its anti-cancer action (Hu & Yao, 2003). The saponin mixture from Chinese origin on Bcap37 breast cancer cells had potent inhibitory effects in a concentration dependent manner (Sun et al., 2003). In addition, it has been reported that the extract of *T. macropterus*, which is in the same family as *T. terrestris*, has cytotoxic activity against a human liver cancer cell line. Since long, *T. terrestris* extracts have been used as anticancer therapy in oriental medicine, however the mechanisms of these effects have not been well elucidated.

Anthelmintic activity:

Anthelmintic activity was observed only in the 50% methanol extracts of Indian *T. terrestris* (whole plant) using the nematode *Caenorhabditis elegans*. Further investigation revealed tribulosin and sitosterol glucoside as active components responsible for Anthelmintic activity (Deepak et al., 2002). Miscellaneous uses: Lin et al., (1999) investigated the use of *T. terrestris* fruits for treatment of vitiligo with positive results. The lyophilized saponin mixture of the plant caused a significant decrease on peristaltic movements of isolated sheep ureter and rabbit jejunum preparations and it was proposed that the saponin mixture may be useful for some smooth muscle spasms or colic pains (Arcasoy et al., 1998). The preventive and therapeutic effects of saponins from *T. terrestris* on diet induced hyperlipidemia in mice have been studied. It was showed that the saponins could lower the levels of serum TC, LDLc and liver TC, TG and increase the activities of superoxide-dismutase in liver (Chu et al., 2003). Hypoglycemic effect of saponins from *T. terrestris* was investigated and the saponins were found to reduce the level of serum glucose significantly (Li et al., 2002).

Miscellaneous uses:

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Lin et al., (1999) investigated the use of *T. terrestris* fruits for treatment of vitiligo with positive results. The lyophilized saponin mixture of the plant caused a significant decrease on peristaltic movements of isolated sheep ureter and rabbit jejunum preparations and it was proposed that the saponin mixture may be useful for some smooth muscle spasms or colic pains (Arcasoy et al., 1998). The preventive and therapeutic effects of saponins from *T. terrestris* on diet induced hyperlipidemia in mice have been studied. It was showed that the saponins could lower the levels of serum TC, LDLc and liver TC, TG and increase the activities of superoxide-dismutase in liver (Chu et al., 2003). Hypoglycemic effect of saponins from *T. terrestris* was investigated and the saponins were found to reduce the level of serum glucose significantly (Li et al., 2002).

MATERIAL & METHOD:

Collection of Plant Material

The plant materials are collected from the surrounding local market area as Powdery form.

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Extraction of plant material

Collected leafs were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried then made into fine powder, this powdered samples (100g/100ml) in ethenol, and distilled water for overnight at room temperature., soxhlet apparatus are used for this extraction. The extract from these solvents are soaked and evaporated under pressure. The leaf extracts were concentrated at 50°C and the residue obtained was stored at 4°C.

Extraction and Isolation

The leaves of about 60 gms of powder was extracted with ethanol and aqueous in soxhlet. All extracts were concentrated on a water bath and residue was dried in a desiccator All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phyto constituents

PHYTOCHEMICAL ANALYSIS OF THE EXTRACT

Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods. In brief, the phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids, and phenols / polyphenols were qualitatively determined as following:

Test for alkaloids (mayer's test)

2.0ml of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids

Test for cardiac glycosides (keller-killani test)

5ml of plant extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. A brown ring of the interface indicates a deoxysugar characteristic of

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cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer which shows the presence of Cardiac glycosides.

Test for tannins

The substance (extracts) mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

Test for saponins

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H₂SO₄. Formation of yellow color observed in each extract indicated the presence of flavonoids.

Test for Steroids

One gram of the test substance (plant extracts) was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of Steroids.

Test for terpenoids (salkowski test)

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5ml of each plant part extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for presence of terpenoids.

Test for Reducing Sugars

One gram of the aqueous extract was weighed and placed into a test tube. This was diluted using 10 ml of deionised distilled water. This was followed by the addition of Fehling's solution. The mixture warmed to 40°C in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of a reducing sugar. Same procedure was repeated using dimethyl sulphoxide (DMSO) as the diluent for the ethanolic extract.

Test for Resins

Two grams of the ethanolic extract was dissolved in 10ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple colour, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material.

Evaluation for Anti-urolithiatic Activity

Step-1: Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous precipitation:

Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H₂SO₄ were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of (2N H₂SO₄), was allowed to react in sufficient quantity of distilled water in a beaker.

The resulting precipitate was calcium phosphate. Both precipitates freed from traces of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60°C for 4 hours.

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Step -2: Preparation of semi-permeable membrane from farm eggs:

The semi-permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely

from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Step-3: Estimation of Calcium oxalate

Weighed exactly 1mg of the calcium oxalate and 10mg of the extract/compound/standard and packed it together in semi evaluation Permeable membrane as mentioned below table. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate).Placed the conical flask of all groups in an incubator, preheated to 37 0C for 2 hours, for about 7-8 hours. Removed the contents of semi-permeable membrane from each group into a test tube. Added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N KMnO₄ till a light pink color end point obtained.1ml of 0.9494 N KMnO₄ equivalents to 0.1898mg of 4 Calcium. The amount of un-dissolved calcium.

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RESULT

Qualitative chemical tests indicated the presence of phenolic compounds, flavnoids, steroids and Saponin in extracts of *Tribulus terrestris* Linn. On basis of this fraction we performed in vitro Anti-Urolithiatic Activity by comparing different extracts of *Tribulus terrestris* with standard. % Dissolution of Calcium oxalate table is given below

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TABLE 2: PHYTOCHEMICAL SCREENING

Phytochemicals	Ethanol extract	Aqueous extract
Alkaloids	+	+
Cardiac Glycosides	+	+
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Steroids	+	+
Terpenoids	-	-

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Reducing Sugars	-	
Resins	-	

These graphical represent shows % Dissolution of calcium oxalate by in vitro Anti-Urolithiatic Activity of extracted fraction of *Tribulus terrestris*. L drug. An aqueous extract at 10mg concentration produced higher dissolution of calcium oxalate as compare to Ethanol fraction. Standard shows higher dissolution as compare to others.

DISCUSSION

This study evaluate that antiurolithiatic activity of different extracts of *Tribulus terrestris* Linn leaves and isolated phenolic compound, steroidal compound. The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. From the study results it is observed that aqueous fraction show highest dissolution of calcium oxalate in comparison to Ethanol. This study has given primary evidence for *Tribulus terrestris* L. as plant which possess anti-litholitic property. This in vitro study has given lead data, and shown that phenolics and steroids form aqueous fraction is quite promising for further work in this regard.

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Summary:

In this busy world no one has enough time to take proper food, even really they does not have time to take the normal amount of water, they are prefer to take the junk foods, cakes, soft drinks, beverages, (Ethylene glycol) and the continuous exposures to a variety of xenobiotics, therapeutic agents and environmental pollution leads to various disorders of organs, especially on kidney to take lake of water in the blood, hence it creates metabolic acidosis due to this the removable calcium on the bone surface nearly 0.5% is eroted which leads to formation of calcium oxalate kidney stone. The number of chemical present in the fast food may the most serious medical consequence of acute renal stone which is common in summer 62.19 percentage peoples per year all over the world facing major problem.

Renal calculi are the most common disorder of the urinary tract. Kidney stone disorder assessed to occur nearly 12% of the population. The stone comprises of 80% of calcium oxalate.

Conclusion:

In vitro urolithiasis has been performed on the selected plant *Tribulus terrestris* by using the standard drug, cystone. The work was performed by using in vitro antiurolithiatic model for calculating percentage dissolution of kidney stone. This study has given primary evidence for *Tribulus terrestris* as the plant which possess antiurolithiatic property and in these aqueous extract is higher dissolution of calcium oxalate as compare to Ethanol fraction. Standard shows higher dissolution as compare to others.

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