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An Overview on Phyto-molecules and Screening Method of Antiurolithiatic Activity

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ABSTRACT

Kidney stones are one of the oldest known and common diseases in the urinary tract system. Kidney stones are a growing global problem. It is also known as Urolithiasis. Lithiasis is a condition where urinary calculus is formed in the kidney and urinary tract. It is a complicated urinary disorder that has gravely troubled the health and quality of human life. It has been associated with an increased risk of end-stage renal failure. Urinary stones affect 10-12% of the population in industrialized countries. There are only a few geographical areas in which the stone disease is rare, *e.g.,* Germany and in the coastal areas of Japan. The etiology of kidney stone is multifactorial. The most common type of kidney stone is calcium oxalate formed at Randall's plaque on the renal papillary surfaces. The mechanism of stone formation is a complex process which results from several physicochemical events including supersaturation, nucleation, growth, aggregation and retention of urinary stone constituents within tubular cells. These steps are modulated by an imbalance between factors that promote or inhibit urinary crystallization. Currently, there is no satisfactory drug to cure and/or prevent kidney stone recurrences. Conventional agents are being used to control kidney stone along with lifestyle management. Medicinal plants are found to be useful in this metabolic disorder from ancient days due to its no or low-toxic nature, easily available in rural areas, cheap; there are fewer chances of recurrence. Thus, further understanding of the pathophysiology of kidney stone formation is a research area to manage urolithiasis using new drugs. Therefore, this review has intended to provide compiled up-to-date information on kidney stone etiology, pathogenesis and prevention approaches and critically review the available literature on various medicinal plants with their antilithiatic activity and screening method of this activity to develop an effective drug to treat the disease.

Keywords: Kidney stones, Urolithiasis, Etiology, Calcium oxalate, Pathophysiology, Medicinal plants

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Introduction

Several therapeutic methods are available in the modern health care system to treat infectious and non-infectious diseases. However, some of these therapies have their own limitations, and also are not available to the majority of the world's population due to their accessibility and affordability reasons. Because of this, approximately 75% of people, mainly from lower economy countries are still dependent on herbal remedies for their basic healthcare requirements[1]. In this regard, substantiation of traditional medicines for the least life threatening diseases, such as urinary stone and hemorrhoids should be considered. Urinary stones (calculi) are the solid crystalline masses that can occur anywhere in the renal tract and the process of formation of urinary stone or appearance of stone anywhere in the urinary tract is termed as urolithiasis[2]. The history of urolithiasis dates back to the early age of civilization. Paleopathological evidences corroborate its incidence around 7000 years ago[3]. Nephrolithiasis is the condition in which the stone is located in the kidney. The term ureterolithiasis refers to the stones that are in the ureter; and cystolithiasis (or vesical calculi) is used to mention stones which form or have passed into the urinary bladder[4]. The formation of stones in the kidneys, is one of the oldest known and widespread diseases in the urinary tract system with a relapse rate of 50% in 5-10 years[5,6]. It is the third most common disorder among urinary diseases[7]. It has been reported that 10-12% of people in industrialized countries (10% of men and 3% of women) have a urinary stone during their lives. The rate of occurrence is three times more in men than women, due to enhancing capacity of testosterone and inhibiting capacity of oestrogen hormone in stone formation[8]. There are only a few geographical areas in which stone disease is rare, e.g., in Greenland and in the coastal areas of Japan[9]. The etiology of this disorder is multifactorial and is related to genetics, diet and low activity[10, 11]. Calcium-containing stones are the most common kidney stones (75-90%), followed by magnesium ammonium phosphate (struvite) (10-15%), uric acid (3-10%) and cystine (0.5-1%) [12]. The mechanisms related to the

development of kidney stones are not completely understood. Generally, it is believed that urolithiasis, the process of stone formation in the urinary tract, causes crystal aggregation, nucleation and growth of insoluble particles[13]. The stones may cause various symptoms, including pain, obstruction, infection and hemorrhage, through the passage of stones in the urinary tract system[14]. Promoters of stone formation are calcium, sodium, oxalate, urates, cystine, low urine pH, Tamm-Horsfall protein and inhibitors of stone formation are divided in two parts, inorganic (magnesium, pyrophosphate and citrate) and organic (nephrocalcin Tamm-Horsfall protein, protease inhibitors, glycosaminoglycans and high urine flow) [15]. Some medical conditions that increase the risk of development of nephrolithiasis include gout, hypertension, diabetes, obesity and primary hyperparathyroidism. Management of stone disease depends on the size and location of the stones. Stones larger than 5mm or stones that fail to pass through should be treated by some interventional procedures such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL) [16] and transureteral lithotripsy[17, 18]. These surgeries are complex and expensive and do not affect the recurrence of stones[17]. Various medicines, including thiazide as diuretic and alkali-citrate, are applied to prevent the frequency of hypercalciuria and hyperoxaluria which cause calculi formation but they are not promising enough due to their limited effectiveness and low tolerability[18-21]. Because of the disadvantages of surgical techniques and limited choice in pharmacotherapy, exploring new pharmacological therapies for the management of kidney stones is worthwhile. Various medicinal plants with diuretic, antispasmodic and antioxidant activities exert inhibitory effects on crystallization, nucleation and aggregation of crystals, making them useful for treatment of urolithiasis. In India, in the Ayurvedic system of medicine, Varuna, Pashanabheda, Gokhru Kulatha were found to be effective in preventing the deposition of the stones in experimental rates. Pharmacotherapy can reduce the recurrence rate. The use of plant is very important as to reduce the side effects related to allopathic medicines and treatments. The purpose of this paper is to critically review about kidney stones and the role of herbal medicines in the management of urolithiasis and could serve as a source of information on the present trends in research on plants having antiurolithiatic activity.

Literature search methodology

Electronic databases, including PubMed, Science Direct, and Scopus, were searched for herbal plants and their bioactive compounds used for prevention and management of urolithiasis from 2005 to act. 2019. The keywords were "kidney stone" or "urolithiasis", or "nephrolithiasis", or "renal calculi", or "renal stone", or "antilithiatic"; and "herbal plant", or "herbal herb", or "phytochemical". The retrieved articles were subclassified into in vitro, in vivo, and clinical studies. The studies included were evaluated with respect to the potential of the plant to be used as a herbal agent, the phytochemical composition of the plant, the kind of kidney stone that the herbal agent is effective on, as well as underlying mechanisms of action.

Epidemiology of kidney stones

Urolithiasis occurs in one out of 20 people at some time in their lives. Calcium stones are mostly found in the patients and the average age of onset is third to fourth decade (30-40years). 5% of populations are affected from kidney stone with a lifetime risk of passing a kidney stone about 8-10%. Looking at the current scenario, our understanding about the disease has improved. Today we have statistics which says; around 12% population worldwide is suffering from kidney stone disease. It affects all ages, sexes and races[7,22] but occurs more frequently in men than in women. However, lifetime recurrence rate is higher in males, although the incidence of nephrolithiasis is growing among females[23]. 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[7]. Global scenario says that this disease is more common in western hemisphere (5-9% in Europe, 13-15% in USA, 12% in Canada) than eastern hemisphere (1-5%). In the Asian countries Saudi Arabia is leading the disease with 20% occurrence rate. Overall Asia shows (4-20%) of kidney stones which include countries like India, China, Pakistan, Myanmar, Thailand, Indonesia, Philippines and UAE[24, 25]. Considering Indian scenario 13-14% of the population has the kidney stones[24]. There are two 'stone belts' more prone to kidney stones, one belt stretched from Amritsar to Uttar Pradesh via Delhi and Agra. Other belt starts from Gujrat to Jabalpur in central India[26]. In south India, Kerala is leading with kidney stone disease with prevalence rate of 2643 per 100,000 adult. Reason for this significant increase in number in Kerala among other states in South India, is life style diseases like obesity and diabetes are more common and steadily increasing with risk 43% in Kerala. There is positive relation between diabetes and kidney stone[27]. 12% of the population is estimated to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage. Repeated stone formation is a frequent problem with all types of stones and consequently an important part of the medical care of patients with stone disease[28].

Etiology

Reasons of development of kidney stone in some people are not totally understood. Beside from the risk factors (intrinsic (such as age, sex and heredity) and extrinsic factors such as geography, climate, dietary, mineral composition and water intake), metabolic condition e.g. cystinuria, hyperuricosuria, xanthinuria, hyperoxaluria, hyperthyroidism and distal tubular acidosis are some common cause of stone formation[29]. A person with a family history of kidney stones may be more likely to develop stones. Urinary tract infections, kidney disorders such as cystic kidney diseases, and metabolic disorders such as hyperparathyroidism are also linked to stone formation. In addition, more than 70% of people with a rare hereditary disease called "renal tubular acidosis" develop kidney stones. Cystinuria and hyperoxaluria are two other rare, inherited metabolic disorders that often cause kidney stones. In cystinuria there is too much of the amino acid cysteine, which does not dissolve in urine and it is voided. This can lead to the formation of stones made of cysteine. In patients with hyperoxaluria, the body produces too much of the salt oxalate. When there is more oxalate that can be dissolved in the urine, the crystals settle out and form stones. Hypercalciuria is inherited. It is the cause of stones in more than half of patients. Calcium is absorbed from food in excess and is lost into the urine. This high level of calcium in the urine causes crystals of calcium oxalate (CaOx) or calcium phosphate (CaPh) to form in kidneys or in the urinary tract. Other causes of kidney stones are hyperuricosuria (a disorder of uric acid metabolism), gout, excess intake of vitamin D, and blockage of the urinary tract. Certain diuretics (water pills) or calcium-based antacids may increase the risk of forming kidney stones by increasing the amount of calcium in the urine. CaOx stones may also form in people who have a chronic inflammation of the bowel or who have had an intestinal bypass operation, or ostomy surgery. As mentioned above, struvite stones can form in people who

have had a urinary tract infection. People who take the protease inhibitor indinavir, a drug used to treat HIV infection and AIDS, are at risk of developing kidney stones[30, 31]. Stones are also classified based on how they are formed in the body e.g by infection, non-infection, genetic or drug related effect[32].

Non-infection stones: Calcium oxalate, Calcium phosphate (including brushite and carbonate apatite), Uric acid.

Infection stones: Magnesium ammonium phosphate, Carbonate apatite, Ammonium urate

Genetic causes: Cystine, Xanthine, 2,8-dihydroxyadenine

Drug stones:

Pathophysiology of urolithiasis diseases

There are basic two aspects in the pathogenesis of kidney stone like as

(a) Increased urinary flow of stone forming constituent elements like calcium, phosphorus, uric acid, oxalate and cysteine.

(b) Physico-chemical change that influence stone formation like pH of urine, stone matrix and protective substance in urine.

For a stone to form within the urinary tract, Urine must be supersaturated for precipitating crystalline component. The agents who can modify nucleation, crystallization, and aggregation, pH of the urine also play important role in stone formation[33].

Mechanism of urolithiasis: crystallization and retention of stones

The diseased state of urolithiasis becomes apparent only when small stone particles are retained within the urinary system and grow into clinically relevant stones[34]. Supersaturation of urine, crystallization and retention of stone within urinary system are the prominent steps involves in the formation of urinary stones.

Crystallization

Crystallization represents the first phase of urinary stone formation and further includes three steps: crystal nucleation, growth and aggregation.

Nucleation

The nucleation is a process of formation of a solid crystal in solution[35]. Generation of a crystal can occur by homogeneous nucleation when local supersaturation allows spontaneous organization of the atoms into the appropriate lattice. However, heterogeneous nucleation is more likely to occur within complex mixtures in which peculiar proteins provide patterns on their surface to organic molecules for the formation of the initial crystal lattice[36]. The organic matrix of the stone compositions constitutes around 2.5% of total weight, which mainly include small proteins such as albumin, Tamm-Horsfall protein and bikunin[37]. Generally, the kidney walls are protected by an anti-adherent glycosaminoglycans layer and because of this, nucleation of stone can only occur at damaged areas or perhaps just at reduced protective layer sites. Nucleation may occur in renal tubules, on bladder walls, on normal or damaged cells, on areas denuded of cells by certain forms of injury, or at interstitial sites[38-40].

Crystal growth

After nucleation, crystal growth is the next major step of stone formation. In this process, several atoms or molecules in supersaturated liquid starts to form clusters. Crystal growth is determined by the molecular size and shape of the molecule, the physical properties of the material, supersaturation levels, pH and defects that may form in the crystal's structure[35].

Aggregation

Aggregation an important step of stone development and is commonly defined as a process in which crystals agglomerate and form larger multicomponent particles in free solution[40]. Aggregation of particles in solution is determined by a balance of forces, including both aggregating and disaggregating effects. Small interparticle distances increase attractive forces and favour particle aggregation[35]. The process of crystallization is influenced by several promoters and inhibitors, as well as some morphoanatomic, dietary and environmental factors. Trace elements in the urine, such as fluoride, iron, iodine, manganese, molybdenum, nickel, selenium, silicone, germanium, vanadium, copper, zinc, chromium, and lithium are found to initiate the process of crystallization[41]. These elements act as a nucleus for the formation of the stone, or influence the external morphology of growing crystals. They may also increase or decrease the speed of the crystallization process[41, 42].

Stone retention

After the crystallization process is complete, the retention of urinary stones within the urinary system is an important step in the development of the disease. Urothelium is generally thought to be resistant to crystal adherence. However, chemical or mechanical urothelial damage may promote crystal binding and aggregation[43]. So far, two hypotheses have been put forwarded for retention of urinary stones: free particle and fixed particle hypothesis. According to the free particle hypothesis, the process of nucleation occurs entirely in the tubular lumen. As crystals move through the renal tubules, rapidly aggregate and grow large enough to get stuck within the tubular lumen[43]. Whereas, in fixed particle hypothesis it has been proposed that crystals gets adhered to a fixed point, such as renal epithelial cells or Randall's plaque[44]. Four different possible modes of stone retention have been identified in the fixed particle hypothesis, namely: 1) growth over white (Randall's) interstitial hydroxyapatite plaque; 2) growth over Bellini duct plugs; 3) formation of microliths within inner medullary collecting ducts and 4) formation in free solution within the calyces or renal collecting system[45].

Kidney stone compositions

The chemical compositions of urinary stones include crystals and noncrystalline 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. 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 calcium oxalate and calcium phosphate stones[46]. Albumin is the major component of the matrix of all stone types[47]. Brushite stone is a hard phosphate mineral with an increasing incidence rate and a quarter of calcium phosphate (CaP) patients form stones containing brushite[48]. In the urinary tract, CaP may be present in the form of

hydroxy-apatite, carbonate apatite, or brushite (calcium monohydrogen phosphate dihydrate, CaHPO4·2H2O). Brushite is resistant to shock wave and ultrasonic lithotripsy treatment[49].

Diagnosis and modern therapeutic strategies for management and cure of urinary stones

After physical examination and considering anticipated symptoms of urinary stone disease, the diagnosis of urinary stone starts with the medical history of the patients; dietary data, complete blood cell count, routine urine analysis and serum creatinine measurement[50]. The further diagnosis mainly includes ultrasound and imaging tests such as an X-ray or computerized tomography (CT) scan. Some of the stone may not be visualized in X-ray, therefore, in this case, ultrasonography is preferred as it detects all types of kidney stones[51]. In many cases, urinary stones can pass spontaneously. Because of this, physicians are usually trying to manage symptoms of stone disease at an initial stage, which includes pain management with analgesia. However, the rate of stone passage is highly variable and depends on its size and location. If stone fails to pass spontaneously, then medical expulsive therapy is generally preferred. This treatment regimen can include prescription of anti-inflammatory drugs, antibiotics, calcium antagonists and α-blockers[52]. However, surgical intervention becomes crucial when conservative therapy fails due to complex composition and larger size of stones, and/or the patient cannot bear the degree of pain until the stone passes[53]. Surgical treatments primarily comprise extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), percutaneous pithotripsy (PCNL) and open surgery[54]. Despite several advantages, these surgical techniques have some limitations, because stone clearance after surgery is dependent on several factors such as patient age, stone size, location and number, radiological renal features and congenital renal anomalies[55]. The recurrence of stones is another major risk factor for surgical and medicament treatments[56]. It has been found that ESWL may cause acute renal injury due to the traumatic effect of shockwave and the possibility of infection after treatment[57].

Screening method of urolithiasis activity

Rat model

Rats represent a well-established, relatively economical model that scientists have utilized since the 19th century. For urinary stone disease, studies utilizing the rat have predominantly focused on reproducing both hypercalciuria and hyperoxaluria, two of the most common pathophysiologic changes associated with urinary stone disease.

In Vivo animal model

Sodium oxalate (NaOx) induced model

As Khan et al. illustrated, different areas of the nephron reflect predictable calcium oxalate crystal formation depending on time elapsed after intraperitoneal injection of sodium oxalate[58]. Varying doses (3, 5, 7, 9, and 10 mg/kg) of sodium oxalate administered to Male Sprague-Dawley rats produced persistent hyperoxaluria and crystals in a dose-dependent fashion[59-61]. Compared to controls, rats receiving 10 mg/kg had over 500% more oxalate excreted with persistent crystals that remained present up to 7 days after injection. Calcium oxalate crystals were appreciated within 15 min of injection and by 6 h there was noticeable crystal aggregation in the ducts of Bellini[62].

Glycolic acid induced model

Using powdered 3% glycolic acid dissolved in drinking water, Ogawa et al. demonstrated that male Wistar-strain rats produce high levels of 24-h urinary oxalate and subsequent calcium oxalate calculi[63]. Interestingly, this study also showed that adding magnesium (Mg) salts to a high glycolic acid diet increased urinary citrate levels despite relatively high levels of urinary oxalate excretion.

Ethylene glycol induced model

Administration of EG in drinkingwater has been shown to result in consistent induction of hyperoxaluria, crystalluria and calcium oxalate nephrolithiasis[64]. Delivering solely 0.75% EG to male rats eventually yielded persistent crystalluria at 12 days and renal crystal deposits at 3 weeks[65]. To enhance the development of crystal deposition, EG often has been combined with other agents such as ammonium chloride (AC) to reduce urinary pH, as well as a vitamin D or calcium chloride to result in subsequent hypercalcemia and hypercalciuria [59,65,66]. This lithogenic combination decreased the time for crystalluria from 12 to 3 days, and detectable calcium oxalate nephrolithiasis from 3 weeks down to 1 week[59]. However, multiple studies have shown EG to be a toxic agent that can cause multi-organ failure[57]. Yamaguchi et al. demonstrated that the combination of EG and AC is detrimental to rat health e with rats having lower weights, worsening renal function, and increased urinary N-acetyl-b-D-glucosaminidase (NAG), an indicator of renal toxicity[68]. Other studies have also found that lipid peroxidation, increased free radicals, and metabolic acidosis also take place as a result of EG Refs[69].

Hydroxy-L-proline induced model

Hydroxy-L-proline (HLP) is derived from the amino acid proline and is a component of collagen that is metabolized to both pyruvate and glyoxalate, primarily in the renal proximal tubule and hepatocyte mitochondria[70]. It is a common ingredient in Western diets and has been shown to be less toxic than other lithogenic agents. Intraperitoneal injection of HLP has resulted in the presence of calcium oxalate crystals within the rat kidney[71]. A large dose of

4-HLP (2.5 g/kg) induced both calcium oxalate dihydrate (Weddellite) and calcium oxalate monohydrate (Whewellite) crystals, detected by scanning electron microscopy. Khan et al. provided 5% HLP (weight/weight HLP/chow) to male Sprague-Dawley rats and compared treated vs controls at 4, 6, and 9 weeks[72]. At 4 weeks, all treated rats were found to have CaOx crystals throughout the regions of the kidney, with the majority present in the tubular lumens of the distal tubules and collecting ducts. By 9 weeks these crystals were mainly located at the tips of the renal papillae. Bushinsky et al. found that the addition of 1%, 3%, and 5% trans-4- HLP to GHS rats altered urine calcium and stone type, with rats receiving 5% HLP having lower urine calcium excretion and consistent calcium oxalate calculi composition[73].

Zinc disc induced model

Rats were anesthetized with sodium pentobarbitone (40 mg/kg, ip). A suprabic incision was made and the urinary bladder was exposed. A small cut was made at the top of the bladder. The urine was then aspirated aseptically into a sterile vial for bacteriological examination and pH determination (using narrow range pH paper BDH). Previously weighted sterile zinc discs were inserted into bladder, and the incision was closed with a single suture using absorbable 4-0 chronic catgut (Ethicon) and the rats were allowed to recover for one week. The implantation of zinc foreign bodies into the urinary bladder induce growth of

urinary stones and hypertrophy of the organ smooth musculature that were greater in males than in female from 4 & 8 w surgery, respectively[74-76].

Dietary manipulation

Aiming for an alternative crystal induction method compared to HLP, Wiessner et al. found that a 5% level of potassium oxalate was required to produce calcium oxalate crystals in both Dahl saltsensitive and Brown Norway male rats[77]. Meanwhile hyperoxaluric rats deprived of dietary Mg demonstrated increased production of calcium phosphate (apatite) stones[78]. Intentional vitamin B6 deficiency can also be employed in rats to enhance hyperoxaluria, hypocitraturia and subsequent calcium oxalate crystal formation[79]. Studies exposing these pyridoxine deficient rats to supplemental Mg demonstrated their ability to counteract the hypocitraturia and effectively prevent calcium oxalate crystal formation[80].

Xenoplantation Model

Stone particles were extracted by PCNL (percutaneous lithotomy) from one male patient with renal stones. The selected stone is cut with a blunt instrument into sections with a diameter of 2-3 mm, weighed and maintained in a sterile environment, before use. Eight-week old male rats weighing about 250-300 gm were selected and randomized into three groups: control, standard and test groups. The rats were anesthetics by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight), and a suprapubic incision exposed the bladder. Following this, a 4-5 mm incision was made at the top of the bladder, and one prepared stone particle was inserted in each rat, and then the bladder and the suprapubic incision was closed respectively. Ethylene glycol was supplied in drinking water at a final concentration of 1% from the second day (day 1) postoperatively for four weeks. After four weeks, kidney and urinary bladder were dissected, and the kidneys were dehydrated in a graded ethanol series and embedded in paraffin. The renal stone formation was assessed by von Kossa histochemical staining. Bladder stone was harvested, weighed and maintained in 75% ethanol for 24 h, before the stone being embedded in auto-polymerizing resin and sectioned transversely with the diamond wire saw to select the best section pane. Sectioned blocks were then fixed on a glass slide with thermoplastic glue and polished successively using a 1, 200 grit sandpaper and a mix of alumina polishing compounds (3, 1 and 0.3 μm) with a small volume of water until it was possible to observe the core clearly under a transmitted light microscope[81].

Chemically induced lithiasis in weanling rats

Calculi is induced in the urinary tract of weanling Fischer-344 rats (postnatal day 28) in less than two weeks by exposure to terephthalic acid (TPA) at 3-5% in the diet or dimethyl terephthalate (DMT) at 1-3% of diet. Specified rats of 24 which randomly divided into four groups each group containing six rats. Group-I, II, III, and IV acts as control received vehicle, disease control (positive control) received (TPA)/(DMT) for two weeks, standard received (TPA/DMT) and cystone 750 mg/kg body weight p.o and the last group, *i.e.* IV serves as a test received (TPA/DMT) plus test drug. After the treatment period, various biological samples are collected and the parameters are measured and compared[82].

Mild tubular damage for hyperoxaluric rats induces renal lithogenesis

It is a two-step or two hit model of lithogenesis used to assess the antilithiatic activity of test drugs. In the first step, it is used to induce crystalluria (hyperoxaluria) which is a necessary step but not sufficient to induce lithiasis. In the second step, it causes tubular damage that induces lithiasis[83].

In vitro models

Determination of effect on CaC_2O_4 crystallization

The CaC_2O_4 Crystallization was determined by the time course measurement of turbidity change due to the crystal formation and aggregation in the metastable situations of Ca2+ and oxalate, stock solutions of $CaCl_2$ (8.5 Mm) and $Na_2C_2O_4$ (1.5 Mm). Containing 200 mM NaCl and 10 mM sodium acetate were adjusted to pH 5.7. An aggregometer devised for platelet aggregation studies based on the measurement of optical density at 620 nm was used to investigate the event of CaC_2O_4 crystallization. The $CaCl_2$ solution (0.5 ml) was stirred constantly both in the absence and presence of different concentrations of the test material or reference drug: potassium citrate at 37 0C. After obtaining a stable base line, crystallization was induced by the addition of $Na_2C_2O_4$ solution (0.5) to obtain the final concentration of Ca2+ as 4.25 and oxalate as 0.75 Mm. The time course measurement of turbidity was simultaneously started on a chart, moving at the speed of 30 mm/h and continued for 15 min with constant stirring of the solutions. All experiments were run in triplicate, Slope of nucleation (SN) and aggregation (SA) phases were calculated using linear regression analysis. Using the slopes, the percentage inhibition was calculated as [(1-Sm/Sc) X 100], where Sm is slope in the presence of modified and Sc is slope of the control experiment. To determine the effect of incubation with the test material on CaC_2O_4 crystal formation, stock solutions of $CaCl_2$ and $Na_2C_2O_4$ having composition similar to those in the kinetic study were used. $CaCl_2$ solutions, containing different concentrations of the test material or potassium citrate, were aliquoted (0.5) to the flat bottomed tubes in a 24 well plate. To each of these tubes $Na_2C_2O_4$ solution (0.5) was added to obtain the final concentration of Ca2+ as 4.25 and oxalate as 0.75 mM. Each concentration of the test material was prepared in triplicate. The plates were then incubated in a shaking water bath at 90 oscillations/min at a temperature of 37^{0}C for 45min. Each tube then observed under an inverted microscope for crystal morphology and count in five randomly selected fields (200x) [84].

Nucleation assay

The method used similar to that descried[85]. The solutions of calcium chloride and sodium oxalate were prepared at the final concentration of 3mmol/l and 0.5 mol/l, respectively, in a buffer containing Tris 0.05 mol/l and Nacl 0.15 mol/l at pH 6.5. Both solutions were filtered through a 0.22μm filter; 33 ml of calcium chloride solution was mixed with 3.3 ml of the test at different concentration. Crystallization was started by adding 33 ml of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm using a PTFE-coated stirring bar. The temperature was maintained at 37 °C. The absorbance of the solution was monitored at 620 nm after every 1 min. The percentage inhibition produced by test was calculated as [1-(Tsi/TSC)]x100, Where Tsc was the turbidity slope in the presence of the inhibitor[86].

Growth assay

Inhibitory activity against CaOx crystal growth was measured using the seeded, solution-depletion assay an TEST solution of 10 mM Tris-HCl containing 90 mM NaCl was adjusted to pH 7.2 with 4 N HCl. Stone slurry (1.5 mg/ml) was prepared in 50 mM sodium acetate buffer (pH 5.7) CaOx monohydrate crystal seed was added to a solution containing 1 mM $CaCl_2$ and 1 mM sodium oxalate ($Na_2C_2O_4$. The reaction of CaCl2 and Na2C2O4 with crystal seed led to deposition of

CaOx (CaC_2O_4) on the crystal surfaces, thereby decreasing free oxalate that is detectable by Spectrophotometer at λ214 nm. When test is added into this solution, Depletion of free oxalate ions will decrease if the test sample inhibits CaOx crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after 30-second incubation with or without test sample. The relative inhibitory activity was calculated as follows: % Relative inhibitory activity =[(C-S)/C]x100, where C is the rate of reduction of free oxalate without any test sample and S is the rate of reduction of free oxalate with a test sample[86, 87].

Calcium phosphate assay

Calcium phosphate (CaP) assay was studied on *in vitro* homogeneous systems of initial mineral phase formation for CaP, its subsequent growth and demineralization by employing 5.0 ml systems which was prepared by adding 0.5 ml of KH_2PO_4 (50 mM), 0.5 of $CaCl_2$ (50 mM), 2.5 ml of Tris buffer (210 mM NaCl + 0.1 mM tris HCl) and increasing volume of test ranging from 0.2 ml to 1.5 ml by subsequently decreasing the volume of water ranging from 1.5 ml to 0.0 ml. This system was centrifuged at 4500 rpm and precipitates so obtained were dissolved in 5 ml of 0.1 N HCl. This 5 ml system for mineralization[88,89]. For the growth, firstly 5 ml systems were prepared using standard protocols then again 5 ml were re-grown on the same tubes with the additions of increasing volumes of the test. Calcium and phosphate were then estimated on the precipitates obtained and dissolved in 0.1 N HCl. In case of control no test was added. T checks the demineralization, again 5 ml system was prepared having no test added to that and precipitates were obtained. To these precipitates, 2.5 ml of Tris buffer (210 mM NaCl+0.1 mM Tris HCl) and increased volumes of test ranging from 0.2 ml to 1.5 ml with subsequently reduced volume of water was added and then centrifuged at 4500 rpm for 15 min. Calcium and phosphate were then estimated in supernatant obtained after centrifuged. The Ca2+ and HPO4-˙ ions were estimated by the methods[90,91] respectively. Percentage inhibition of mineral phase in the presence of test was calculated as % inhibition= ((C-T)/C)x100, where T is the concentration of Ca2+ or HPO42˙ ion of the precipitates formed in test having test ranging from 0.2 ml to 1.5 ml in the assay systems and C is the concentration of Ca2+ or HPO42˙ ion of the precipitate formed in control systems which had distilled water (Millipore) and no test[92].

Calcium oxalate crystal assay

Inhibitory activity of test was also checked on calcium oxalate crystal growth. A 4 ml system was prepared to check the effect of the test in inhibiting growth of calcium oxalate crystals. In this systems, 1 ml each of 4 mM calcium chloride and 4 mM sodium oxalate were added to a 1.5 ml solution, containing NaCl (90 Mm) buffered with Tris HCl (10 mM) pH 7.2. To this 30 µl of calcium oxalate monohydrate (COM) crystal slurry (1.5 mg/ml acetate buffer) was added consumption of oxalate begins immediately after COM slurry addition and was monitored for 600 sec by disappearance of absorbance at 214 nm. When test is added into this solution, depletion of free oxalate ions will decrease if test inhibits calcium oxalate crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after 30 sec incubation with or without the test. The relative inhibitory activity was calculated =((C-S)/C)x100, where C is the rate of reduction of free oxalate without any test sample and S is the rate of reduction of free oxalate with a test sample[92].

Lactate dehydrogenase leakage assay

LDH leakage assay was performed by the methods described[93]. The 6.6 mM NADH and 30 mM sodium pyruvate were prepared in Tris (0.2M, pH 7.3) reaction was initiated with the addition of 50 µl of the test sample and the disappearance of NADH was monitored at 340 nm, for 5 min at an interval of 1 min. The percentage of LDH release was calculated by dividing the activity of LDH in the supernatant by the LDH activity measured after complete cell lysis achieved by sonication[86].

Herbal drugs used in treatment of urolithiasis

Herbal medicines have lesser side effects than the modern medicines and reduce the recurrence rate of renal stone[94]. The complete mechanism of action of plant based remedies is lacking but plant based phytotherapeutic agents are more efficacious in urolithiasis treatment. Allopathic medicines target only one aspect of urolothiatic pathophysiology while plant based therapy is known to be effective against different stages of stone pathophysiology. Medicinal plant extracts show antiurolithiatic property exert by altering the ionic composition of urine, decreasing the calcium ion concentration or increasing magnesium and citrate excretion. Herbal medicines have several phytoconstituents and exert their beneficial effects in urolithiasis by multiple action like diuretic or lithotriptic properties. Diuretic action is also needed to increase the amount of fluid going through the kidneys and flush the deposits[95]. Drug with multiple mechanisms of protective action may be one way forward in minimizing tissue injury to human disease[96]. Most of the traditional remedies were taken from plants and their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects. Some of the plants which showed promising results in *in vivo* antiurolithiatic models are given in Table 1.

Table 1. Details regarding part used, plant extracts and study designs which include model used[97-126].

Plant	Part	Extract	Experiment model	References
Adiantum capillus veneris	Whole plant	50% ET	0.75% Ethylene glycol and 1% Ammonium chloride	Ahmed *et al*., 2013
Bergenia ligulata	Rhizome	70% E	0.75% Ethylene glycol and 1% Ammonium chloride	Bashir & Gilani, 2009
Berberis vulgaris	Root Bark	B, AQ	0.75% Ethylene glycol and 1% Ammonium chloride	Bashir *et al*., 2010
Boerhaavia diffusa	Root	AQ	0.5 % Ethylene glycol	Pareta *et al*., 2011
Citrus medica	Fruit	FJ	0.75% (v/v) Ethylene glycol	Shah *et al*., 2014
Costus igneus	Stem	ET	0.75% (v/v) Ethylene glycol	Manjula *et al*., 2012
Cynodon dactylon	Rhizome	AQ	0.75% Ethylene glycol and Ammonium chloride	Atmani *et al*., 2009
Desmodium styracifolium and *Pyrrosiae petiolosa*	Whole plant	AQ	Calculi producing diet (CPD)	Mi *et al*., 2012
Flos carthami	Whole plant	CH	0.75% Ethylene glycol	Lin *et al*., 2012
Foeniculum vulgare and *Cymbopogon proximus*	Whole plant	FB	70 mg/ kg Sodium oxalate	Ibrahim and El-Khateeb, 2013
Helichrysum plicatum	Flower	50% ET	1% Ethylene glycol and 1% Ammonium chloride	Bayir *et al*.,2011
Helichrysum graveolens and *Helichrysum stoechas*	Flower	Decoction extract	70 mg/kg Sodium oxalate	Orhan *et al*., 2015
Hibiscus sabdariffa	Calyces	AQ	0.75% Ethylene glycol and 1% Ammonium chloride	Laikangbam &Devi, 2011
Holarrhena antidysenterica	Seed	70% ET	0.75% Ethylene glycol and 1% Ammonium chloride	Khan *et al*., 2012
Launaea procumbens	Leaf	ME	0.75% Ethylene glycol	Makasana *et al*.,2014
Lemon	Juice	LJ	0.75% Ethylene glycol and 2%Ammonium chloride	Touhami *et al*.,2007
Melia azedarach	Leaf	70% ET	0.75 % Ethylene glycol	Dharmalingam *etal*., 2014
Moringa oleifera	Root wood	AQ, ET	0.75 % Ethylene glycol	Karadi *et al*.,2006
Orthosiphon grandiflorus, Hibiscus sabdariffa and *Phyllanthus amarus*	Whole plant	AQ	Feed a diet containing 3% glycolic acid to produce hyperoxaluria and kidney stones.	Woottisin *et al*.,2011
Orthosiphon grandiflorum	Leaf	AQ	0.5% Ethylene glycol and vitamin D3 in salad oil	Akanae *et al*.,2010
Quercus salicina Blume /*Quercus stenophylla* Makino	Leaf	AQ	0.5% Ethylene glycol	Moriyama *et al*.,2009
Paronychia argentea	Aerial part	AQ, B	IP Sodium oxalate (7 mg/100 g of bw)	Bouanani *et al*.,2010
Punica granatum	Fruit ME	ME, CH	0.75% Ethylene glycol	Rathod *et al*.,2012
Phyla nodiflora	Whole plant	95% ET	Gentamycin and Calculi producing diet.	Doddola *et al*.,2010
Psidium guajava	Leaf	95% ET	0.75% Ethylene glycol	Nagar *et al*.,2015
Rubia cordifolia	Root	70% ET	0.75% Ethylene glycol and 1% Ammonium chloride	Divakar *et al*.,2010
Rosa canina	Fruit	50% ME	1% Ethylene glycol	Nasrabadi *et al*.,2012
Sesbania grandiflora	Leaf	FJ	Rat Pellet Feed with 5 % Ammonium oxalate	Doddola *et al*., 2008
Solanum xanthocarpum	Fruit	50% ET	0.75% Ethylene glycol	Patel *et al*., 2012
Trachyspermum ammi	Seed	IP	0.4 % Ethylene glycol and 1 % Ammonium chloride	Kaur *et al* ., 2009

AQ: Aqueous extract, B: Butanol, CH: Chloroform, EA: Ethyl acetate, ET: Ethanol, FB: Formulated beverage, FJ: Fresh juices, HE: Hexane, IP: Isolated protein, LJ: Lemon juice, ME: Methanol.

Different polyherbal formulations in urolithiasis

1. Cystone
2. Rilith
3. Renomet
4. Polyherbal I
5. Polyherbal II
6. Neeri –KFT
7. Polyherbal formulation III
8. Gokshuradi polyherbal Ayurvedic formulation
9. Crashcal
10. Lithocare[127]

Marketed herbal formulations having antiurolithiatic activity

There are many marketed formulations which are having antiurolithiatic activity; some of them are Cystone (Himalaya Drug Company, India), Calcuri (Charak Pharmaceuticals, Bombay, India) and Chandraprabha bati (Baidyanath, India). These formulations have been widely used clinically to dissolve urinary calculi in the kidney and urinary bladder. Pharmacological and clinical studies carried out on a composite herbal formulation, Trinapanchamool consisting of five herbal drugs namely *Desmostachya bipinnata, Saccharum officinarum, Saccharum nunja, Saccharum spontaneum* and *Imperata cylindrica* was found to be effective both as prophylactic in preventing the formation and as curative in dissolving the pre-formed stones in albino rats. The antiurolithiatic activity of this formulation has been attributed to its diuretic activity[128].

Dietary plants for the prevention of kidney stones

Green tea (Camellia sinensis), Raspberry (Rubusidaeus, from Rosaceae family), Rubia cordifolia (madder or Indian madder), Parsley (Petroselinum crispum), Pomegranate (Punica granatum), Pistacia lentiscus, Solanum xanthocarpum, Urtica dioica or "Stinging Nettle", Dolichos biflorus (horse gram), Ammi visnaga, Nigella sativa, Hibiscus sabdariffa, Origanum vulgare[129].

Conclusion

The present review states the different steps involved in kidney stone disease. It explains the mechanism of formation of the kidney stones. As there is no proper medicine in Allopathy for the management of anti-lithiasis and also the surgical treatment has the more chances of recurrence, these two factors particularly diverted the large population toward the use of herbal medicines. Medicinal plants have wide acceptance due to a large number of advantages such as lesser toxic effects safe, effective, cheap (cost effective), fewer chances of recurrence of disease, easily available in rural areas. The present paper provides information regarding the potential medicinal plants used in the management of anti-lithiasis and also about the screening models of anti-lithiasis in order to develop a new drug for the management of anti-lithiasis to overcome the various disadvantages faced by the wide range of population and get relieved from the disease. Most of these studies were preliminary, carried out in animals and are not sufficient for the development of a pharmaceutical product. Still, intensive preclinical and clinical studies are required to evaluate the efficacy and toxicity of these plant products. Further, chemical studies of the plants are needed to isolate the active principles and investigate them in order to identify a promising Lead compound. Let us hope for the development of the safe and effective drug for the management of anti-lithiasis.

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