Review on Plants extract as anti-urolithiatic and pathogenesis of urolithiasis induced by ethylene glycol

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Abstract:

Renal calculi have become a crucial health issue everywhere on the world due to the adjustments in way of life and nutritive propensities for the present time. As of recently, People medication or folklore treatment is the significant reliance for the lithogenesis other than the endoscopic or shock wave lithotripsy. Numerous therapeutic plants were discovered that have antagonistic effect to urolithiatic properties and have been utilized by Ayurveda, homoeopathic medicine, or unani framework. In this paper, we analysed some generally known and as of lately found plant extract which act against lithiasis are discussed. It is mainly focused on lithiasis induced by ethylene glycol utilizing in-vivo experiments. Various experiment shows synergic effect of hyperoxaluria, hypercalciuria, hypocitraturia, decrease in magnesium level and antioxidant properties, change in pH, and elevation in phosphorus and uric acid level leads to the development of lithiaisis. Concentration and the type of the extracting solvent are important for the effectiveness of the extract as it contains different phytochemicals like saponin, flavonoids, phenolic compounds, alkaloids, etc. Generally plants extract which are anti-microbial and have antioxidant properties are helpful in reducing stone formation and protecting from renal injury.

Keywords: Ethylene glycol, anti-urolithiatic, renal calculi, plant extract, calculogenesis

Introduction

Kidney stone also known as Urolithiasis is a quite common diseases accounting for the third most predominant infections and over 15% of the total population is affected making it a crucial public health issue [1,2] and it is expanding more in India. It is considered to be a minor problem but however if it is left untreated, at that point it might prompts serious renal issue. Numerous factors lead to the genesis of renal calculi in addition with the habit or the lifestyle, hereditary factors, dietary propensities, contamination in urinary tract, climatic condition or hypertension [2, 3]. Clinical or medicinal therapy mainly promotes to drink more volume of water (more than 3-4L) to normally release the stone , reduction of protein rich food or to perform a medical procedure i.e. surgery. Nowadays, many progressed technique like extracorporeal shock wave lithotripsy is utilized to breakdown the renal calculi formed. But this is costly and has some traumatic effects or remains fragments of stone leading to many side effects on the body like having renal fibrosis, haemorrhage or acute tubular necrosis [4]. Besides, the reoccurrence of stone is seen as a rule with reoccurrence rate as high as 50% [5].

Comparing to the synthetic method, Medicinal Plant extract is more efficient and harmless in the treatment of Renal Calculi. Some traditional treatment system like Unani, Ayurveda has been effectively using indigenous plant to treat renal calculi without any side effects known till date although the logical behind the cure is not yet established systematically. [6]. Some natural sources of citrate fruits like lemon, orange, mandarins are used to help reduced the stone formation and recently tomato juices is known to have a high citrate and magnesium concentration which can be used for treatment [7].

Formation of Renal Calculi or kidney stone is an elaborate and intricate process which is difficult to follow as it includes different stages which lead to the final development of stone. Starting with the accumulation of mineral salts like calcium followed by crystal nucleation, growth, aggregation and adherence leading to stone formation [8]. Crystal formation mainly occurs in the collecting system of the body or the distal tubule loop of nephron. Small size crystals can easily pass with the urine unnoticed but if the size is large then it remained adhered to the walls of the epithelial cells and later it turns into stone. Some common nephrolithiasis indication includes severe pain in the lower part of abdomen, painful urination, emesis, nausea, blood in urine [9].

The molecular structure, composition, dynamics and types of minerals involved in the formation of renal stones can be explored using Solid Stare Nuclear Magnetic Resonance Spectroscopy. For the characterisation of renal stones, ^{13}C and ^{31}P NMR is being used and for differentiating between Ca^{2+} in the stone and minerals containing calcium in the urinary tract, ^{43}Ca NMR can be used [10]. Thus, a thorough investigation and understanding of the pathophysiology is essential for treatment and prevention of stone formation.

Kidney Stone Classification

There are different types of renal calculi depending on the type of mineral it is formed from.

1. Calcium stones- They mainly constitute of minerals like calcium oxalate and rarely, calcium phosphate like Brushite [$Ca(HPO_4).2H_2O$] were also found. This type of stone is most regularly found in urinary tract and predominantly made up of calcium oxalate monohydrate but dihydrated and trihydrated also occurs infrequently. In the pathogenesis of urolithiaisis $CaC_2O_4.H_20$ crystals is more threatening due to its high affinity to cells of renal tubule and can easily break down the inner lining of the renal cells [11, 12]
2. Uric acid stone- they are formed when the Uric acid excretion is high in urine or when the urine is acidic (estimated about below pH 5.5). Sodium urate and ammonium urate might also present along with uric acid. Since they are invisible under X-ray, it can be diagnosed by using computerized tomography scan (CT scan). Uric acid stone are mostly accompanied with people with obesity and diabetes. In this, pH of the urine plays a vital role and a slight increase in pH will break the stone formation [13, 14].
3. Struvite stones- Bacterial species like Proteus, *Corynebacterium, Pseudomonas, Klebsiella* etc. which released Urease also infect the urinary tract. Urea present in the urine is being decomposed into ammonia and CO_2 by the urease making the urine more basic in nature [11]. This basicity of the urine enhances the accumulation of NH_4^+, CO_3^{2-}, and PO_4^{3-} as well as Mg^{2+} in favour of struvite stone formation [15, 16]. In women, most of the infection occurs because of shorter urethra [17].
4. Cystine Stone- It is genetic disorder caused by cystine leakage in urine. They are often called as ‘cystinuria’ [12]

80% of kidney stone is composed of Calcium oxalate with some calcium phosphate stone and 15-20% of other mixed stone accounts. The main principle behind renal stone aggregation and growth is believed to be the non-oxalate organic materials which are associated with calcium on the surface of the stone [12, 18].

Ethylene glycol induced Urolithiasis

The urinary system of Rat is human-like. The male rat model is thus often used for in vivo studies to imitate the human renal system. Ethylene glycol (EG) is used to induce urolithiasis in these rats to study the pathophysiology of formation of renal calculi. Drosophila is also often used and rodent is rarely used because they are less vulnerable to nephrolithiasis and a particular condition is needed to get a sustained hypercalciuria [19]. Sometimes ammonium chloride is also used along with ethylene glycol as it aids in metabolic acidosis.

EG is a component of many antifreeze, surfactants, surface coatings, industrial coolants emulsifiers etc. It is also an intermediate during synthesis of many chemical products like polyethylene terephthalate resins (PET), polyester fibres. Its exposure to human is mainly from using antifreeze. There is acute poisoning due to inhale of EG and kidney is majorly affected form it [20]

EG is toxic as it causes metabolic acidosis, cardiac arrest and renal failure in humans and animals. EG is swiftly absorbed and metabolized to form glycolic acid in liver. Glycolic acid is further oxidised to form glyoxylic acid and upon further oxidation of glyoxylic acid by glycolate oxidase producing oxalic acid as the end metabolite that precipitates with calcium to form calcium oxalate monohydrate (CaOx) (Fig 1) [21].

Hyperoxaluria or unrestricted urinary discharged of oxalate is induced by using ethylene glycol which increases the renal retention and oxalate concentration facilitating the formation of stones. Due to increase retention of oxalate, crystal deposition in intra-tubular epithelial cells, tubular injury and inflammation are often observed [22]. Hyperoxaluria is typically of two kinds:-

A) Primary hyperoxaluria: - It occurs when there is excessive secretion of oxalate by certain enzyme due to genetic disorder. The liver doesn't secrete enough enzymes that prevent overproduction of oxalate.
B) Secondary hyperoxaluria: - It occurs when the dosage of oxalate is high in the body due to consumption of food rich in oxalate like chocolate, coffee, berries, spinach [23]. Elevation in discharge of oxalate in urine facilitates the engagement of surplus oxalate with calcium increase the development of renal stone. The crystals of calcium oxalate are indissoluble and expand swiftly, blocking the urinary tract [22].

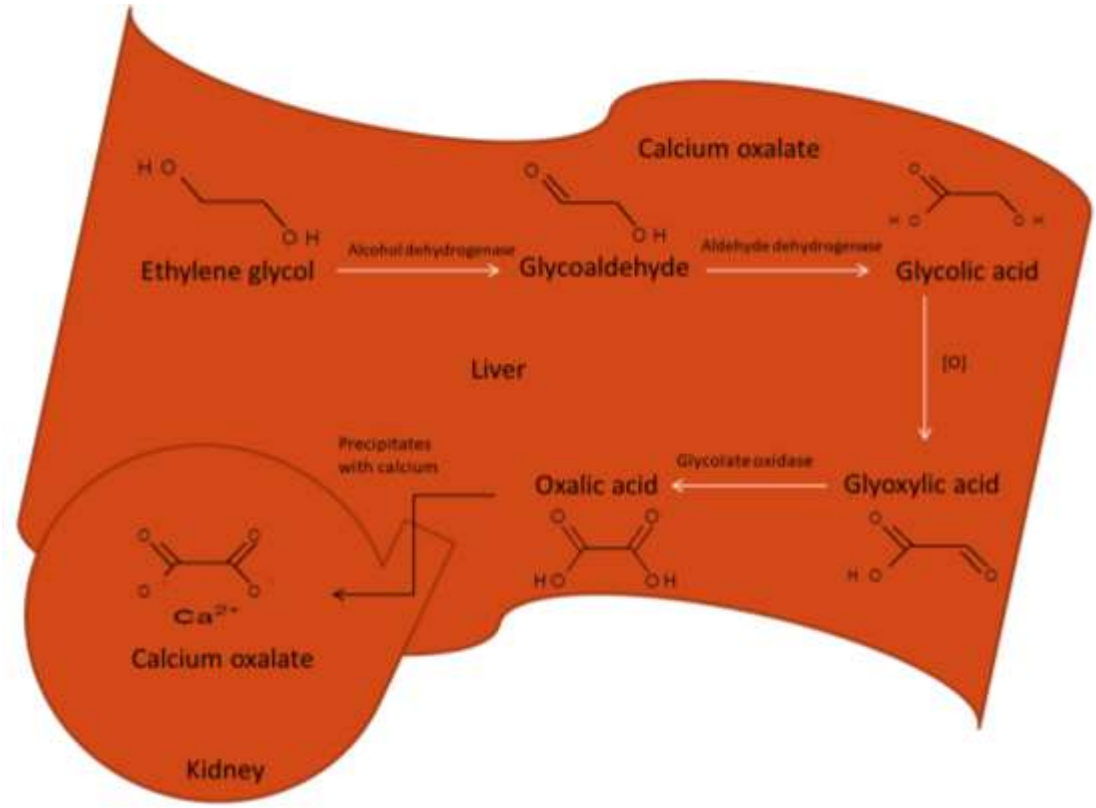

Fig 1: - Ethylene glycol induced lithiasis

Formation of free radical and peroxidation of lipids is also related to toxicity produce by renal stone formation. Crystal deposition brings damage to kidney epithelial cell and sometimes causes cell necrosis which leads to renal cell inflammation. Asymmetrical rise in dimethyl arginine level indicates demolition of renal endothelial cells [24, 25].

Reactive oxygen species (ROS) are produced by exposure of the renal tubular epithelial cells to Calcium oxalate crystals, causing oxidative stress that ultimately leads to cell damage. Cells generate several oxygen- scavenging enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, etc. to regulate the overloaded ROS. These function as a marker for indicating ischemia or damage in kidney tissue [26].

Inducing of lithiasis in Uninephrectomized state using ethylene glycol promotes damage in renal tissue and renal hypertension making it more vulnerable to cause urolithiasis. In order to retain a steady equilibrium composition of fluid in uninephrectomized animals, the remaining nephron raises the filtration load of urine, which could result in glomeruli dysfunction. In addition, secretion of lithogenic substances by urine also increases causing hypercalciuria, accompanied by renovascular hypertension. These suggest that elevated blood pressure is also associated with renal calculi [27].

Crystal growth is enhanced when there is super saturation of CaOx (calcium oxalate) in urine which increases nucleation for forming crystals and also produces oxidative stress when renal epithelial cell is exposed to the formed crystals [28]. Kidney oxidative stress has been revealed to develop from damage in mitochondria caused by Calcium oxalate monohydrate when oxygen consumption is low [29].

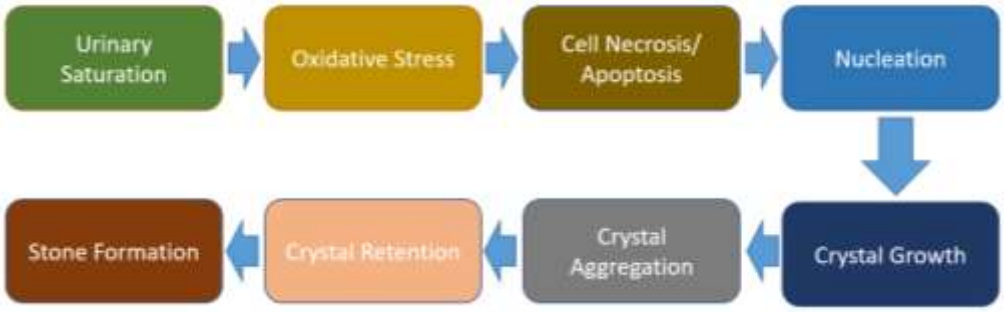

Fig 2: - General pathway for formation of stone [11]

Calculogeneisis

Renal calculi formation or calculogenesis is a complex mechanism with multistep and entangled process. The exact scientific mechanism has not been established, however, below are some interconnected events that occurs during the induced urolithiasis by ethylene glycol in vivo experiment (Fig 3)[8, 11, 18]: -

- Supersaturation of urine is one of the causative factors.
- An ideal environment for the growth and development of calcium oxalate stone is provided by forming a base with calcium phosphate crystal when there is increase in concentration of oxalate and calcium along with phosphate concentration [22]
- The rate of filtration in glomeruli is decrease due to blockage of urine outflow by stone in the urinary tract, resulting in an increase amount of waste product particularly nitrogenous waste product like uric acid, urea, BUN (blood urea nitrogen) in the serum. Lithiasis is encourage by uric acid as its binding protein binds to CaOx increasing the process of crystallization [14,20]
- Lipid peroxidation is also increased and the antioxidant potential of the kidney is reduced. Oxalate reacts with polyunsaturated fatty acid present in the renal cell membrane to facilitate in lipid peroxidation and also injures the renal tissues. Increase in serum creatinine shows the renal cell damage [21].
- In induced lithiasis, the urinary citrate level also reduced due to citrate reabsorption by renal tubular cells [23].
- In EG induced lithiasis, there is an increase in the release of glycolic acid oxidase (GAO) and Lactate dehydrogenase (LDH). The function of oxalate synthesizing enzyme is increased by GAO by elevating the supply of the substrate and LDH assist in deposition of oxalate in the renal cells due to its ability to catalysed oxidation coupling reaction and can reduced glyoxylate into glycolate and oxalate with the help of pyridine nucleotide coenzyme. LDH is a subtle predictor for severe renal injury [26-28]
- There is increase in malondialdehyde (MDA) and decrease in glutathione (GSH) and anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT) [30]
- Hypercalciuria also tends to nucleate and deposit calcium-oxalate crystals against the calcium phosphate formed base. But compared to hyperoxaluria, it is slightly significant in renal stone pathogenesis.
- There is remarkable decrease in body weight due to disturbance of fat, protein and carbohydrates metabolism by EG.
- The level of magnesium decreases while sodium and chloride ions concentration increases. While not noticeable, there is increase in urine volume and reversal of urine pH [27, 29].

- There is polymorphic irregular deposition of crystal on the inside of distal tubules causing the proximal tubules to dilate with interstitial inflammation [31]

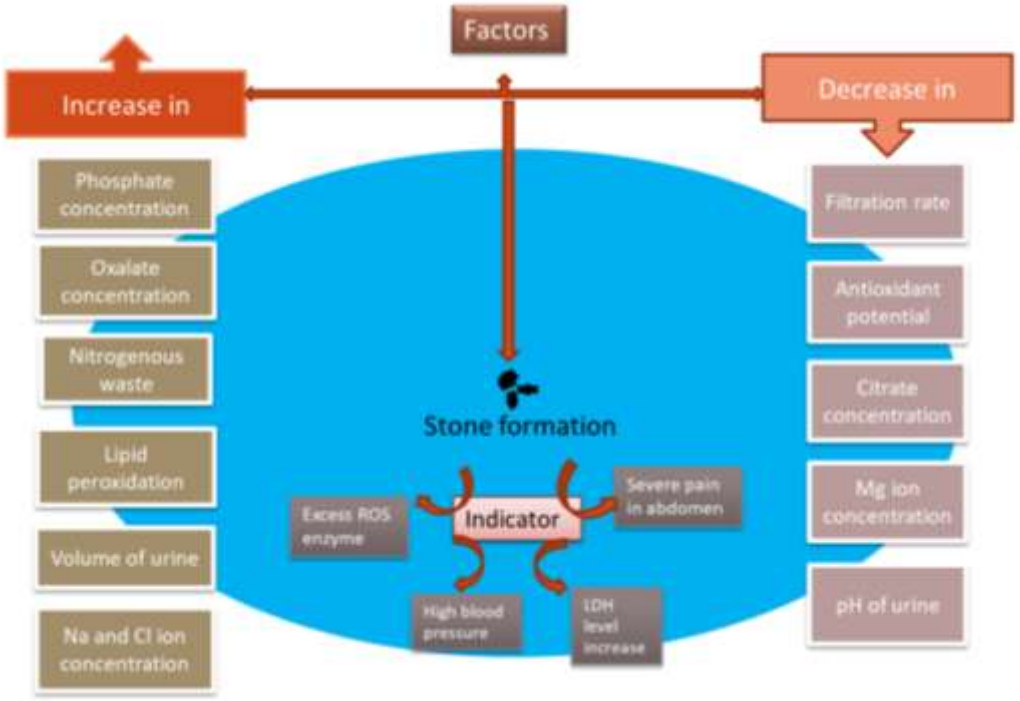

Fig 3: - Factors which helps in renal calculi formation.

Effect of Sex hormones in lithaisis.

In urolithiasis, role of gender is substantial. The rate of male stone occurrence is 2 to 3:1 ratio range relative to female [32]. Orchidectomy in male rats has been shown to decrease renal calculi development and there is no formation of stone in female rats whose ovaries have removed. This has shown that testosterone, a male sex hormone plays a significant role in development of renal calculi [33].

In women, citrate excretion is more so the formation of calcium stone is less prevalent in women as compared to males. Citrate forms a calcium complex, thus reducing calcium stone crystallization. In other words, with an elevation in concentration of citrate, the saturation of calcium oxalate and calcium phosphate decreases [34].

The function of Glycolic acid oxidase which is to form oxalate is intensified by testosterone while Estrogen hormone, estradiol decreases the oxalate formation rendering the female uninjured rats less prone to calcium stone. Moreover, reabsorption of calcium in blood from the renal cells is enhanced by estrogen while testosterone and androgens oppose to it 35].

Effect on Endoplasmic Reticulum

The synthesis of proteins transpires primarily in the lumen of Endoplasmic reticulum (ER). Increase in oxalate level or Hyperoxaluria caused ER stress by affecting protein folding in ER. This leads to accumulation of proteins in ER lumen which are unfolded or mis-folded. This change the expression of osteopontin, an enzyme to reduced crystal growth produced by inflammatory environment and injured cells [36].

ER is also vulnerable to any extra cellular stimuli that interfere with its normal cell function. Stone inhibition protein is mostly N-glycosylated, so they primarily experience protein folding malfunction in ER and eventually assist in stone forming progression [37].

A small molecule known as chaperon is used to restore the protein folding ability of ER to stabilise the protein conformation. 4-Phenylbutyric acid, a chaperone has been found to mediate the oxidative disorder of ER stress by reinstating the function of antioxidant proteins, thus decreasing the potency of renal calculi [38].

Ascorbic acid for inducing Urolithiasis

L-Ascorbic acid/ Vitamin C are also used for inducing lithaisis in rat model. It is an important vitamin present in fruits and vegetables that is soluble in water. In order to eradicate harmful reactive oxygen species (ROS), ascorbic acid acts as an antioxidant and also serves as a catalyst, a cofactor for many enzymatic reactions. Vitamin C deficiency causes scurvy and 480 mg/day is the minimum requirement. If the intake is more than 2 g/ day lithiasis may be caused, leading to formation of stone in kidney [39]

Oxalate is formed from ascorbic acid by series of oxidation of ascorbic acid to dehydroascorbic acid and diketogulonic acid which at last breakdown to form oxalate (Fig 4). With the aid of urine oxalate is excreted out but if the quantity is high, supersaturation occurs, which eventually contributes to stone formation [40]. Lithiasis induced by ascorbic acid can be serious if not properly treated. This could lead to persistent infection in renal, chronic hemodialysis, transplantation or even death [41].

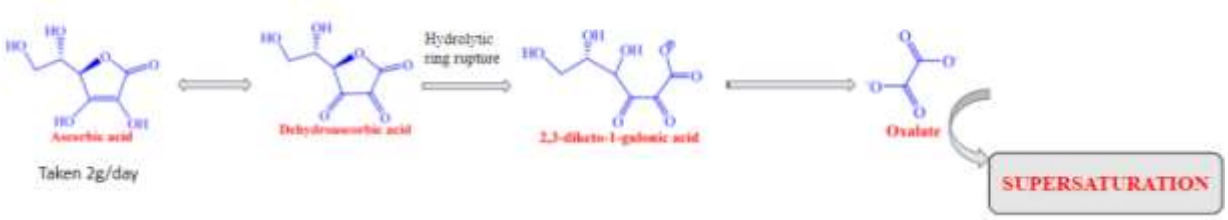

Fig 4:- Pathway of forming oxalate from ascorbic acid

Table: -Some Plants extract which can be used as anti-lithogenic are as follows:

S.No.	Parts used	Plant	Solvent used	Effects	Ref.
1.	Leaves	*Pavania lasiopetala*	Aqueous extract	By breaking up into small fragments, it prevents supersaturation, nucleation and crystallization of CaOx crystals.	42
		Cannabis sativa			43
		Euphorbhia hirta	Hexane extract		44
		Bauhinia variegata L	Ethanolic extract		45
		Duranta erecta	Methanolic extract	Antimicrobial activity protects the urinary tract infection and inhibits CaOx formation	46
		Copaifera lucens	n-butanoic	Galloylquinic acid of	47

			Extract	the extract's act as antiurolithiatic	
		Kalanchoe pinnata	Ethyl acetate extract	Prevent kidney tissue oxidative damage	48
		Vitis vinifera	Ethanolic extract	Prevent formation of stone and injury of renal cells	49
2.	Aerial parts	*Eryngium campestre*	Aqueous extract	Reduce CaOx crystal deposition	50
		Hygrophila spinosa	Methanolic extract		51
3.	Roots	*Pituranthos scoparius*	Hydro methanolic extract	Treatment of lithasis by dissolving the retaining CaOx stone in the lumen	52
		Cyperus rotundus L			53
		Rubia cordifolia			31
		Daucus carota	Ethanolic extract		54
		Salvia miltiorrhiza	Aqueous extract	Decreasing the rate of crystals formation	55
		Moringa oleifera		Reduce and prevent growth of urinary stone	70
		Angelica sinensis	Polysaccharide extract	Maintained pathological changes of kidney and prevent crystallization	56
4.	Fruits	*Emblica officinialis*	Ethyl acetate extract	Inhibit CaOx crystals growth	48
		Solanum xanthocarpum	Aqueous extract	Saponin fraction decreases stone formation.	71
		Tribulus terrerstris		Protects from injuring renal cells and maintained the ion concentratiom	57
		Viburnum opulus L (Gilaburu)		Aids in easily stone passage urine which are less than 10mm	58
5.	Intact plant	*Peladium murex* L	Ethyl acetate extract	Inhibiting and dissolving the renal calculi formed	59
		Cynodon dactylon			48
		Ceterach officinarum	Aqueous extract	decrease the size of CaOx crystals	60
		Pericampylus glaucus	Ethanol extract	Ionic concentration is maintained reducing the calculi formed and preventing the renal wall from damage	61
		Solanum virginianum			62
		Leea macrophylla		Normalized renal	20

				impairments and reduce crystal deposits	
		Mucuna pruriens	Methanol extract	Inhibit crystals aggregation and growth	63
6.	Seeds	*Prunus mahaleb L*	Methanol extract	Prevent deposition and formation of stone	64
		Dolichos biflorus	Aqueous extract		65
		Parsley crispum	Ethanol extract	It decrease the calcium ion level in serum	66
7.	Stem/ bark	*Bambu sanutans*	Ethyl acetate extract	Reduce the calculi formed and ion concentration	48
		Cinnamomum zeylanicum	Hydro alcoholic extract	Inhibits crystallization of stone	67
		Mimusops elengi			30

Phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, and steroids were predominantly found to be present in the qualitative and quantitative analysis of all the extracts. The synergistic effect of all the phytochemicals may contribute to the anti-lithogenic activity of the plant extracts described before. Saponin has the ability to destroy the crystal formed during lithiasis as it can demolish the accumulation of mucoprotein abeyance which is regarded as promoter for stone formation [68]. Terpenoid also helps in reducing the size of the crystal inhibiting stone formation [69].

The extract of plants acts on different stone forming stages and their effect is dose dependent. Various phytochemicals have different solvent affinity and hence the anti-oxidant often depends on the kind of solvent used for extraction [72]. It is revealed that extract of E. *hirta* in hexane and ethyl acetate extract of O. *stamineus* with high alkaloid content as contrast to other solvent have a greater effect on inhibiting CaOx crystallization [44]. Methanolic extract of M. *pruriens* and ethanolic extract of B. *variegate* are more effective in inhibiting crystals formation then the same plant extract on other solvent like aqueous extract [45, 63].

Some plant also produces inhibitors that prevent the crystallization of calcium oxalate, such as Glycosaminoglycans (GAGs) [45]. The renal cells are protected by Lanostane which have anti-inflammatory properties. In the treatment of renal stone caused by bacterial infection, the antimicrobial property of the extract also helps [57].

Brief discussion on effect of plant extract

- It mediated urine ionic concentration by decreasing calcium ion concentration and increasing concentration of magnesium and citrate. Magnesium prevents the development of stones by forming soluble oxalate complex thus preventing the calcium to bind with the oxalate.
- Treatment with extract control the uric acid level thereby fastening the process dissolving the formed stone.
- Plant extract restored the oxygen scavenging activity of a cell and minimise renal tissue damage thus reducing crystal deposition.

- The stone formed is disintegrate and promotes easy passage of nitrogenous waste through urine.
- Tissue pathology of kidney is normalized to minimize renal stone damage and also prevent body weight loss.
- Volume of urine is maintained by the diuretic potential of extract and pH of urine is also restored [68]

Thus, an imbalance between inhibitors and promoters like oxalate, calcium, phosphate act as a potential factor for renal calculi pathogenesis.

Regulators in Urolithiasis

- **Ferulic acid**
  It is a phenolic compound which is a secondary metabolite present in almost all vegetables and fruits cell wall. It has antioxidant effect and has many pharmacologic effects that can cure cancer, diabetes, inflammation or ageing.
  Experiment on rats with a commercially accessible ferulic acid was conducted by Zhao *et al* (2019). He found that a higher dose of ferulic acid (about 80 mg/kg) developed a greater effect and prevented of renal calculus by controlling the anti-oxidant enzyme against ethylene glycol induced oxidative stress. Free radicles produced during EG metabolism are removed by reducing the degree of lipid peroxidation. Ferulic acid's anti-inflammatory properties also helps in decreasing the the potential to develop renal calculi.
  There is increased in the release of enzyme like ALP (alkaline phosphates), gamma glutamyl transferase (GGT) and LDH (lactate dehydrogenase) during structural cell damage in kidney due to oxidative stress caused by it which decreases the function of oxygen scavenging enzymes [73].
- **Vinegar**
  Acetic acid, the main ingredient of vinegar, has been found to help minimize CaOx crystal deposition in renal cells by altering androgen receptors [74].
- **Caffeic acid (3, 4-dihydroxycinnamic acid)**
  It is a phenolic compound with high antioxidant property, primarily classified in some fruits and herbs (B. *nutans*, P. *murex*, C. *officinarum*) as hydroxycinnamic acid. It regulates osteopontin expression. Caffeic acid treatment preserved the ionic concentration and inhibits peroxidative damage in renal cells [60, 75].
- **Pentoxifylline**
  It is a xantenes derivative drug which is used for the treatment of PAD (peripheral artery diseases). It decreases inflammation and functions as a non-specific phosphodiesterase inhibitor that controls TNF i.e. tumour necrosis factor α. It has recently shown to have anti-oxidant effect that is capable of regulating ROS (reactive oxygen species) [76-78].
- **L-arginine**
  L-arginine, a semi-essential nitrogen rich amino acid, reduces the risk from developing renal calculi by controlling citrate concentration, uric acid level and pH of urine. It also has anti-oxidant property that neutralizes the oxidative stress caused by ROS during lithiasis [79].
- **Galloylquinic acids**
  Hydrolysable tannins, also known as gallotanins are galloylquinic acid derivatives. They have anti-oxidant or ROS scavenging property which depend on the amount of galloyl moieties present. The highest anti-oxidant property are those of 3, 4, 5-tri-O-

galloylquinic acid and has been shown that it reduces size and nucleation of CaOx crystal by preventing the formation on the cell surface [80, 81].

Conclusion

The causes and precise mechanism of Urolithiasis are still not understood in these current scenarios. But based on the changes in pathophysiology of the induced lithiasis in vivo experiment, it has been concluded that stone formation is responsible by the synergic effect of all factors discussed above. About more than 200 conventional therapeutic plants were investigated to have antilithiatic property and studies have been carried out on how it affects renal stone pathology. But the secondary metabolites responsible for anti-lithogenic activity is still remain elusive, although a relative understanding of how alkaloids, flavonoids, phenolic compounds etc. cease some stages in the development of stone by its anti-oxidant potential. The treatment was found to be dependent on the quantity of extract used and also rely on the form of extracting solvent used. Further work is required to be done to understand the chemistry behind lithogenesis so that a very refine and more reliable medication will be found in future to treat the renal calculi without fear of reoccurrence after the procedure. And hopefully this analysis will be of great benefit to those working in this area.

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