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Urolithiasis is a broad term describing the concretions formed in the whole urinary system, predominantly in kidneys and ureter. These abnormalities may also be formed in the lower urinary system i.e. bladder or urethra (Bernier and Sims, 2009). In clinical context, nephrolithiasis refers to the presence of calculi specifically in the kidneys. Though metabolic and environmental risk factors had been proposed for the formation of kidney stones, yet the mechanism of stone formation is multifaceted (Sakhaee et al., 2012). Kidney stone diseases are the third most common type of urinary tract related ailments to the mankind since ages. The evidence of kidney stones had been found by researchers in a 7,000 year old Egyptian mummy (Prien and Frondel, 1947) advocating the possibilities that only modern life style and eating habits are not the culprit for kidney stones.

Urolithiasis is a global health issue, spanning all geographic regions with an estimated 1% annual incidence rate with 3-5% prevalence and 15-25% lifetime risk of developing kidney stones. In majority of the patients urolithiasis is a recurrent clinical morbidity (Lotan et al., 2004). Moe had described that the occurrence of kidney stone at some time in their life is twice in men (12%) as compared to women (5%) in America in 2006 (Moe, 2006). Rizvi et al., (2002) had reported a prevalence rate of 15% in India. There are two high incidence stone belts reported in India. Majority of the cases had been reported from Amritsar to Agra in UP while passing through Delhi in North India (Ganesamoni and Singh, 2012). The second belt ranges from Jamnagar in west coast extends inwards towards Jabalpur in central India. Very low incidence areas have been reported in West Bengal and coastal areas of Andhra Pradesh, Maharashtra, Karnataka, Tamilnadu and Kerala (Aggarwal et al., 2014a). Nephrolithiasis is largely a recurrent disease with a relapse rate of 50% in 5–10 years and 75% in 20 years (Trinchieri et al., 1999). Considered once a male dominant disorder, the prevalence of renal stone disease has been on the rise in both females and males. Urolithic disorders remain the major economic and health burden worldwide. The comorbidities include but not limited to
bone loss and fractures, chronic kidney disease (CKD), coronary artery disease, type 2 Diabetes mellitus, hypertension and the metabolic syndrome (Sakhaee et al., 2012).

Symptoms of urolithiasis depend mostly on the size and location of calculi in the urinary tract. These symptoms generally include acute ureteral or renal colic, hematuria (microscopic or gross blood in urine), urinary tract infection (UTI) or vague abdominal or flank pain. The calculus may be lodged at ureteropelvic junction (the junction of the kidney and ureter) and hence may result in acute ureteral obstruction with severe intermittent colic flank pain (Colella et al., 2005). While the calculi that can pass into the ureter may lead to ureteral colic (an acute, sharp, spasm-like pain) located in the flanking region. Finally if the calculus reaches into the bladder the only signs are the urgency, dysuria and frequency of urination.

There are different types of renal calculi based on their chemical composition. The most common type of stones is the combination of calcium oxalate and calcium phosphate stones (Coe et al., 2010). The most common type of calculi are composed of calcium oxalate (CaOx) and calcium phosphate (CaP) (80%). The other minor types reported are Struvite stones (10%) and uric acid stones (9%). About 1% of the calculi are made up of either cystine or ammonium acid urate or may be diagnosed as drug-related stones (Coe et al., 2005).

Despite detection of urinary stones hundreds of years ago, their pathogenesis and prevention/cure are not fully understood (Hou, 2013). As described earlier the pathogenesis of CaOx stone formation is a multifaceted process which involves multiple steps. The steps are generally categorised as nucleation, crystal growth, crystal aggregation and crystal retention. It is a multifactorial disease resulting from the complex interaction of genetic predisposition and environmental factors which regulate calcium salt precipitation in the urinary system. Stone formation usually results from an imbalance between factors that promote urinary crystallization and those that inhibit crystal formation and growth (Coe et al., 2005). Both Cystinuria and hyperoxaluria are inherited metabolic disorders which may lead to nephrolithiasis. Especially, hyperoxaluria is predominantly involved as a risk factor of human idiopathic calcium oxalate disease. This enhances CaOx supersaturation and stone formation (Sharma et al., 2015). In renal
epithelial cell cultures, oxalate toxicity is accompanied by the generation of reactive oxygen species (ROS) (Jonassen et al., 2003; Thamilselvan et al., 2003). High oxalate concentration imposes oxidative stress on renal cells by stimulating the accretion of lipid peroxides while decreasing the accessibility of major cellular antioxidants, such as reduced glutathione (GSH) (Selvam, 2002). Inflammation and intracellular morphological changes via oxidative stress are substantive to renal calcium crystallization (Khan, 2005). Unknown infections are also been associated with the risk of urolithiasis especially the struvite stones. The drug related stone formation is reported to be associated with the use of calcium-based antacids (diuretics). These chemicals may enhance the risk of nephrolithiasis by increasing the calcium excretion in urine. Mineral metabolism is important in formation of urinary stones or calculi (Halabe and Sutton, 1992). Drug induced urolithiasis has been reported to be associated with ephedrine and its derivatives (Blau, 1998). Acidic urine which might be due to the deficiency of xanthine oxidase, causes crystal precipitation, resulting in stone formation (Bernier and Sims, 2009). Renal injury promotes crystal retention and the development of a stone nidus on the renal papillary surface and further supports crystal nucleation at lower supersaturation (Fasano and Khan, 2001).

As the disease is multifactorial so are the treatment options. The wide range of therapeutic options includes allopathic, homeopathic, ayurvedic management besides surgical interventions. All of these options have seen revolutionary changes over the years. Modern day intervention tools smooth the progress of passage of endoscopes up the ureter into the kidney pelvis and facilitate local stone rupture using high powered lasers (Bagley, 2002). Many techniques such as extracorporeal shock wave lithotripsy (ESWL) and Percutaneous Nephrolithotomy (PCNL) have revolutionized the management of kidney stones but are still associated with side effects and kidney damage (Michel et al., 2007). Quite a few synthetic chemicals have also been identified as inhibitors of crystallization and oxalate biosynthesis. Many of them are under clinical trials like α-blocker or calcium-channel blockers (Hollingsworth et al., 2006), bendroflumethiazide (Wolf et al., 1983), hydrochlorothiazide (Laerum and Larsen, 1984; Scholz et al., 1982), trichlormethiazide (Ohkawa et al., 1992), allopurinol (Ettinger et al., 1986) etc. These compounds need to be evaluated to prove their potential as prophylactic
efficacy for lowering incidence of urolithiasis. Besides, these treatments cause undesirable side effects such as haemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation (Manjula et al., 2012), therefore the focus is now shifting from synthetic chemicals to herbal compounds for treatment of urolithiasis. Infusions of Paeonia officinalis, Mentha spp and Cicer arietinum for dissolving kidney stones had been reported since 23-79 AD. Many of the different herbal preparations are documented in hindu writings for dissolving the stone.

Pashanbheda is an important most widely used herbal medicine reported in Ayurvedic system of medicine as a diuretic and lithotriptic formulation. Experiments have shown that supplementation of agents which could decrease oxidative stress was able to rescue the cells from oxalate-induced toxic effects (Hovda et al., 2010). Now-a-days various phytotherapeutic agents have been proposed as useful alternative or complementary therapies for the management of urolithiasis, in part due to their antioxidative effects. In the last few years the potential efficacies of natural compounds and herbs have been documented for the treatment of urolithiasis (Aggarwal et al., 2013a). There are various antioxidants which have been shown to reduce oxidative stress. Recently, N-acetyl cysteine (NAC) and vitamin E have been found to be effective agents in treating hyperoxaluria in rat model (Sharma et al., 2015). Similarly, 1,2,3,4,6-Penta-O-galloyl-beta-D-glucose, a water soluble gallotannin can also reduce the urinary oxalate crystal excretion and curtailing reactive oxygen species (ROS) production in ethylene glycol (EG) induced human primary renal epithelial cells. Some of the popular marketed composite antiurolithiatic herbal formulations such as Cystone (Himalaya Herbal Healthcare, India), Calcury (Charak Pharma Pvt. Ltd.), Neeri (Aimil Pharmaceuticals, India), Uriflow (BioNeutrix Healthcare of Brooklyn, New York), Uriflush (Global Biosciences, Indonesia) and Culdisol (Ganga Pharmaceuticals, India), Divya Vrikkdoshar Kwath (Swami Ramdev's Divya Pharmacy) have been used globally (Chitme et al., 2010). The use of the term ‘Pashanbheda’ has been documented for many diuretic and other plants including Rotula aquatic, Alternanthera sessalis in Mysore (Chauhan et al., 2011), Aerva sp. in South India (Yasir and Waqar, 2011), Ammaunia baceifera in Kerala, Bauhinia racemosa, Coleus spp., Bryophyllum spp., Didymocarpus
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pedicellata, Ocimum basilicum in Bengal (Tiwari et al., 2012). Fruits of Tribulus terrestris (Gokhru) had been documented valuable as diuretic and in the treatment of nephrolithiasis (Aggarwal et al., 2012a). Also, an animal study reported the effectiveness of Varuna and Kulatha in preventing the deposition of the stones (Kamboj et al., 2011). The role of coconut water as an antiurolithiatic agent had been reported recently (Gandhi et al., 2013). The mechanism of action of this herbal remedy includes the inhibition of oxidative stress related genes and hence reduced mineral deposition in kidney tissue.

Bergenia ligulata syn. Saxifrega ligulata, is a perennial herb. The morphology of the plant highlighted its thick root stock, short and fleshy stem with white, pink or purple flowers. This plant has been found in the temperate regions of Himalayas at the altitudes ranging from 2,000 and 2,500 meters which include the regions from Kashmir to Bhutan. Many of the traditional pharmacological uses of B. ligulata had been documented in the past several years. The medicinal use of its roots are the most commonly used plant part and the pharmaceutical analysis revealed the presence of important agents including tannic acid, gallic acid, starch, mineral salt, albumin, glucose, mucilaginous matter, wax and aromatic substances. The presence of these molecules are the reason behind their usage as diuretic, an antidiabetic, wound healer, cardiotonic, astringent, expectorant, antipyretic and anti-haemorrhoidal drug (Kirtikar and Basu, 1983). Also, the roots have been used as an antipyretic tonic, in diarrhoea and also as an anti-scorbutic agent. Its extract has skin whitening ability, anti-oxidant, anti-aging and anti-inflammatory abilities. Also, the alcoholic extract has significant analgesic, antibacterial and diuretic properties (Sajad et al., 2010; Sinha et al., 2001a). Its composition makes it effective as a treatment option for the skin around the eyes (Solanki, 2011). The systematic studies and the trails are scarce for highlighting the scientific evidences for the usefulness of ‘Pashaanbheda’. More studies will surely help in identifying novel pharmaceutical remedies for the effective treatment of urolithiasis.

In the indigenous medicine system, B. ligulata is the topmost botanical source of ‘Pashaanbheda’ drug. Bergenia ligulata as a whole plant or parts such as roots and rhizomes are used for kidney and bladder stones and other problems related to urinary system. Rhizome is the primary constituent or source of drug. It is light, cool, bitter, has useful effect in cough and cold. Some preliminary studies had evaluated the antiurolithic
potential of *B. ligulata* rhizome. The aqueous extract of *B. ligulata* rhizome inhibited not only the homogenous precipitation of calcium oxalate crystals (Garimella *et al.*, 2001) but also inhibited *in vitro* growth of calcium oxalate and calcium hydrogen phosphate dihydrate crystals (Joshi *et al.*, 2005a; Joshi *et al.*, 2005b). *Bergenia ligulata* has also been shown to possess antilithiatic properties both *in vitro* and *in vivo* system (Bashir and Gilani, 2009) but there is no report about its active antilithiatic metabolites. The identification of the active metabolites from *Bergenia ligulata* is important as it can provide a base for the formation of an effective antilithiatic drug.

The present work has been designed to understand the role of *Bergenia ligulata* rhizome in preventing and treating kidney stones and to further explore the effective metabolites from the crude extract.

**OBJECTIVES**

1. To study the effect of rhizome extract of *Bergenia ligulata* on the calcium phosphate and calcium oxalate crystallisation.
2. To study the *in vivo* antilithiatic potential of crude extract.
3. Isolation of effective antilithiatic metabolite(s) from *Bergenia ligulata* rhizome.
4. Characterization and structural elucidation of the effective antilithiatic metabolite.
5. Validation of the active metabolite(s) against calcium oxalate crystal formation in rat urolithiatic model.
6. To study the effect of potent metabolite(s) on CaOx induced lipid peroxidation in liver and kidney of experimental rats.