**Research Article**

**SAPONIN RICH FRACTION OF BAUHINIA VARIEGATA LINN. AMELIORATES KIDNEY STONE FORMATION IN RATS**

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ABSTRACT: The present study was planned to know the effect of saponin-rich extract of Bauhinia variegata Linn. (SREBV) in an ethylene glycol induced urolithiasis model. The qualitative and quantitative analysis of SREBV revealed presence of β-sitosterol, stigmasterol and lupeol. Administration of ethylene glycol (0.75 % v/v in distilled water) to male Wistar rats for 28 days successfully induced urolithiasis with hyperoxaluria, hypercalciuria as well as an increased renal excretion of uric acid and inorganic phosphate. Supplementation with SREBV significantly decreased these levels of stone promotor via diuresis and increased levels of stone inhibitor like magnesium. There was a significant reduction in creatinine, uric acid, and blood urea nitrogen in rats administered with SREBV. Moreover, reno-protective effect is evident by decreased deposits of calcium-oxalate crystals in the kidney tissue of SREBV treated rats. The antilithiatic activity of SREBV is also supported by its radical scavenging activity portrayed in DPPH assay in-vitro as well as alleviation in lipid peroxidation and improvement in antioxidant enzyme levels in-vivo. With this study, it was clinched that the SREBV supplementation safeguarded EG-induced urolithiasis as it abbreviated the growth of urinary stones. The mechanisms attributing to this effect might be due to its antioxidant, diuretic, nephroprotective and curbing in stone-forming constituents.

**Key words:** Antiurolithiatic effect, Antioxidant, Bauhinia variegata, Calcium oxalate, Saponin.

**INTRODUCTION**

Urolithiasis, *i.e.*, formation of kidney stone, is one such disease which remains incurable even after tremendous research contributed in the field of urology. Urolithiasis being the third most (Raheem *et al.* 2017) afflicted urological diseases in the world, has an important effect on the health care system with high recurrence rate up to 50% in the first 5 years of the initial stone episode (Khan *et al.* 2016). Calcium oxalate (75%–90%) is the most predominant component of calculi, followed by uric acid (5%-20%), calcium phosphate (6%-13%), struvite (2%-15%), apatite (1%) and cystine (0.5%-1%) (Liu *et al.* 2018). The highest occurrence of stone disease has been reported in men than women (Alelign and Petros 2018).

The lithogenesis is multifactorial (Knoll 2010) and influenced by epidemiological, biochemical and genetic risk factors. It involves formation of all types of urinary calculi in the renal, which possibly will deposit throughout the entire urogenital tract from the renal pelvis to the urethra. The pathogenesis differs mainly based on the stone phenotype. Even though a prodigious convalesce has been occurring into the core insight of stone pathogenesis; the significance of urine biochemistry is still questionable, and this is the main reason for failure in development of antilithiatic agents. Several lithiasis promoters such as oliguria, low pH, calcium, oxalate etc., and inhibitors (organic inhibitors/macromolecules *viz.* glycosaminoglycans, uromodulin, etc., and inorganic inhibitors *viz.* magnesium, citrate, etc.) plays the major role in crystal formation. Currently in modern medicine, the treatment comprises of either administration of symptomatic drugs like thiazide diuretics, alkalizing agents, allopurinol, anti-inflammatory etc., or use of other invasive procedures like extracorporeal shock wave lithotripsy, retrograde intrarenal surgery (RIRS), laparoscopic ureterolithotomy or robotic surgery and percutaneous nephrolithotomy (Shafi *et al.* 2016). However, these therapies come with many risks like decrease in renal function, acute renal injury, and an increase in stone recurrence. Moreover, the adverse
effects of oral drugs compromise long term usage of them (Onaran et al. 2016). So, the physicians remain dependent on alternative systems of medicine for better relief. The recent era has observed dramatic expansion regarding the knowledge of molecular mechanism of plant derived therapeutic agents. With elucidation of the mechanism of action they have secured place in current regimen as alternative or adjunct therapy. The multicomponent plant system may exert their effect by many mechanisms like diuresis, anti-inflammatory, analgesic and anti-oxidant activity. However, the rational for their use need to be established through systematic pharmacological and clinical studies. Hence, the present study intends to establish the scientific rationality of the antiurolithiatic activity of one such herbal plant, Bauhinia variegata Linn. (Caesalpiniaceae) is a medium-sized deciduous tree, commonly known as (Sanskrit) kanchanara, (Hindi) kovidara. All the parts of the plant are used to treat like asthma, ulcer, liver complaints, cancer, diabetes, inflammation, infections. The leaves of B. variegata consists of lupeol, saponins, terpenoids, β-sitosterol, alkaloids, oils, fat glycoside, phenolics, lignins, tannins, kaempferol- (Fouada et al. 2006) 3-glucoside, rutin, quercetin, quercitrin, apigenin, apigenin-7-O-glucoside, amides (Mamillapalli et al. 2016, Yadav et al. 2016). Previously, Mamillapalli et al. reported in-vitro inhibitory activity of aqueous and ethanolic extracts of leaves of Bauhinia variegata Linn. on calcium oxalate crystallization (Mamillapalli et al. 2016). In the present study, we have used saponin rich extract of Bauhinia variegata (SREBV). Saponins are a diverse group of compounds extensively distributed in the plant kingdom, which is characterized by their structure comprising a triterpene or steroid aglycone and one or more sugar chains glycone (Güçlü-Ustündað and Mazza 2007). Numerous herbs rich in saponins themselves have the potency to prevent the formation of stone (Fouada et al. 2006, Joshi et al. 2005) by blocking their adherence to renal cells averting them from overgrowth and formation of plaques through coating the crystals at initial step and provides the smoothness to crystal surface, along with this it displaced pre-bound crystals from the tissue (Fouada et al. 2006). Moreover, pentacyclic triterpenoidal saponins also found to be efficient in reducing the risk of stone formation by way of preventing crystal-induced tissue damage, dilution of urinary stone-forming constituents, and restore the normal status of antioxidant enzymes (Malini et al. 2000). With the above-mentioned facts, it was thought worthwhile to evaluate antiurolithiatic activity of SERBV on an experimentally induced in-vivo lithiasis in male Wistar rats.

MATERIALS AND METHODS
Collection and identification of plant material
Bauhinia variegata Linn. (English: Mountain ebony, Gujarati: Kanchnar) leaves were collected from Anand, Gujarat in the month of December 2018. The sample was authenticated by the Pharmacognosy specialist, Anand Pharmacy College, Anand, Gujarat, India. A voucher specimen of the plant was deposited in the herbarium (APCH-56).

Drugs and chemicals
Standard β-sitosterol (≥95%), lupeol (≥94%), stigmasterol (~95%), bovine albumin, epinephrine, thiobarbituric acid (TBA), tris buffer, 5-5’-dithiobis [2-nitrobenzoic acid] (DTNB, Ellman’s Reagent), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5-thionitrobenzoic acid (TNB), were used in this project were of analytical grade and were obtained from Sigma Aldrich Pvt. Ltd, Hyderabad. Ethylenediaminetetraacetic acid (EDTA), sodium citrate, carboxy methyl cellulose (CMC), sodium dihydrogen phosphate (NaH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄), sodium bicarbonate (NaHCO₃), sodium carbonate (Na₂CO₃) were of analytical grade and were obtained from Merck Pvt. Ltd., Hyderabad. Calcium chloride (CaCl₂), magnesium sulphate (MgSO₄), manganese sulphate (MnSO₄), sodium chloride (NaCl), and potassium permanganate (KMnO₄) were of analytical grade and were obtained from Chemdyes Cop., Rajkot. Standard drug ‘Cystone’ was procured from Himalaya Drug Company Ltd., India. All the biochemical tests had been performed using commercially available kits purchased from i-Chem, Jeev Diagnostic Pvt. Ltd., Chennai, India.

Preparation of SREBV from dried leaves of B. variegata
Saponin rich-extract of leaves of B. variegata was prepared according to method reported by Obadoni and Ochuko (2001) with few modifications. After authentication of plant, fresh leaves were dried at room temperature in the shed, powdered, and then forty grams of the dried powder was extracted with n-butanol. The mixture was heated at 95°C under reflux for 5 hours. The resultant extract was filtered and concentrated to dryness by evaporation at 55°C. The resulting extract was weighed and stored at room temperature.

Qualitative analysis of SREBV
The phytochemical tests namely Foam test for saponins (Fouada et al. 2006), Salkowski test for triterpenoids, Liebermann burchard test for steroids (Patel et al. 2016), Dragendorff test for alkaloids, FeCl₃ test for phenols and
tannins (Bargah 2015), Shinoda and fluorescence test for flavonoids, and Keller killiani test for glycosides (Panchal 2012) were performed.

**Quantitative analysis of SREBV**

Estimation of total saponin content (TSC): vanillin-sulphuric acid assay

The TSC in SREBV was determined according to reported method by Le et al. (2018).

**High performance thin layer chromatography**

The β-sitosterol, lupeol and stigmasterol present in SREBV were determined by HPTLC (Karthika et al. 2014). Standard α-sitosterol, lupeol, and stigmasterol were used as a reference standard. Test sample (15 µL) and standards (5 µL) were applied on pre-coated silica gel 60 F<sub>254</sub> TLC plate of thickness 0.2 mm using automatic TLC applicator. The plate was developed in 20 x 10 cm<sup>2</sup> twin trough glass chamber previously saturated with mobile phase. Two mobile phases, n-hexane: ethyl acetate: formic acid: acetic acid (6.8:2.94:0.09:0.09) for detection of β-sitosterol, and Toluene: ethyl acetate (7:3) for detection of stigmasterol and lupeol were used for system development. The developed plate was dried, derivatized with Anisaldehyde H<sub>2</sub>SO<sub>4</sub> reagent, and scanned using Camag HPTLC scanner at 525 nm. The quantification analysis and Rf values were recorded.

**DPPH assay**

The free radical scavenging activity (RSA) of the plant extract was performed to measure antioxidant activity using the DPPH assay method (Thaipong et al. 2006). In brief, 24 mg of DPPH was dissolved in 100 ml methanol to make stock solution (24 mg/mL). For preparation of working solution, 10 ml solution was withdrawn from stock solution and mixed with 45 ml methanol. Three different concentrations of extract solutions (10–100 µg/mL) in methanol was prepared and 150µL of test solution was added to 2.850 ml of DPPH (working) solution. The mixtures were kept in a dark place for 24 hour and absorbance was measured at 517 nm against an equal amount of DPPH and methanol as a blank. Ascorbic acid (vitamin C) was used as standard control (10–100 µg/mL). The percentage of free radical scavenging activity (RSA%) was calculated using the equation:

\[
\text{Percentage of scavenging of DPPH} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100
\]

Where, A<sub>0</sub> is absorbance of the control and A<sub>1</sub> is absorbance of the test extracts. The antioxidant activities were expressed as IC<sub>50</sub> which defined as the concentration of the test substance required to produce a 50% reduction in the concentration of DPPH.

**Experimental design**

**Animals**

Healthy male Wistar rats (250-300 g) procured from Zydus Research Centre, Ahmedabad were used in experiment after 5 days of acclimatization. The animals were kept in a group of 3 per cage under well-controlled environment (Temperature: 22 ± 2°C, humidity: 55 ± 5 % and 12h /12h light-dark cycle). The animals had free access to rat chow diet (purchased from Pranav Argo Pvt. Ltd) and water ad libitum. All experimental animals were examined and handled following the guiding principles for the Care according to committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Anand Pharmacy College with protocol No. 2018-IAEC/1847.

**Dose selection and drug preparation**

The dose of SREBV (100 mg/kg) was selected on basis of previously reported anti-inflammatory activity (Saha 2011). Standard drug Cystone (A marketed polyherbal formulation) and SREBV extract were suspended in 0.5% Carboxymethyl cellulose (CMC).

**Induction of urolithiasis**

The rats were randomly divided into following four groups:

- **Group I:** Normal group (n=6).
- **Group II:** Model control, received 0.75% v/v ethylene glycol in drinking water for 28 days (n=6).
- **Group III:** Standard control, received 0.75% v/v ethylene glycol in drinking water for 28 days and Cystone (750 mg/kg body, p.o., once a day) (n=6).
- **Group IV:** Treatment control, received 0.75% v/v ethylene glycol in drinking water and SREBV (100 mg/kg body weight, p.o., once a day) for 28 days (n=6).

Except Group 1, all groups received lithiatic agent Ethylene glycol (0.75% w/v) in drinking water for 28 days. The study duration was of 28 days.

**Urine, serum and kidney parameters**

On day 28, all rats were housed in separate metabolic cages for collection of 24 hrs urine sample. The 24 hrs samples were preserved at -20°C until urine analysis. Urine was analysed for uric acid, inorganic phosphate, calcium, and magnesium in an autoanalyzer (Turbochem...
100, USA) using commercially available kits, according to manufacturer’s instructions. Urinary oxalate was measured by direct precipitation followed by titration method (Hodgkinson and Williams 1972). The urine volume and pH were also measured.

All rats were weighed on 29th day. The blood was collected from retro-orbital plexus under anaesthesia-containing mixture of Ketamine and Xylazine (50 mg/kg and 10 mg/kg, i.p. respectively). The blood samples were centrifuged at 10,000 rpm for 10 min after collection. The serum was stored in -80°C until serum analysis. Serum creatinine, uric acid, BUN, inorganic phosphate, and calcium were analysed by autoanalyzer (Turbochem 100, USA) using commercially available kits, according to manufacturer’s instructions.

All animals were humanely euthanised and both the kidneys of animals were excised. The right kidney was fixed in 10% neutral formalin, processed, embedded in paraffin wax, tissue sectioned at 5 µm, and stained with haematoxylin and eosin (H&E), for microscopic examination. The left kidney tissues were homogenized with (pH 7.4) phosphate buffer and whole homogenates were centrifuged at 11,000 rpm for 15 min at 4°C to discard any cell debris. The supernatant was used for estimation of catalase, MDA (Camkurt et al. 2017, Beyer and Fridovich 1987) and oxalate (Hodgkinson and Williams 1972).

**Statistical analysis**

The values were expressed as mean ± SEM. The statistical analysis was carried out by one-way ANOVA followed by Dunnett’s post hoc test using GraphPad Prism 6.01. The values with p<0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

**Qualitative analysis of SREBV**

The yield of extract was found to be 6.7 g (16.75% w/w) of dried leaf powder. The phytochemical screening of SREBV showed the presence of saponins, triterpenoids, and steroids (Table 1).

**HPTLC fingerprint analysis**

HPTLC fingerprint patterns have been evolved for SREBV. Standard β-sitosterol was detected at 525 nm accurately using silica gel F<sub>254</sub> HPTLC pre-coated plates with mobile phase n-hexane: ethyl acetate: formic acid: acetic acid (6.8:2.94:0.09:0.09), the Rf value of standard β-sitosterol was about 0.70. The chromatographs of β-sitosterol and SREBV are shown in below Fig. 2 to 4. The Rf value of β-sitosterol nearly matched with the Rf value of SREBV was about 0.74 and the peak corresponding to β-sitosterol from the sample solution had same retention factor as that from standard β-sitosterol. In simultaneous detection, standard stigmasterol and lupeol were detected at 525 nm accurately using silica gel F<sub>254</sub> HPTLC pre-coated plates with mobile phase Toluene: ethyl acetate (7:3), the Rf value of standard stigmasterol and lupeol was about 0.74 and 0.88 respectively. The chromatographs of stigmasterol, lupeol and SREBV are shown in below Fig. 2 to Fig. 8. The Rf value of lupeol exactly matched with the Rf value of SREBV was about 0.88 and the peak corresponding to lupeol from the SREBV had same retention factor as that from standard lupeol. The peak of stigmasterol was not detected in the SREBV by HPTLC scanner though it was visually seen on HPTLC plate.

**Determination of total saponin, β-sitosterol and lupeol content**

The total saponin content in SREBV was found to be 100.16% (y = 0.0001x + 0.0008; R<sup>2</sup> = 0.999). Based on the area under curve (AUC), the total quantity of β-sitosterol present in SREBV was found to be 2.60 µg. Based on the AUC, the total quantity of lupeol present in SREBV was found to be 61.71 µg. Several studies have shown beneficial effects of lupeol and steroid in experimentally induced urolithiasis (Joshi et al. 2005, Manjula et al. 2012, Naikal James and Shilpika 2020).

**In-vitro antioxidant activity**

Free radical scavenging activities of SREBV and ascorbic acid were assessed by DPPH free radical assay (Fig. 1 and Table 2). The results suggest that SREBV possess free radical scavenging activity in concentration dependent manner. The calculated IC<sub>50</sub> for SREBV and ascorbic acid was 0.382 µg/ml and 0.309 µg/ml respectively. The main mechanism behind this action involves transfer of hydrogen to free radical to produce nonreactive species. Hence, addition of this hydrogen eradicates the potentiality of electrons which is responsible for radical reactivity (Kachkoul et al. 2020). It is hypothesized that supersaturation of urine with promoter ions leads to generation of free radicals. This is exposed to renal tubules leading to oxidative stress and damage. Also, crystal promoters activate inflammatory mediators which further damages tubules. This vicious cycle of oxidative stress and inflammation finally leads to development of stones (Khan 2014).
In-vivo antilithiatic activity

Urinary output and pH

To determine the characteristic or types of calculi, urine analysis plays an imperative role. EG administration in rats for 28 days caused significant decrease in urine volume (p<0.0001) and pH (p<0.01). (Table 3)

The decrease in urine output suggests reduced GFR which ultimately accounts for supersaturation of urine with promoter ions. The SREBV significantly (p<0.05) increased urinary output and thus improved GFR as compared to model control animals. This diuretic effect quickens the process of preformed stone dissolution (Karadi et al. 2006), removes tiny particles of crystal and prevent the crystal aggregation at initial step by coating the crystal to make smooth surface thereby obstructs the new stone formation. Moreover, diuresis can also dilute the stone components which reduce the super-saturation step in urine (Selvam et al. 2001). This effect might be due to the presence of two identified saponins in SREBV.

The pH plays major role in nucleation, aggregation and type of stones which are formed (Patel and Acharya 2020). In general, in acidic pH (approximately <5.5), uric acid crystals are more predominant whereas calcium oxalate and/or calcium phosphate calculi occur at pH >7.2. The alteration of normal urinary pH was not observed with treatment of SREBV, suggesting positive effect in antilithiasis activity. It may be accountable for dissolving the complexes of calcium and oxalate thereby

decreasing supersaturation process in urine.

The standard drug Cystone significantly (p<0.001) increased urinary output and did not disturb normal pH.

Urinary calculi promoters and inhibitors

It is well-defined that the major cause for urolithiasis is believed to be hyperoxaluria, hypercalciuria, hyperuricosuria and hypomagnesuria. Various studies presented that EG caused these alterations in urine of animals, which lead to the retention and excretion of oxalate (Patel and Acharya 2020). Also, oxalate is produced by the metabolism of ethylene glycol in the liver (Green et al. 2005). Increased levels of these urinary calcium and oxalate act as nidus for growth and development of new calcium oxalate stone. Also,

Table 1. Qualitative phytochemical analysis of SREBV.

| Phytochemical components | SREBV  |
|--------------------------|--------|
| Saponins                 | +      |
| Triterpenoids            | +      |
| Steroids                 | +      |
| Alkaloids                | -      |
| Tannins                  | -      |
| Phenols                  | -      |
| Flavonoids               | -      |
| Glycosides               | -      |

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increased inorganic phosphate excretion and oxalate stress provide favourable environment for formation of calcium phosphate crystals, which in turn epitaxially stimulate deposition of calcium oxalate crystals (Ashok et al. 2010). The concentration of calculi promoters namely calcium (p<0.0001), oxalate (p<0.0001), uric acid (p<0.0001) and inorganic phosphate were significantly increased in EG induced urolithiatic animals as compared to normal control. (Table 3) The enhanced levels caused aggregation, and crystal growth. However, this development was hindered by significant decreased in oxalate, calcium (p<0.0001), and phosphate levels in urine after SREBV treatment. The plant extract rich in saponins may act by inhibiting steps of oxalate synthesis which in turn halt the cascade of stone formation. This can be attributed to its diuretic activity which flushed out these excessive stone promoting ions. Along with this, uric acid levels were also significantly higher in EG treated animals as compared to normal control animals. This may be due to alteration in urinary pH. It is proven that the solubility of calcium oxalate gets to interfere in the presence of uric acid (Patel et al. 2012). Treatment with SREBV significantly (p<0.0001) reduced the excretion of uric acid and reduced the risk of stone formation.

Also, in EG treated model group, levels of magnesium were significantly (p<0.0001) lowered as compared to normal control animals. Magnesium inhibits calcium oxalate crystallization by forming complex with oxalate and thus decreases the availability for calcium to bind which results in inhibiting supersaturation of calcium oxalate (Selvam et al. 2001). Treatment group restored

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### Table 3. Effects of SREBV extract on rat urine parameters in EG induced urolithiasis.

| Parameter              | Normal control | Model control | Standard control | Treatment control |
|------------------------|----------------|---------------|------------------|-------------------|
|                        | Group 1        | Group 2       | Group 3          | Group 4           |
| **Urine Parameters**   |                |               |                  |                   |
| Urine volume (ml)      | 9.33±0.401     | 5.31±0.834*** | 9.0±0.288***     | 7.41±0.436        |
| Urine pH               | 7.1±0.230      | 5.8±0.146***  | 8.0±0.00****     | 7.2±0.211***      |
| Calcium (mg/dl)        | 9.24±0.446     | 14.62±0.181***| 10.3±0.057****   | 8.53±0.853****    |
| Oxalate (mg/24h urine) | 0.027±0.000    | 0.201±0.013***| 0.013±0.002****  | 0.074±0.006****   |
| Uric acid (mg/dl)      | 6.245±0.108    | 21.52±0.092***| 3.827±0.149****  | 7.165±0.774****   |
| Magnesium (mg/dl)      | 8.25±0.317     | 0.503±0.168***| 9.75±0.086****   | 0.940±0.641       |
| Inorganic phosphate (mg/dl) | 2.903±0.176 | 7.067±2.289 | 1.7±0.288*** | 5.217±1.576 |

[Results presented as mean ± SEM, n=6 in each group. One-way ANOVA test followed by Dunnett’s post hoc test was used to determine the statistical significant difference between the groups by using graph pad prism 6.01. Whereas, *p<0.05 value compared to normal control, **p<0.01 value compared to model control, ***p<0.001 value compared to model control, ****p<0.0001 value compared to normal control and p<0.0001 value compared to model control].
the magnesium excretion compared to model control and henceforth reduced the growth of calcium oxalate crystals in animals.

The standard drug Cystone significantly restored all levels of urinary calculi promoters and inhibitors.

**Serum levels of creatinine, BUN, uric acid, calcium and inorganic phosphate**

It has been reported that urinary obstruction due to presence of large crystals leads to decrease in glomerular filtration rate which eventually leads to accumulation of nitrogenous substances such as creatinine, BUN and uric acid in blood (Patel et al. 2012). This was also apparent in current study where levels were significantly elevated in EG treated model group animals as compared to normal control animals. The significant (p<0.0001) beneficial effect is observed in Cystone and SREBV treated groups. This can be attributed to antilithiatic and diuretic activity of lupeol and β-sitosterol present in SREBV (Table 4).

Also, serum levels of calcium and inorganic phosphate were significantly (p<0.0001) increased in model group as compared to normal animals. The increase in calcium levels were significantly (p<0.0001) prevented by treatment with SREBV and Cystone. This might be due to increase in urine output and improved GFR. However, the non-significant effect was observed on levels of inorganic phosphate after treatment with SREBV.

**Kidney homogenate levels of oxalate, MDA and Catalase**

It is well documented that EG administration contributes to the augmented generation of ROS, oxidative stress, renal injury and inflammation while decreased the antioxidant enzymes in the kidney (Patel et al. 2012). Therefore, restoration of antioxidant enzymes

### Table 4. Effects of SREBV extract on rat serum parameters in EG induced urolithiasis.

| Parameter               | Normal control | Model control | Standard control | Treatment control |
|-------------------------|----------------|---------------|------------------|-------------------|
| Group 1                 | Group 2        | Group 3       | Group 4          |
| Creatinine (mg/dl)      | 0.586±0.028    | 2.133±0.107*** | 0.575±0.032**** | 0.762±0.033****  |
| BUN (mg/dl)             | 23.2±0.057     | 45.2±0.043***  | 30.1±0.058****  | 16.5±1.59****    |
| Uric acid (mg/dl)       | 2.005±0.337    | 4.294±0.215*** | 0.983±0.119**** | 0.3±0.044****    |
| Calcium (mg/dl)         | 7.326±0.575    | 19.25±1.181*** | 2.533±0.121**** | 10.48±1.427****  |
| Inorganic phosphate (mg/dl) | 3.224±0.008 | 4.417±0.174*** | 3.851±0.010'    | 4.2±0.235        |

(Results presented as mean ± SEM, n=6 in each group. One-way ANOVA test followed by Dunnett’s post hoc test was used to determine the statistical significant difference between the groups by using graph pad prism 6.01. Whereas, * p<0.05 value compared to model control, **** p<0.0001 value compared to model control, #### p<0.0001 value compared to normal control).

Fig. 5. HPTLC chromatoplate of before derivatization and after derivatization of SREBV comparison with standard stigmasterol and lupeol (Stigmasterol-Lupeol- SREBV).

Fig. 6. Densitometric chromatogram of standard stigmasterol detected at 525 nm after derivatization of chromatoplate.
and reduction of oxidative stress could be an effective approach in the treatment of renal stone diseases. The levels of MDA were significantly (p<0.01) increased in model control animals as compared to normal animals along with significant (p<0.0001) decrease in Catalase levels. The treatment groups significantly inhibited lipid peroxidation (p<0.01) and restored Catalase enzyme levels. Various studies reported antioxidant effect of lupeol depicted by reduced lipid peroxidation and oxidative injury in rat kidney (Malini et al. 2000). Alleviation of lipid peroxidation, in turn, rectifies the oxidative stress and increased activity of antioxidant enzyme. It is worth noting that Catalase enzyme levels are higher in SREBV treated animals than Cystone. (Table 5) Also, the oxalate levels were significantly increased in model control animals as compared to normal group which was significantly (p<0.0001) prevented by treatment with SREBV and Cystone.

### Histopathology

Microscopic examination of kidney section in lithiatic rats presented amassing of CaOx crystals in tubular and interstitial spaces which causes significant changes in the histology of kidneys such as glomerular congestion, tubular dilation, and necrosis. (Fig. 9) Treatment with SREBV and Cystone prevented this renal tissue damage and inflammation with very few crystals in different parts of the renal tubules. Treatments accelerating the dissolution of pre-formed stone and/or avoiding the formation of new crystals which shows the protection against renal damage. This protection probably due to the presence of both identified saponins owing to its potent antioxidant effect.

### CONCLUSION

The present investigation indicates that saponin rich extract of Bauhinia variegata is endowed with anti-urolithiatic activity. The mechanism underlying this effect was mediated by lowering the concentration of stone forming components, promoting stone inhibitor, stone dissolving characteristic, diuretic as well as antioxidant effect and inhibiting the process of lithogenesis, thereby protecting from nephritic damage. The effect may be due to lupeol and α-sitosterol present in saponin rich fraction.

### Table 5. Effects of SREBV extract on rat kidney homogenate parameters in EG induced urolithiasis.

| Parameter               | Normal control | Model control | Standard control | Treatment control |
|-------------------------|----------------|---------------|------------------|------------------|
|                         | Group 1        | Group 2       | Group 3          | Group 4          |
| Oxalate (mg)            | 0.013±0.002    | 0.853±0.151*** | 0.065±0.001**** | 0.022±0.001**** |
| MDA (µg/ml)             | 0.068±0.008    | 0.192±0.034**  | 0.098±0.013**   | 0.084±0.006**   |
| Catalase (µM/g of tissue) | 1.089±0.133  | 0.039±0.005*** | 0.148±0.019     | 1.144±0.165**** |

(Results presented as mean ± SEM, n=6 in each group. One-way ANOVA test followed by Dunnett’s post hoc test was used to determine the statistical significant difference between the groups by using graph pad prism 6.01. Whereas, ## p<0.01 value compared to normal control, ** p<0.01 value compared to model control, #### p<0.0001 value compared to normal control, ****p<0.0001 values compared to model control).
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REFERENCES
Alelign T, Petros B (2018) Kidney stone disease: an update on current concepts. Adv Urol 2018: 01-12.

Ashok P, Koti BC, Vishwanathswamy AHM (2010) Antiurolithiatic and antioxidant activity of Mimusops elengi on ethylene glycol-induced urolithiasis in rats. Indian J Pharmacol 42: 380-383.

Alelign T, Petros B (2018) Kidney stone disease: an update on current concepts. Adv Urol 2018: 01-12.

Bargah RK (2015) Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn. J Pharmacogn Phytochem 4: 07-09.

Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 559-566.

Camkurt MA, Fýndýklý E, Bakacak M, Tolun FI, Karaaslan MF (2017) Evaluation of malondialdehyde, superoxide dismutase and catalase activity in fetal cord blood of depressed mothers. Clin Psychopharmacol Neurosci 15: 35-39.

Fouada A, Yamina S, Nait MA, Mohammed B, Abdlekrim R (2006) In vitro and in vivo antilithiasic effect of saponin rich fraction isolated from Herniaria hirsuta. J Bras Nefrol 28: 199-203.

Fig. 9. Histopathological appearance of kidney tissues in rats (H&E, 10X).
[(A) Normal group: Normal renal tubules with no dilation and inflammation; (B) Model group (EG): Degenerative changes in architecture of glomeruli (straight arrow) and marked tubular dilation and inflammation (segmented arrow); (C) EG+STD: less tubules dilation (straight arrow) with increasing number of glomerulus compared to model group; (D) EG+SREBV-100 mg/kg: same changes as seen with standard group].
Green ML, Hatch M, Freel RW (2005) Ethylene glycol induces hyperoxaluria without metabolic acidosis in rats. Am J Physiol Renal Physiol 289: F536-F543.

Güçlü-Ustündağ O, Mazza G (2007) Saponins: properties, applications and processing. Crit Rev Food Sci Nutr 47: 231-258.

Hodgkinson A, Williams A (1972) An improved colorimetric procedure for urine oxalate. Clin Chim Acta 36: 127-132.

Joshi VS, Parekh BB, Joshi MJ, Vaidya AB (2005) Herbal extracts of Tribulus terrestris and Bergenia ligulata inhibit growth of calcium oxalate monohydrate crystals in vitro. J Cryst Growth 275: e1403-e1408.

Karthika K, Jamuna S, Paulsamy S (2014) TLC and HPTLC fingerprint profiles of different bioactive components from the tuber of Solena amplexicaulis. J Pharmacogn Phytochem 3: 198-206.

Kachkoul R, Houssaini TS, Mohim M, El Habbani R, Lahrichi A (2020) Chemical compounds identification and antioxidant and calcium oxalate antocrystallization activities of Punica granatum L. Evid Based Complement Alternat Med 2020: 9424510.

Karadi RV, Gadge NB, Alagawadi KR, Savadi RV (2006) Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats. J Ethnopharmacol 105: 306-311.

Khan SR (2014) Reactive oxygen species, inflammation and calcium oxalate nephrolithiasis. Transl Androl Urol 3: 256-276.

Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK et al. (2016) Kidney stones. Nat Rev Dis Primers 2: 16008-16008.

Knoll T (2010) Epidemiology, pathogenesis, and pathophysiology of urolithiasis. Eur Urol Suppl 9: 802-806.

Le AV, Parks SE, Nguyen MH, Roach PD (2018) Improving the vanillin-sulphric acid method for quantifying total saponins. Technologies 6: 01-12.

Liu Y, Chen Y, Liao B, Luo D, Wang K et al. (2018) Epidemiology of urolithiasis in Asia. Asian J Urol 5: 205-214.

Malini MM, Lenin M, Varalakshmi P (2000) Protective effect of triterpenes on calcium oxalate crystal-induced peroxidative changes in experimental urolithiasis. Pharmacol Res 41: 413-418.

Mamillapalli V, Khantamneni PL, Mohammad Z, Mathangi A, Nandigam N et al. (2016) Phytochemical and In vitro antirolithic studies on the leaf extracts of Bauhinia variegata Linn. Int J Pharm Sci Res 7: 4074-4084.

Manjula K, Rajendran K, Eevera T, Kumaran S (2012) Effect of Costus igneus stem extract on calcium oxalate urolithiasis in albino rats. Urol Res 40: 499-510.

Modh KM, Parmar PT, Panigrahi B, Anand IS, Patel CN (2011) Pharmacognostical and phytochemical evaluation of leaves of Bauhinia variegata Linn. Pharmacogn J 3: 45-49.

Naikal James PS, Shilpika N (2020) Antirolithic activity of natural constituents isolated from Aerva lanata flowers. Int J Pharm Sci Drug Res 12: 668-673.

Obadoni BO, Ochuko PO (2001) Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. Glob J Pure Appl Sci 8(2): 203-208. DOI: 10.4314/gjpas.v8i2.16033.

Onaran M, Orhan N, Farahvash A, Ekin HN, Kocabıyık M et al. (2016) Successful treatment of sodium oxalate induced urolithiasis with Helichrysum flowers. J Ethnopharmacol 186: 322-328.

Panchal PM (2012) Pharmacognostical and phytopharmacological investigation of Peltophorum pterocarpum (DC) Backer ex. Heyne. Int J Ayurveda Med 3: 196-217.

Patel MR, Panchal HS, Saluja AK (2016) Identification of terpenoids and steroidal compounds in Caryota urens leaves by column chromatography and various spectroscopic techniques. World J Pharm Pharm Sci 5: 1610-1622.

Patel PK, Patel MA, Vyas BA, Shah DR, Gandhi TR (2012) Antirolithic activity of saponin rich fraction from the fruits of Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) against ethylene glycol induced urolithiasis in rats. J Ethnopharmacol 144: 160-170.

Patel VB, Acharya N (2020) Effect of Macrotyloma uniflorum in ethylene glycol induced urolithiasis in rats. Heliyon 6: e04253-e04260.

Raheem OA, Khandwala YS, Sur RL, Ghani KR, Denstedt JD (2017) Burden of urolithiasis: trends in prevalence, treatments, and costs. Eur Urol Focus 3: 18-26.

Saha S (2011) In vivo study for anti-inflammatory activity of Bauhinia variegata L. leaves. Pharm crop 2: 70-73.

Selvam R, Kalaiselvi P, Govindaraj A, Bala Murugan V, Kumar AS (2001) Effect of Aerva lanata leaf extract and Vediappu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol Res 43: 89-93.
Shafi H, Moazzami B, Pourghasem M, Kasaeian A (2016) An overview of treatment options for urinary stones. Caspian J Intern Med 7: 1-6.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compost Anal 19: 669-675.

Yadav M, Gulkari VD, Wanjari MM (2016) Bryophyllum pinnatum leaf extracts prevent formation of renal calculi in lithiatic rats. Anc Sci Life 36: 90-97.

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