Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Evaluation of Anti-urolithiatic Activity of *Bougainvillea spectabilis* Willd. against Sodium Oxalate-induced Nephrolithiasis in Rats

Prabhat K. Das*, Jai S. Vaghela

B. N. College of Pharmacy, Bhupal Nobles', University, Udaipur, Rajasthan, India

ARTICLE INFO

Article history:
Received: 03 November, 2022
Revised: 30 December, 2022
Accepted: 10 January, 2023
Published: 30 January, 2023

Keywords:
Bougainvillea spectabilis, Flavonoids, Oxalate, Sodium oxalate, Urolithiasis

DOI:
10.25004/IJPSDR.2023.150109

ABSTRACT

As there is no effective treatment for kidney stones, the development and progression of renal calculi continue to be a cause for concern despite developments in contemporary medicine. In the current study, the anti-urolithiasis activity of *Bougainvillea spectabilis* Willd. against sodium oxalate (NaOx)-induced urolithiasis in rats was studied. Leaves of *B. spectabilis* were extracted with various solvents, i.e., petroleum ether, chloroform, ethyl acetate, ethanol, and water. Albino wistar male rats were divided into five groups of six animals each. All groups except the positive control were treated intraperitoneally with sodium oxalate (70 mg/kg) for 10 days. Treatment with the standard drug cystone (5 mL/kg), ethanol, and aqueous extracts (200 mg/kg) was given to the respective groups for 10 days of study. On the 10th day, serum creatinine, calcium, uric acid, blood urea nitrogen, urine calcium, oxalate, and uric acid levels were estimated, along with urine microscopy to confirm crystals. Kidneys were isolated and used for histopathological studies. The results suggested that the administration of *B. spectabilis* to rats with sodium oxalate-induced lithiasis reduced and prevented the formation of urinary stones. Also, the treatment of lithiasis-induced rats with the toxic control drug and extracts of *B. spectabilis* restored all the elevated urine and serum biochemical parameters, including creatinine, calcium, oxalate, uric acid, and blood urea nitrogen, and significantly increased the urine volume. The histopathology showed depositions of a large number of calcium oxalate crystals in the kidney in the calculi-induced group with enlargement of glomeruli and necrosis of tubules, while in the standard and extract-treated groups, small and fewer deposits were seen with the recovered architecture of the kidney tubules. The result indicates the anti-urolithiasis activity of *B. spectabilis* mediated possibly by the presence of flavonoids, calcium oxalate crystal inhibitors, and antioxidant properties which maintain the balance between stone promoters and inhibitors constituents and this study rationalized its medicinal use in urolithiasis.

Introduction

Herbal 'renaissance' is a worldwide phenomenon. In contrast to synthetics, which are viewed as hazardous to humans and the environment, herbal products represent safety today. Although herbs had been valued for millennia due to their medicinal, flavorful, and aromatic properties, synthetic products of the modern era temporarily eclipsed their significance. On numerous grounds, traditional medical practices are nonetheless commonly utilized. Population growth, an inadequate drug supply, the prohibitive cost of treatments, the adverse effects of several allopathic drugs, and the emergence of resistance to currently-used drugs for infectious diseases have led to a greater emphasis on the use of plant materials as a source of medicines for a wide range of human ailments. However, the mindless reliance on synthetics has ended, and people are returning to natural substances in the expectation of safety and security.

Urolithiasis has been a distinct problem from the beginning of recorded history, when Egyptian mummies discovered the condition in 4800 B.C. Urolithiasis refers to the production of stones in the kidney, urethra, ureters, or urinary bladder. In India, 12% of the population is anticipated to have urinary stones, of which 50% may

***Corresponding Author:** Mr. Prabhat K. Das

Address: B. N. College of Pharmacy, Bhupal Nobles', University, Udaipur, Rajasthan, India

Email ✉**:** +91-9098095524

Tel.: kumar_prabhat3027@yahoo.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

result in kidney failure or damage to the kidneys.[1] Complex and variable are the underlying causes of urinary calculus illness. The involvement of genetic, metabolic, and nutritional factors, anatomical abnormalities[2] and temporal, geographical, and individual differences,[3] further complicates it. Therapy with lowered calcium and oxalate consumption, thiazides (diuretics), phosphate salts, and allopurinol in various combinations have significantly reduced the occurrence of recurring kidney stones. However, these medications' side effects restrict their usage in long-term medical treatment.[4] Despite considerable studies to determine the processes of stone formation, dietary control, and the evaluation of medicinal plants and other agents in the treatment of urinary stones, no standard medicine is now available. Numerous medications, diuretics, and antispasmodics are utilized to assist the passage and expulsion of urinary calculi; however, the management of urinary calculi is still inadequate. Urolithiasis treatment largely depends on stone size and placement in the urinary system. In the majority of cases, stones are removed using surgical procedures such as extracorporeal shock wave lithotripsy, percutaneous nephrolithotomy, and ureteroscopy; however, stone recurrence was reported in approximately 50% of patients after surgical stone removal.[5] Surgical therapies have adverse effects, including hypertension, tubular necrosis, bleeding, and renal fibrosis.[6] Despite the limitations of medicinal treatment, surgical stone removal is still regarded as the most effective technique to alleviate symptoms, despite its high cost and high recurrence rate. In light of the aforementioned information, the hunt for the optimum anti-urolithiasis medication continues, and indigenous medicines should be tested. There is a growing interest in using natural products and ayurvedic medicines to manage urolithiasis, as stone production is a continuous process, and recurrence is noticed frequently.

Green plants synthesize and store a wide range of biochemical compounds, many of which are extractable and employed as chemical feedstocks or raw materials in various scientific experiments. In the current context, it becomes imperative to do fundamental scientific research on indigenous medicinal herbs. So, we have selected *Bougainvillea spectabilis* for the present study. The leaves were subjected to screening for their anti-urolithiasis activity to investigate and justify the claim of their anti-urolithiasis potential.

The genus *Bougainvillea* is indigenous to South America and derives its name from Louis Antoine de Bougainville (1729–1811), an admiral in the French Navy who encountered the plant in Brazil in 1768 and first introduced it to the rest of the world, where it quickly spread throughout tropical and warm climates.[7]

In the plant family Nyctaginaceae, the genus *Bougainvillea* has 18 species, three of which are horticulturally significant: *B. spectabilis*, *B. glabra,* and *B. peruviana*. From the stem, flowers, and leaves of *B. spectabilis*, the following phytochemical compounds are extracted: alkaloid, flavonoids, furanoids, glycosides, phenols, phlobotannins, quinones, saponins, steroids, tannins, and terpenoids.[8]

From review literature regarding the traditional uses or phytochemical properties of *B. spectabilis* are antibacterial,[9-11] anticancer,[12] antidiabetic,[13-15] antifertility[16,17] anti- fungal, [18] anti-inflammatory,[19,20] antihyperlipidemic,[21,22] antioxidant,[23-25] antiulcer,[26] antiviral,[27] hepatoprotective,[28] and thrombolytic activities.[29]

Srivastava S.(1962) indicated the presence of the enzyme oxalic acid oxidase in the leaves of *B. spectabilis*.[30] Malomo (2006) reported some beneficial effect of the plant on liver and kidney function.[28] Rajeswari P (2018) also indicated from their *in-vitro* study that Aqueous extract of another species of *Bougainvillea i.e., B. glabra* showed anti-urolithiatic potential.[31] In another study Das *et al.* (2021) reported the *in-vitro* anti-urolithiatic activity of the plant *B. spectabiils*.[32]

To our knowledge, *B. spectabilis'* anti-urolithiasis potential has not been investigated in *in-vivo* investigations. This plant's litholytic potential has only been determined through *in-vitro* research. Due to the presence of phytochemical components flavonoids and the antioxidant, hepatoprotective, and antihyperlipidemic capabilities, we were motivated to screen and evaluate the *in-vivo* anti-urolithiatic activity of ethanolic and aqueous extracts of *B. spectabilis* leaves.

Materials And Methods

Collection and Authentication of Plant Material

In the month of January, the leaves of *B. spectabilis* Wild. were collected from the local areas in and around Madhya Pradesh. Retired Professor of Botany, PG govt college Khargone Dr. S. K. Mahajan has validated the herbarium specimen of the plant that was deposited in the department of Pharamacognosy. The registration number for *B. spectabilis*'s plant authentication certificate was SKM/PGC/2018/X-10.

Processing of Plant Material

The freshly collected leaves were thoroughly washed many times to wipe out any type of dirt, debris or crystals found on the leaves, shade-dried, and then grinded using a mechanical pulverizer. After passing it through a sieve, the fine powder was collected.

Chemicals and Reagents

Sodium oxalate was purchased from Fisher Scientific, Mumbai. All other chemicals used in experiment were of analytical grade and were purchased from authorized scientific labs. Standard drug Cystone (Himalaya Drug Company) purchased from local market of Indore. Calcium,

blood urea nitrogen, creatinine, uric acid (Accucare kits) estimation kits were procured from Span Diagnostics Pvt Ltd India.

Preparation of the Plant Extracts

Total of 200 g of dried and coarsely chopped leaves were extracted in a soxhlet extractor with petroleum ether, chloroform, ethyl acetate, ethanol, and water using continuous hot percolation for 18 to 24 hours. Finally, the concentrated extracts were evaporated to dryness, and the solvent-specific extracts were weighed.

Animals

Wistar albino male rats weighing 140–200 g were used. The members of CPCSEA approved the experimental protocol in the meeting held in the institute and according to the regulation animals were maintained under standard conditions for an acclimatization period of 15 days before performing the experiment. All rats were housed individually in metabolic cages and the temperature was maintained at 22 ± 2°C. The members of CPCSEA approved the condition in the animal house (Regd. No. 870/PO/Re/S/05/CPCSEA) maintained by B.N. college of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan.

Acute Oral Toxicity Study[33]

An acute oral toxicity study was carried out to determine the minimum lethal dose of ethanolic and aqueous leave extract of *B. spectabilis* Wild. Wistar albino rats of male weighing 140–200 gm were used. The experimental animals were kept fasting overnight providing only water. The acute oral toxicity study was done according to Organization for Economic Cooperation and Development (OECD) No. 425 guideline at dose range 100 to 2000 mg/kg. No mortality of animals was observed at the dose range. They were observed continuously for any behavioral changes and toxic manifestations like hyperactivity, changes in the skin, fur, convulsions, excretion, dilation of the pupil, sedation, hypothermia, and mortality during the first 4 hours, periodically during the first 24 hours. Thereafter the animals were continuously monitored at regular intervals for 7 days. No deaths or hazardous signs were detected in the rats during the 7 days of observation. Hence 200 mg/kg dose of the ethanolic as well as aqueous extracts were taken for the following experiment.

Experimental Design

Induction of Urolithiasis by Sodium Oxalate[34]

Rats were placed individually in metabolic cages. During the experiment, food and water were given freely in cages and body weights, water, and diet intake was determined. After 1 week of acclimatization, sodium oxalate (70 mg/kg) was administered intraperitoneally for 10 days to induce renal calculi. The Wistar male albino rats were divided into five groups, each consisting of six animals (n = 6): Group I served as positive control, received 0.5% (w/v) gum acacia solution (5 mL/kg p.o.), group II served as toxic control, received only sodium oxalate (70 mg/kg, i.p.), group III served as standard, received standard drug cystone (5 mL/kg, p.o.) + sodium oxalate (70 mg/kg, i.p.), and groups IV received sodium oxalate (70 mg/kg, i.p.),+ aqueous extract of *B. spectabilis* (200 mg/kg, p.o.), Groups V received sodium oxalate (70 mg/kg, i.p.),+ ethanolic extract of *B. spectabilis* (200 mg/kg, p.o.), respectively.

Evaluation of Anti-Urolithiatic Activity

Collection and Analysis of Urine

On the tenth day, 24 hour urine samples were obtained from each animal housed in an individual metabolic cage. During the urine collection time, animals will have unrestricted access to potable water. Before storing the urine at 4°C, a single drop of strong hydrochloric acid was added. Urine was analyzed for calcium, uric acid and oxalate content by calorimetric method according to Hodgkinson, 1970.[35]

Change in Body Weight

The change in body weight of various groups in individual animal were taken and calculated by using initial weight minus final body weight.

Microscopic Study of Urine

On 28th day, the collected urine sample volume were measured followed by centrifugation at 3000 rpm for 10 minutes. After centrifugation, the urine samples were examined under an electron microscope using to ensure the presence of crystals.

Serum Analysis

After the experimental period, blood was collected from retro-orbital under anesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 3000 rpm for 10 minutes and analysed for creatinine, uric acid, calcium and blood urea nitrogen (BUN). The creatinine, uric acid, and BUN diagnostic kits (Span Diagnostics Ltd., India) were used and calcium was analysed according to Lorentz, 1982. [36]

Kidney Histopathology

The abdomen was incised and opened, and both kidneys were removed from each animal. Isolated kidneys were cleaned off extraneous tissue, weighed and rinsed with ice-cold normal saline. The left kidney was fixed with 10% v/v neutral formalin and after harvesting, sliced horizontally and sent to advanced pathology department (SAIMS Indore) for hematoxylene and eosin staining. Calcium oxalate crystal depositions were observed by electron microscope. Same histopathology slides were subjected to microscopic examination for the presence of glomerular enlargement, hydrophobic and necrotic changes in tubules, peritubular congestion, blood vessel

congestion, interstitial edema and presence of hyaline casts and inflammatory cells.

Statistical Analysis

Results were indicated in terms of mean ± SEM. Statistical significance of data were assessed by analysis of variance (One way-ANOVA), followed by comparison between different groups using Dunnett's multiple comparison test. The significance was considered at the level of $p<0.05$.

Results

The results are indicated in Table 1 and 2 and Figs. 1 and 2. During the treatment, the toxic control group and *B. spectabilis*-treated groups had considerably lower body weight than the positive control group. However, the standard group gained more weight than the toxic control group. Weight of kidneys in toxic control group was also increased significantly (1.70 ± 0.05) than positive control group (1.53 ± 0.06) (Table 1). In toxic control group, a significant increase in the levels of Serum creatinine, calcium, uric acid and BUN (3.48 ± 0.31, 11.01 ± 0.67, 4.92 ± 0.34, 39.97 ± 0.79 mg/dl) was observed than positive control group (1.40 ± 0.24, 8.25 ± 0.43, 2.82 ± 0.29, 31.9 ± 0.93 mg/dl). While in standard drug the levels of Serum creatinine, calcium, uric acid and BUN were decreased significantly than toxic control group (1.94 ± 0.43, 8.41 ± 0.36, 3.25 ± 0.45, 33.50 ± 0.71 mg/dl). In ethanolic extract treated group a significant decrease in the levels of serum creatinine, calcium, uric acid and BUN (2.52 ± 0.49, 9.61 ± 0.55, 3.72 ± 0.35, 35.10 ± 0.73 mg/dl) was observed than aqueous treated group (3.09 ± 0.43, 10.53 ± 0.48, 4.28 ± 0.47, 37.47 ± 0.80 mg/dl). The urolithiasis in sodium oxalate-induced model showed a significant elevation in urine biochemical parameters along with the reduced urine output compared to the normal rats. Treatment with standard and ethanolic extract resulted remarkable

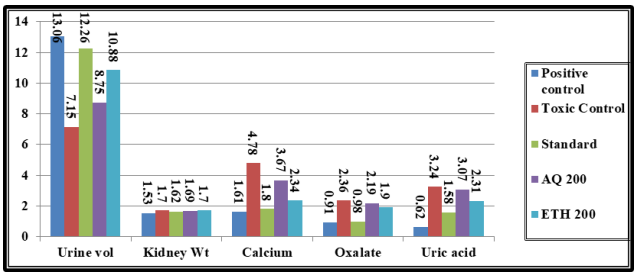

Fig. 1: Urine Biochemistry of Sodium Oxalate induced Urolithiasis. Results are express on mean ± SEM from our observations. p^* :-< 0.05 p^{**} :-< 0.01 p^{***} :-< 0.001 significantly different from control.

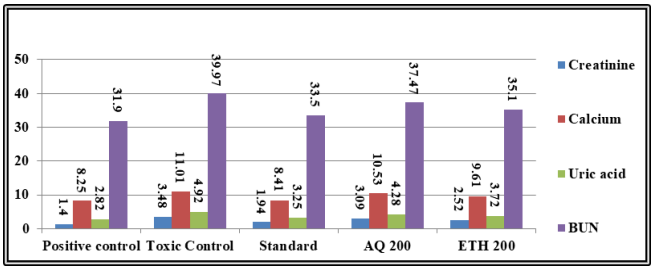

Fig. 2: Serum Biochemistry of Sodium Oxalate induced Urolithiasis Results are express on mean ± SEM from our observations. p^* :-< 0.05 p^{**} :-< 0.01 p^{***} :-< 0.001 significantly different from control

Table 1: Urine Biochemistry of Sodium Oxalate induced urolithiasis on 10th Day

Group	*Sex*	*Body Wt. in gm.*		*Urine vol. in mL*	*Kidney .Wt in gm.*	*Calcium mg/dl*	*Oxalate mg/dl*	*Uric acid mg/dl*
		I	*F*					
Positive control	M	149.33 ± 4.40	154.83 ± 5.13	13.06 ± 0.81	1.53 ± 0.06	1.61 ± 0.47	0.91 ± 0.26	0.62 ± 0.23
Toxic Control	M	169.33 ± 6.21	156.83 ± 6.49	7.15 ± 0.59	1.70 ± 0.05	4.78 ± 0.47	2.36 ± 0.58	3.24 ± 0.63
Standard	M	157 ± 8.38	166.83 ± 7.50	12.26 ± 0.86	1.62 ± 0.06**	1.80 ± 0.47	0.98 ± 0.23	1.58 ± 0.34***
Aqueous (200 mg/kg)	M	173.16 ± 8.91	162.16 ± 7.52	8.75 ± 0.63	1.67 ± 0.03	3.67 ± 0.37	2.19 ± 0.23	3.07 ± 0.37
Ethanolic (200 mg/kg)	M	178.66 ± 7.79	173.83 ± 7.52	10.88 ± 0.81	1.64 ± 0.05***	2.34 ± 0.57	1.90 ± 0.29	2.31 ± 0.43**

Table 2 : Serum Biochemistry of Sodium Oxalate-induced Urolithiasis on 10th Day

Group	*Sex*	*Body Wt. in gm.*		*Creatinine mg/dl*	*Calcium mg/dl*	*Uric acid mg/dl*	*BUN mg/dl*
		I	*F*				
Positive control	M	149.33 ± 4.40	154.83 ± 5.13	1.40 ± 0.24	8.25 ± 0.43	2.82 ± 0.29	31.9 ± 0.93
Toxic Control	M	169.33 ± 6.21	156.83 ± 6.49	3.48 ± 0.31	11.01 ± 0.67	4.92 ± 0.34	39.97 ± 0.79
Standard	M	157 ± 8.38	166.83 ± 7.50	1.94 ± 0.43***	8.41 ± 0.36**	3.25 ± 0.45*	33.50 ± 0.71
Aqueous (200 mg/kg)	M	173.16 ± 8.91	162.16 ± 7.52	3.09 ± 0.43	10.53 ± 0.48	4.28 ± 0.47	37.47 ± 0.80
Ethanolic (200 mg/kg)	M	178.66 ± 7.79	173.83 ± 7.52	2.52 ± 0.49***	9.61 ± 0.55	3.72 ± 0.35**	35.10 ± 0.73

rise in urine volume (12.26 ± 0.86 and 10.88 ± 0.81) at the end of the experiment, however aqueous extract produced in minimal raise in volume of urine (8.75 ± 0.63) collected as analyzed to the toxic control group (7.15 ± 0.59). Sodium oxalate demonstrated considerable raise in urinary calcium levels of toxic control group (4.78 ± 0.47) as analyzed to positive control (1.61 ± 0.47). Treatment with standard drug cystone reduced urinary calcium levels remarkably (1.80 ± 0.47). Treatment with ethanolic extract remarkably lower urinary calcium levels (2.34 ± 0.57) compared to aqueous extract treated group (3.67 ± 0.37). Remarkable higher urinary oxalate levels were reported in toxic control (2.36 ± 0.58) animals as analyzed to the positive control group (0.91 ± 0.26). Pretreatment with standard drug and particularly ethanolic extract for 10 days remarkably reduced urinary oxalate levels (0.98 ± 0.23 and 1.90 ± 0.29) which was significantly much lower than aqueous extract (2.19 ± 0.23). After 10 days of sodium oxalate treatment, urinary uric acid levels increased in the toxic control group (3.24 ± 0.63) as analyzed to positive control animals (0.62 ± 0.23). Pretreatment with cystone and ethanolic extract demonstrated notably reduction in the uric acid levels (1.58 ± 0.34 and 2.31 ± 0.43) when compared with aqueous treated group (3.07 ± 0.37).
Histopathological examination revealed that the positive control group showed no changes in renal tubules and glomerulus. There was no deposition of CaOx crystals in renal tissues [Figs. 3A & 4A]. The Toxic control group showed marked deposition of CaOx crystals, significant glomerular and tubular necrosis, congestion, blood vessel rupture with presence of large sized hyaline casts [Figs. 3B & 4B]. The standard drug-treated group exhibited recovered size of glomeruli and tubules [Figs. 3C & 4C]. There was no deposition of CaOx crystals. In 200 mg of aqueous extract-treated group exhibited marked tubular necrosis and inflammation with enlarged glomeruli with a mixed and medium sized crystal deposits [Figs. 3D & 4D]. In 200 mg ethanolic-treated group also exhibited small sized crystals compared to aqueous extract [Figs. 3E & 4E]. In ethanolic extract, recovery of glomeruli and tubule structure in renal tissues was evident.

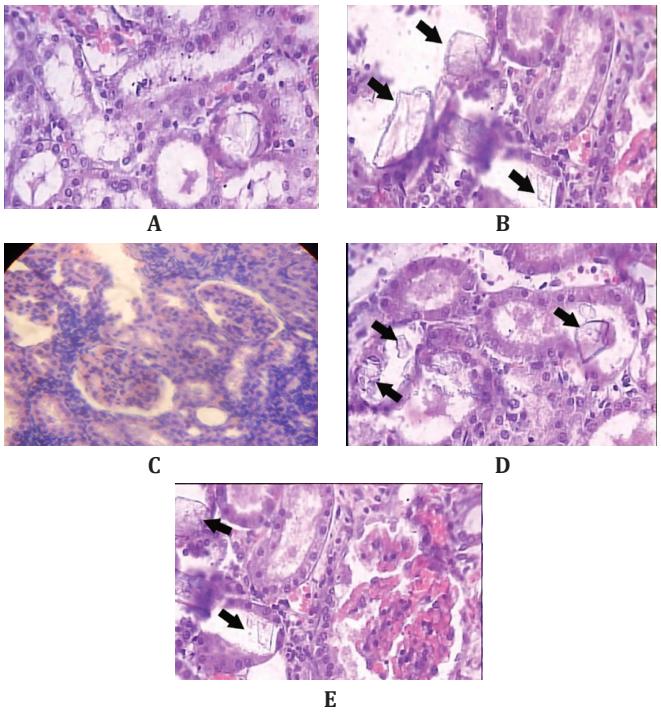

Fig. 3: Histopathological Section of Kidney after 10 days of treatment. A: Positive control group showed normal structure of glomerulus and tubules. B: Toxic control group showed enlarged glomeruli, tubular necrosis with large sized crystals. C: Standard group showed decreased glomeruli size without presence of crystals. D: Aqueous group showed enlarged glomeruli size with medium sized crystals. E: Ethanolic extract group showed decreased glomeruli size with mild sized crystals.

Discussion

The production of renal stones is a biological process involving a physicochemical ingredient and crystallisation. The nucleation that leads to crystal growth and crystal aggregation, which are responsible for stone creation, is an essential aspect of crystallisation. For the treatment of urolithiasis, medicines that block crystallisation and modify these processes or lower oxalate supersaturation are of great interest.

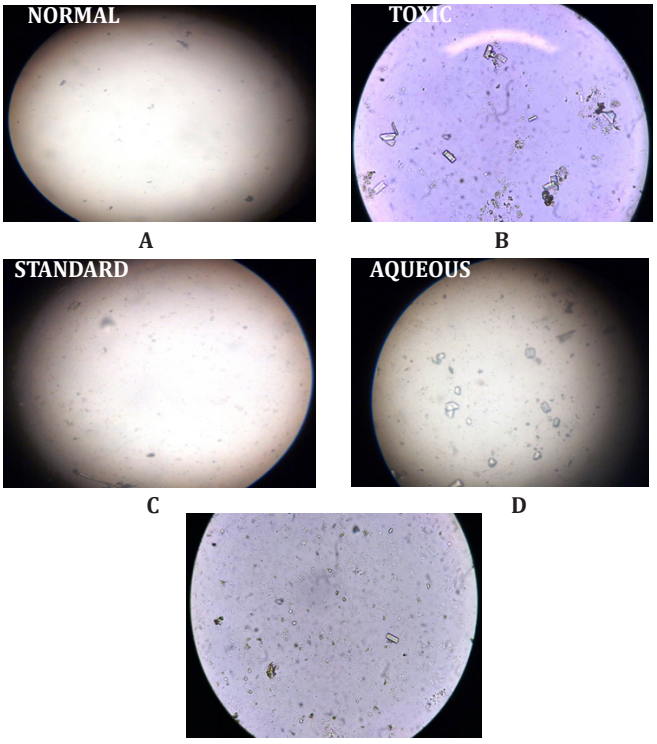

Fig. 4: Urine Microscopy under electron microscope after 10 days of treatment. A: Positive Control group showed no present of crystals. B: Toxic control group showed large sized crystals. C: Standard group showed absent of crystals. D: Aqueous extract group showed medium quantity sized crystals. E: Ethanolic extract group showed small sized crystals.

In the production of kidney stones, calcium oxalate, calcium phosphate, or other compounds in the urine form crystals on the inner surfaces of the kidneys. This step is known as the formation of the early mineral phase. Over time, crystals can join to create small, hard masses known as stones; this process is known as crystal growth. Crystals of calcium oxalate begin to develop, aggregate with other crystals, and are retained in the kidney. This is the aggregation process that leads to kidney damage. According to statistics, kidney stones have been one of the leading causes of renal failure for a very long time.

As there is currently no one effective medication for urolithiasis, surgery is regarded as the best option, particularly when other treatments fail. However, it is pricey and unaffordable for the average person. Consequently, natural medications are viewed as the next available option. The components isolated from the leaves of *B. spectabilis* demonstrated substantial ability to suppress crystal formation. This could be due to the high flavonoid and saponin content of *B. spectabilis*.[37] Calcium oxalate urolithiasis has been induced in rats using a variety of animal models. Among these models, the sodium oxalate-induced hyperoxaluria rat model results in the rapid development of calcium oxalate crystals in the renal tubules of experimental animals, and is therefore frequently used for quick screening of anti-urolithiatic medicines.[38]

Increased urinary oxalate concentration is associated with the biochemical pathways of sodium oxalate-induced lithiasis. Rats develop hyperoxaluria after intraperitoneal injection of sodium oxalate.[39,40] Due to its limited solubility, this result in the precipitation of calcium oxalate in urine. High oxalate levels and calcium oxalate crystals in the nephron, in particular, harm epithelial cells, resulting in heterogeneous nucleation and aggregation of calcium oxalate crystals in the renal tubules of experimental mice. [41] Because the urinary system of male rats is more similar to that of humans, male rats were used to develop urolithiasis. In addition, previous research has indicated that the amount of stone deposition in female rats is substantially smaller than in male rats.[42]

The increased urine volume is anticipated to aid in the early elimination of urinary stones. In the present investigation, treatment with *B. spectabilis* resulted in a considerable increase in the volume of urine in comparison to toxic control animals. According to reports, diuresis accelerates the process of dissolving prefabricated stones.[42] It also accelerates the elimination of small crystals and minimises the likelihood of these crystals growing and aggregating, hence preventing the production of new urinary system stones. Diuresis also leads to urine dilution of components, which reduces the probability of stone formation by decreasing the calcium oxalate saturation product. The induction of calculi with sodium oxalate leads in hyperxaluria. Consistent hyperoxaluria is a greater risk factor in the aetiology of renal stone development.[43,44] In addition, hyperoxaluria may result in the deposition of calcium oxalate in many organs.

Nevertheless, treatment with *B. spectabilis* extracts significantly decreased urinary oxalate excretion compared to toxic control rats. Compared to the aqueous extract, the ethanolic extract was the most powerful. In the current investigation, toxic control rats exhibited elevated urine and serum calcium levels. According to reports, hyperoxaluria results in calcium oxalate precipitation in the urine.[41] This calcium oxalate precipitation also causes damage to the renal architecture over a varied range, causing functional impairment as well. Precipitation and long-term deposition of calcium oxalate render the system more vulnerable and provoke the development of problems. Therefore, the lowered renal oxalate level indicates that *B. spectabilis* has the capacity to prevent the retention of stones, which is consistent with the study's other findings. However, *B. spectabilis* treatment resulted in a significant drop in urine and serum calcium levels. In this sense, ethanolic extract was found to be the most efficacious of all extracts. The improvement in urine calcium excretion, suggesting *B. spectabilis*'s potential to avoid damage, halts any potential functional abnormalities, which is the most frequently anticipated outcome of any therapy. This action may be a result of either the avoidance of hyperoxaluria or the suppression of calcium-oxalate binding. In the treatment of urolithiasis, avoidance of stone formation is regarded as the first step, particularly during recurrence, as it can prevent the development of additional symptoms and consequences.

Patients with kidney stones and rats with hyperoxaluria have been reported to excrete more uric acid.[44] There is evidence that uric acid inhibits the solubility of calcium oxalate. Furthermore, it binds to and inhibits urinary glycosaminoglycans. It has been shown that glycosaminoglycans suppress the nucleation and crystallisation of calcium oxalate.[45] Therefore, the binding of uric acid to free urinary glycosaminoglycans inhibits the inhibitory effect of these molecules on calcium oxalate crystallization. The preponderance of uric acid crystals in calcium oxalate stones and the fact that uric acid binding proteins may bind to calcium oxalate and control its crystallization suggest that uric acid plays a fundamental role in the production of stones. In the current investigation, the uric acid excretion in the urine of lithiatic control rats was substantially higher than that of vehicle control rats. Nevertheless, therapy with cystone and *B. spectabilis* decreased this increase in urine uric acid excretion. It was discovered that ethanolic extract was the most effective at reducing urinary uric acid concentrations. This suggests that *B. spectabilis* decreased the excretion of uric acid and, consequently, decreased the likelihood of stone formation.

The high serum levels of creatinine, calcium, uric acid, and

blood urea nitrogen (BUN) are indicative of renal injury caused by the nucleation and subsequent formation of renal stones. In the present investigation, therapy with the standard drug and *B. spectabilis* prevented the rise of these serum markers, indicating the prevention of kidney injury. Compared to aqueous extract, ethanolic extract was determined to be the most efficacious among all extracts in this regard. This effect of the ethanolic extract of the plant *B. spectabilis* after standard medication was regarded as one of the most valuable because renal damage can result in a wide range of functional abnormalities and the onset of a number of complications that make the condition more difficult to manage.

The presence of hyaline cast in the kidneys and tubular congestion and necrosis due to calculi-inducing therapy produced severe kidney injury. Treatment with cystone and *B. spectabilis* avoided these histological changes in addition to restoring the structure, resulting in a considerable decrease in renal damage. This suggests that *B. spectabilis* reduces renal tubule damage significantly. The plant's antioxidant[23-25] and hepatoprotective[28] properties may be primarily responsible for this effect. Histological alterations are regarded as a direct technique for determining the efficacy of a treatment. These results demonstrated conclusively that *B. spectabilis* has the potential to prevent and reverse pathogenic alterations, supporting earlier histological studies.

Conclusion

Overall, this considerable improvement in urine parameters, serum parameters, and kidney histopathology parameters by *B. spectabilis* in sodium oxalate-induced urolithiasis animals demonstrates its efficacy in all phases of pathogenesis. The quantity of action exerted by plant material is largely determined by the predominance of phytochemicals and their interaction with one another. The summary of the results suggests the anti-urolithiatic potential of ethanolic extract of the plant *B. spectabilis*. The presence of both flavonoids and saponins in the plant suggests that it could be the active principle of the plant for urolithiasis. However, the coexistence of other physiologically active constituent(s) cannot be overlooked. Herbal remedies are helpful in treating urolithiasis and have fewer adverse effects than current drugs. In addition, herbs are frequently used as an adjunct to modern drugs to prevent illness recurrence; hence, herbal medicines may interact with these modern medicines administered concurrently for urolithiasis. This study merely demonstrates the potential of *B. spectabilis* as an anti-urolithiatic agent. For a better understanding of the safety, efficacy, and mechanism of action of these active phytoconstituents of *B. spectabilis* as an anti-urolithiatic agent, additional research is required on the isolation of these active phytoconstituents.

Acknowledgement

I am extremely thankful to my research supervisor Dr. Jai Singh Vaghela, Associate Professor, Department of Pharmacology, Bhupal Nobles' University, Udaipur. I would like to extend my sincere thanks to Dr. Amit, Head of Pathology department, SAIMS Indore for their valuable time to complete this research work.

Conflict of Interest

Authors do not have any conflict of interest.

Reference

1. Mohamed N, Farook P, Mozhiyarasi and Nalini R. Inhibition of Mineralization of Urinary Stone Forming Minerals by Medicinal Plants. *E-Journal of Chemistry* 2006; 3: 182-5.
2. Gangwal K. Current concepts on aetiology of urolithiasis. 1971; 8:58.
3. Singh P, Singh L, Prasad S, Singh M. Urolithiasis in Manipur (North Eastern Region of India). Incidence and chemical composition of stones. *Am J Clin Nutr* 1978; 31: 1519-25.
4. Preminger G. Management of lower pole renal calculi: Shock Wave Lithotripsy versus Percutaneous Nephrolithotomy versus Flexible Ureteroscopy. *Urol Res* 2006; 34: 108-11.
5. Nabi G, Downey P, Keeley FX, Watson GM, McClinton S. Extracorporeal shock wave lithotripsy (ESWL) versus ureteroscopic management for ureteric calculi. Cochrane Database Syst Rev. 2012; 5 :1095-103.
6. Aeckart KSJ, Schroder FH. Effect of extra corporeal shock wave lithotripsy (ESWL) on renal tissue. Urol Res. 1989; 17(1):3-7.
7. Mahajan MM, Dudhgaonkar S, Deshmukh SN. Antidiabetic and hypolipidemic effects of the aqueous leaf extract of *Bougainvillea* species. Int J Basic Clin Pharmacol 2015; 4:596-7.
8. Rashid F, Sharif N, Ali I, Sharif S, Nisa FU, Naz S. Phytochemical analysis and inhibitory activity of ornamental plant (*Bougainvillea spectabilis*). Asian J Plant Sci Res 2013; 3:1-5.
9. Umamaheswari A, Shreevidya R, Nuni A. In vitro antibacterial activity of *Bougainvillea spectabilis* leaves extracts. Adv Biol Res 2008; 2:1-5.
10. Kumara Swamy M, Sudipta KM, Lokesh P, Neeki MA, Rashmi W, Bhaumik SH, Phytochemical screening and in vitro antimicrobial activity of *Bougainvillea spectabilis* flower extracts. Int J Phytomed 2012; 4:375-9.
11. Hajare CN, Inamdar FR, Patil RV, Shete CS, Wadkar SS, Patil KS, et al. Antibacterial activity of the leaves of *Bougainvillea spectabilis* against E. coli NCIM 2832 and M. aureus NCIM 5021. Int J Pharm Sci Rev Res 2015; 34:194-6.
12. Kumar DJ, Sonia K, Madhan R, Selvakumar K. Antiyeast, antioxidant and anticancer activity of *Tribulus terrestris* Linn and *Bougainvillea spectabilis* Linn. Res J Pharm Technol 2011;4:1483-9.
13. Narayanan CR, Joshi DD, Mujumdar AM. Hypoglycemic action of *Bougainvillea spectabilis* leaves. Curr Sci 1984; 53:579-81.
14. Saikia H, Das S. Antidiabetic action of *Bougainvillea spectabilis* (leaves) in normal and alloxan induced diabetic albino rats. Indian Drugs 2009;46: 391-7.
15. Jawla S, Kumar Y, Khan MS. Hypoglycemia activity of *Bougainvillea spectabilis* stem bark in normal and alloxan-induced diabetic rats. Asian Pac J Trop Biomed 2012;2:919-23.
16. Mishra N, Joshi S, Tandon VL, Munjal A. Evaluation of antifertility potential of aqueous extract of *Bougainvillea spectabilis* leaves in swiss albino mice. Int J Pharm Sci Drug Res. 2009;1: 19-23.
17. Hembrom AR, Pragya S, Kumar J, Singh VN. Effects of aqueous leaf extract of *Bougainvillea spectabilis* on seminal quality of mice. J Adv Zool. 2011; 32:119-22.
18. Ali MS, Ibrahim SA, Ahmed F, Pervez MK. Colour versus bioactivity in the flowers of *Bougainvillea spectabilis* (Nyctaginaceae). Nat Prod Res. 2005; 19:1-5.

19. Joshi DD, Mujumdar AM, Narayanan CR. Anti-inflammatory activity of *Bougainvillea spectabilis* leaves. Indian J Pharm Sci. 1984; 46:187-8.
20. Mandal G, Chatterjee C, Chatterjee M. Evaluation of anti-inflammatory activity of methanolic extract of leaves of *Bougainvillea spectabilis* in experimental animal models. Pharmacogn Res 2015; 7:18-22.
21. Adebayo JO, Adesokan AA, Olatunji LA, Buoro DO, Aoladoye AO. Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. Biochemistry 2005; 17:45-50.
22. Saikia H, Lama A. Effect of *Bougainvillea spectabilis* leaves on serum lipids in albino rats fed with high fat diet. Int J Pharm Sci Drug Res 2011; 3:141-5.
23. Chaires-Martinez L, Monroy-Reyes E, Bautista-Bringas A, Jimenez-Avalos HA, Sepulveda-Jimenez G. Determination of radical scavenging activity of hydroalcoholic and aqueous extracts from *Bauhinia divaricata* and *Bougainvillea spectabilis* using the DPPH assay. Pharmacognosy Res. 2009; 1:238-44.
24. Venkatachalam RN, Singh K, Marar T. *Bougainvillea spectabilis*, a good source of antioxidant phytochemicals. Res J Pharm Biol Chem Sci. 2012;3: 605-13.
25. Dhankhar S, Sharma M, Ruhil S, Balhara M, Kumar M, Chhillar AK. Evaluation of antimicrobial and antioxidant activities of *Bougainvillea spectabilis*. Int J Pharm Pharm Sci. 2013;5:178-82.
26. Malairajan P, Gopalakrishnan G, Narasimhan S, Jessi KV. Antiulcer activity of crude alcoholic extracts of *Bougainvillea spectabilis* Willd. Jundishapar J Nat Pharm Prod. 2007;2:1-6.
27. Bolognesi A, Polito L, Olivieri F, Valbonesi P, Barbieri L, Battelli MG, et al. New ribosome-inactivating proteins with polynucleotide:adenosine glycosidase and antiviral activities from Basella rubra L. and *Bougainvillea spectabilis* Willd. Planta 1997; 203: 422-9.
28. Malomo SO, Adebayo JO, Arise RO, Olorunniji FJ, Egwim EC. Effects of ethanolic extract of *Bougainvillea spectabilis* leaves on some liver and kidney function indices in rats. Phytochem Pharmacol 2006; 17:261-72.
29. Sherwani SK, Khan MM, Zubair A, Shh MA, Kazmi SU. Evaluation of in vitro thrombolytic activity of *Bougainvillea spectabilis* leaf extract. Int J Pharm Sci Rev Res 2013; 21:6-9.
30. Srivastava SK, Krishnan PS. An Oxalic Acid Oxidase in the Leaves of *Bougainvillea spectabilis*. Biochem. J, 1962; 85: 33-38.
31. Rajeswari. P, Keziya priya, Bhanusree. In Vitro Evaluation of Anti-Urolithiatic Activity of Aqueous Extract of *Bougainvillea glabra* (Leaves) Ijppr. Human Journals, 2018; Vol. 12 (3): 409-421.
32. Das et al., Evaluation Of *In- Vitro* Litholytic Activity of Plant *Bougainvillea spectabilis*. J Adv Sci Res, 2021; 12 (4): Suppl 1: 311-315.
33. Smith QE, Pharmacological screening tests progress in medicinal chemistry, Butterworths, London, 1960; 16: pp. 1-5.
34. Aisha Shehzad, et al. Antiurolithic Evaluation of *Cucurbita pepo* Seeds Extract against Sodium Oxalate-Induced Renal Calculi. Pharmacognosy Magazine, Volume 16, Issue 68, January-March 2020 (Supplement 1).
35. Hodgkinson A. Determination of oxalic acid in biological material. *Clin. Chem.* (1970); 16: 547.
36. Lorentz K. Improve determination of calcium with ortho-cresolphthalein complexone. Clin Chim Acta 1982; 126: 327-33.
37. Das P, Vaghela J, Badore N, Pharmacognostical, Phytochemical and Fluorescence analysis of the plant *Bougainvillea spectabilis* (Willd.). Research J. Pharm. and Tech.2021; 14(7): 3733-3738.
38. Gupta P, Patel N, Bhatt L, et al. Anti-urolithiatic effect of petroleum ether extract stem bark of *Crataeva adansonii* in rats. Pharm Biol. 2006; 44: 160-165.
39. Takawale RV, Mali VR, Kapase CU, et al. Effect of *Lagenaria siceraria* fruit powder on sodium oxalate induced urolithiasis in Wistar rats. J Ayurveda Integr Med. 2012; 3:75-79.
40. Patel N, Badole S, Gupta P, et al. Effect of ethanolic extract of leaves of *Cocculus hirsutus* (L.) diels on experimentally induced urothiliasis in rats. J Nat Rem. 2008;8:24-31.
41. Scheid CR, Cao LC, HoneymanT,. How elevated oxalate can promote kidney stone disease: changes at the surface and in the cytosol of renal cells that promote crystal adherence and growth? Front Biosci. 2004; 9:797-808.
42. Karadi RV, Gadge NB, Alagawadi KR. Effect of *Moringa oleifera* Lam. Rootwood on ethylene glycol induced urolithiasis in rats. J Ethnopharmacol. 2006; 105: 306-311.
43. Ingale KG, Thakurdesai PA, Vyawahare NS. Effect of *Hygrophila spinosa* in ethylene glycol induced nephrolithiasis in rats. Indian J Pharmacol. 2012; 44: 639-642.
44. Divakar K, Pawar AT, Chandrasekhar SB, et al. Protective effect of the hydroalcoholic extract of *Rubia cordifolia* roots against ethylene glycol induced urolithiasis in rats. Food Chem Toxicol. 2010;48:1013-1018.
45. Grases F, Gil JJ, Conte A. Glycosaminoglycans: inhibition of calcium oxalate crystalline growth and promotion of crystal aggregation. Colloids Surfaces. 1989; 36:29-38.

HOW TO CITE THIS ARTICLE: Das PK, Vaghela JS. Evaluation of Anti-urolithiatic Activity of *Bougainvillea spectabilis* Willd. against Sodium Oxalate-induced Nephrolithiasis in Rats. Int. J. Pharm. Sci. Drug Res. 2023;15(1):57-64. **DOI:** 10.25004/IJPSDR.2023.150109