Research Article

An Exploration for Protective Effect of Rhizome Extract *of Iris pseudacorus* L. and Seed Extract of *Dolichos biflorus* L. in Sodium Oxalate Induced Urolithiasis in Rat Model

Sarita Sharma[1]*, Dr. (Prof) Amit J. Raval[2], Dr. (Prof) Raju Koneri[3], Dr. Gaurav Kumar Sharma[4], Dr. (Prof) Kaushal K. Chandrul[5]

1. PhD Research Scholar, Pacific Academy of Higher Education and Research University, Udaipur, India.
2. Professor, Pacific Academy of Higher Education and Research University, Udaipur, India.
3. Professor and Dean, Karnataka College of Pharmacy, Bangalore, India.
4. Associate Professor, Dept of Pharmacy, Mewar University, Gangrar, Rajasthan, India.
5. Professor and Principal, Dept of Pharmacy, Mewar University, Gangrar, Rajasthan, India.

***Corresponding author's E-mail:** sharmasarita.4416@gmail.com

Received: 14-02-2022; **Revised:** 26-04-2022; **Accepted:** 05-05-2022; **Published on:** 15-05-2022.

ABSTRACT

The aim of our presented study is to assess the effect of Rhizome extract of *Iris pseudacorus* L. and seed extract of *Dolichos biflorus* L. as preventive agent in experimentally induced urolithiasis model in rats. Rats were administered Sodium Oxalate (70 mg/kg, i. p.) in drinking water for 28 days *in drinking water.* In addition to this, Saponin extract of *Iris pseudacorus* and *Dolichos biflorus* of low dose and high dose were administered along with Sodium Oxalate on 14-28th day. After the experimental period, blood samples were collected by cardiac puncture to analyse for Creatinine, Calcium, Blood Urea Nitrogen (BUN), Phosphorus, Uric acid, Alkaline Phosphate, Potassium, and Alanine Amino Transferases followed by various antioxidants and kidney histopathology. The ethylene glycol feeding resulted in an increased level of all parameters evaluated compared to normal rats. All these conditions were reversed with plant extract treatment. Histopathological analysis also showed that rats treated with Sodium Oxalate had large deposits of calcium oxalate crystals, and that deposits were reduced in rats treated with plant extract. Results were also compared with the marketed product cystone as a standard. These data suggest that *Iris pseudacorus* and *Dolichos biflorus* Saponin extracts has a protective activity against urolithiasis.

Keywords: Calcium oxalate, Kidney stone, Antiurolithiatic, *Iris pseudacorus*, *Dolichos biflorus*.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2022.v74i01.029

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v74i01.029

INTRODUCTION

The widespread usage of herbal remedies in recent years has presented India with an excellent opportunity to search for therapeutic lead compounds from an old system of medicine, namely Ayurveda, that can be used in the development of novel drugs. Natural products account for more than half of all modern medications, and they play an essential part in the pharmaceutical industry's drug research programmes.[1]

Urolithiasis is a widespread issue that has afflicted humans for generations. The production of urinary calculi in the urinary system is known as urolithiasis.[2] Although the overall chances of producing stones differ around the world, it is a global public health concern.[3] Kidney calculi have risen in occurrence over the last three decades.[4]

Urinary calculi are the third most common urinary system problem. Urinary tract stone disease affects almost 10% of the population of the industrialised world, according to estimates. In developed countries, kidney stones represent for 0.5 to 1.9 percent of clinical cases.[5] Urinary calculi can lead to urinary tract blockage, hydronephrosis, infection, and bleeding.[6] To remove the calculi, surgical procedures, lithotripsy, and local calculus disruption with a high-power laser are commonly utilised. These operations, however, are costly, and recurrence is prevalent.[7]

Various therapies are being employed to try to prevent recurrence, including thiazide diuretics and alkali-citrate, but empirical evidence for their efficiency is lacking.[8] Traditional remedies, on the other hand, have supplied a substitute for many ailments as well as some additional information on disease pathogenesis.[9] As a result, the hunt for new antilithiatic therapies derived from natural sources has become more important, as herbal medicines are less expensive and have less adverse effects.[10]

Iris pseudacorus (Iridaceae) and *Dolichos biflorus* (Fabaceae) is reported to be used in Urinary complaints.[11] There is no work reported on the antiurolithiatic activity of *Iris pseudacorus* and *Dolichos biflorus*, hence the present investigation has been undertaken.

1. MATERIALS AND METHODS

Collection of plant material

The *Iris pseudacorus* L. Rhizome was procured from Iran and *Dolichos biflorus* Linn Seeds were procured from Bangalore, Karnataka. Dr.Geetanjali (HOD of Botany

Department Sree Siddaganga College Tumkur University, India.) has identified and authenticated the sample (Reference No. 507/20-21).

Extraction of the plant material and sample preparation

The Rhizome of *Iris pseudacorus* L. is sliced into small parts and dried under shades for 7 days at room temperature. The dried rhizome of *Iris pseudacorus* and seed of *Dolichos biflorus* were powdered, then the sieved (10/40). The powder was used for preparation of methanol extraction. The 1000 ml methanol reflux condenser extracted every 100 g powder for 3 periods of 7 hours till it gets half. After completion of extraction, the extract was filtered by using Whattman No.1 paper and evaporated to get dryness at room temperature. Methanolic extract was subjected to preliminary phytochemical screening.[12-15]

Isolation and purification of Saponin from Methanolic extraction of *Iris pseudacorus* L. Rhizome and *Dolichos biflorus* Linn Seeds

Extraction of Saponin was done by TLC fractionation method. 5gms of methanolic extract was subjected to saponification in 50ml of 20% ethanol. Followed by filtrations and residues was once again extracted with 20%/50ml ethanol and filtered. Both the filtrates combined together and heated to residue the volume to 40ml at 900C. Fractioned with 40ml of diethyl ether in separating funnel (Repeated twice) and ether layer was recovered. Aqueous layer was fractionated with 60ml of n-Butanol in separating funnel (Repeated twice) and aqueous layer recovered. N-Butanol layer was washed with 5% NaCl solution, dried and weighed. Finally, 10 ml methanol was added and methanol layer and white powder separated. White powder assumed that highly purified. Solubility and chemical tests were conducted to confirm the presence of Saponin. And this sample (Saponin) is used for all further experimentations.

Experimental animals

Wistar rats (Both sex, 5-6 weeks old) weighing 150-200 gm and Albino mice (Male, 5-6 weeks old) 20-25 gm have been used for the current study. All the work carried out on the animals was in accordance with the CPCSEA guidelines and the Research protocols have been approved by the IAEC, KCP, and the Sl. No. was KCP/IAEC/08/20-21/16/13-03-21.

Drugs and Chemicals

All the chemicals used for the study was procured from Himedia, Mumbai and Merck, India. Equipment's were used was purchased from Analytical Technologies limited, India and Thermo Scientific, USA.

Acute toxicity test on the pure active Saponin *Iris pseudacorus L. Rhizome* and *Dolichos biflorus Linn* Seedsas per OECD guidelines No. 425

Albino mice (Female, 5-6 weeks old) with a weight of 20-25 gm were fasted overnight and limit and test is carried out with an initial dose of 175mg/kg/b.w. The following order is followed: 175, 550, 1750 and 5000 mg/kg /b.w.

All the Animals have been observed during the time being especially first 30 minutes to 24 hours. The special attention is required during the first 4 hours and then every day up to 14 days.

***Model: Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rat Model*[16]**

Experimental Methods

42 Wistar rats age 5 to 6 weeks weighing (150-200g) have been divided in to following groups, with 6 animals in each group (n=6), in the following manner:

Group 1	Normal control	Vehicle for 28 days.
Group 2	Disease control	Sodium Oxalate (70 mg/kg, i. p.) in drinking water for 28 days.
Group 3	Standard group	Sodium Oxalate (70 mg/kg, i. p., 28 days) + Cystone (750 mg/kg, p.o.) on 14th -28th day.
Group 4	Test group 1	Sodium Oxalate (70 mg/kg, i. p., 28 days) + Saponin of Iris pseudacorus at low dose (X mg/kg, p.o.) on 14-28th day.
Group 5	Test group 2	Sodium Oxalate (70 mg/kg, i. p., 28 days) in drinking water for 28 days + Saponin of Iris pseudacorus at high dose (Y mg/kg, p.o.) on 14-28th day.
Group 6	Test group 3	Sodium Oxalate (70 mg/kg, i. p., 28 days) + Saponin of Dolichos biflorus at low dose (X mg/kg, p.o.) on 14-28th day.
Group 7	Test group 4	Sodium Oxalate (70 mg/kg, i. p., 28 days) in drinking water for 28 days + Saponin of Dolichos biflorus at high dose (Y mg/kg, p.o.) on 14-28th day.

Parameters to be Evaluated

Biochemical Parameters

Collection of Blood Samples

After the experimental period, blood samples were collected by cardiac puncture under mild pentobarbital anesthesia. Collected blood samples were allowed to clot for 10 mins at room temperature and Serum was separated by centrifugation at 10000×g for 10 minutes and analysed for Creatinine, Calcium, Blood Urea Nitrogen (BUN), Phosphorus, Uric acid, Alkaline Phosphate, Potassium, and Alanine Amino Transferases.

Histopathology Studies & Kidney Homogenate Analysis

At the end of the experiment, on day 28th the rats were sacrificed by high dose of pentobarbital and kidneys excised, isolated kidneys have been cleaned off extraneous tissue and rinsed in ice cold physiological

saline. After paraffin infiltration the Tissue pieces were sectioned at 5µm and stained with haematoxylin and eosin for Histopathological examination.[17-19]

Analysis of Tissue Antioxidant Enzyme:

The remaining half portion of the right kidney was used for the estimation of various marker enzymes like MDA or LPO, GSH and LDH. 10% homogenate of the tissues were prepared in 0.1M Tris HCL buffer (pH 7.4) in a homogenizer. The homogenate was centrifuged at 12000 × g for 30 minutes. The supernatant obtained after centrifugation were used for the estimation of various marker enzymes.[20, 21]

Statistical Analysis

The data were presented as Mean ± S.E.M. from N = 6 rats in each group and analyzed using one way of Variance ANOVA followed by Tukey multiple comparison tests. P value <0.05 was considered statistically significant. Graph pad Prism 5.0 and Excel software were used for statistical analysis.

RESULTS

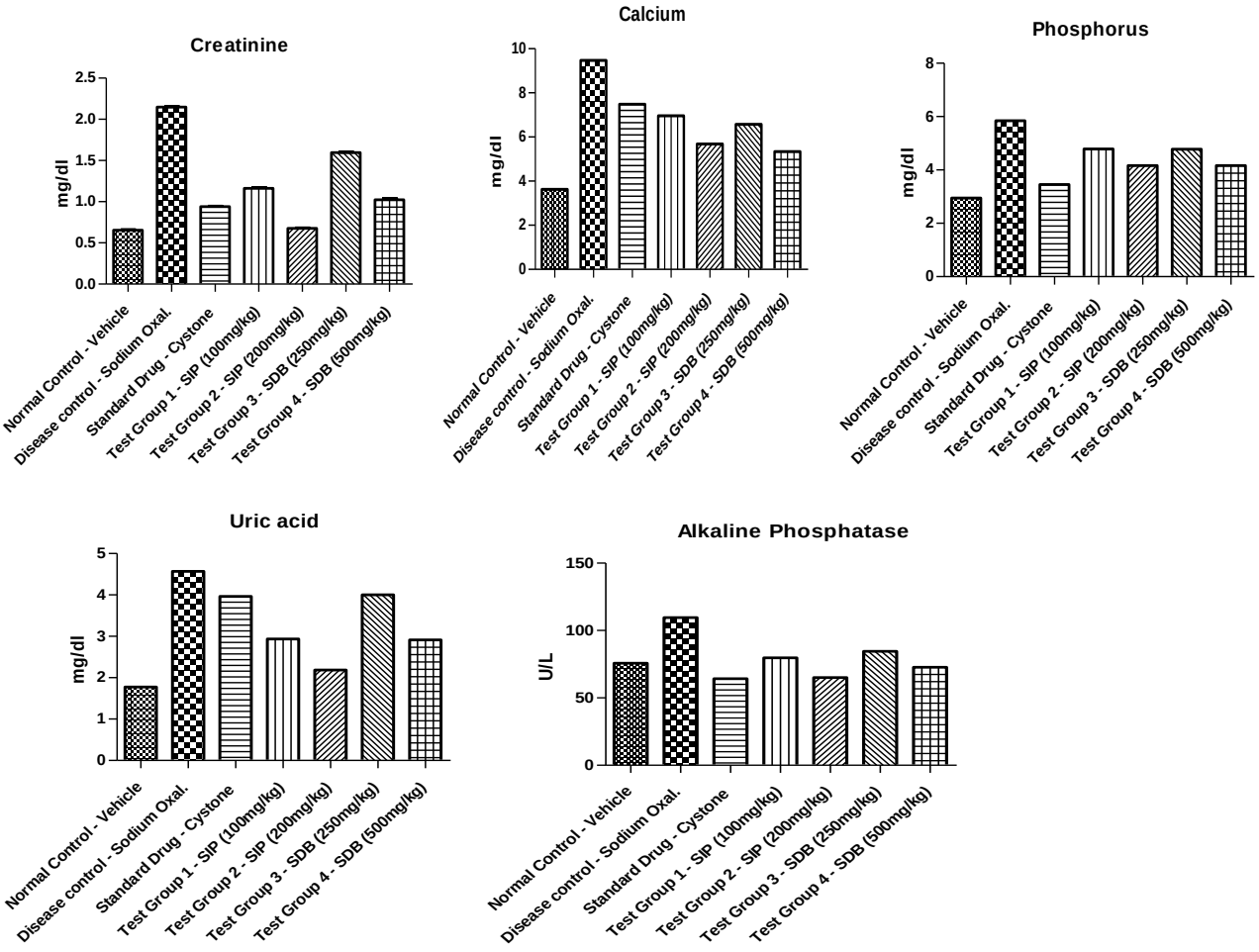
Extraction, Isolation and Purification of Phytoconstituents

Yield of Saponin of Methanolic extract of Iris pseudacorus L. Rhizome and Dolichos biflorus Linn Seeds.

The Yield of crude extracts of;

- *Iris pseudacorus* L. Rhizome was 6.87%. And from 5gms of crude extract 0.5185gm of Saponin was obtained by quantitative determination. The % of Saponin was found to be 10.37.
- *Dolichos biflorus* Linn Seed was 16.14%. And from 5gms of crude extract 0.7549 of Saponin was obtained by quantitative determination. The % of Saponin was found to be 15.09.

Model: Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rat Model



Values are expressed as Mean ± SEM, n = 6 in each group

Figure 1: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on Creatinine, Ca, Phosphorus, Uric acid and ALP Analysis after 28 days of treatment

Table 1: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on Renal Analysis (Creatinine, Ca, Phosphorus, Uric acid and ALP) after 28 days of treatment

Groups	Test Description				
	Creatinine (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Uric acid (mg/dl)	Alkaline Phosphatase (U/L)
Normal Control -*Vehicle*	0.65±0.007	3.62±0.006	2.94±0.014	1.77±0.009	75.67±0.47
Disease control - Sodium Oxalate (70 mg/kg, i. p.)	2.15±0.004	9.47±0.012	5.84±0.014	4.568±0.017	109.6±0.75
Standard Drug - *Cystone (750 mg/kg/b.w./p.o.)*	0.94±0.005	7.48±0.009	3.45±0.017	3.965±0.03	64.31±0.6
Test Group 1 - SIP (100mg/kg/b.w./p.o.)	1.162±0.009	6.96±0.014	4.78±0.007	2.93±0.008	79.81±0.6
Test Group 2 - SIP (200mg/kg/b.w/p.o.)	0.675±0.007	5.68±0.008	4.16±0.014	2.185±0.01	65.13±0.54
Test Group 3 - SDB (250mg/kg/b.w./p.o.)	1.597±0.003	6.57±0.013	4.77±0.009	4.0±0.02	84.63±0.41
Test Group 4 - SDB (500mg/kg/b.w/p.o.)	1.023±0.012	5.33±0.024	4.167±0.01	2.91±0.08	72.71±0.32

Values are expressed as Mean ± SEM, n = 6 in each group

Table 2: Statistical Comparison Test between All the Groups

Groups/Parameters	Creatinine (mg/dl)		Calcium (mg/dl)		Phosphorus (mg/dl)		Uric acid (mg/dl)		Alkaline Phosphatase (U/L)	
Tukey's Multiple Comparison Test	Significant? P < 0.05?	Summary	Significant? P < 0.05?	Summary	Significant? P < 0.05?	Summary	Significant? P < 0.05?	Summary	Significant? P < 0.05?	Summary
Normal Control - Vehicle vs Disease control - Sodium Oxal.	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Standard Drug - Cystone	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 1 - SIP (100mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 2 - SIP (200mg/kg)	No	ns	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	**
Disease control - Sodium Oxal. vs Standard Drug - Cystone	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 1 - SIP (100mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***

Disease control - Sodium Oxal. vs Test Group 2 - SIP (200mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 1 - SIP (100mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 2 - SIP (200mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	No	ns
Standard Drug - Cystonevs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***	No	ns	Yes	***
Standard Drug - Cystonevs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 2 - SIP (200mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	No	ns	Yes	***	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***	No	ns	Yes	***
Test Group 2 - SIP (200mg/kg) vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***
Test Group 2 - SIP (200mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	No	ns	Yes	***	Yes	***
Test Group 3 - SDB (250mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***	Yes	***

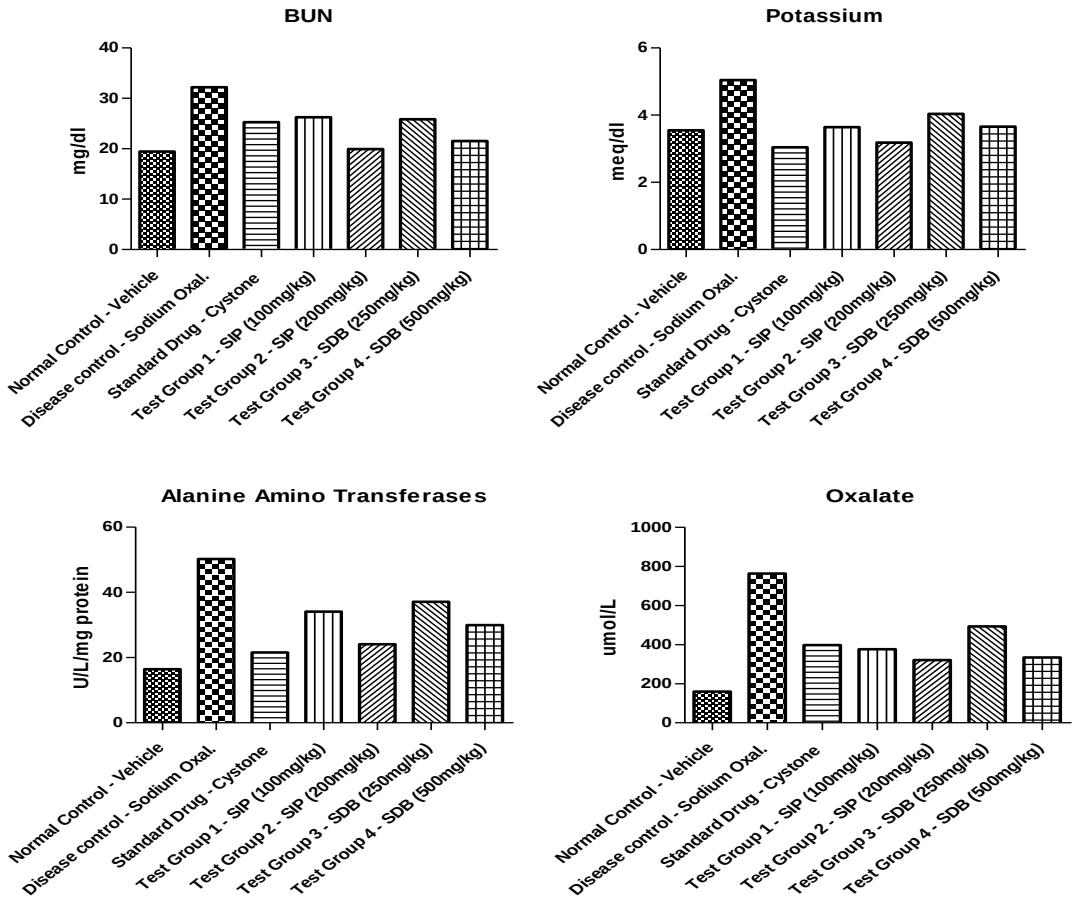
Table 3: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on Renal Analysis (BUN, K, AAT, and Oxalate) after 28 days of treatment

Groups	Test Description			
	BUN (mg/dl)	Potassium (meq/dl)	Alanine Amino Transferases (U/L/mg protein)	Oxalate (µmol/L)
Normal Control -*Vehicle*	19.46±0.42	3.54±0.096	16.46±0.24	159.9±0.48
Disease control - Sodium Oxalate (70 mg/kg, i. p.)	32.21±0.45	5.047±0.024	50.21±0.58	763.2±2.08
Standard Drug - *Cystone (750 mg/kg/b.w./p.o.)*	25.29±0.43	3.04±0.02	21.59±0.73	397.3±2.84
Test Group 1 - SIP (100mg/kg/b.w./p.o.)	26.24±0.94	3.64±0.11	34.07±0.58	375.8±6.08
Test Group 2 - SIP (200mg/kg/b.w/p.o.)	19.94±0.58	3.185±0.02	24.12±0.58	320.5±1.78
Test Group 3 - SDB (250mg/kg/b.w./p.o.)	25.85±0.79	4.03±0.024	37.10±0.58	492.3±2.10
Test Group 4 - SDB (500mg/kg/b.w/p.o.)	21.56±0.41	3.65±0.012	29.95±0.58	333.5±2.14

Values are expressed as Mean ± SEM, n = 6 in each group

Table 4: Statistical Comparison Test between All the Groups

Groups/Parameters	BUN (mg/dl)		Potassium (mg/dl)		Alanine Amino Transferases (U/L/mg protein)		Oxalate (µmol/L)	
Tukey's Multiple Comparison Test	Significant? P < 0.05?	Summary	Significant? P < 0.05?	Summary	Significant? P < 0.05?	Summary	Significant? P < 0.05?	Summary
Normal Control - Vehicle vs Disease control - Sodium Oxal.	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Standard Drug - Cystone	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 1 - SIP (100mg/kg)	Yes	***	No	ns	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 2 - SIP (200mg/kg)	No	ns	Yes	**	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 4 - SDB (500mg/kg)	No	ns	No	ns	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Standard Drug - Cystone	Yes	***	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 1 - SIP (100mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 2 - SIP (200mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 1 - SIP (100mg/kg)	No	ns	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 2 - SIP (200mg/kg)	Yes	***	No	ns	No	ns	Yes	***
Standard Drug - Cystonevs Test Group 3 - SDB (250mg/kg)	No	ns	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 4 - SDB (500mg/kg)	Yes	**	Yes	***	Yes	***	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 2 - SIP (200mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 3 - SDB (250mg/kg)	No	ns	Yes	***	Yes	*	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	No	ns	Yes	***	Yes	***
Test Group 2 - SIP (200mg/kg) vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***	Yes	***
Test Group 2 - SIP (200mg/kg) vs Test Group 4 - SDB (500mg/kg)	No	ns	Yes	***	Yes	***	No	ns
Test Group 3 - SDB (250mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	**	Yes	***	Yes	***



Values are expressed as Mean ± SEM, n = 6 in each group

Figure 2: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on BUN, Potasium, AAT, and Oxalate Analysis after 28 days of treatment.

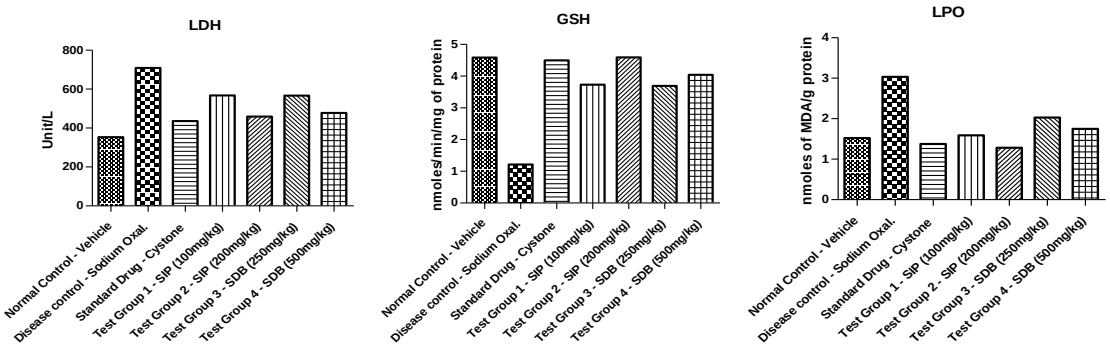
Table 5: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on Assay of tissue (Kidney homogenate) enzyme after 28 days of treatment

Groups	Test Description		
	Lactate dehydrogenase (LDH) (Unit/L)	Glutathione Stimulating Hormone (GSH) (nmoles/min/mg of protein)	Lipid Peroxidation (LPO) (nmoles of MDA/g protein)
Normal Control -*Vehicle*	353.6±0.58	4.583±0.008	1.523±0.006
Disease control - Sodium Oxalate (70 mg/kg, i. p.)	708.5±1.6	1.21±0.011	3.032±0.024
Standard Drug - *Cystone (750 mg/kg/b.w./p.o.)*	436.3±1.74	4.49±0.12	1.373±0.02
Test Group 1 - SIP (100mg/kg/b.w./p.o.)	567.5±1.43	3.73±0.077	1.587±0.004
Test Group 2 - SIP (200mg/kg/b.w/p.o.)	459.3±2.4	4.593±0.068	1.282±0.013
Test Group 3 - SDB (250mg/kg/b.w./p.o.)	566.8±0.87	3.69±0.023	2.03±0.01
Test Group 4 - SDB (500mg/kg/b.w/p.o.)	477.3±0.714	4.04±0.015	1.74±0.02

Values are expressed as Mean ± SEM, n = 6 in each group

Table 6: Statistical Comparison Test between All the Groups

Groups/Parameters	Lactate dehydrogenase (LDH)		Glutathione Stimulating Hormone (GSH)		Lipid Peroxidation (LPO)	
Tukey's Multiple Comparison Test	**Significant? P < 0.05?**	**Summary**	**Significant? P < 0.05?**	**Summary**	**Significant? P < 0.05?**	**Summary**
Normal Control - Vehicle vs Disease control - Sodium Oxal.	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Standard Drug - Cystone	Yes	***	No	ns	Yes	***
Normal Control - Vehicle vs Test Group 1 - SIP (100mg/kg)	Yes	***	Yes	***	No	ns
Normal Control - Vehicle vs Test Group 2 - SIP (200mg/kg)	Yes	***	No	ns	Yes	***
Normal Control - Vehicle vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***
Normal Control - Vehicle vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Standard Drug - Cystone	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 1 - SIP (100mg/kg)	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 2 - SIP (200mg/kg)	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***
Disease control - Sodium Oxal. vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 1 - SIP (100mg/kg)	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 2 - SIP (200mg/kg)	Yes	***	No	ns	Yes	**
Standard Drug - Cystonevs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***
Standard Drug - Cystonevs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 2 - SIP (200mg/kg)	Yes	***	Yes	***	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 3 - SDB (250mg/kg)	No	ns	No	ns	Yes	***
Test Group 1 - SIP (100mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	*	Yes	***
Test Group 2 - SIP (200mg/kg) vs Test Group 3 - SDB (250mg/kg)	Yes	***	Yes	***	Yes	***
Test Group 2 - SIP (200mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	***	Yes	***
Test Group 3 - SDB (250mg/kg) vs Test Group 4 - SDB (500mg/kg)	Yes	***	Yes	**	Yes	***



Values are expressed as Mean ± SEM, n = 6 in each group

Figure 3: Effect of oral administration of SIP and SDB on Ethylene Glycol (0.75%v/v) Induced Urolithiasis Rats on Assay of tissue (Kidney homogenate) enzyme (LDH, GSH & LPO) after 28 days of treatment.

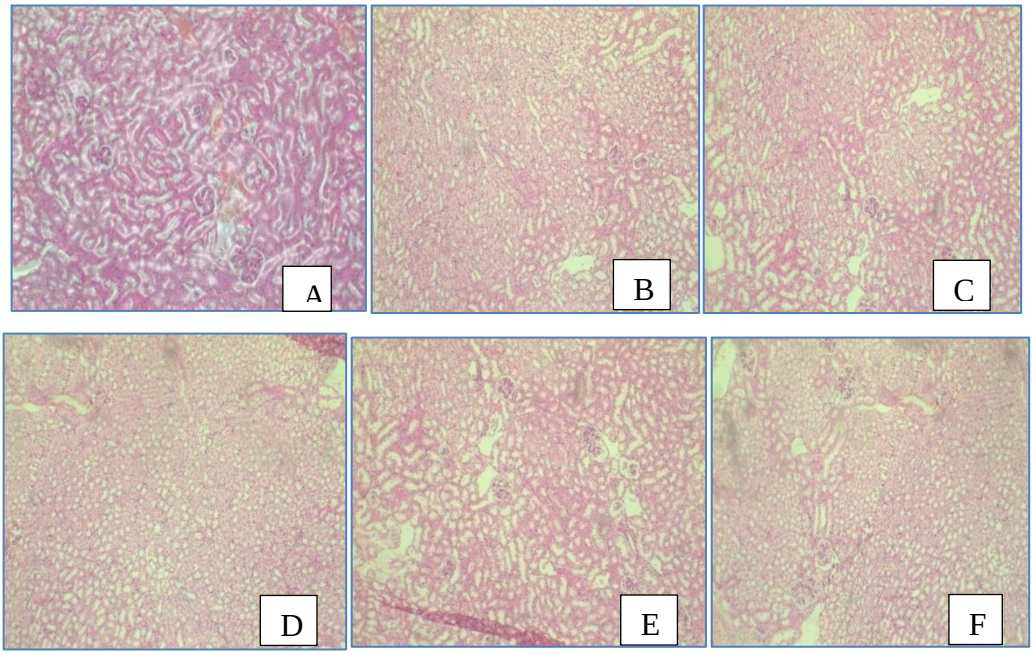


Figure 4: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on tissue histology (Kidney) after 28 days of treatment

A. ***Disease control - Sodium Oxalate:*** Architecture – Loss of normal architecture.
Glomerulus – Severe damage and edema formation inside Bowman's capsule
Tubules –Degeneration & cast formation in lumen of tubule
Congestion of blood vessels
Mononuclear inflammatory infiltration with decrease in stromal cells

B. **Standard Drug – *Cystone:*** Architecture – Mild degeneration.
Glomerulus – loss of glomerulus & infiltration of mononuclear cells. Mild improvement in necrosis.
Tubules – Loss of tubular epithelial cells.
Blood Vessels – Congestion of blood vessels

C. **Test Group 1 - SIP (100mg/kg):** Architecture – Mild Intact
Glomerulus – Tough capillaries surrounded by Bowman's capsule.
Tubules –Intact with mild cast & mild infiltration of mononuclear cells in tubuler interstitium.
Blood Vessels – Moderate congestion of blood vessels

D. **Test Group 2 - SIP (200mg/kg):** Architecture – Intact
Glomerulus – Tough capillaries surrounded by Bowman's capsule.
Tubules – Unremarkable
Blood Vessels – Unremarkable
Interstitium – Unremarkable

E. **Test Group 3 - SDB (250mg/kg):** Architecture – Mild Intact
Glomerulus – Moderate infiltration.

Tubules – Mild degeneration with presence of luminal cast.
Blood Vessels – Congestion of blood vessels.

F. **Test Group 4 - SDB (500mg/kg):** Architecture – Intact
Glomerulus – Tough capillaries surrounded by Bowman's capsule.
Tubules – Unremarkable
Blood Vessels – Unremarkable
Interstitium – Unremarkable
Haematoxylin and Eosin stain, scale bar = 100µm

DISCUSSION

Elevated creatinine, Serum ALP levels and presence of Uric acid crystals signifies impaired kidney function. As the kidneys become impaired for any reason, all these parameters in the blood will rise due to poor clearance of creatinine by the kidneys.

Calcium and phosphorous usually keep each other in check. With the progression of kidney disease, high phosphorus levels may lead to low serum calcium by depositing it onto the bones and other tissues.

An excess BUN, potassium and serum enzyme (Alanine amino Transferases) indicates the decline in kidney function due to a disease or kidney damage which can be advanced stages of chronic kidney disease. Elevated lipid peroxides, LDH and decreased glutathione (GSH) indicates some form of tissue damage.

An excess amount of oxalate can combine with calcium in the urine and cause kidney stones and crystals to form. Recurrent kidney stones and crystals can damage the kidney and lead to kidney failure.

CONCLUSION

In conclusion, the presented data indicate that administration of Saponin *Iris pseudacorus* L. Rhizome and *Dolichos biflorus* Linn Seeds to rats with Sodium Oxalate induced lithiasis reduced the growth of urinary stones by reversing all the abnormal parameters, thus supporting folk information regarding the antiurolithogenic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone constituents as detergent nature of saponines.

ABBREVIATIONS

ANOVA: Analysis of variance

BUN: Blood Urea Nitrogen

CaOx: Calcium Oxalate

CKD: Chronic Kidney Disease

EG: Ethylene glycol

GSH: Glutathione Stimulating Hormone

LDH: Lactate dehydrogensae

LPO: Lipid peroxidation

MDA: Malondialdehyde

ROS: Reactive Oxygen Specie

SDB: Saponin of Dolichosbiflorus L.

SIP: Saponin of Iris pseudacorus L.

X dose: Low dose

Y dose: High dose

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The animal husbandry procedures and experimental protocol were in accord with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Before beginning the experiment, ethical clearance was taken (Sl. No. KCP/IAEC/08/20-21/16/13-03-21.)from Institutional Animal Ethics Committee (IAEC).

ACKNOWLEDGMENT

The authors are thankful to the Dean and HOD, Department of Pharmacology, Karnataka College of Pharmacy, Bangalore-64 for providing laboratory facility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pendse AK. Urolithiasis in Udaipur and Jodhpur: A comparative study on prevalence and urinary profile. Bull III, Annual Conference of Urology Society of India., 1985; 12.
2. Alelign T, Petros B. Kidney stone disease: An update on current concepts. AdvUrol 2018;18:3068365.
3. Ramaswamy K, Killilea DW, Kapahi P, Kahn AJ, Chi T, Stoller ML. The elementome of calcium based urinary stones and its role in urolithiasis. Nat Rev Urol 2015;12:543 57.
4. Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. Rev Urol. 2010;12:e86-96.

5. Hadjzadeh MA, Khoei A, Hadjzadeh Z and Parizady M. Ethanolic extract of *Nigella sativa* L. seeds on ethylene glycol-induced kidney calculi in rats. Urology Journal, 2007; 4: 86-90.
6. Prasad K, Sujatha D, Bharathi K. Herbal drugs in urolithiasis – a review. Pharmacognosy Review, 2007; 1: 175-79
7. Bashir S, Gilani AH. Antiurolithiatic effect of *Bergeniali gulata* rhizome: an explanation of the underlying mechanisms. Journal of Ethnopharmacology, 2009; 122: 106-116.
8. Kirtikar KR, Basu BD. Indian Medicinal Plants. Dehradun: International Book Distributors, (2nd ed) 1983; 3: 1615-17.
9. Ezeonwu VU, Dahiru D. Protective Effect of Bi-Herbal Formulation of *Ocimum gratissimum* and *Gongronemalati folium* Aqueous Leaf Extracts On Acetaminophen-Induced Hepato Nephrotoxicity In Rats. American Journal of Biochemistry, 2013; 3: 18-23.
10. Baker JT, Borris RP, Carte B, Cordell GA, Soejarto DD. Natural product drug discovery and development: New perspective on international collaboration. Journal of Natural Products, 1995; 58: 1325-57.
11. Ahmed S., Ahsan Maliick I., Hasan MM. Exploring globally used antiurolithiatic plants of A to L families: Asteraceae, Fabaceae and *L amiaceae* revisited. Journal of Pharmacognosy and Phytochemistry 2017; 6(5): 1780-1787
12. Sharada LD, Somshekhar SK: Isolation and characterization of phytoconstituents from *Chlorophytum borivilianum*. Pharm. Res 2010; 2: 343.
13. Gini TG, Jothi GJ: Preliminary Phytochemical Screening. Int. J. Pharm. Phytochem. Res 2013; 5(3): 200-214.
14. Anjamma M and Bhavani NL: Comparative Phytochemical Constituents Evaluation from the Fruit Extracts of *Momordica charantia* L. and *Momordica dioica* Roxb. Int. J. Curr. Biotechnol 2015; 3(8): 17-21.
15. Burke RW, Diamondstone BI, Velapoldi RA, Menis O: Mechanisms of the Liebermann-Burchard and Zak Color Reactions for Cholesterol. Clin.Chem 1974; 20(7): 794-801.
16. Pandhare RB, Shende RR, Avhad MS et al: Anti-urolithiatic activity of *Bryophyllum pinnatum* Lam. hydroalcoholic extract in sodium oxalate-induced urolithiasis in rats. Journal of Traditional and Complementary Medicine, 2021;11(6):545-551.
17. Mukharjee TB, N. Aulakh GS, Jain HC. Herbal drugs for urinary stones – literature appraisal. Indian Drugs; 1984; 21:224-8.
18. Bahuguna Y, Rawat MSM, Juyal V, Gupta V. Antilithiatic effect of flowers of *Jasminum auriculatum* Vahl. Int J Green Pharm 2009;3(2):155-158.
19. Kalyan Betanabhatla S, Christina AJM, Syama Sundar B, Selvakumar S. Antilithiatic activity of Hibiscus sabdariffa Linn. on ethylene glycol-induced lithiasis in rats. Nat Prd Rad 2009; 43-7.
20. Karadi R, Gadge N, Alagawadi K, Savadi R. Effect of *Moringa oleifera* Lam. root- wood on ethylene glycol induced urolithiasis in rats. J Ethnopharmacol 2006; 21:306-11.
21. Anonymous. The wealth of India.Raw material. (Vol.7). New Delhi: CSIR; 1952: 68.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.