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Research Article

PHARMACOLOGICAL EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF *DOLICHOS BIFLORUS* SEEDEXTRACT IN RAT'S MODEL

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ABSTRACT

Objectives: The main aim of the present study was to investigate the antiurolithiatic activity of *Dolichos Biflorus* methanolic seed extract in a rat's model. In the phytochemical screening, it was found that *Dolichos Biflorus* seed extract showed the presence of tannins steroids, protein, flavonoids, terpenoids, mucilage, saponin, and carbohydrate and the absence of alkaloids fixed oil. Hence, this plant has highly diuretic activity.

Methods: Model: Sodium oxalate induced urolithiasis in rats. A total of 30 rats were used for this study and the animals were divided into five groups. Each group contains six rats: Normal control group, disease control group (sodium oxalate 75 mg/kg, IP), standard group (Cystone, 750 mg/kg, PO), treatment group1 (*Dolichos Biflorus* 150 mg/kg, PO), treatment Group 2 (*Dolichos Biflorus* 300 mg/kg, PO). Urolithiasis was induced by using sodium oxalate (75 mg/kg, IP) for 28 days.

Results: At the end of the experiment, all the animal blood samples were collected to check the various biochemical parameters. Animals were sacrificed by giving a high dose of pentobarbitone and kidneys were collected for antioxidant and histopathological study. From the renal function test, it was found that the drug is showing a potent effect when compared to the disease control group and standard group. Moreover, from the antioxidant and histopathology study, it was found that the drug is showing a potent effect when compared to the disease control and standard group and control group.

Conclusion: After all the investigation, it was found that oral administration of *Dolichos Biflorus* seed extract at the low dose of 150 mg/kg and the high dose of 300 mg/kg against the sodium oxalate-induced urolithiasis and it was found that high is more effective as compared to low dose. Drug was able to suppress oxalate synthesizing enzymes and minerals. Moreover, histopathology study in the treatment group showed recovery and normal architecture of glomerulus with a tuft of capillaries surrounded by Bowman's capsule. The most of tubules are showing normal architecture and recovery. After seeing all the results, it is confirmed that the test drug *Dolichos Biflorus* has potent antiurolithiatic activity.

(*Dolichos Biflorus short form mentioned below as DB)*

Keywords: *Dolichos Biflorus,* Antiurolithiasis activity, Sodium oxalate, Kidney stone.

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INTRODUCTION

The medicinal plant plays an important role in the traditional system. Herbal medicine is the formulation in which plants are used for formulation [1].

In the history of mankind, so many infectious diseases have been treated using a medicinal plant. Mostly, in developing countries, herbal medicines are used. Herbal medicine becomes a very important subject for pharmacological studies in the last few decades. After all, those pharmacological studies researcher provides an evidence for a plant that the plant has those effects and also provides safety of utilizing traditional plant. Herbal medicine provides conventional treatments. It provides safe and well-tolerated remedies for chronic illness. Since many existing synthetic drugs cause various side effects [1,2].

Benefits of using herbal medicine:

- Uses of herbal medicine have a long history because they have better patient tolerance well as acceptance.
- In a developing country like India, we can easily get or grow a plant because India has rich agro-climatic cultural and ethnic biodiversity.
- Now, the world population is growing rapidly for a growing population cheaper and more sustainable supply is required for these herbal medicines are a better option.

Throughout the world, herbal medicine has provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science both in crude form and as a pure chemical on which modern medicines are structured [3].

Urolithiasis is one of the most common diseases of the urinary tract which has been affecting humankind since antiquity. Urolithiasis is associated with calculus or stone formation in the urinary collecting system but kidney calculus often arises in the kidney. Kidney stones form when urine containsa more crystal-forming substance such as calcium, oxalate, and uric acid. These crystal-forming agents come together to form crystals [4,5].

Formation of renal crystal is a multifactorial process that may relate to diet, urinary tract infection, altered urinary solutes and colloids, decrease urinary drainage and urinary stasis, prolonged immobilization of Randall's plaque, and microlith [6].

When urea splitting organisms infect the urinary tract, bacteria disintegrate the urea excreted in the urine in the presence of the urease enzyme, which subsequently triggers the formation of ammonia rendering the urine alkaline. In an alkaline state, urine leads to contain precipitated crystals of calcium oxalate, magnesium phosphate, and calcium carbonate in large amounts thereby leading to a strong tendency to form calculi. Bacterial infection may induce stone formation by crystal adherence [5,7].

Most of the urea splitting organism belongs to the species proteus but organism such as pseudomonas, staphylococcus, *Escherichia coli,* and even mycoplasma even reported of producing urease [7].

Now, coming to the model of how inducing agents induce kidney stones in the body ethylene glycol and sodium oxalate are the inducing agents that I am using in the experiment [6].

How does ethylene glycol produce kidney stones, when ethylene glycol is metabolized by the body it produces toxic metabolites such as glycolaldehyde, glycolate, and glyoxylate. These metabolites cause tissue destruction primarily form calcium oxalate deposition and metabolic abnormalities. Especially, a high anion-gap causes metabolic acidosis, lactic acidosis, and hypocalcemia. Oxalate acid combines with calcium to form calcium oxalate crystals, which deposit in the kidney. This can result in hematuria and protein urea, increase creatinine, and renal failure [6].

The main reason thought to be the formation of stones in the kidney is the uncontrolled growth of calcium oxalate and uric acid in the urinary tract which leads to a decreased level of citrate in the urinary. Citrate and magnesium are the main inhibitors of stone formation in the urinary tract. When there is a lack of citrate level in the urinary tract, it causes stone formation [2].

Same as the previous model when sodium oxalate induced to the body through IP in the body.

Oxalate levels will increase in the body and oxalate will bind to calcium in the urinary tract and form calcium oxalate stones.

There are so many factors that are directly or indirectly involved in the formation of a kidney stone-

- Epidemiological factor
- Biochemical factor
- Genetic factor [2,8].

MATERIALS AND METHODS

Source of data

The experiment was performed as described in the standard bibliography, kinds of literature and textbooks. The reputed journals and publications are obtained from the college library and through a web search.

Collection of materials

The seeds of the plant were obtained from the authorized commercial dealer (Shree Mahadev enterprises, 35, Radhika Vihar, Krishna Nagar, Mathura-281004). Order id-OD123736981623169000, Invoice number- FACOSE2200005491.

Preparation of extract

The seeds of the plant *Dolichos Biflorus* were collected and washed using fresh water and dried under shade [5]. The seeds are crushed to a fine powder after drying. The chemical compounds present in the seeds were extracted with methanol by soxhlation. The solvent was then evaporated using a rotary evaporator and the phytochemicals were collected and stored for further analysis [7,9].

Phytochemical investigation

The methanolic extract was investigated for the presence of secondary metabolite [5,9].

Drugs and chemicals

All the drugs and chemicals of pure analytical grade were obtained from the local suppliers.

In vivo studies

Experimental animals:

- Wistar albino rats of either sex weighing (150–200g) were used in the study. The animals were housed in polypropylene cages in groups of six to rats per cage and kept under controlled environmental conditions [1]. Care of animals according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [3]. The study is approved by the Institutional Animal Ethics Committee. IAEC Registration number: KCP-IAEC/09/21-22/10/18/12/21

Experimental Methods

Total of 30 Wistar rats aged 6–8 weeks weighing (150–200 g) were divided into five groups, with six animals in each group (n=6), in the following manner [1]:

Model: Sodium oxalate induced [1]:

- Group 1: Normal control – Vehicle, that is, Normal Saline (10 ml/kg, p.o.) [1] for 28 days.
- Group 2: Disease control – Sodium oxalate (75 mg/kg, i.p) [1] for 28 days.
- Group 3: Standard group – Sodium oxalate (75 mg/kg, i.p) [1]+cystone (750 mg/kg, p.o.) [3] for 28 days.
- Group 4: Treatment group – Sodium oxalate (75 mg/kg, i.p) [1] for 28 days + methanolic extract of *Dolichos biflorus* at (150 mg/kg, p.o.) [3] for 28 days.
- Group 5: Treatment group – Sodium oxalate (75 mg/kg, i.p) [1] for 28 days + methanolic extract of *Dolichos biflorus* at (300 mg/kg, p.o.) [3] for 28 days.

Collection and analysis

At the end of the treatment, urine and serum samples will be collected and the animals will be sacrificed using a high dose of pentobarbitone sodium for histopathology and antioxidant analysis of the kidney. Blood samples will be withdrawn by cardiac puncture and retroorbital routes [1].

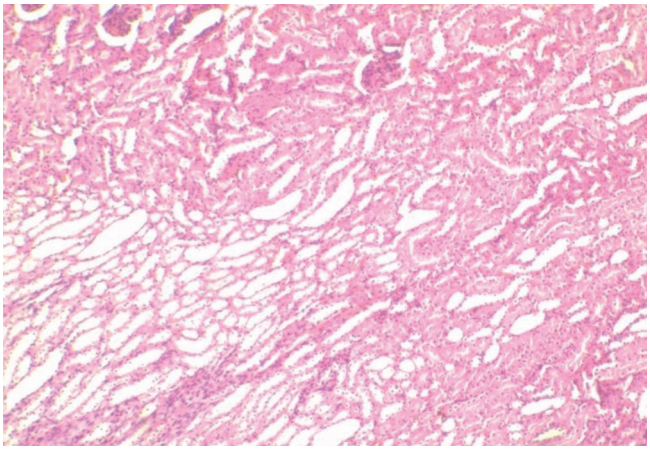
The following biochemical parameters will be determined:

I. Serum analysis: Calcium, BUN, uric acid, creatinine, sodium chloride, potassium, microalbumin, magnesium, oxalate, and alanine immune transferee [1,6].
II. Antioxidant study [1,3]: Assay of tissue enzyme: All the animals shall be sacrificed at the end of the treatment period. Kidney homogenates shall be prepared and the following enzyme levels shall be analyzed using suitable methods.
    - Kidney homogenate: Lactate dehydrogenase (LDH) (Oxalate synthesizing enzyme)
    - LPO (Lipid peroxidation)
    - Glutathione [1]
III. Histopathological study of kidney [1].

RESULTS

Histopathological study

Normal control group



Showing normal glomerulus with a tuft of capillaries surrounded by Bowman's capsule with tubules is lined by columnar epithelial cell

Table 1: Estimation of creatinine

S. No.	Groups	Treatment	Creatinine level
1.	Normal control	Vehicle only	0.6660±0.0163
2.	Disease control	Sodium oxalate (75 mg/kg , IP)	0.9760±0.0354
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + cystone (750 mg/kg, P.O)	0.7100±0.0089
4.	Low dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* 150 mg/kg, po)	0.7320±0.0128
5.	High dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* 300 mg/kg, po)	0.6100±0.0130

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance.

Tukey's multiple comparisons tests	Mean difference	Below threshold?	Summary	Adjusted p value
NC-vehicle only versus DC-sodium oxalate 75 mg/kg	−0.3100	Yes	****	<0.0001
NC-vehicle only versus Cystone 750 mg/kg	−0.04400	No	ns	0.5254
NC-vehicle only versus DB 150 mg/kg	−0.06600	No	ns	0.1642
NC-vehicle only versus DB 300 mg/kg	0.05600	No	ns	0.2960
DC-sodium oxalate 75 mg/kg versus cystone 750 mg/kg	0.2660	Yes	****	<0.0001
DC-sodium oxalate 75 mg/kg versus DB 150 mg/kg	0.2440	Yes	****	<0.0001
DC-sodium oxalate 75 mg/kg versus DB 300 mg/kg	0.3660	Yes	****	<0.0001
Cystone 750 mg/kg versus DB 150 mg/kg	−0.02200	No	ns	0.9304
Cystone 750 mg/kg versus DB 300 mg/kg	0.1000	Yes	*	0.0139
DB 150 mg/kg versus DB 300 mg/kg	0.1220	Yes	**	0.0024

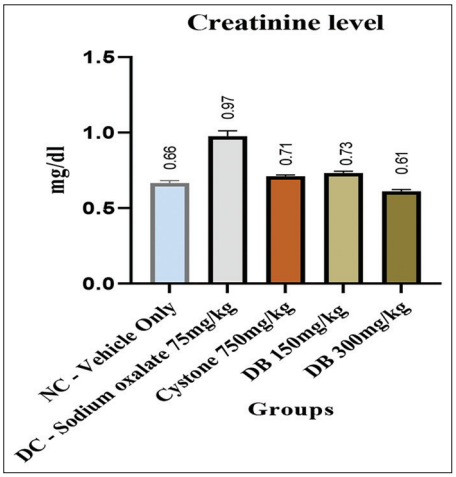


Table 2: Estimation of calcium

S. No.	Groups	Treatment	Calcium level
1.	Normal control	Vehicle only	3.725±0.1064
2.	Disease control	Sodium oxalate (75 mg/kg, IP)	8.513±0.1568
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + Cystone (750 mg/kg, P.O)	4.302±0.1289
4.	Low dose	Sodium oxalate (75 mg/kg, IP) + *Dolichos Biflorus* 150 mg/kg, po)	5.850±0.0991
5.	High dose	Sodium oxalate (75 mg/kg, IP) + *Dolichos Biflorus* 300 mg/kg, po)	4.267±0.1054

Tukey's multiple comparisons tests	Mean difference	Below threshold?	Summary	Adjusted p value
NC-Vehicle only versus DC-NaOX 75 mg/kg	−4.788	Yes	****	<0.0001
NC-Vehicle only versus standard cystone 750 mg/kg	−0.5767	Yes	*	0.0191
NC-Vehicle only versus DB 150 mg/kg	−2.125	Yes	****	<0.0001
NC-Vehicle only versus DB 300 mg/kg	−0.5417	Yes	*	0.0305
DC-NaOX 75 mg/kg versus standard cystone 750 mg/kg	4.212	Yes	****	<0.0001
DC-NaOX 75 mg/kg versus DB 150 mg/kg	2.663	Yes	****	<0.0001
DC-NaOX 75 mg/kg versus DB 300 mg/kg	4.247	Yes	****	<0.0001
Std-Cystone 750 mg/kg versus DB 150 mg/kg	−1.548	Yes	****	<0.0001
Std-Cystone 750 mg/kg versus DB 300 mg/kg	0.03500	No	ns	0.9996
DB 150 mg/kg versus DB 300 mg/kg	1.583	Yes	****	<0.0001

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance.

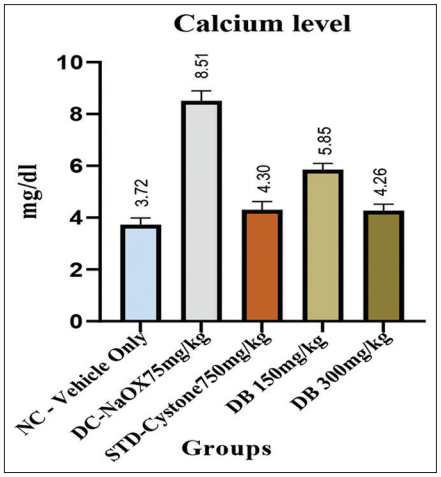


Table 3: Estimation of phosphorus

S. No.	Groups	Treatment	Phosphorus level
1.	Normal control	Vehicle only	3.018 ± 0.0697
2.	Disease control	Sodium oxalate (75 mg/kg, IP)	5.640 ± 0.1281
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + cystone (750 mg/kg, P.O)	4.400 ± 0.1414
4.	Low dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* 150 mg/kg, po)	5.283 ± 0.1014
5.	High dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* 300 mg/kg, po)	4.352 ± 0.0757

Tukey's multiple comparisons tests	Mean difference	Below threshold?	Summary	Adjusted p value
NC-vehicle only versus dc Naox 75 mg/kg	−2.622	Yes	****	<0.0001
NC-vehicle only versus standard-cystone (750 mg/kg)	−1.382	Yes	****	<0.0001
NC-vehicle only versus DB-150 mg/kg	−2.265	Yes	****	<0.0001
NC-vehicle only versus DB-300 mg/kg	−1.333	Yes	****	<0.0001
DC-Naox 75 mg/kg versus standard cystone (750 mg/kg)	1.240	Yes	****	<0.0001
Dc-naox 75 mg/kg versus DB-150 mg/kg	0.3567	No	ns	0.1609
Dc-naox 75 mg/kg versus DB-300 mg/kg	1.288	Yes	****	<0.0001
Std-cystone (750 mg/kg) versus DB-150 mg/kg	−0.8833	Yes	****	<0.0001
Std-cystone (750 mg/kg) versus DB-300 mg/kg	0.04833	No	ns	0.9976
DB-150 mg/kg versus DB-300 mg/kg	0.9317	Yes	****	<0.0001

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance.

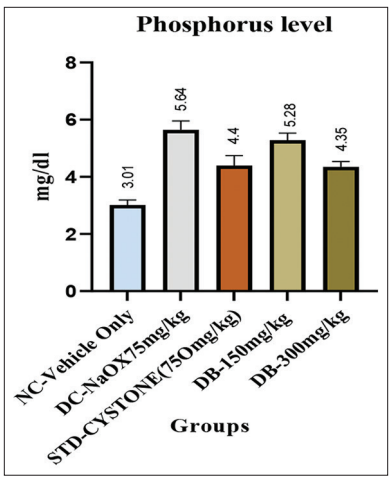


Table 4: Estimation of uric acid

S. No.	Groups	Treatment	Uric acid level
1.	Normal control	Vehicle only	1.633±0.1189
2.	Disease control	Sodium oxalate (75 mg/kg , IP)	3.623±0.1052
3.	Standard group	Sodium oxalate (75 mg/kg, IP)+Cystone (750 mg/kg, P.O)	2.407±0.0926
4.	Low dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* 150 mg/kg, po)	2.452±0.1217
5.	High dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* 300 mg/kg, po)	2.288±0.0629

Tukey's multiple comparisons tests	Mean difference	Below threshold?	Summary	Adjusted p value
NC-vehicle only versus DC-naox 75 mg/kg	−1.990	Yes	****	<0.0001
NC-vehicle only versus Std-cystone 750 mg/kg	−0.7733	Yes	***	0.0001
NC-vehicle only versus DB-150 mg/kg	−0.8183	Yes	****	<0.0001
NC-vehicle only versus DB-300 mg/kg	−0.6550	Yes	**	0.0011
DC-naox 75 mg/kg versus Std-cystone 750 mg/kg	1.217	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-150 mg/kg	1.172	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-300 mg/kg	1.335	Yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-150 mg/kg	−0.04500	No	ns	0.9978
Std-cystone 750 mg/kg versus DB-300 mg/kg	0.1183	No	ns	0.9231
DB-150 mg/kg versus DB-300 mg/kg	0.1633	No	ns	0.7911

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance.

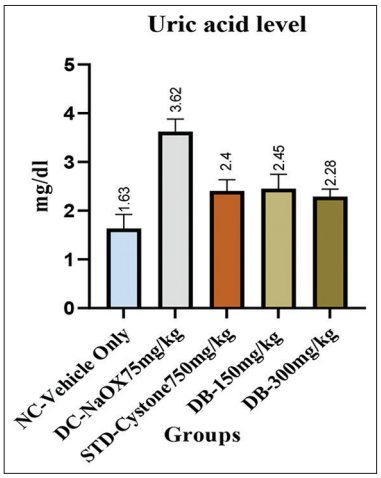


Table 5: Estimation of alkaline phosphatase

S. No.	Groups	Treatment	Alkaline phosphatase
1.	Normal control	Vehicle only	75.79 ± 0.9879
2.	Disease control	Sodium oxalate (75 mg/kg , IP)	96.73 ± 1.101
3.	Standard group	Sodium oxalate (75 mg/kg, IP)+cystone (750 mg/kg, P.O)	77.42 ± 1.044
4.	Low dose	Sodium oxalate (75 mg/kg, ip)+*Dolichos Biflorus* 150 mg/kg, po)	78.68 ± 0.8641
5.	High dose	Sodium oxalate (75 mg/kg, ip)+*Dolichos Biflorus* 300 mg/kg, po)	66.41 ± 1.172

Tukey's multiple comparifdhfegjgjnsons tests	Mean difference	Below threshold?	Summary	Adjusted p value
NC-vehicle only versus DC-naox 75 mg/kg	−20.94	Yes	****	<0.0001
NC-vehicle only versus Std-cystone 750 mg/kg	−1.632	No	ns	0.7996
NC-vehicle only versus DB-150 mg/kg	−2.885	No	ns	0.3122
NC-vehicle only versus DB-300 mg/kg	9.378	Yes	****	<0.0001
DC-naox 75 mg/kg versus Std-cystone 750 mg/kg	19.31	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-150 mg/kg	18.05	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-300 mg/kg	30.32	Yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-150 mg/kg	−1.253	No	ns	0.9111
Std-cystone750 mg/kg versus DB-300 mg/kg	11.01	Yes	****	<0.0001
DB-150 mg/kg versus DB-300 mg/kg	12.26	Yes	****	<0.0001

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance.

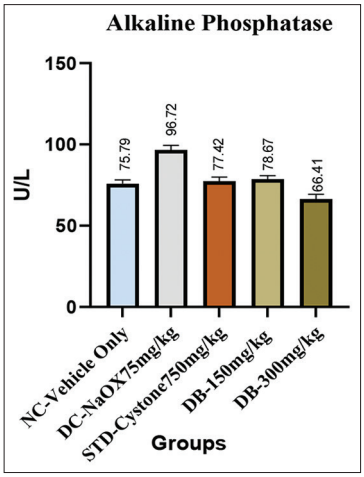


Table 6: Estimation of BUN

S. No.	Groups	Treatment	BUN level
1.	Normal control	Vehicle only	19.32±0.5746
2.	Disease control	Sodium oxalate (75 mg/kg, IP)	28.56±0.6385
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + cystone (750 mg/kg, P.O)	21.92±0.7903
4.	Low dose	Sodium oxalate (75 mg/kg, IP) + dolichos biflorus 150 mg/kg, po)	26.12±0.7881
5.	High dose	Sodium oxalate (75 mg/kg, ip) + dolichos biflorus 300 mg/kg, po)	20.15±0.5356

Tukey's multiple comparisons test	Mean difference	below threshold?	Summary	Adjusted p value
NC-vehicle only versus DC-naox 75 mg/kg	−9.238	Yes	****	<0.0001
NC-vehicle only versus Std-cystone 750 mg/kg	−2.600	No	ns	0.0777
NC-vehicle only versus DB-150 mg/kg	−6.805	Yes	****	<0.0001
NC-vehicle only versus DB-300 mg/kg	−0.8350	No	ns	0.9029
DC-naox 75 mg/kg versus Std-cystone 750 mg/kg	6.638	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-150 mg/kg	2.433	No	ns	0.1106
DC-naox 75 mg/kg versus DB-300 mg/kg	8.403	Yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-150 mg/kg	−4.205	Yes	**	0.0015
Std-cystone 750 mg/kg versus DB-300 mg/kg	1.765	No	ns	0.3679
DB-150 mg/kg versus DB-300 mg/kg	5.970	Yes	****	<0.0001

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance.

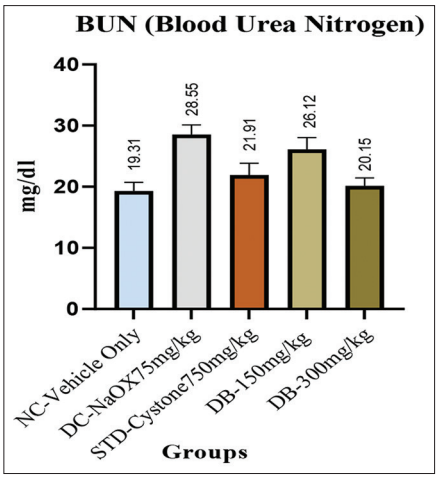


Table 7: Estimation of potassium

S. No.	Groups	Treatment	Potassium level
1.	Normal control	Vehicle only	4.018 ± 0.1263
2.	Disease control	Sodium oxalate (75 mg/kg , IP)	5.202 ± 0.1077
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + cystone (750 mg/kg, P.O)	3.777 ± 0.1061
4.	Low dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (150 mg/kg, po)	4.620 ± 0.168
5.	High dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (300 mg/kg, po)	3.910 ± 0.0825

Tukey's multiple comparisons test	Mean difference	Below threshold?	Summary	Adjusted p value
NC-vehicle only versus dc-naox 75 mg/kg	−1.183	Yes	****	<0.0001
NC-vehicle only versus Std-cystone 750 mg/kg	0.2417	No	ns	0.6300
NC-vehicle only versus DB-150 mg/kg	−0.6017	Yes	*	0.0139
NC-vehicle only versus DB-300 mg/kg	0.1083	No	ns	0.9687
DC-naox 75 mg/kg versus Std-cystone 750 mg/kg	1.425	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-150 mg/kg	0.5817	Yes	*	0.0183
DC-naox 75 mg/kg versus DB-300 mg/kg	1.292	Yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-150 mg/kg	−0.8433	Yes	***	0.0004
Std-cystone 750 mg/kg versus DB-300 mg/kg	−0.1333	No	ns	0.9353
DB-150 mg/kg versus DB-300 mg/kg	0.7100	Yes	**	0.0030

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance

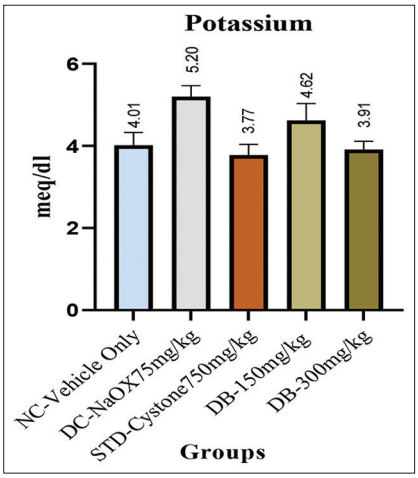


Table 8: Estimation of LDH (Lactate Dehydrogenase)

S. No.	Groups	Treatment	LDH level
1.	Normal control	Vehicle only	351.0±1.561
2.	Disease control	Sodium oxalate (75 mg/kg , IP)	751.7±0.819
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + cystone (750 mg/kg, P.O)	457.1±1.172
4.	Low dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (150 mg/kg, po)	579.0±1.077
5.	High dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (300 mg/kg, po)	474.7±0.7738

Tukey's multiple comparisons test	Mean difference	Below threshold?	Summary	Adjusted p value
NC-vehicle only versus DC-naox 75 mg/kg	−399.7	yes	****	<0.0001
NC-vehicle only versus Std-cystone 750 mg/kg	−105.2	yes	****	<0.0001
NC-vehicle only versus DB-150 mg/kg	−227.1	yes	****	<0.0001
NC-vehicle only versus DB-300 mg/kg	−122.7	yes	****	<0.0001
DC-naox 75 mg/kg versus Std-cystone 750 mg/kg	294.5	yes	****	<0.0001
DC-naox 75 mg/kg versus DB-150 mg/kg	172.6	yes	****	<0.0001
DC-naox 75 mg/kg versus DB-300 mg/kg	277.0	yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-150 mg/kg	−121.9	yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-300 mg/kg	−17.56	yes	****	<0.0001
DB-150 mg/kg versus DB-300 mg/kg	104.3	yes	****	<0.0001

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance

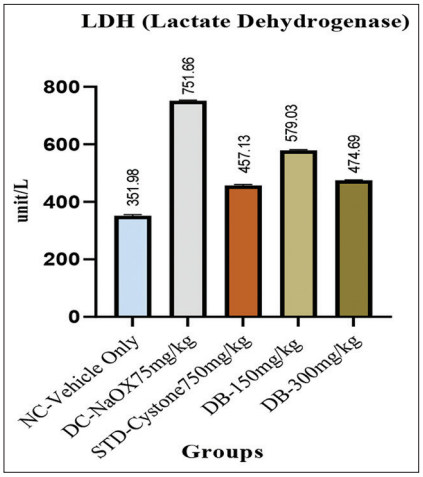


Table 9: Estimation of GSH (glutathione)

S. No.	Groups	Treatment	GSH level
1.	Normal control	Vehicle only	4.307 ± 0.1673
2.	Disease control	Sodium oxalate (75 mg/kg , IP)	1.782 ± 0.1120
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + cystone (750 mg/kg, P.O)	4.148 ± 0.1590
4.	Low dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (150 mg/kg, po)	3.460 ± 0.1034
5.	High dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (300 mg/kg, po)	4.442 ± 0.1563

Tukey's multiple comparisons test	Mean difference	Below threshold?	Summary	Adjusted p value
NC-vehicle only versus DC-naox 75 mg/kg	2.525	Yes	****	<0.0001
NC-vehicle only versus Std-cystone 750 mg/kg	0.1583	No	ns	0.9317
NC-vehicle only versus DB-150 mg/kg	0.8467	Yes	**	0.0024
NC-vehicle only versus DB-300 mg/kg	−0.1350	No	ns	0.9607
DC-naox 75 mg/kg versus Std-cystone 750 mg/kg	−2.367	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-150 mg/kg	−1.678	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-300 mg/kg	−2.660	Yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-150 mg/kg	0.6883	Yes	*	0.0165
Std-cystone 750 mg/kg versus DB-300 mg/kg	−0.2933	No	ns	0.5967
DB-150 mg/kg versus DB-300 mg/kg	−0.9817	Yes	***	0.0004

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance.

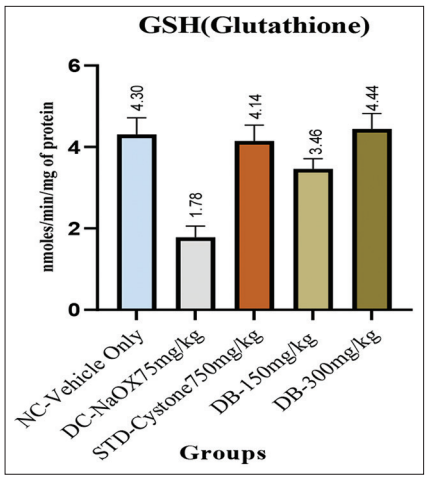


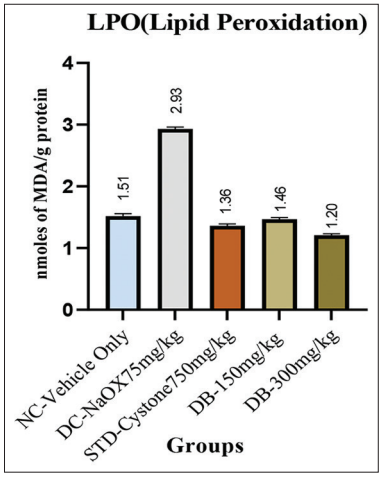
Table 10: Estimation of LPO

S. No.	Groups	Treatment	LPO level
1.	Normal control	Vehicle only	1.518±0.01641
2.	Disease control	Sodium oxalate (75 mg/kg , IP)	2.930±0.01342
3.	Standard group	Sodium oxalate (75 mg/kg, IP) + Cystone (750 mg/kg, P.O)	1.360±0.01238
4.	Low dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (150 mg/kg, po)	1.467±0.01174
5.	High dose	Sodium oxalate (75 mg/kg, ip) + *Dolichos Biflorus* (300 mg/kg, po)	1.208±0.00980

LPO: Lipid peroxidation

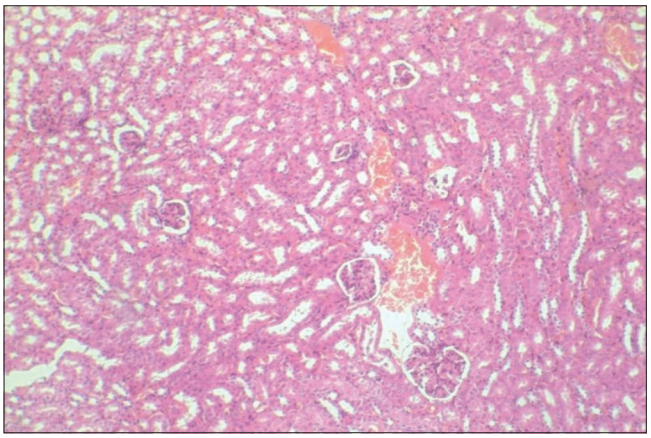
Tukey's multiple comparisons test	Mean difference	Below threshold?	Summary:	Adjusted p value
NC-vehicle only versus DC-naox 75 mg/kg	−1.412	Yes	****	<0.0001
NC-vehicle only versus Std-cystone 750 mg/kg	0.1583	Yes	****	<0.0001
NC-vehicle only versus DB-150 mg/kg	0.05167	No	ns	0.0636
NC-vehicle only versus DB-300 mg/kg	0.3100	Yes	****	<0.0001
DC-naox 75 mg/kg versus Std-cystone 750 mg/kg	1.570	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-150 mg/kg	1.463	Yes	****	<0.0001
DC-naox 75 mg/kg versus DB-300 mg/kg	1.722	Yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-150 mg/kg	−0.1067	Yes	****	<0.0001
Std-cystone 750 mg/kg versus DB-300 mg/kg	0.1517	Yes	****	<0.0001
DB-150 mg/kg vs. DB-300 mg/kg	0.2583	Yes	****	<0.0001

*p<0.05, **p<0.01 values are mean±SEM, n=6, when compared with control using one-way ANOVA followed by Tukey's multiple comparison test. ANOVA: Analysis of variance



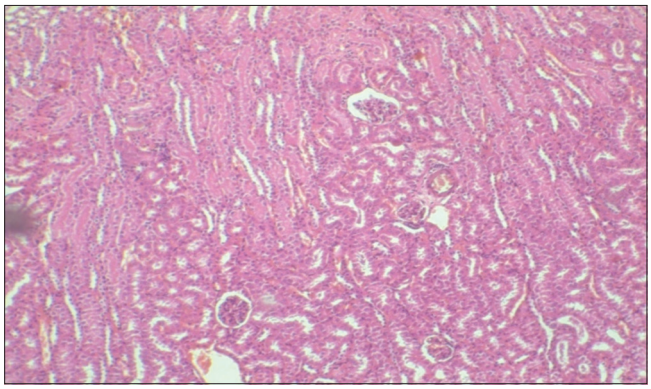
cytoplasm staining pink color and normal architecture. Hematoxylin and Eosin stain, scale bar = 100 µm.

Disease control



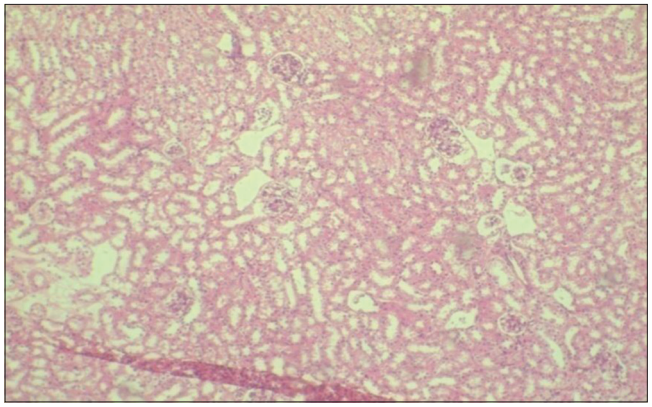
Showing glomerular degeneration with loss of capillaries surrounded by Bowman's capsule. The tubules are showing toxicity with severe tubular degeneration and loss of tubular architecture which is also evident by accumulation in the center of the tubules. Hematoxylin and Eosin stain, scale bar = 100 µm

Standard group



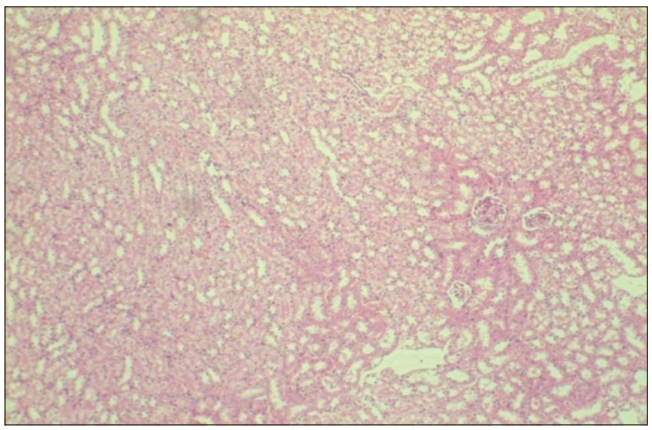
Showing glomerulus with loss of capillaries surrounded by Bowman's capsule. The tubules show recovery from toxicity and appear to be normal architecture with mild tubular degeneration evident by accumulation in the center of the tubules. (Hematoxylin and Eosin stain, scale bar = 100 µm.

Low dose group



Showing recovery and normal architecture of glomerulus with a tuft of capillaries surrounded by Bowman's capsule. The most of tubules are showing normal architecture and recovery. However, they were few tubules showing mild degeneration evident by accumulation in the center of the tubules. Hematoxylin and Eosin stain, scale bar = 100 µm.

High dose group



Showing recovery and normal architecture of glomerulus with a tuft of capillaries surrounded by Bowman's capsule. The tubules are showing normal architecture and moderate recovery. Hematoxylin and Eosin stain, scale bar = 100 µm.

DISCUSSION

The antiurolithiatic activity of *Dolichos biflorus*is confirmed after Comparision of the test group with the standard group, disease control group, and the control group. The serum analysis test group can suppress the level of calcium, BUN, potassium, creatinine, and alkaline phosphatase when compare to the standard and disease control group. From the kidney homogenate study, it is also found that the test drug has potent antiurolithiatic activity [1,3].

The antiurolithiatic effect was further confirmed by kidney histopathological studies. Indeed, kidney sections of untreated rats showed abundant crystal depositions. Furthermore, renal epithelial cells had more tubular dilatation and damage shown by large spaces in the tissue. In treated rats, fewer crystal depositions were seen compared to untreated animals and the necrosis, as well as the tubule dilatation, was very limited [3]. Renal stone deposition damages the renal tissue and deteriorates renal function [1]. Lithogenic treatment caused impairment of renal functions of the untreated rats as evident from the markers of glomerular and tubular damage: Raised BUN, uric acid, urea, and serum creatinine that was lowered in animals receiving plant extract [4]. Tissue injury and inflammation in these animals are due to exposure to phosphate and calcium phosphate crystals, leading to the generation of reactive oxygen species, development of oxidative stress, lipid peroxidation, and depletion of antioxidant enzymes [7,9]. The renal epithelial injury further promotes crystal retention, as epithelial injury exposes a variety of crystal adhesion molecules on epithelial surfaces and promotes stone formation. Probably antioxidant constituents of the plant restore the renal antioxidant enzyme and prevent renal cell injury [1,8].

CONCLUSION

From the present study, we conclude the preliminary phytochemical analysis of *Dolichos Biflorus Lin*. indicated the presence of alkaloids, flavonoids, proteins, saponins, terpenoids, phytosterols, carbohydrates, and fatty acids.

In vivo anti-atiurolithiatc activity of *Dolichos Biflorus* methanolic seed extract, we evaluated in one model sodium oxalate-induced urolithiasis.

From the above investigation, it is proved that the drug is showing significant activity as compared to the standard drug cystone and the disease control group. The high dose of *Dolichos biflorus* (300 mg/kg) is showing a better effect as compared to the low dose of 150 mg/kg and the standard drug cystone 750 mg/kg.

After seeing all the results, we can conclude that the drug is having potent atiurolithiatic activity.

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AUTHORS CONTRIBUTIONS

All the authors contributed to the preparation of the final manuscript.

CONFLICTS OF INTEREST

The author declared that there are no conflicts of interest.

AUTHORS FUNDING

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