



Evaluation of Anti Urolithiatic Activity of *Dolichos biflorus* Seed Extract by Using Ethylene Glycol Induced Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

In the phytochemical screening, it was found that *Dolichos Biflorus* seed extract contained the presence of tannins steroids, protein, flavonoids, terpenoids, mucilage, saponin, and carbohydrate, but no alkaloids fixed oil. So, this plant has highly diuretic activity. Which can use for the treatment of urolithiasis.

Objective: The Present study aims to investigate the anti-Urolithiatic activity of *Dolichos Biflorus* methanolic seed extract in a rat model.

Methods: *Dolichos Biflorus* Seeds Extract were evaluated for anti-Urolithiatic activity. Urolithiasis has been induced in Wistar rats through Ethylene glycol (0.75%v/v, p.o). Upon completion of the experiment, animals in all groups have been sacrificed and biochemical parameters such as; calcium, creatinine, Phosphorus, Uric acid, Alkaline Phosphatase BUN (Blood Urea Nitrogen), and histology of Kidney have been observed. Furthermore, Potassium, Oxalate levels, and Alanine Amino Transferases were measured.

Results: The extract of *Dolichos Biflorus* was proven to be safe in the toxicity findings.

It has been shown that in-vivo significant effect of plant extract was able to manage the urolithiatic markers such as calcium, creatinine, phosphorus, uric acid, alkaline phosphatase, BUN (Blood Urea Nitrogen), and potassium. In the urolithiatic rats it has been observed that abundant crystal depositions, renal epithelial cells had more tubular dilatation and damage shown by large spaces in the tissue, renal stone deposition damages the renal tissue and deteriorate the renal function.

Urolithiatic markers such as calcium, creatinine, phosphorus, uric acid, alkaline phosphatase, BUN (Blood Urea Nitrogen), and potassium were normal in animals receiving plant extract and prevent renal cell injury.

Conclusion: All its findings and phytoconstituents existing inside the extract must stay the viable chemical materials involved in the prevention of Urolithiatic.

Keywords: *Dolichos biflorus*; ethylene glycol; anti-urolithiatic activity; renal calculi.

1. INTRODUCTION

The stones in the kidneys are characterised by solid particles formed in the kidneys and urinary system. However, in some cases, these stones can be minute and go through the blood circulation and pass out the body through the urinary tract with urine. In this case, the formation of kidney stones has no harm or damage to the body organs or tissues. In the contrast, if the stones are bigger, they will block the urine flow and cause pain that required medical treatment. Urolithiasis is one of the most common diseases of the human urinary tract [1] and is associated with calculus or stone formation in the urinary system but kidney calculus often arises in the kidney. Kidney stones form when urine contains a more crystal forming substance such as calcium, oxalate, and uric acid. These crystal forming agents come together to form crystals [2].

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, and prolonged immobilization of Randall's plaque and microlith [3].

The metabolism of ethylene glycol metabolized by the human body results in toxic compounds such as glycolaldehyde, glycolate and glyoxylate which result in destroying of tissue due to the deposition of the calcium oxalate deposition. Specifically, a high anion gap causes metabolic acidosis, lactic acidosis and hypocalcaemia. Oxalate acid is associated with calcium to form calcium oxalate crystals, which deposit in the kidney causing haematuria and the presence of the protein urea, increasing creatinine and eventually renal failure [4].

Decoction of seeds is used in diarrhoea, and haemorrhage from bowels, and is given to females during parturition to promote the discharge of the lochia. Pulse is a demulcent in calculus affection and coughs. A soup is a diet in sub-acute cases of enlarged liver and spleen. As

a home remedy, kulthi has been used in dysuria, bleeding piles, vaginal bleeding, and leucorrhoea. Its use in reducing obesity is also recognized [5]. In short, there is a good level of traditional and experimental evidence to support various claims and advantages of this widely available plant [6]. Several medications prescribed for preventing the recurrence include thiazide diuretics and alkali citrate. However, the effectiveness of these drugs is still not clinically proven [7].

Using herbal medicine and plant extracts has been widely known as an alternative to chemical or synthetic drugs with a lesser side effect. This leads to the fact that using herbal medicine techniques is corresponding with the return to natural remedies with less or absent side effects. The urinary stones disease is known throughout history and is very common in the Northern part of India [8].

2. MATERIALS AND METHODS

2.1 Plant Information

- **Organism Name:** *Dolichos Biflorus*
- **Genus:** Dolichos
- **Family:** Fabaceae.
- **Kingdom:** Plantae
- **Common name:** Horsegram, Catjang
- It is widely distributed in Africa and Asia.
- **Macroscopic Characters of the seeds:** The drug comprises of compressed seeds that are reinforced, shining, finely polished grey or brownish grey or reddish brown.
- These are herbs and shrubs growing upright, sometimes with climbing stems. The leaves have single blades or are pinnately divided into three leaflets. The plants sometimes produce their leaves after flowering. The flowers are white or purple, or occasionally yellow. The fruit is a flattened legume pod.

It is also known as kulthi beans or horse gram seeds. In Ayurveda the seed is used in the treatment of piles, pain, constipation,

wounds, urinary calculi, cough, oedema, asthma etc [9].

It is also used to reduce crystalluria and to lyse stones. The powdered seeds are used as a poultice to induce sweating [10].

Dolichos biflorus is commonly used in herbal and traditional medicine with remarkable healing properties. Seeds of this plant are widely used treatment of several ailments besides being used as a tonic, astringent, and diuretic [11].

2.2 Plant Collection and Extract Preparation

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

2.3 Extraction of *Dolichos biflorus* Seed by Using Soxhlet Extractor and Sample Preparation [12]

2.3.1 Preparation of extract

The seeds of the plant *Dolichos biflorus* were collected and washed using fresh water and dried under shade. 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 is then evaporated by using a rotary evaporator and the phytochemicals were collected and stored for further analysis.

2.4 Experimental Animals

Wistar rats Male (8-10 weeks old) weighing 150–200g were included in this study. Before starting the experiment, the rats were maintained in the laboratory for adjustment and adaptation for 7 days. The experimental rats were maintained in ventilated cages at a photoperiod of 12:12 and a temperature of $25 \pm 2^\circ\text{C}$. The animals had easy access to *ad libitum* standard chewable food and water. The procedures of the experiments were carried out following the general standards and protocols based on the approval of the IAEC (Institutional Animal Ethical Committee) of Karnataka College of Pharmacy, Bangalore (Reg. Number: IAEC/ 09/21-22/10/18/12/21).

2.5 Model for Antiurolithiatic Activity

2.5.1 Ethylene glycol-induced urolithiasis

Groupings were done in the following manner, Where N = 6 animals in each group (Chart 1).

At the end of the treatment, serum samples were collected and the animals were sacrificed by using a high dose of pentobarbitone sodium for histopathology and antioxidant analysis of the kidney. Blood samples were withdrawn by cardiac puncture and retroorbital routes and samples were centrifuged at 2500 rpm for 15 minutes and examined. The parameters;

2.5.2 Observed parameters

Calcium, BUN (Blood Urea Nitrogen) uric acid, creatinine, potassium, alanine immune transferase, Phosphorus, oxalate, Alkaline Phosphatase [13,14].

Chart 1. Ethylene glycol-induced urolithiasis

01. Ethylene glycol induced Urolithiasis in Rat's Model	Group I: Normal Control Group – Vehicle. i.e. Normal Saline (10 ml/kg, p.o.) for 28 days	6 rats
	Group II: Disease Control, Received – Ethylene glycol (0.75%v/v) in drinking water for 28 days	6 rats
	Group III: Standard drug, Received Ethylene glycol (0.75% v/v) + Cystone (750mg/kg,p.o.)for 28 days	6 rats
	Group IV: Test drug (Low dose), Received Ethylene glycol (0.75%v/v) in drinking water for 28 days + methanolic extract of <i>Dolichos biflorus</i> at (150mg/kg, p.o.)	6 rats
	Group V: Test drug (High dose), Treatment group – Ethylene glycol (0.75%v/v) in drinking water for 28 days + methanolic extract of <i>Dolichos biflorus</i> at (300mg/kg, p.o.)	6 rats

The euthanized rats were conducted through a high dose of Pentobarbital followed by scarification. Kidneys of each animal were dissected and chopped into small parts followed by preservation and fixation in 10% formalin for 48 hours. Alcohol was used for tissue dehydration, embedded in paraffin, cut in a microtome and stained with Haematoxylin-Eosin dye. The mounted slides were examined under the microscope and compared to the slides from the control group. Determination of biochemistry markers was carried out following the standard protocols and procedures in the manufacturer's instructions.

2.6 Statistical Analysis

The data of the present study was analysed descriptively for the mean and standard error

mean (Mean ± S.E.M.) of the 6 replicates in each experimental group. All statistical analyses were carried out using the Graph Pad Prism software package (version 5). The one-way analysis of variance (ANOVA) was utilized to examine the significant differences in the means at the significance level of P<0.05. The multiple comparison test of the Tukey HSD test was used for pairwise comparison.

3. RESULTS

The Control group received an equivalent volume of "vehicle" only, the Disease control group received "Ethylene glycol" (0.75%v/v) in water, the Standard group received "Cystone" (750mg/kg,p.o.) and the Test drug, "dolichos biflorus" extract received low dose 150mg/kg & high dose 300 mg/kg p.o. respectively.

4. THE FOLLOWING PARAMETERS WERE OBSERVED POST-TREATMENT

4.1 Estimation of Calcium

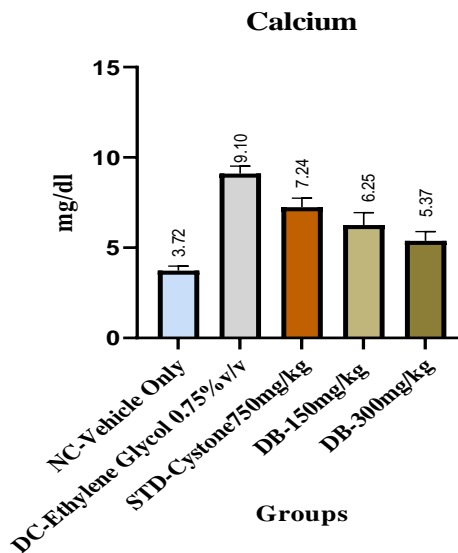


Fig. 1. Calcium

Results of calcium presented as Mean ± S.E.M (n=6)

Table 1. Statistics of calcium-> Comparison between the groups

Tukey's multiple comparisons tests	Mean diff.	Below threshold?	Summary	Adjusted P value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-5.378	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	-3.520	Yes	****	<0.0001
NC-Vehicle Only vs. DB-150mg/kg	-2.533	Yes	****	<0.0001
NC-Vehicle Only vs. DB-300mg/kg	-1.653	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	1.858	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	2.845	Yes	****	<0.0001

Tukey's multiple comparisons tests	Mean diff.	Below threshold?	Summary	Adjusted P value
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	3.725	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	0.9867	Yes	*	0.0164
STD-Cystone750mg/kg vs. DB-300mg/kg	1.867	Yes	****	<0.0001
DB-150mg/kg vs. DB-300mg/kg	0.8800	Yes	*	0.0383

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

4.2 Estimation of Creatinine

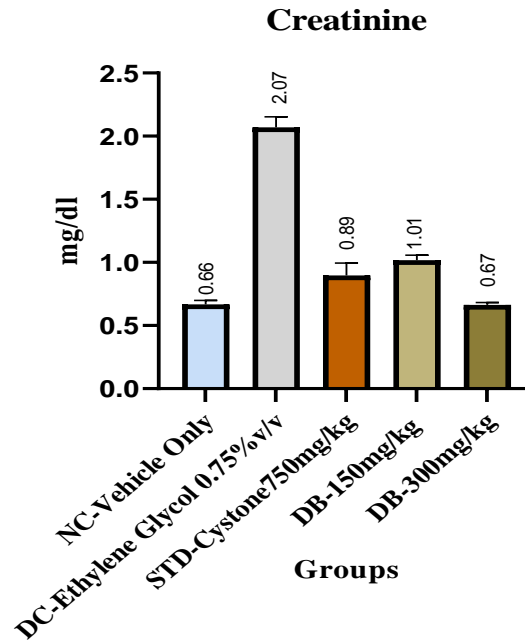


Fig. 2. Creatinine
Levels of creatinine presented as Mean ± S.E.M (n=6)

Table 2. Statistics of creatinine -> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean diff.	Below threshold?	Summary	Adjusted P value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-1.403	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	-0.2300	Yes	****	<0.0001
NC-Vehicle Only vs. DB-150mg/kg	-0.3517	Yes	****	<0.0001
NC-Vehicle Only vs. DB-300mg/kg	0.003333	No	ns	>0.9999
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	1.173	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	1.052	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	1.407	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	-0.1217	Yes	*	0.0176
STD-Cystone750mg/kg vs. DB-300mg/kg	0.2333	Yes	****	<0.0001
DB-150mg/kg vs. DB-300mg/kg	0.3550	Yes	****	<0.0001

*P < 0.05, **P < 0.01 values are mean ± SEM, n=6, when compared with control by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test

4.3 Estimation of Phosphorus

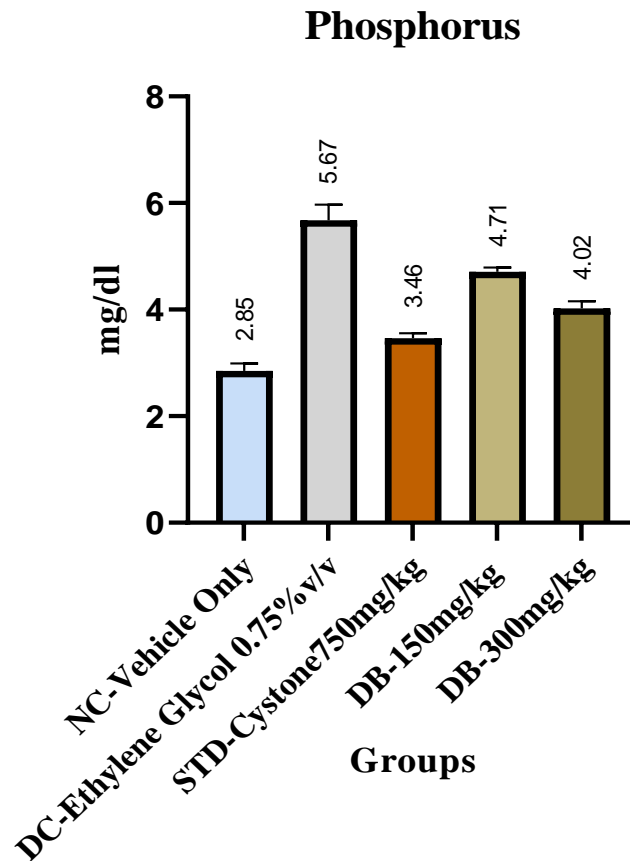


Fig. 3. Phosphorus
Levels of phosphorus presented as Mean ± S.E.M (n=6)

Table 3. Statistics of Phosphorus -> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean Diff.	Below threshold?	Summary	Adjusted P Value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-2.8267	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	-0.61000	Yes	****	<0.0001
NC-Vehicle Only vs. DB-150mg/kg	-1.8583	Yes	****	<0.0001
NC-Vehicle Only vs. DB-300mg/kg	-1.1700	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. STD Cystone750mg/kg	2.2167	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	0.96833	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	1.6567	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	-1.2483	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-300mg/kg	-0.56000	Yes	****	<0.0001
DB-150mg/kg vs. DB-300mg/kg	0.68833	Yes	****	<0.0001

*P < 0.05, **P < 0.01 values are mean ± SEM, n=6, when compared with control by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test

4.4 Estimation of Uric Acid

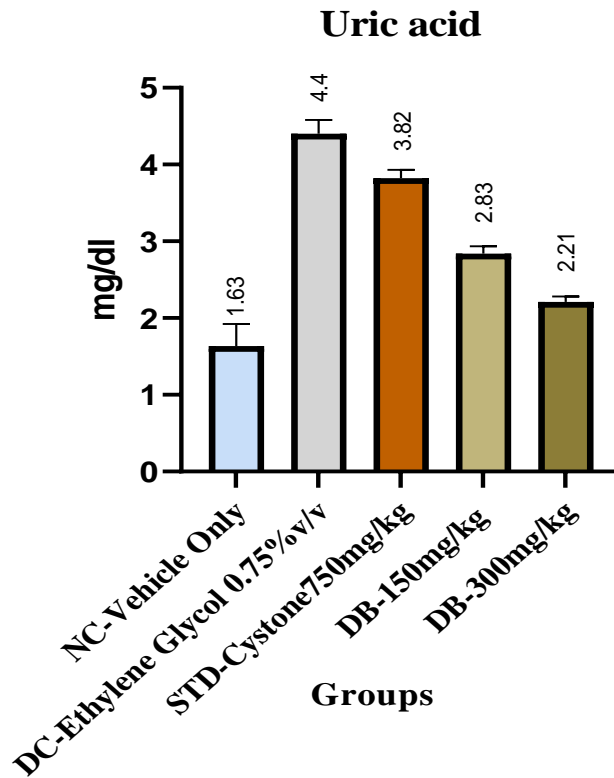


Fig. 4. Uric acid
Results of uric acid levels were presented as Mean ± S.E.M (n=6)

Table 4. Statistics of Uric acid -> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean diff.	Below threshold?	Summary	Adjusted P Value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-2.767	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	-2.190	Yes	****	<0.0001
NC-Vehicle Only vs. DB-150mg/kg	-1.205	Yes	****	<0.0001
NC-Vehicle Only vs. DB-300mg/kg	-0.5783	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	0.5767	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	1.562	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	2.188	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	0.9850	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-300mg/kg	1.612	Yes	****	<0.0001
DB-150mg/kg vs. DB-300mg/kg	0.6267	Yes	****	<0.0001

*P < 0.05, **P < 0.01 values are mean ± SEM, n=6, when compared with control by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test

4.5 Estimation of Alkaline Phosphatase

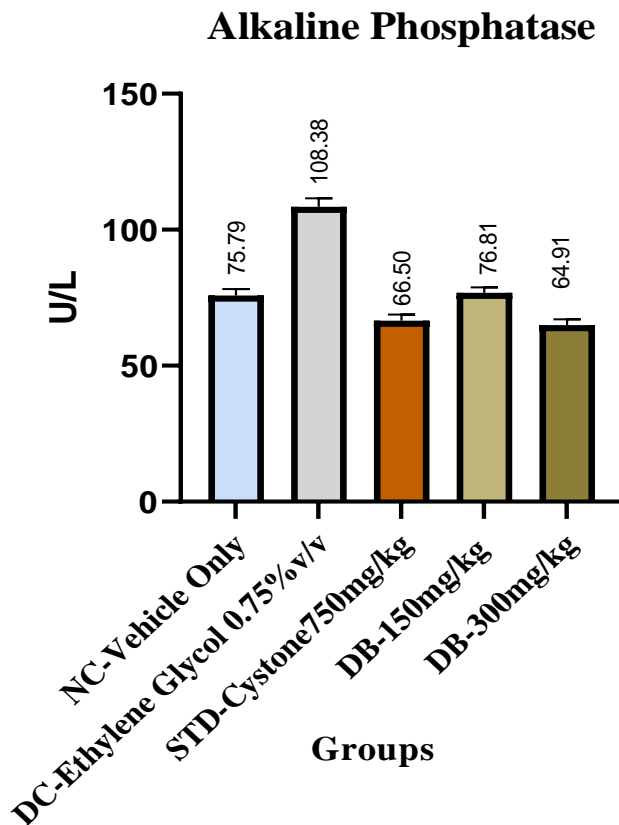


Fig. 5. Alkaline Phosphatase
Results of alkaline phosphatase presented as Mean \pm S.E.M (n=6).

Table 5. Statistics of Alkaline Phosphatase -> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean Diff.	Below threshold?	Summary	Adjusted P value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-32.59	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	9.282	Yes	****	<0.0001
NC-Vehicle Only vs. DB-150mg/kg	-1.025	No	ns	0.9484
NC-Vehicle Only vs. DB-300mg/kg	10.88	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	41.87	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	31.57	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	43.47	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	-10.31	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-300mg/kg	1.598	No	ns	0.7883
DB-150mg/kg vs. DB-300mg/kg	11.91	Yes	****	<0.0001

*P < 0.05, **P < 0.01 values are mean \pm SEM, n=6, when compared with control by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test

4.6 Estimation of BUN (Blood Urea Nitrogen)

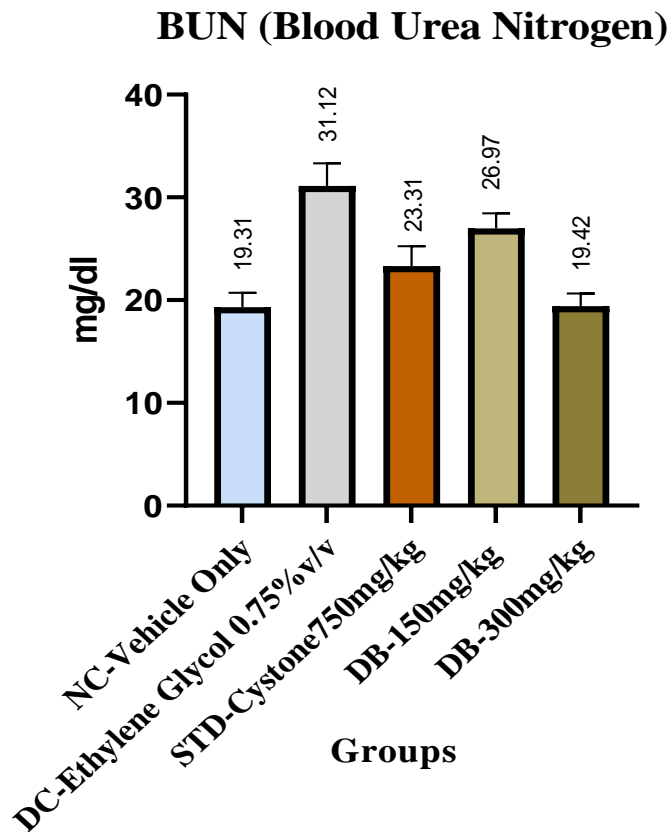


Fig. 6. BUN (Blood Urea Nitrogen)
Levels of blood urea nitrogen presented as Mean ± S.E.M (n=6)

Table 6. Statistics of Blood Urea Nitrogen-> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean diff.	Below threshold?	Summary	Adjusted P value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-11.81	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	-3.993	Yes	**	0.0033
NC-Vehicle Only vs. DB-150mg/kg	-7.657	Yes	****	<0.0001
NC-Vehicle Only vs. DB-300mg/kg	-0.1033	No	ns	>0.9999
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	7.813	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	4.150	Yes	**	0.0022
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	11.70	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	-3.663	Yes	**	0.0075
STD-Cystone750mg/kg vs. DB-300mg/kg	3.890	Yes	**	0.0043
DB-150mg/kg vs. DB-300mg/kg	7.553	Yes	****	<0.0001

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

4.7 Estimation of Potassium

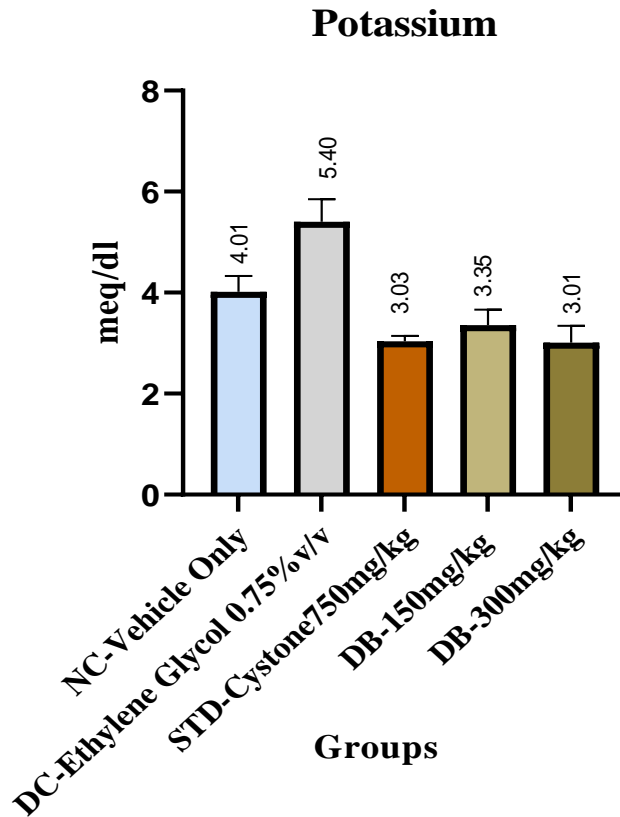


Fig. 7. Potassium
Potassium levels presented as Mean ± S.E.M (n=6)

Table 7. Statistics of Potassium -> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean Diff.	Below threshold?	Summary	Adjusted P value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-1.387	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	0.9800	Yes	***	0.0002
NC-Vehicle Only vs. DB-150mg/kg	0.6633	Yes	*	0.0112
NC-Vehicle Only vs. DB-300mg/kg	1.008	Yes	***	0.0001
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	2.367	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	2.050	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	2.395	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	-0.3167	No	ns	0.4435
STD-Cystone750mg/kg vs. DB-300mg/kg	0.02833	No	ns	0.9999
DB-150mg/kg vs. DB-300mg/kg	0.3450	No	ns	0.3595

*P < 0.05, **P < 0.01 values are mean ± SEM, n=6, when compared with control by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test

4.8 Estimation of Oxalate Level

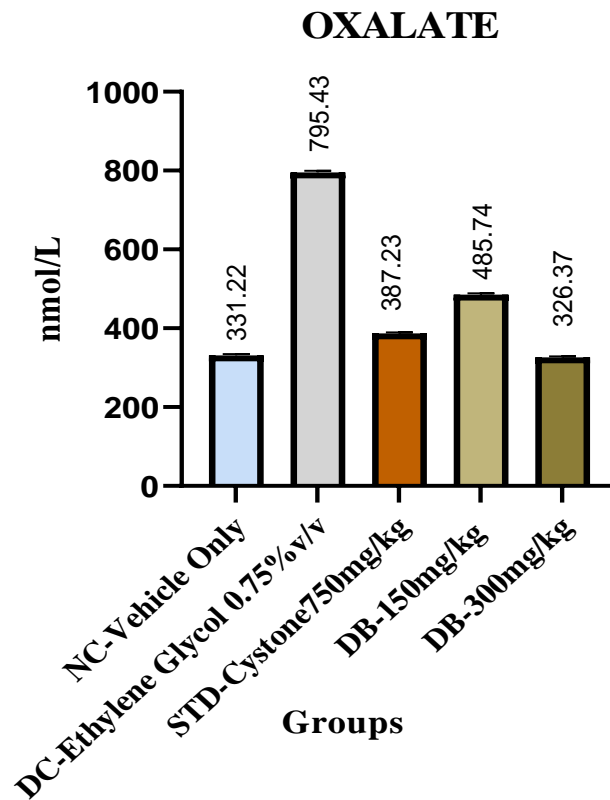


Fig. 8. Oxalate
Oxalate concentrations presented as Mean ± S.E.M (n=6)

Table 8. Statistics of Oxalate -> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean diff.	Below threshold?	Summary	Adjusted P value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-464.2	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	-56.00	Yes	****	<0.0001
NC-Vehicle Only vs. DB-150mg/kg	-154.5	Yes	****	<0.0001
NC-Vehicle Only vs. DB-300mg/kg	4.853	No	ns	0.0871
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	408.2	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	309.7	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	469.1	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	-98.52	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-300mg/kg	60.86	Yes	****	<0.0001
DB-150mg/kg vs. DB-300mg/kg	159.4	Yes	****	<0.0001

*P < 0.05, **P < 0.01 values are mean ± SEM, n=6, when compared with control by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test

4.9 Estimation of Alanine Amino Transferases

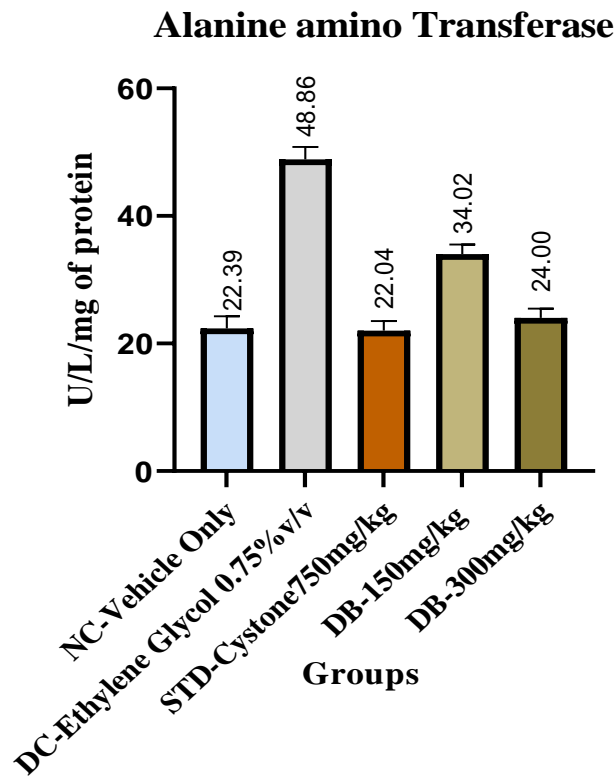


Fig. 9. Alanine amino transferases
Levels of alanine aminotransferase presented as Mean ± S.E.M (n=6)

Table 9. Statistics of Alanine Amino Transferases -> Comparison between the groups: Tukey's Multiple Comparison tests

Tukey's multiple comparisons tests	Mean diff.	Below threshold?	Summary	Adjusted P value
NC-Vehicle Only vs. DC-Ethylene Glycol 0.75%v/v	-26.47	Yes	****	<0.0001
NC-Vehicle Only vs. STD-Cystone750mg/kg	0.3467	No	ns	0.9961
NC-Vehicle Only vs. DB-150mg/kg	-11.63	Yes	****	<0.0001
NC-Vehicle Only vs. DB-300mg/kg	-1.613	No	ns	0.4634
DC-Ethylene Glycol 0.75%v/v vs. STD-Cystone750mg/kg	26.82	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-150mg/kg	14.84	Yes	****	<0.0001
DC-Ethylene Glycol 0.75%v/v vs. DB-300mg/kg	24.86	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-150mg/kg	-11.98	Yes	****	<0.0001
STD-Cystone750mg/kg vs. DB-300mg/kg	-1.960	No	ns	0.2764
DB-150mg/kg vs. DB-300mg/kg	10.02	Yes	****	<0.0001

*P < 0.05, **P < 0.01 values are mean ± SEM, n=6, when compared with control by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test

5. HISTOPATHOLOGICAL ANALYSIS

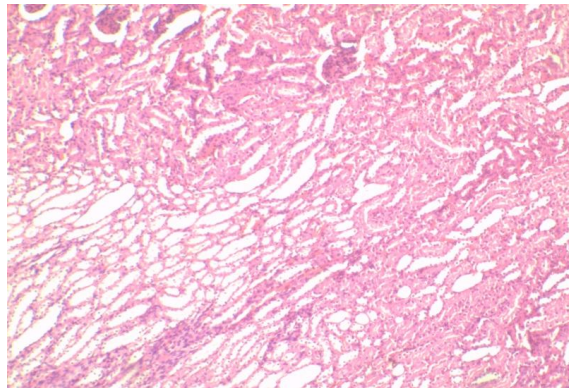


Image 1. Normal control group

Showing normal glomerulus with a tuft of capillaries is bounded by Bowman's capsule with tubules lined by columnar epithelial cell cytoplasm staining pink colour and normal architecture. Haematoxylin and Eosin stain, scale bar = 100µm

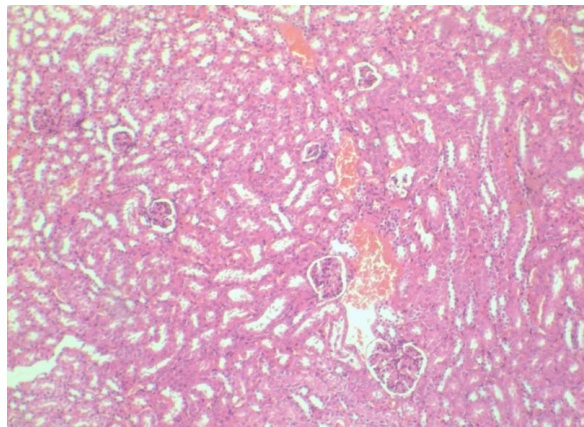


Image 2. Disease control

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 centre of the tubules. Haematoxylin and Eosin stain, scale bar = 100µm

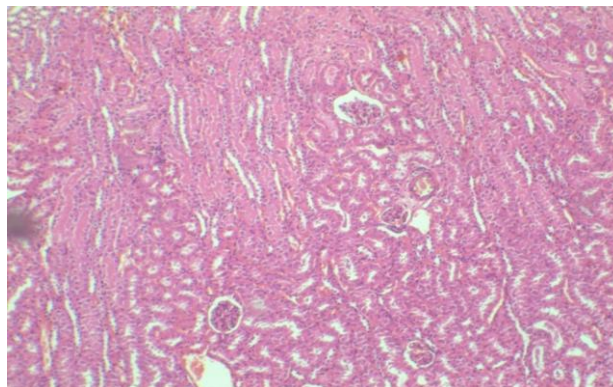


Image 3. Standard group

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. (Haematoxylin and Eosin stain, scale bar = 100µm

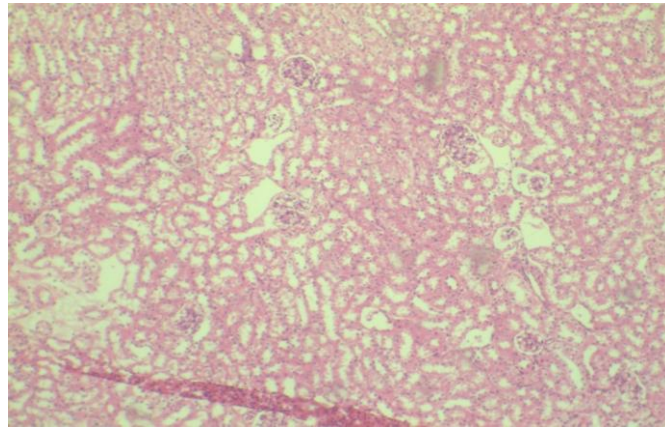


Image 4. Low dose group

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. Haematoxylin and Eosin stain, scale bar = 100µm

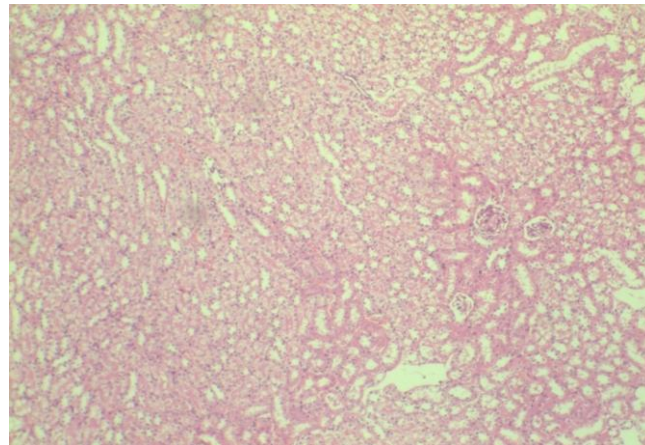


Image 5. High dose group

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. Haematoxylin and Eosin stain, scale bar = 100µm

6. DISCUSSION

This study presents data on antiurolithiatic activity of *Dolichos Biflorus* is confirmed after Comparison 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.

The histological profile of the kidney tissue proved the antiurolithiatic effect. Interestingly, the

sections of the rat's kidneys in the control group showed the formation of crystals. In addition to that, epithelial cells of the kidney showed remarkable tubular dilatation and damage as illustrated by the gaps in the tissue. However, in the treated group, the rat's kidney exhibited less crystal formation. Furthermore, less necrosis and tubule dilatation were scarce. The damage caused by the formation of the kidney stones is ultimately affecting the renal function in the control group as proven by the markers of glomerular and tubular damage: elevated levels of BUN, uric acid, urea, and serum creatinine were lower in the treated animals. Inflammation and tissue damage are because exposing them to phosphate and calcium phosphate crystals

results in the formation of reactive oxygen species, development of oxidative stress, lipid peroxidation, and depletion of antioxidant enzymes. In the same context, the damage to the epithelial tissue of the kidney enhances crystal retention and eventually promotes the formation of kidney stones on the epithelial tissue surface. One important explanation of the plant extract mechanism is that antioxidant compounds can enhance the antioxidant enzymes in the kidney and constrain cell injury or damage.

The herbal or traditional medicines are used through oral intake similar to the procedure of anti urolithiatic activity evaluation of *L. procumbens* against ethylene glycol-induced renal calculi in rats. The justification for this study using rat males is the urinary system of the males is quite similar to humans in inducing and formation of urolithiasis. In addition, several studies reported lesser kidney stone formation in rat females compared to males [15].

7. CONCLUSION

This study describes the anti-urolithiatic activity of *Dolichos biflorus* methanolic seeds extract in Ethylene glycol induced urolithiatic in a rat's model.

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 and it has a potent diuretic effect.

BUN, uric acid, urea and serum, and creatinine that was lowered in animals receiving plant extract. The protection of the Kidney with this treatment is ideal for urolithiasis patients.

All other urolithiasis markers like calcium, BUN, potassium, and creatinine showed a dose-dependent manner concerning their control group. The alkaline phosphatase, calcium, and uric acid also improved significantly.

Histology of kidneys in urolithiatic animals showed that necrosis, as well as tubule dilatation, was very limited.

The high dose of *Dolichos biflorus* (300mg/kg, PO) is showing a better effect as compared to the low dose of (150mg/kg, PO) and the standard drug (750 mg/kg, PO).

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study had been ethically approved by the (Institutional Animal Ethical Committee) of Karnataka College of Pharmacy, Bangalore. Which conducted the meeting for M.pharm Students on the date (18/12/2021) before taking part in the study, the committee approved for use of the animals in the experiment and provided the registration number (Reg. Number: IAEC/09/21-22/10/18/12/21) to start the experiment on animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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