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Anti Urolithiatic Activity of *Dolichos biflorus* by Using Wistar Albino Rats in Ethylene Glycol Induction Method

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Abstract

Objective: *Dolichos biflorus* has been used in the Indian indigenous system of medicine, Ayurveda, for the treatment of various health ailments including renal calculi. The present study was undertaken to investigate the Anti-urolithic activity of ethanolic extract of *Dolichos biflorus* in Wistar Albino Rats.
Method: *Dolichos biflorus* extract was prepared by using 98% ethanol by maceration method and phytochemical analysis was performed. The samples were examined for antiurolithiatic action by determining phosphate, calcium, and oxalate levels in urine; Urea, uric acid, and creatinine in serum with the Automatic Laboratory Chemistry Research System. The histopathology of the kidney was analyzed.
Result: *Dolichos biflorus* extract at a dosage of 200mg/kg, 400mg/kg in Wistar Albino Rats Significantly, decreased the level of phosphate, calcium as well as oxalate in urine; decreased the serum level of urea nitrogen and creatinine, and uric acid. Comparing the observed outcomes at various dosages to the control groups revealed significant differences. The tubules exhibit normal morphology and have recovered to some extent. These results revealed that the seed extract of *Dolichos biflorus* decreased calculi levels.

Keywords: Urolithiasis, Dolichos biflorus, calculi, EDS

INTRODUCTION

The development or deposition of calculi in the urinary tract is known as urolithiasis, and it is thought to be the third most common illness in the world, affecting about 12% of the population.[1] The physiochemical process that results in crystal nucleation, aggregation, and growth of calcified renal stones is aided by numerous biological processes, such as urine volume, pH, elevated calcium or sodium oxalate, and urates.[2] With a success rate of 68 to 86%, over 90% of patients with upper urinary tract stones are currently being treated based on the size, kind, and location of the stones. [3]
This study's objective is to assess the anti-urolithiatic activity of *Dolichos biflorus* in Wistar Albino Rats. Kidney calculus frequently develops in the kidney, whereas stone or calculus development in the urine collection system is associated with urolithiasis. When uric acid, calcium, and oxalate—elements that can form crystals—are present in higher amounts in urine, kidney stones can develop. Together, these crystal-forming

substances produce crystals. [4,5] The multifactorial process of renal crystal formation may be influenced by dietary factors, urinary tract infections, changed urine particles and molecules, decreased urine outflow and microlith, stasis of the urinary tract, and extended immobility of Randall's plaque.[6] Although proteus species comprise the majority of urea-splitting organisms, urease production has also been documented. These include Escherichia coli, Staphylococcus, the bacterium Pseudomonas, and even Mycoplasma species.[7] Urinary calculi are tiny, concrete particles of acidic & mineral sodium chloride. The majority of the typical kind contains calcium in addition to phosphate or oxalate.[8]All parts of the urinary tract, including the kidney, ureter, and bladder which can vary substantially in size have calculi formation issues that have been observed and recorded.[9]Men are three times as likely than women to experience this occurrence rate.[10]The primary focus of current research, which seeks a safer and more effective management strategy for urolithiasis, is an indigenous medicine with fewer side effects.[11]According to epidemiological studies, nephrolithiasis is more typical among men (12%) compared to women (6%) and is more prevalent in both between the ages of 20 and 40 sexes.[12]10-12% of people have urinary stones in developed countries.[13] *Dolichos biflorus*(Fabaceae) is well-known for its antioxidant action and is frequently used to treat kidney stones, leukorrhea, urinary diseases, and menstrual problems. Native to most of India, *Dolichos biflorus*, also known as Muthira, is a branching, suberect, and downing herb that can be found up to 1000 meters above sea level. Synonyms are horse grain, poor man's pulse, and kulattha in Ayurveda. It is distributed in Africa, Asia, and India.[14] The plants are believed to contain the following chemical constituents: linoleic acid, polyphenols, oxalates, bulbiformin, streptogenin, and β-sitosterol. Horse gram seeds are infusion-cooled (Hima) and administered 80–100 ml daily to treat kidney calculi, micturition difficulties, and bladder pain.

MATERIAL AND METHODS

Collection and authentication of Plant material

The seeds of *Dolichos biflorus* were selected for investigation and were procured from the nearest area of our college. Authentication of plant material was done by Dr.Chaithra G K, BAMS, MD (Ayu), Associate Professor, Heading Centre for Herbal Gardens, the University of Trans-Disciplinary Health Sciences & Technology, Bangalore 560064, Karnataka, India.

Preparation of plant extract

The seeds were dried, and then they were ground with an electric grinder to a coarse powder. The powdered seeds were macerated in 98% ethanol for three days at room temperature, stirring occasionally. A rotary evaporator operating at 40°C was used to filter and eliminate the extracted solvent. After freeze-drying, the extract was kept at -20°C to maintain its antiurolithiatic properties.

Preliminary phytochemical studies

The extract was subjected to phytochemical analysis to indicate the presence of polyphenols, flavonoids, polysaccharides, and saponins.

Chemicals and apparatus

All chemicals and reagents used were of analytical grade and procured from approved suppliers. Apparatus such as the metabolic cage (Dolphin, Mumbai), the biochemistry analyzer (Benspera Clinical Chemistry Analyzer C-61), the UV-spectrophotometer (Jasco model V- 630) and the centrifuge (Remi) were used in the study.

Experimental Animals

Adult Wistar Albino Rats (200-250gm) were used to evaluate the Antiurolithiatic activity. The animals were housed in conventional settings with a 12-hour light and dark cycle, room temperature (20 $\pm 2^0$ C), and relative humidity (60% ± 5%). The animals were acclimatized to the laboratory conditions for at least one week before the experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee of Aditya Bangalore Institute of Pharmacy Education &Research, Bangalore (Project proposal no. 80/1611/CPCSEA) and studies were carried out according to current CCSEA regulations.

Acute toxicity studies
An acute toxicity assay was conducted for ethanolic extract as per OECD guideline 423 (limit test-standard protocol). Six male Wistar albino rats (three animals in each step) and a total of 12 animals were randomly selected. The extract was found to be safe up to 2000 mg/kg b.w. [15]

Evaluation of antiurolithiatic activity of *Dolichos biflorus* on ethylene glycol (0.75% v/v) induced Wistar albino rats
Group – I: Normal control
Group – II: Diseases Control (Ethylene glycol 0.75% in drinking water was administered)
Group – III: Standard (Ethylene glycol 0.75% in drinking water was administered + Febuxostat 20mg/kg)
Group – IV: Low Dose (Ethylene glycol 0.75% in drinking water + *Dolichos biflorus* 200 mg/kg)
Group – V: High Dose (Ethylene glycol 0.75% in drinking water + *Dolichos biflorus* 400 mg/kg)
The above treatment was given up to 28 days.

Collection and analysis of urine
Every animal was housed in a separate metabolic cage, and on day 28, urine samples were taken every 24 hours. Animals had unrestricted access to water to drink while the waste was being gathered. An automated system was used to determine the levels of calcium, phosphate, and oxalate in urine. [16,17,18]

Serum analysis
On the 28th day, blood was extracted from the retro-orbital plexus using a capillary procedure under mild ether anesthesia. The serum was separated using centrifugation at 10,000 g for 10 minutes, and the Computerized Clinical Laboratory Analysis System was used to measure the levels of creatinine, urea, nitrogen, and uric acid.[19]

Kidney histopathology analysis
The kidneys were extracted from the sacrificed rats. The left kidney was removed and thoroughly cleaned in 0.1 M phosphate-buffered saline (pH 7.4), which was extremely cold. Each animal's right kidney was preserved in 10% neutralized formalin to verify the lithiasis incidence. Haematoxylin and eosin dye was used to colour the paraffin-embedded isolated kidney, which was then sliced into thin sections measuring 5 mm. Under a binocular microscope, the slides were inspected for the presence of calcium oxalate, renal cellular and tubular necrosis, and histopathological abnormalities in the kidney architecture.[20]

RESULTS

Phytochemical Studies
The phytoconstituents were extracted by using 98% of Ethanol as a solvent. The active constituents of various extracts are alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, and starch. % yield of extract was 13.56%.

Table 1: Phytochemical Analysis

Test for Constituents	*Dolichos biflorus*
Alkaloids	++
Flavonoids	+
Phenols	-
Proteins	+
Carbohydrates	++
Glycosides	-
Saponins	-
Triterpenoids	-
Tannins	+
Steroids	-
Starch	-

Urine biochemistry
Elevated urinary calcium promotes the nucleation and precipitation of CaOx, or apatite (calcium phosphate), from urine and the subsequent crystal formation. By producing calcium phosphate crystals, which epitaxially

stimulate CaOx deposition, increased urinary phosphate excretion combined with oxalate stress appears to provide an environment conducive to stone formation.

Table 2: Effect of *Dolichos biflorus* extract (seed) on urine calcium, phosphate, and oxalate levels in ethylene glycol-induced urolithiasis in rats

Groups	Calcium(mg/dl)	Phosphate(mg/dl)	Oxalate(mg/dl)
Normal control	4.95 ± 0.65	5.01 ± 0.24	6.09±0.20
Disease control	8.86 ± 0.36	17.6 ± 0.44	9.02 ± 0.09
Standard (Febuxostat)	6.028 ± 0.08***	13.5 ± 0.41***	6.04 ± 0.14***
EDS 200mg/kg	7.44 ± 0.35*	15.3 ± 0.19**	7.89 ± 0.43*
EDS 400mg/kg	6.82 ± 0.11**	14.1 ± 0.48***	6.92 ± 0.31***

Every value is mean ± SEM, with n = 6 and ns = not significant. *Dolichos biflorus* ethanolic extract *p< 0.05 and **p<0.01 versus the control group and a ***p<0.001 vs normal group were tested using one-way analysis of variance (ANOVA) and Dunett's multiple comparison test.

Serum biochemistry

In urolithiasis, waste products, particularly nitrogenous chemicals like urea, creatinine, and uric acid corrosive, accumulate in blood and impede the flow of urine through stones in the urinary system, lowering the glomerular filtration rate (GFR). The creatinine and uric acid levels in the serum were remarkably elevated in calculi-induced animals.

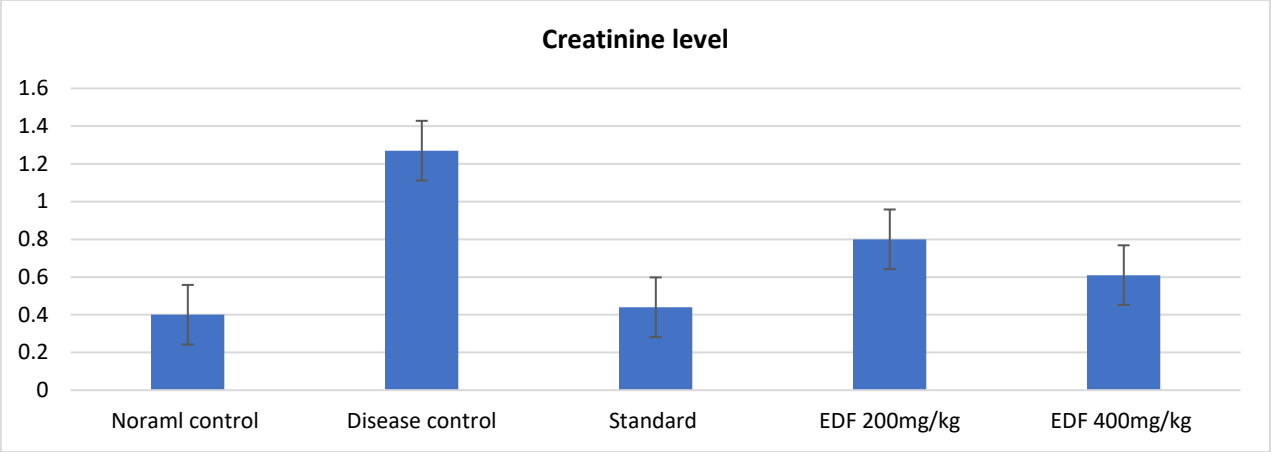

Fig 1: Histogram showing the effect of *Dolichos biflorus* seeds extract on urinary excretion of creatinine

Every value is mean ± SEM, with n = 6 and ns = not significant. *Dolichos biflorus* ethanolic extract *p< 0.05 and **p<0.01 versus the control group and a ***p<0.001 vs normal group were tested using one-way analysis of variance (ANOVA) and Dunett's multiple comparison test.

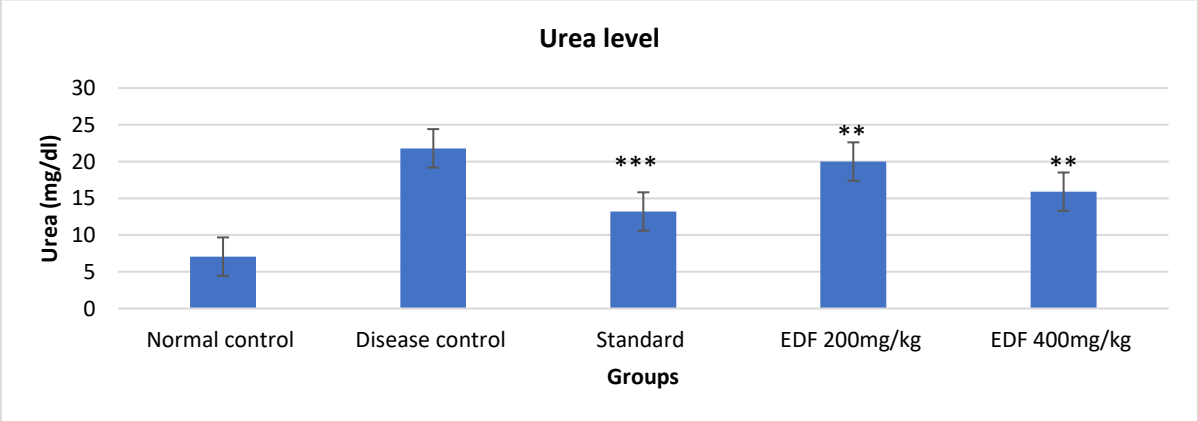

Fig 2: Histogram showing the effect of *Dolichos biflorus* extract on urinary excretion of urea

Every value is mean ± SEM, with n = 6 and ns = not significant. *Dolichos biflorus* ethanolic extract *p< 0.05 and **p<0.01 versus the control group and a ***p<0.001 vs normal group were tested using one-way analysis of variance (ANOVA) and Dunett's multiple comparison test.

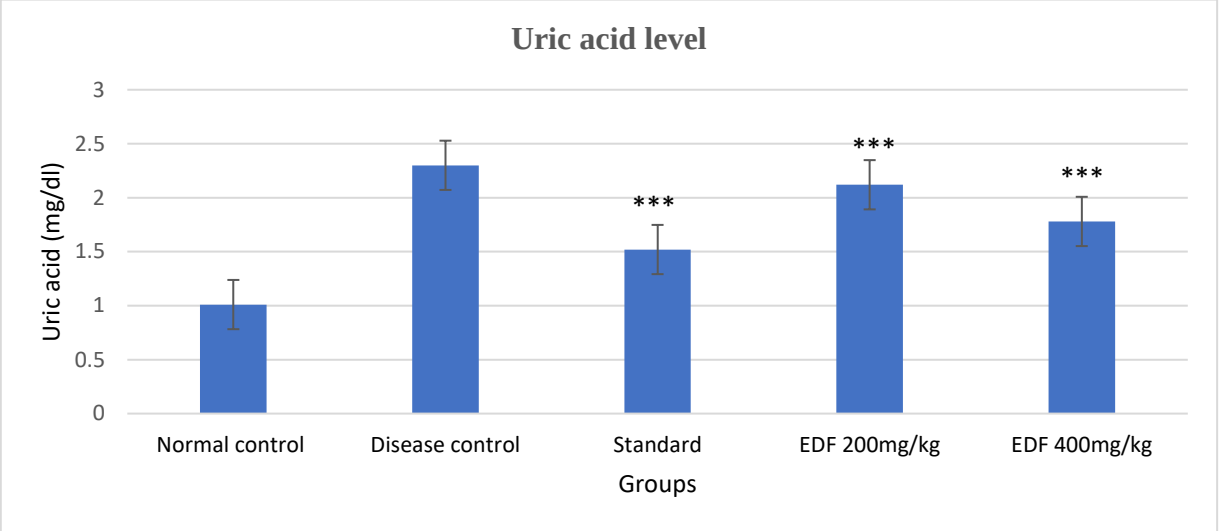

Fig3: Histogram showing the effect of *Dolichos biflorus* seeds on urinary excretion of uric acid

All the values are mean ± SEM, n=6, ns= not significant, one-way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test, *p< 0.05 and **p<0.01 vs control group and a ***p<0.001vs normal group

Histopathological examination of the kidney

The histopathological study of the kidney sections was performed utilizing a compound microscope equipped with a digital camera. A general score was run to observe the calculi by visualizing several fields. Support for the biochemical data was also provided by the histological examination. By comparison with the normal mouse kidney design, the collecting system, peritubular interstitium, and the final segment of the nephron were severely damaged in all of the stone-producing rodents.

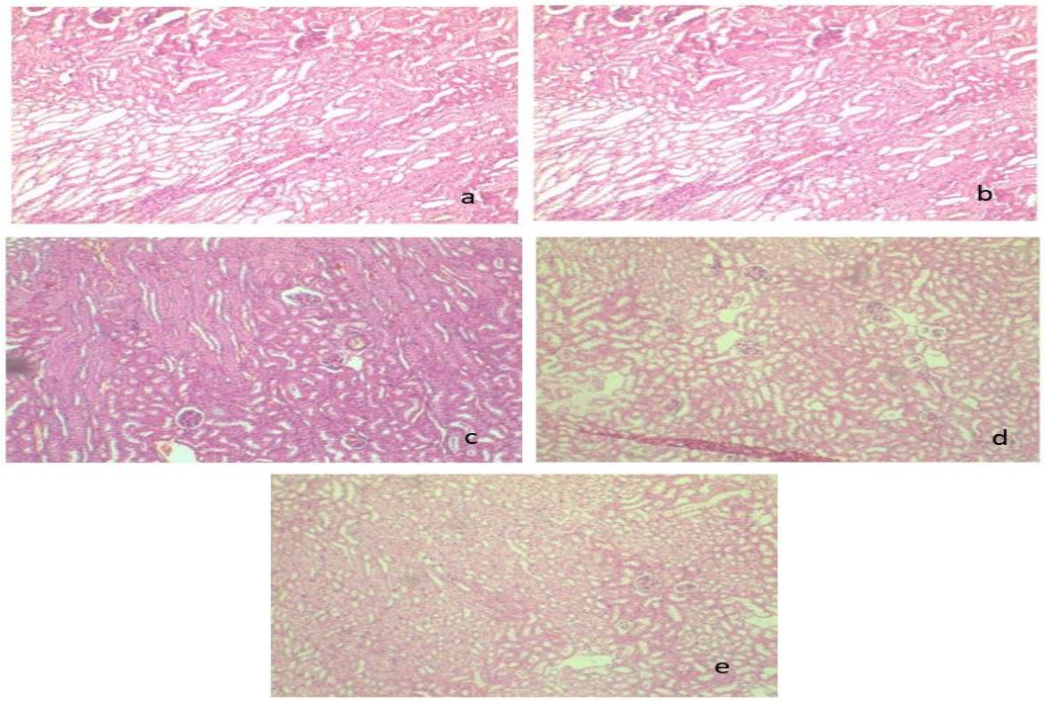

Fig 4: Histopathological study of the kidney sections ;(a) Control group (b) Disease control (c)Standard group (d) Low dose of extract (e) High dose of extract.

DISCUSSION

The antiurolithiatic activity of *Dolichos biflorus* was 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 compared to the standard and disease control group. From the kidney homogenate study, it is also found that the test drug has potent antiurolithiatic activity.

One of the main causes of calculogenesis is widely thought to be urinary hypersaturation about the substances that form stones. Evidence from earlier research revealed that young male albino rats developed renal calculi, primarily constituted of calcium oxalate, following 14 days of ethylene glycol (0.75% v/v) administration.[21] Rats fed ethylene glycol develop stones as a result of hyperoxaluria, which increases renal retention and excretes an excessive amount of oxalate in the urine. In this investigation, animals with calculi had higher excretions of calcium and oxalate. The production of calcium phosphate crystals, which cause calcium oxalate deposition, appears to be a result of increased urine phosphate excretion in conjunction with oxalate stress, creating an environment conducive to stone formation. Stone formation risk was decreased by using the *Dolichos biflorus* treatment, which raised the phosphate level.[22] Due to stones obstructing the urinary system's ability to evacuate pee, urolithiasis causes a reduction in the glomerular filtration rate (GFR). Waste products, especially nitrogenous compounds like urea, creatinine, and uric acid, build in the blood as a result. Rats given a diet high in calculi-producing supplements were shown to have kidneys with increased lipid peroxidation and lower antioxidant capability. Studies have shown that oxalate reacts with polyunsaturated fatty acids in cell membranes to produce damage to renal tissue and induce lipid peroxidation. Increased blood urea nitrogen, uric acid, and creatinine levels in the serum of the calculi-induced rats (Group II) indicated significant kidney injury.[23] Histopathological analysis of kidney sections from rats treated with ethylene glycol to produce urolithic revealed dilatation of the proximal tubules and interstitial inflammation, which may be related to elevated levels of calcium and oxalate in the kidney.[24] The administration of *Dolichos biflorus* ethanolic extract in the current investigation prevented damage to the tubules and calyxes and reduced the amount and size of calcium oxalate deposits in several renal tubule locations.

CONCLUSION

Based on the current investigation, we deduce that *Dolichos biflorus* Lin. contained alkaloids, flavonoids, proteins, saponins, terpenoids, phytosterols, carbohydrates, and fatty acids. According to the aforementioned analysis, the medication is significantly more active than the control group and the usual medication febuxostat. Compared to the low dose of 200 mg/kg, the high dose of *Dolichos biflorus* (400 mg/kg) is demonstrating a greater effect. We may infer from all the data that the medication possesses strong antiurolithiatic effect.

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