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Safety evaluation of *Dolichos biflorus* seed extract against ethylene glycol induced renal stone in experimental rats

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Abstract
This study was designed to elucidate the toxicity of *Dolichos biflorus* seed extract in ethylene glycol induced renal stone in experimental rats. In toxicity evaluation, no mortality or any toxic reaction was recorded in any group after 28 days of administering the extracts at (300 and 3000 mg Kg^{-1} BW). The extracts did not cause any behavioural or physical changes in experimental rats. The results shows that the extract has nephroprotective effect and lowers the serum concentration of creatinine, BUN, calcium and phosphorus significantly (p<0.001) as compared to untreated group. In untreated group II, the concentration of serum creatinine, BUN, calcium and phosphorus was recorded to be between the positive group and extract treated group (300mg/kg group). The study contributes to establishing the nontoxic quality parameters of *Dolichos biflorus* seed extract and the results suggest the safety of the extracts in upto 10 times of therapeutic use.

Keywords: Toxicity, *Dolichos biflorus*, biochemical, BUN, creatinine, calcium

Introduction
Traditional medicines have been used in the treatment of urinary stones from ancient times, and they need scientific evaluation to understand their mode of action. The ripe kernel juice of *Musa paradisiacal* is popular among the rural people in South India and has been used to dissolve urinary stones (Nadkarni, 1976) [1]. It has no toxic effect, is readily available and easily consumable. In the indigenous system of medicine (Ayurveda), many plants have been claimed to be useful in urinary stones or calculus. Among these, stem juice of Musa species is most commonly used as lithotriptic. The Ayurvedic physicians of Dakshina Kannada district of Karnataka and Northern Kerala recommend a particular variety of *Musa paradisiaca*, locally known as Puttubale which is claimed to be very effective in dissolving urinary stones. Earlier studies on Musa stem juice have shown a lowering of liver glycolic acid oxidase activity and glycolic acid content of hyperoxaluric acid (Kailash *et al*., 1992) [2]. Ash of the peel of *Musa sapientum* showed an increase in urine volume and K+ as well as other electrolyte excretion than normal saline in a study in rats. Successive ethanolic extract also give this diuretic effect (Jain *et al.,* 2007) [3]. Phytochemicals such as saponin, flavonoids and terpenoids are known to be responsible for this effect (Rizvi *et al*., 1980; Sood *et al*., 1985 and Chodera *et al*., 1991) [4, 5, 6]. The aqueous extracts of seeds of *Dolichos biflorus* inhibited homogenous precipitation of calcium hydrogen phosphate dihydrate crystals (Garimella *et al*., 2001) [7]. However, the effects of these extracts have not been evaluated in animal models. The seeds of *D. biflorus* have been reported to show anti-hepatotoxic (Laskar *et al*., 1998) [8], anti-nephrotoxic (Rao *et al*., 1999) [9], free radical scavenging activity (Kanaka *et al*., 2012) [10], antioxidant (Rao *et al*., 2011) [11] and hypolipidemic activity. The traditional Ethnoveterinary practitioners consider decoction of *Dolichos biflorus* seed as an excellent natural product that has litholytic potential and in many pathological conditions of renal disorders. However, scientific validation of such claims are lacking. The current study is therefore proposed to evaluation Safety at two different doses of the *Dolichos biflorus* seed extract.

Materials and Methods
Hyperoxaluria and Calcium oxalate deposition in the kidney was induced by mixing ethylene glycol (EG) in the drinking water to a final concentration of 0.75% with 2% ammonium chloride (NH4 Cl). For this experiment a total of 36 rats were used. The rats were divided into six groups of six animals each and subjected to different treatments as mentioned in Table 1. The treatment was continued for 28 consecutive days once daily.

The rats were continuously observed for 6 hours, thereafter hourly for another 6 hours for any adverse reactions or symptoms or mortality. There after these experimental rats were observed closely one hour at morning for all 28 days of experiment for clinical symptoms, mortality etc. At the end of the experiment each rat was sacrificed using pentobarbital sodium. Kidneys were removed and dissected into two equal halves. One part was taken into a polyethylene packer without any preservatives/ fixative for estimation of tissue mineral content.

Table 1: Experimental protocol to assess the efficacy and safety of test extract

Treatment	Ethylene glycol	Ammonium chloride	Extract (*Dolichos biflorus)*	Standard (Cystone 500mg/kg)	placebo
Negative control(Healthy))	Nil	Nil	Nil	Nil	Nil
Untreated group I (Diseased)	0.7% in drinking water	2 % in drinking water	Nil	Nil	Nil
Untreated group II	0.7% in drinking water	2 % in drinking water			Distilled water fro day 10th
Standard(Cystone) 500mg/kg bw	0.7% in drinking water	2 % in drinking water		500mg/kg	nil
300mg/kg	0.7% in drinking water	2 % in drinking water	300mg/kg		
3000mg/kg	0.7% in drinking water	2 % in drinking water	3000 mg/kg		

The samples of blood were collected from individual rats on day '0' of the experiment and thereafter at 7 days intervals for 28 days from the orbital plexus using micro haematocrit capillaries piercing through the outer canthus from each animal without anticoagulant and allowed to clot at room temperature. Serum was separated by centrifugation at 2000 rpm for 20 minutes in refrigerated centrifuge and stored at 4^0C till analysis

Biochemical Analysis

The serum collected was utilized to measure the following parameters:

a) Serum urea nitrogen was determined using Diactyl monoxime method (Wybenga *et al*., 1971) [12] and results were expressed as mg/dl of serum.
b) Creatinine (Frankel *et al*., 1970) [13] and results were expressed as mg/dl.
c) Calcium concentrate ion was estimated using o-Cresolphthalein complexioned method (Miller *et al*., 1994) [14] and results were expressed as mg/dl.
d) Phosphorus concentration was expressed following the UV Molybdate method (Miller *et al*., 1994) [14] using specific commercial diagnostic kits manufactured by Span Diagnostic (India) Pvt. Ltd. Surat. The results were expressed as mg/dl.

Tissue calcium content

The kidneys stored at -20º C for determining the calcium content. The total calcium contents of renal tissue was estimated according to Economou *et al*. (1987) [15] using atomic absorption spectroscopy. Briefly the kidney tissues were dried at 100º C for 24h and weighed. About 500 mg kidney tissues were wet digested using double acid mixture (perchloric acid: nitric acid =1:5). The mixture was digested until the liquid became transparent. The calcium concent rat ion in the acid digest was estimated using atomic absorpt ion spectrophotometer (Analyst 200, Perkin Elmer, and Switzerland). The quality control criteria was strictly adhered to by repeated analysis of reference sample. The calcium content of the kidney was expressed as mg/g wet tissue of the kidney (Economou *et al*., 1987) [15].

Statistical Analysis

The data were expressed as mean±SEM. Standard error of mean and p-values were used to determine any significant difference among different treatment groups using two-way analysis of variance (ANOVA) following standard protocol (Snedecor and Cochran, 1994) [16].

Result and discussion

Mean± SE values of serum creatinine of diseased and extract treated rats were presented in table 2. Highly significant (p<0.001) increase in creatinine levels (mg/dl) of untreated control rats (1.83±0.26 mg/dl) was recorded as compared to healthy rats (1.17±0.07 mg/dl). The treated groups i.e. the rat groups who were given aqueous extract of *Dolichos biflorus* seeds also showed significant (p<0.001) decrease in the serum levels of creatinine compared to untreated rats. The serum levels of creatinine of extract treated rats were estimated as 1.42±0.18 mg/dl in rats 300 mg/kg and 1.22±0.08 mg/dl for 3000 mg/kg group. In cystone treated rats (standard group) the level of creatinine was 1.32±0.06 mg/dl which is significantly p<0.001 lower than untreated group.

This clearly shows that the extract has nephroprotective effect and lowers the serum concentration of creatinine significantly (p<0.001) as compared to untreated groups. In untreated group II, the concentration of serum creatinine was recorded to be between the positive groups and extract treated group (300mg/kg), i.e. 1.57±0.11 mg/dl (figure 6).

Table 2: Concentration of creatinine (mg/dl) in serum of normal, Urolithic, Cystone treated and *Dolichos biflorus* extract treated rats

Groups	Days post treatment				
	0th day	7th day	14th day	21st day	28th day
Negative control(Healthy)	$0.29\pm0.05^{A,}$	$0.45\pm0.18^{AB,ab}$	$0.82\pm0.32^{BC,ab}$	$1.00\pm0.05^{C,ab}$	$1.17\pm0.07^{C,a}$
Untreated group I (Diseased)	$0.29\pm0.03^{A,}$	$0.72\pm0.13^{B,b}$	$1.08\pm0.05^{B,ab}$	$1.01\pm0.10^{B,ab}$	$1.83\pm0.23^{C,b}$
Untreated group II	$0.29\pm0.05^{A,}$	$0.36\pm0.03^{A,a}$	$1.64\pm0.47^{B,b}$	$1.35\pm0.12^{B,bc}$	$1.57\pm0.11^{B,ab}$
Standard (cystone) 500mg/kg	$0.55\pm0.26^{A,}$	$0.30\pm0.07^{A,a}$	$1.01\pm0.06^{B,ab}$	$1.03\pm0.08^{B,ab}$	$1.32\pm0.06^{B,a}$
EC_{50}	$0.32\pm0.07^{A,}$	$0.37\pm0.11^{A,a}$	$1.05\pm0.26^{B,ab}$	$1.47\pm0.28^{B,c}$	$1.42\pm0.14^{B,a}$
10 times EC_{50}	$0.36\pm0.05^{A,}$	$0.39\pm0.05^{A,a}$	$0.55\pm0.12^{AB,a}$	$0.71\pm0.11^{B,a}$	$1.22\pm0.08^{C,a}$

Values (Mean±SE) bearing different uppercase superscript vary significantly (p<.001) between periods and lowercase superscript between groups

Mean± SE values of serum urea nitrogen levels of urolithiatic and extract treated rats are presented in table 3. Highly significant (p<.001) increase in serum urea nitrogen levels (46.81±4.10 mg/dl) was recorded in untreated rats compared to healthy control rats (24.57±0.0.49 mg/dl). The treated group, i.e. rat receiving aqueous extract of *Dolichos biflorus* seeds showed significant (p<0.001) decrease in the serum levels of urea nitrogen compared to untreated (diseased) rats. Serum levels of urea nitrogen in extract treated groups on day 28 were estimated as 35.89±1.76 mg/dl for 300 mg/kg and 31.35±0.08 mg/dl for 3000 mg/kg group. In cyst one treated rats (standard group) the level of blood urea nitrogen was 37.90±3.33 mg/dl which was significantly (p<0.001) lower than untreated group. This clearly shows that the extract has nephroprotective effect and lowers the serum concentration of blood urea nitrogen significantly (p<0.001) as compared to untreated control groups. In untreated control group, the concentration of serum blood urea nitrogen was found to be between the positive group and extract treated rats (300 mg/kg) i.e. 36.32±4.62 mg/dl.

Table 3: Concentration of urea nitrogen (mg/dl) in serum of normal, Urolithic, Cystone treated and *Dolichos biflorus* extract treated rats

Groups	Days post treatment				
	0TH day	**7th day**	**14th day**	**21st day**	**28th day**
Negative control (Healthy)	35.37±3.55^{b,C}	30.29±2.08^{BC}	20.98±2.74^{a,A}	23.40±3.66^{a,AB}	24.57±0.49^{a,AB}
Untreated group I (Diseased)	18.101.07^{a,A}	33.36±3.72^{B}	41.05±2.97^{d,BC}	45.34±3.40^{c,C}	46.81±4.10^{c,C}
Untreated group II	22.39±2.09^{a,A}	27.62±1.16^{AB}	31.19±1.44^{bc,BC}	37.40±2.19^{bc,C}	36.32±4.62^{b,C}
Standard (Cystone) 500mg/kg bw	19.82±1.19^{a,A}	30.41±1.42^{B}	34.00±1.00^{c,BC}	39.14±2.52^{bc,C}	37.90±3.33b,^{C}
300mg/kg	20.93±0.73^{a,A}	27.13±1.91^{B}	27.47±1.92^{b,B}	30.98±3.37^{ab,BC}	35.89±1.76^{b,C}
3000mg/kg	29.63±2.14^{b,A}	29.70±2.02^{AB}	25.28±1.16^{ab,AB}	26.50±1.38^{a,AB}	31.35±0.84^{ab,B}

Values (Mean±SE) bearing different uppercase superscript vary significantly (p<.001) between periods and lowercase superscript between groups

Mean± SE values of serum calcium levels (mg/dl) of untreated and extract treated rats were presented in table 4. Highly significant (p<0.001) increase in calcium levels of untreated rats (11.64±0.15 mg/dl) was recorded as compared to healthy rats (6.15±0.58 mg/dl). The treated groups, i.e. the rat given aqueous extract of *Dolichos biflorus* seeds showed significant (p<0.001) decrease in the serum levels of calcium compared to untreated rats. The serum levels of Calcium in extract treated rats were estimated as 10.51±1.31 mg/dl for 300 mg/kg on day 28 and 9.61±1.22 mg/dl for 3000 mg/kg group. In cystone treated rats (standard group) the level of calcium was estimated as 9.71±0.44 mg/dl which is significantly (p<0.001) lower than untreated group. This clearly shows that the extract has nephroprotective effect and lowers the serum concentration of calcium significantly (p<0.001) as compared to untreated control groups. In untreated control II, the concentration of serum calcium was found to be between the untreated control group and extract treated group i.e. 10.56±0.28 mg/dl.

Table 4: Concentration of calcium (mg/dl) in serum of normal, Urolithic, Cystone treated and *Dolichos biflorus* extract treated rats

Groups	Days post treatment				
	0th day	**7th day**	**14th day**	**21st day**	**28th day**
Negative control (healthy)	6.65±0.45^{ab,}	6.87±0.21^{a,}	7.70±0.43^{a},	7.84±1.10^{a,}	6.15±.58^{a,}
Untreated group I (diseased)	6.60±0.24a^{b,A}	9.15±0.31^{d,B}	10.32±0.22^{ab,BC}	11.66±0.97^{b,C}	11.64±0.15^{b,C}
Untreated group II	7.74±0.46^{b,A}	7.85±0.19^{bc,A}	10.20±1.11^{ab,B}	10.22±0.91^{ab,B}	10.56±028^{b}
Standard (Cystone) 500mg/kg	5.81±0.50^{a,A}	8.69±0.53^{cd,B}	10.48±0.28^{ab,CD}	11.53±0.75^{b,D}	9.71±0.44^{b,BC}
300mg/kg	6.93±0.28^{ab,A}	8.54±0.17^{cd,A}	11.03±0.46^{ab,B}	11.38±0.42^{b,B}	10.51±1.31^{b,B}
3000mg/kg	7.21±0.48^{b,A}	7.00±0.23^{ab,A}	7.79±0.30^{a,AB}	7.95±0.24^{a,AB}	9.61±1.22b^{,AB}

Values (Mean±SE) bearing different uppercase superscript vary significantly (p<.001) between periods and lowercase superscript between groups

Mean±SE values of serum phosphorus (mg/dl) of urolithiatic and extract treated rats are presented in table 5. Highly significant (p<0.001) increase in phosphorus levels of untreated rats (10.35±0.48 mg/dl) was recorded as compared to healthy rats (5.56±0.54 mg/dl). The treated groups i.e. rat given aqueous extract of *Dolichos biflorus* seeds also showed significant p<0.001 decrease in the serum levels of phosphorus compared to untreated rats. The serum levels of phosphorous of extract treated groups were estimated as 8.15±0.49 mg/dl for 300 mg/kg and 8.41±0.64 mg/dl for 3000 mg/kg group, respectively. In cystone treated (standard group) the level of phosphorus was 7.97±1.25 mg/dl which is significantly p<0.001 lower than untreated control group. This clearly shows that the extract has nephroprotective effect and lowers the serum concentration of phosphorous significantly (p<0.001) as compared to untreated groups. In untreated II group, the concentration of serum phosphorous was estimated between the posit ive group and extract treated group (300mg/kg group), i.e. 10.35±0.48 mg/dl. Phosphorus levels (mg/dl) increase significantly (p<0.001) on different periods.

Table 5: Concentration of phosphorous (mg/dl) in serum of normal, Urolithic, Cystone treated and *Dolichos biflorus* extract treated rats

Groups	Days post treatment				
	0th day	**7th day**	**14th day**	**21st day**	**28th day**
Negative control(Healthy))	4.72±0.40^{AB}	4.38±0.07^{a,A}	4.78±0.09^{a,AB}	5.96±0.49^{a,C}	5.56±0.54^{a,BC}
Untreated group I (Diseased)	5.30±0..45^{A}	6.43±0.36^{c,AB}	7.50±0.57^{b,cB}	9.83±.69^{cd,C}	10.69±1.20^{c,C}
Untreated group II	6.01±0.37^{A}	6.71±0.32^{c,AB}	7.82±0.31^{c,B}	10.26±1.07^{,dC}	10.35±0.48^{bc,C}
Standard(Cystone) 500mg/kg bw	6.03±0.57^{AB}	5.25±0.13^{b,A}	6.23±0.75^{b,ABC}	8.47±0.56^{bcd,C}	7.97±1.25^{b,BC}
300mg/kg	5.65±0.78^{AB}	4.53±0.14a^{,A}	6.60±0.65^{bc,BC}	7.80±1.01^{abc,C}	8.15±0.49^{bc,C}
3000mg/kg	5.12±0.44^{A}	4.36±0.30^{a,A}	4.71±0.18^{a,A}	7.41±0.04^{ab,B}	8.41±0.64^{bc,B}

Values (Mean±SE) bearing different uppercase superscript vary significantly (p<.001) between periods and lowercase superscript between groups

The concentration of calcium in acid digested kidney tissue is presented in table 6. The untreated control is having highest calcium level per gram of tissue and the calcium level in kidney is decreased in extract treated rats and standard treated rats. The inducing agent is promoting the excretion and deposition of calcium oxalate and therefore it is evident that extract is reducing the deposition of calcium oxalate crystals in kidney.

Table 6: Kidney Calcium content (mg/gm of wet tissue) of healthy, untreated, standard and *Dolichos biflorus* extract treated rats

Group	Healthy control	Untreated group I	Untreated group II	Standard Cystone	300mg/kg	3000mg/kg
Calcium mg/gm	2.57±0.01	3.67±0.01	3.38±0.01	2.66±0.01	2.74±0.02	2.68±0.01

Values (Mean±SE) of calcium content in kidney tissue

Scientific published report is available on *Dolichos biflorus* against urolithiasis however Anturolithiatic effect of some other individual plant extract in ethylene glycol induced urolithiasis are on record. The extract showed the graded response against the ethylene glycol induced nephrotoxicity in rats and increase in the efficacy of the extract was found dose dependent. The same results were found by Krishna *et al.* (2013) [17], when he found that serum creatinine and BUN levels were reduced in rats which was dose dependent by treating them with plant extract of *Solanum virginianum*. They treated rats with two different dose of 200 mg/kg and 400 mg/kg and found that higher doses reduce the serum creatinine and BUN more compared to lower doses. Same results were found by Narumalla *et al.* (2012) [18] by feeding different doses of ethanolic extract of Aspergillus racemosus to rats at doses rate of 200, 400, 800, 1600 mg/kg bw. Higher dose has lowered serum creatinine and BUN values more than lower doses.

For safety study the extract was treated at EC50 and 10 times of EC50 dosage to record the biochemical and histopathological changes. The rats treated with the extract and standard drug showed less increase of serum urea nitrogen, creatinine, calcium and phosphoirus compard to untreated control group. Based on the findings it is found to be safe. The extract showed neither mortality nor any visible clinical signs of general weakness in the animals during observation period. Results of Toxicity studies suggested nontoxic nature of the plant extract which is evidenced through normal urine volume production and other biochemical parameters recorded in the experimental animals. In the present study, it was observed that there is no drug related toxicity by *Dolichos biflorus* seed extract and concluded that the maximum tolerated dose is greater than 3000 mg/kg. The animals does not suffer any mortality even after feeding for 28 days through oral guvage.

References

1. Nadkarni KM. The medicinal and nutritional properties of *Musa* species. Indian Materia Medica. 1976; 1:822-827.
2. Kailash P, Varalakshmi P. Effect of banana stem juice on biochemical changes in liver of normal and hyperoxaluric rats. Indian J Exp. Biol. 1992; 30:440-442.
3. Jain DL, Baheti AM, Parakh SR, Ingale SP, Ingale PL. Study of antacid and diuretic activity of ash and extracts of Musa sapientum L. fruit peel. Phcog. Mag. 2007; 3(10):116-119.
4. Rizvi SH, Shoeb A, Kapil RS, Satya PP. Two diuretic triterpenoids from Antiderma menasu. Phytochemistry. 1980; 19:2409-2410
5. Sood AR, Bajpai A, Digits M. Pharmacological and biological studies on saponins. Indian J Pharmacol. 1985; 17:178-179.
6. Chodera A, Dabrowska K, Sloderbach A, Skrzypczak L, Budzianowski J. Effect of flavanoid fractions of Solidago virgaurea L. ondiuresis and levels of electrolytes. Acta. Pol. Pharm. 1991; 48:35-37.
7. Garimella TS, Jolly C, Narayanan S. *In vitro* studies on antilithiatic activity of seeds of *Dolichos biflorus* Linn. and rhizomes of *Bergenia ligulata* Wall. Phytother Res. 2001; 15:351-355.
8. Laskar S, Bhattarcharyya UK, Sinhababu A, Basak BK. Antihepatotoxic activity of kulthi *Dolichos biflorus* seed in rats. Fitoterapia. 1998; 69:401-402.
9. Rao M, Rao P, Kamath R, Rao MN. Reduction of cisplastin induced nephrotoxicity by cystone, a polyherbal Ayurvedic reparation, in C57BL/6J mice bearing B16F1 melanoma without reducing its antitumor activity. J Ethnopharmacol. 1999; 68:77-81.
10. Kanaka KD. Variability and divergence in horsegram Dolichos uniflorum. J Arid Land. 2012; 4(1):71-76.
11. Rao KN, Somasundarm G, Kumar DS, Manavalan R, Muthu AK. Antioxidant potential of various extracts from whole plants of *Dolichos biflorus* Linn evaluated by three *in vitro* methods. Int. Res. J Pharm. 2011; 2:252-256.
12. Wybenga DR, Di Giorgio J, Pileggi VJ. Manual and automated methods for urea nitrogen measurement in whole serum. Clin. Chem. 1971; 17(9):891.
13. Frankel S, Reitman S, Sonnenwirth AC. Gradwohl's Clinical Laboratory Methods and Diagnosis. 1970; 1(7):340-343.
14. Miller RD, Smith DA, Williams SA, Hochstrasser RM. Near-field time- resolved fluorescence studies of poly phenylmethyl silane with subwavelength resolution. J Fluoresc. 1994; 4(2):137-40.
15. Economou C, Thomus J, Tombelem G, Arvis G. Predominance gauche de la lithiase renale. Sem Hop Paris. 1987; 63:277-80.
16. Snedecor GW, Cochran WG. Statistical methods (Iowa: Iowa State University Press), 1994.
17. Krishna MC, Srinivas S, Madhan ME. Antiurolithiatic activity of the plant extracts of Solanum virginianum on ethylene glycol induced urolithiasis in rats. IJPBS. 2013; 3(4):328-334.
18. Narumalla J, Somashekara S, Chikkannasetty Damodaram G, Golla D. Study of antiurolithiatic activity of Asparagus racemosus on albino rats. Indian J Pharm. 2012; l44(5):576-579.