Journal of Animal Research: v.5 n.4, p. 773-777. December 2015

DOI: 10.5958/2277-940X.2015.00128.X

Evaluation of Diuretic Effect of Aqueous Extract of *Dolichos biflorus* Seeds against Ethylene Glycol Induced Renal Stone in Experimental Rats

Azhar Shuaib Batoo[1*], Sahadeb Dey[2], Manzoor Ahmad Bhat[3] and Ananya Dan[4]

[1]*Division of Veterinary Medicine, Indian veterinary research Institute, Izatnagar, INDIA*
[2]*Division of Medicine Indian Veterinary Research Institute, Izatnagar, INDIA*
[3]*Division of Veterinary Surgery, Indian Veterinary Research Institute, Izatnagar, INDIA*

**Corresponding author: AS Batoo, Email: azharshuaib@gmail.com*

Received: 30 August, 2015 **Accepted:** 10 October, 2015

ABSTRACT

Aqueous extracts of seeds of *Dolichos biflorus* was evaluated for its diuretic activity against experimentally induced renal stone in rats. The animals were grouped into seven groups of six animals each. Hyperoxularia was induced by giving ethylene glycol and ammonium chloride to a final concentration of 0.75 % and 2% in drinking water for a period of 28 days. The first group of animals served as negative (healthy) control and received normal distilled water. The second group served as positive (untreated) control and no extract was given to this group. Rest groups received aqueous extract of *Dolichos biflorus* at a dose of 100, 150, 200, 250 and 300 mg/kg. The urine volume was recorded for all the groups after every seven days for about 28 days. The extracts treated groups showed increase in total urine production and the extract treated group (300 mg/kg) showed significantly and markedly increased the urine output ($p < 0.01$). There was significant ($p < 0.01$) decrease in pH of positive control rats as compared to extract treated rats. Urinary pH also showed dose dependent effect, the higher the dose the lesser the decrease in pH of urine. The extract treated rats showed lesser decrease in pH as compared to positive control rats. Crystals in urine also varied with dose of extract and lesser crystals were found in rats treated with higher dose of extract and maximum crystals were found in positive control group. These findings suggest the possible traditional use of this plant in nephrolithiasis as diuretics.

Keywords: *Dolichos biflorus,* renal stone, hyperoxularia, ethylene glycol

Diuretic agents have very wide application in the treatment of various chronic diseases associated with edema. They are generally prescribed for the treatment of hypertension, congestive heart failure, glaucoma, diabetes insipidus and liver aliments. The modern era of diuretic therapy began in 1949 when sulphanilamide was discovered to possess diuretic and natriuretic properties (Schwartz., 1949). The seeds of *D. biflorus* have been reported to show anti-hepatotoxic (Laskar *et al.* 1998), anti-nephrotoxic (Rao *et al.* 1999), free radical scavenging activity (Kanaka *et al.* 2012), antioxidant (Rao *et al.* 2011; Hazra *et al.* 2009) and hypolipidemic activity.

The traditional Ethnoveterinary practitioners consider decoction of *Dolichos biflorus* seed as an excellent natural product that has potential to ameliorate urolithiasis by it diuretic effect and in many pathological conditions of renal disorders. However, scientific validation of such claims is lacking. The current study

is therefore proposed to evaluate the efficacy of *Dolichos biflorus* seed extract in ameliorating kidney stones in experimental rats by its diuretic effect as no systematic pharmacological studies have been carried out in order to confirm its diuretic activity. Hence, in the present study diuretic activity of aqueous extracts of seeds of *dolichos biflorus* was investigated to justify the rationale behind using this plant as diuretic in hypertension and kidney stone. The present investigation was undertaken to confirm traditional medicinal use of the plant.

MATERIALS AND METHODS

The *Dolichos biflorus* seeds were collected from Bareilly district of northern India. These were identified and authenticated from Botanical Survey of India, Central National Herbarium, Howrah, India where voucher specimen was deported (voucher No. CNH/I-I/51/2011/Tech II/248).

Extract preparation

The material was uniformly powdered using an electric grinder. Aqueous extract of the powdered seeds was prepared using distilled water as solvent for 6 h in a soxhlet apparatus at 70^{O}C. The extract was filtered using filter paper (Whatman No. 40). The solvent was removed by using rotary evaporator. The extract was dried *in vacuo* and stored refrigerated condition until use.

Animals and treatments

Fourty two albino waster rats of either sex, 12 to 13 weeks old, weighing about 150 to 200 g bred in the Laboratory Animal Research Division of the Institute were used for experiment after obtaining permission from Institute Animal Ethics Committee. The rats were housed in clean polypropylene cages at nearly about normal physiological conditions. They were provided with standard ration and *ad libitum* water. They were acclimatized in experimental animal house for 7 days before starting the experiment. The animal care procedures and experimental protocol was in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Experiment protocol

Hyperoxaluria and Calcium oxalate deposition in the kidney was induced by oral administration of ethylene glycol (EG) and ammonium chloride in the drinking water to a final concentration of 0.75% and 2%, respectively for 28 days. The animals were divided into seven groups of six animals each and subjected to treatment for 28 days as follows:

Group	Ethylene glycol	Ammonium chloride	Test extract
Negative control	NIL	NIL	NIL
Positive control	0.75% in drinking water	2% in drinking water	NIL
100 mg/kg bw wt	0.75% in drinking water	2% in drinking water	100 mg/kg b.wt
150 mg/kg bw	0.75% in drinking water	2% in drinking water	150 mg/kg b.wt
200 mg/kg bw	0.75% in drinking water	2% in drinking water	200 mg/kg b.wt
250 mg/kg bw	0.75% in drinking water	2% in drinking water	250 mg/kg b.wt
300 mg/kg bw	0.75% in drinking water	2% in drinking water	300 mg/kg b.wt

COLLECTION OF URINE

For assessment of urinary parameters the rats were housed in the individual metabolic cages upto 24 hours and urine samples were collected on day 0, 7, 14, 21 and 28 post treatment. Volume was determined using measuring cylinder while as urinary pH was measured using digital pH meter (Orion,USA). Microscopic examination of urine was done following the methods of Stockham and Scott (2008).

RESULTS

Urine volume

The total urine of each rat was collected in metabolic cages at weekly interval for 4 weeks. The volume of urine produced by different group of rats was shown in Table 1. The rats treated with extract showed diuretic effect as volume of urine was significantly ($p<0.01$) higher compared to untreated group. The extract increased urine volume production perhaps because of increase in glomerular filtration. There is variation of urine production within extract treated group and it was evident that increase in urine production was highest in rats received 300 mg/kg bw extract. The volume of urine produced increases as the dose of test extract increases. This shows dose dependent effect of aqueous extract of *dolichos biflorus* seeds on urine output hence as the dose of extract increases the volume of urine output also increases.

Table 1. Volume of urine produced by negative control, positive control and extract treated rats at different time intervals

Group	Days post treatment				
	O day	7th day	14th day	21st day	28th day
Negative control	4.50 ± 0.04^{B}	4.40 ± 0.04^{Aab}	4.40 ± 0.00^{Ab}	4.40 ± 0.00^{Ab}	4.50 ± 0.04^{Bb}
Positive control	4.30 ± 0.04^{C}	4.00 ± 0.18^{BCa}	4.00 ± 0.00^{BCa}	3.80 ± 0.07^{ABa}	3.50 ± 0.18^{Aa}
100mg/kg	4.40 ± 0.07^{A}	4.33 ± 0.06^{Ab}	4.60 ± 0.04^{Bbc}	4.40 ± 0.07^{Ab}	4.70 ± 0.04^{Bbc}
150mg/kg	4.50 ± 0.18	4.50 ± 0.00^{ab}	4.50 ± 0.18^{bc}	4.60 ± 0.04^{c}	4.80 ± 0.07^{cd}
200mg/kg	4.30 ± 0.04^{A}	4.40 ± 0.00^{Aab}	$4.50\pm0.18^{ABb}c$	4.70 ± 0.07^{Bc}	5.00 ± 0.04^{Cd}
250mg/kg	4.40 ± 0.00^{A}	4.60 ± 0.07^{Bbc}	4.80 ± 0.00^{Cc}	$4.90\pm.04^{Cd}$	5.30 ± 0.04^{De}
300mg/kg	4.50 ± 0.00^{A}	4.80 ± 0.00^{Bc}	5.20 ± 0.04^{Cd}	5.30 ± 0.04^{De}	5.60 ± 0.04^{Ef}

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

Table 2. pH of urine of negative control, positive control and extract treated rats at different time intervals

Days post treatment	O day	7th day	14th day	21st day	28th day
Healthy control	6.68 ± 0.01^{Cc}	6.65 ± 0.00^{Aa}	6.66 ± 0.00^{ABd}	6.70 ± 0.00^{Dd}	$6.67\pm0.00B^{Cb}$
Untreated control	6.70 ± 0.00^{Ed}	6.65 ± 0.01^{Da}	6.54 ± 0.00^{Ca}	6.39 ± 0.00^{Ba}	6.29 ± 0.00^{Aa}
100mg/kg bw	6.67 ± 0.00^{Bbc}	6.66 ± 0.00^{Bab}	6.62 ± 0.00^{Ab}	6.66 ± 0.01^{Bb}	6.66 ± 0.00^{Bb}
150mg/kg bw	6.70 ± 0.00^{Dd}	6.70 ± 0.00^{Dc}	6.64 ± 0.00^{Ac}	6.68 ± 0.00^{Cc}	6.66 ± 0.00^{Bb}
200mg/kg bw	6.65 ± 0.00^{Aa}	6.66 ± 0.01^{Aab}	6.67 ± 0.00^{Be}	6.68 ± 0.00^{Cc}	6.69 ± 0.00^{Dc}
250mg/kg bw	6.66 ± 0.00^{Aab}	6.67 ± 0.00^{Bb}	6.68 ± 0.00^{Cf}	6.71 ± 0.00^{Dd}	6.73 ± 0.00^{Ed}
300mg/kg bw	6.70 ± 0.00^{Bd}	6.67 ± 0.00^{Ab}	6.71 ± 0.00^{Bg}	6.73 ± 0.00^{Ce}	6.78 ± 0.00^{De}

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

Table 3: effect of aqueous extract of *Dolichous biflorous* seeds on crystal excretion in different group of rats

Group	Urine Microscopy
Negative control	No crystals were found in the urine
Positive control	There were abundant crystals in the urine
100 mg/kg bw	Crystals were present but less no per field than positive control
150 mg/kg bw	Less number of crystals were found per field than 100mg/kg bw group.
200 mg/kg bw	Less number of crystals were found per field a s compared to 150 mg/kg bw group.
250 mg/kg bw	Less number of crystals were found per field a s compared to 200 mg/kg bw group.
300 mg/kg bw	Less number of crystals were found per field a s compared to 250 mg/kg bw group.

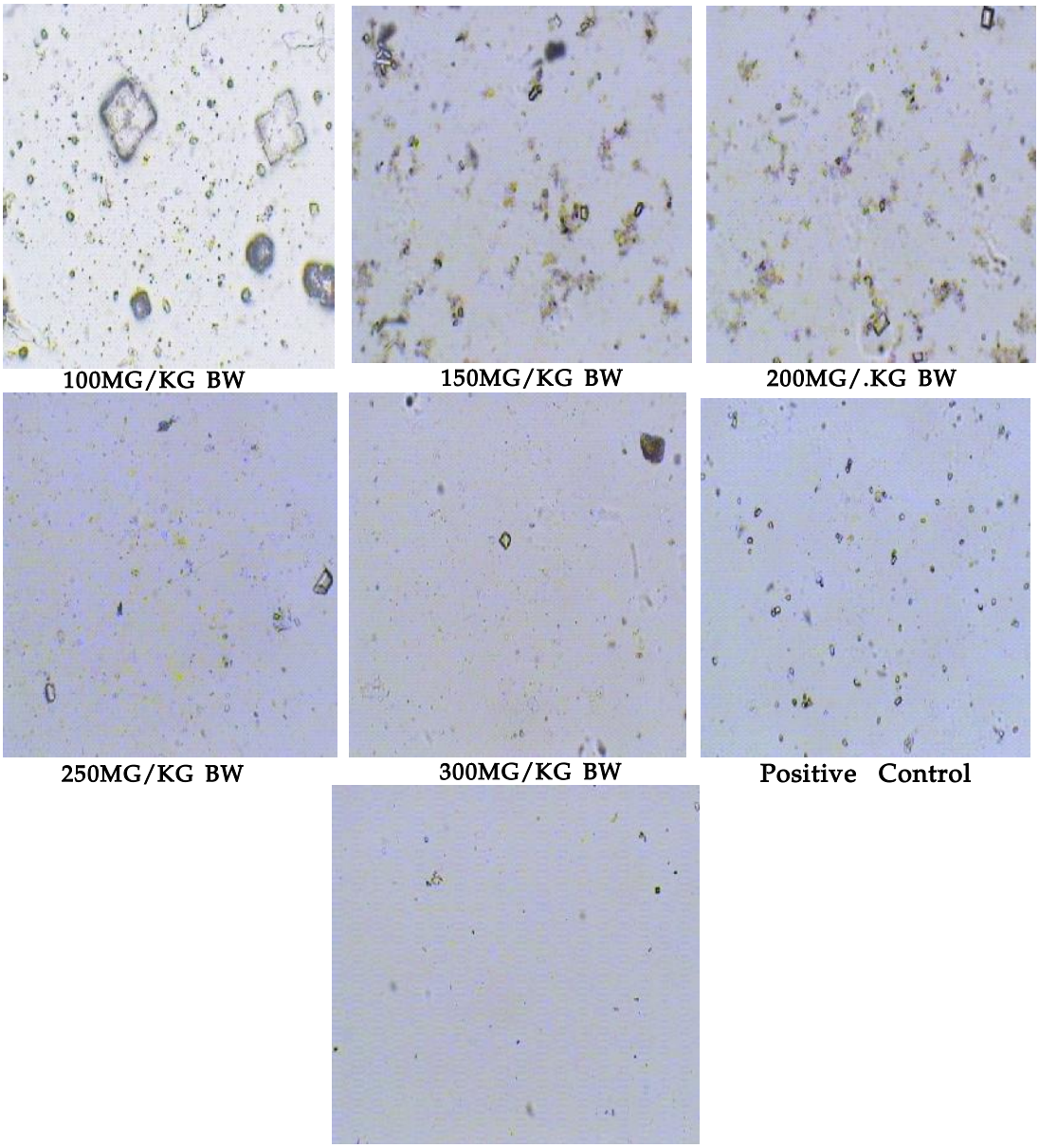

Fig. 1. Urine microscopy of negative control, positive control and extract treated rats

Urine pH

The pH of urine was assessed using digital pH meter. The results are shown in the Table 2. The effect of the extract clearly indicated that it increased the pH of urine. The untreated control group shows more decrease in urine pH as compared to extract treated groups .Within the extract treated groups the decrease in ph increases as the dose of extract decreases. Hence the effect of extract on pH is dose dependent and increase in ph of urine is associated with increase in dose of extract.

Urine microscopy

The urine collected from individual rats was then subjected to microscopic examination. The slides showed the presence of crystals in the positive control group and variable results were shown by extract treated groups. The table 3 shows the effect of aqueous extract of *Dolichous biflorous* seeds on crystal excretion in different group of rats. The Figure 1 shows the crystals in different groups of extract treated rats.

DISCUSSION

Urine samples were collected on days 0, 7, 14, 21 and 28 of the experiment using metabolic cages. Our results in effect of the extract on urine volume recovered found that the extract is having the diuretic effect and urine

produced is depending on dose. The urine volume increased with dose of the test extract. The increase in urine production may be one effect that influences antiurolithiatic activity. Similiar type of response was found by Maksana *et al*. (2014) where they have given dried extract of leaves of *Launaea procumbens* to ethylene glycol induced renal calculi in rats at two different doses of 150 and 300 mg/kg bw and found that rats received 300 mg/kg extract produced more urine. Untreated animals showed statistically significant decrease in urine volume ($p<0.001$) and pH ($p<0.001$) in comparison to normal control. The pH of urine varies with different doses. The lowest pH was found in positive control group and with the increase in dose of test extract pH also increased towards alkaline side. The highest effect was shown by 300 mg/kg bw group.

The microscopic examination of the urine samples of negative control animals was found to be devoid of crystals or crystal like structures. Positive control group animals showed abundant and large Calcium oxalate crystals. Rats received extract (300 mg/kg bw) showed very few and small (almost dissolved) crystals, when compared to that of the other groups (100 mg/kg bw, 150 mg/kg bw, 200 mg/kg bw and 250 mg/kg bw). Krishnaveni *et al*. 2013 got the same results when they found that Extract treated rats showed fewer crystals in the urine when compared to the positive control rats.

REFERENCES

Hazra, B., Sarkar, R., Mandal, S., Biswas, S. and Mandal, N. 2009. Studies on antioxidant and antiradical activities of *Dolichos biflorus* seed extract. *Afr. J. Biotechnol*, **8**: 3927-3933.

Kanaka, K.D. 2012. Variability and divergence in horsegram *Dolichos uniflorus*. *J. Arid Land.,* **4**(1): 71-76.

Krishnaveni, J., Rajkiran, E., Manjula, P. and Sudheer, K. 2013. Antiurolithiatic activity of cucumis sativus. *IJPR* **3**(2)**:** 46-52.

Laskar, S., Bhattarcharyya, U.K., Sinhababu, A. and Basak, B.K. 1998. Antihepatotoxic activity of kulthi *Dolichos biflorus* seed in rats. *Fitoterapia.*, **69**: 401-402.

Makasana, A.,Ranpariya, V., Desai, D., Mendpara, J. and Parekh, V. 2014. Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycolinduced renal calculi in rats *Toxicology Reports.*, **1:** 46–52.

Rao, K.N., Somasundarm, G., Kumar, D.S., Manavalan, R., Muthu, A.K. 2011. Antioxidantpotential of various extracts from whole plants of *Dolichos biflorus* Linn evaluated by three *in vitro* methods. *Int. Res. J. Pharm.,* **2**: 252-256.

Rao, M., Rao, P., Kamath, R. and Rao, M.N. 1999. 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*. **68**: 77-81.

Schwartz, W.B. 1949. The effect of sulphonamides on salt and water excretion in congestive heart failure. *N Engl J Med.*, **240**: 173-177.

Stockham, S.L. and Scott, M.A. 2008. Urinary system. In Fundamentals of Veterinary Clinical Pathology, 2nd ed. Stockham SL, Scott MA, eds., pp. 415-94. Ames, IA : Blackwell Publishing.