*Int.J.Curr.Microbiol.App.Sci* (2018) *7*(6): 368-375

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706* Volume 7 Number 06 (2018)**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2018.706.041

# Determination of EC50 of Aqueous Extract of *Dolichos biflorus* Seeds against Ethylene Glycol Induced Renal Stone in Wistar Rats

**Azhar Shuaib Batoo[1*], Sahadeb Dey[1], Mir Mudasir[2], Zamir Ali Ganaie[3], Afaq Amin Najar[4] and Burhan Nabi[4]**

*[1]Division of Veterinary Medicine, Indian Veterinary Research Institute, Izatnagar-243122, India*
*[2]Division of Veterinary Pathology, G.B.P.U.A.T, Pantnagar, Uttrakhand-263145, India*
*[3]Division of Veterinary Parasitology, SKUAST - Kashmir-180004, India*
*[4]Division of Veterinary Medicine, SKUAST - Jammu-181102, India*

**Corresponding author*

**Keywords**

*Dolichos biflorus*, Ethylene glycol, Histopathology, Renal parenchyma

**Article Info**

***Accepted:***
02 May 2018
***Available Online:***
10 June 2018

**A B S T R A C T**

The present study was undertaken to study the $EC_{50}$ of aqueous extract of *Dolichos biflorus* seeds against ethylene glycol induced renal stone in rats. 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 5 groups, viz. healthy control, untreated control, 150, 250 and 300 mg test extract groups. Efficacy of treatment was assessed by estimating serum concentrations of urea nitrogen, Creatinine and histopathology. The serum urea nitrogen and serum creatinine was lower in extract treated rats than the untreated rat. Histopathology of kidney revealed lesions in the renal parenchyma of untreated rats and kidney lesions were almost absent in 300 mg/kg body weight dose. The aqueous extract of *Dolichos biflorus* seeds was found to be effective against renal stone in wistar rats. The EC50 of the extract was found to be 300mg/kg body weight.

## Introduction

Urolithiasis is defined as the formation of urolith anywhere in the urinary system. The disease is reported worldwide and occurs in all species of the animals but most frequently recorded in feeder steer and lambs[1]. Urinary calculi formation usually results from a combination of various physiological, nutritional and management factors. It may occur due to excessive or imbalanced intake of minerals in feedlot cattle or in fattening cattle receive rations high in cereal grain and oil meals[2]. Urolithiasis in ruminants, especially in cattle, is of considerable economic importance as losses inflicted by this melody are considered very high[3]. Treatment of obstructive urolithiasis has been found to vary depending upon clinical status of animal and duration of obstruction[4,5]. Standard pharmaceutical drugs on the other hand not fully effective in all cases, costly, quite

common reoccurrences, risks of long term infertility, potential side effects and no guarantee[6]. Surgical treatment causes some problems like long term renal damage, hypertension and reoccurrence of stones. In India, many 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 aqueous extracts of seeds of *Dolichos biflorus* inhibited homogenous precipitation of calcium hydrogen phosphate dihydrate crystals[7].

However, the effects of the extract have not been evaluated in animal models. The seeds of *D. biflorus* have been reported to show anti-hepatotoxic[8], anti-nephrotoxic[9], free radical scavenging activity[10,11] and antioxidant activity[12]. 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 validations of such claims are lacking. Therefore the present study as planned to study the $EC_{50}$ of aqueous extract of *Dolichos biflorus* seeds against ethylene glycol induced renal stone in experimental rats

## Materials and Methods

The present study was undertaken to survey the anti-urolithiatic activity of aqueous extract *Dolichos biflorus* seeds. The study was done on rats which were used only after obtaining permission from Institute Animal Ethics Committee.

## Plant material

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.

## 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˚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

Thirty albino wistar rats of either sex, 12-13 weeks old, weighing about 150-200 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 3 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).

## Determination of $EC_{50}$

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 five groups of 6 animals each and subjected to treatment for 28 days (Table 1).

## Sampling

The anti-urolithiatic activity of the test extract was determined on the basis of changes in

biomarkers like serum urea nitrogen, creatinine and histopathology of kidney.

## Biochemical analysis

The blood samples were collected from individual rats on day 0 of the experiment and thereafter at 7 days interval for 28 days from the orbital plexus using microhaematocrit 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. The serum was harvested from the blood of rats for determining the following parameters. Serum urea nitrogen was determined using Diactyl monoxime method[13].

Serum creatinine was assessed calorimetrically by using specific commercial diagnostic kits manufactured by Span Diagnostic (India) Pvt. Ltd.Surat[14].

## Tissue calcium content

The kidneys were stored at -20˚C for determining the calcium content. The total calcium contents of renal tissue were estimated using atomic absorption spectroscopy[15].

Briefly the kidney tissues were dried at 100˚C for 24h and weighed. About 500 mg kidney tissues were wet digested using double acid mixture (perchloric acid: nitric acid =1:5)[16]. The mixture was digested until the liquid became transparent. The calcium concentration in the acid digest was estimated using atomic absorption 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[15].

## Histopathological analysis

The rats were sacrificed on day 28 post treatment. The kidney and urinary bladder was fixed with 10% solution of buffered formalin (pH 7.4). The tissue was embedded in paraffin and the sections of 5 µm were taken using microtome and stained with haematoxylin and eosin. The slides were examined for histopathological lesions and presence of crystals using binocular microscope.

## Statistical analysis

The data were expressed as mean±SE. 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[17].

## Results and Discussion

Since the aqueous extract of seeds of *Dolichos biflorus* have shown renoprotective effect, the $EC_{50}$ was determined using biomarkers of urolithiasis.

## Biochemical analysis

### Serum creatinine levels

Mean±SE. values of serum creatinine levels of diseased and extract treated rats were presented in Table 2. Highly significant ($p<0.001$) increase in creatinine concentration of untreated control rats was recorded as compared to the values recorded in healthy control rats. The treated groups, i.e., rat received aqueous extract of *Dolichos biflorus* seeds have shown significant decrease ($p<0.001$) in the serum levels of creatinine compared to untreated control rats. The serum levels of creatinine in extract treated rats after a period of 28 days were estimated as 4.13±0.70 mg/dl for 150 mg/kg, 3.03±0.18

mg/ dl for 250 mg/kg and 2.04±0.11 mg/dl for 300 mg/kg group.

### Serum blood urea nitrogen

Highly significant ($p<0.001$) increase in serum urea nitrogen levels in untreated control rats was recorded as compared to healthy control rats.

The treated groups, i.e. rat given aqueous extract of *Dolichos biflorus* seeds have shown significant ($p<0.001$) decrease in the serum levels of urea nitrogen compared to untreated control rats. The serum levels of urea nitrogen of extract treated groups after a period of 28 days were 32.79±0.70 mg/ dl for 150 mg/kg, 31.34±2.40 mg/dl for 250 mg/kg and 23.19±0.58 mg/dl for 300 mg/kg group, respectively (Table 3).

### Kidney calcium level

The concentration of Calcium in acid digested kidney tissue is presented in Table 4. The untreated control is having highest Calcium level per gram of tissue and as dose is increased, the calcium level in kidney decreased.

### Histopathological analysis

After a period of 28 days treatment all rat as per the experiment protocol were sacrificed after proper anaesthesia and their kidneys and urinary bladders were collected, fixed with 10% solution of buffered formalin (pH 7.4). The histopathological changes were recorded and shown in Figure 1. In healthy control group normal kidney architecture was found without any cellular infiltration (Figure 1a). Mononuclear cell infiltration and haemorrhages were found renal medulla in positive control group (Figure 1b). Rats receiving test extract dose 150 mg/kg bw showed mononuclear cell infiltration, medullary degeneration and hemorrhages (Figure 1c). In case of rats receiving test extract dose 250 mg/kg bw, mild haemorrhages in medulla were found (Figure 1d). Rats receiving test extract dose 300 mg/kg bw showed normal kidney architecture similar to that of negative control group (Figure 1e)

**Table.1** Protocol to determine the $EC_{50}$ in rats

| Group | Ethylene glycol | Ammonium chloride | Test extract |
|---|---|---|---|
| **Healthy control** | Nil | Nil | Nil |
| **Positive control** | 0.75% in drinking water | 2 % in drinking water | Nil |
| **150 mg/kg bw** | 0.75% in drinking water | 2 % in drinking water | 150 mg/kg bw |
| **250 mg/kg bw** | 0.75% in drinking water | 2 % in drinking water | 250 mg/kg bw |
| **300 mg/kg bw** | 0.75% in drinking water | 2 % in drinking water | 300 mg/kg bw |

**Table.2** Serum creatinine concentration (mg/dl) in normal, urolithiatic and *Dolichos biflorus* extract treated rats

| Groups | Days post treatment | | | | |
|---|---|---|---|---|---|
| | 0th day | 7th day | 14th day | 21st day | 28th day |
| Healthy control | $1.02\pm0.01^{A}$ | $1.06\pm0.22^{A,a}$ | $1.49\pm0.32^{AB,a}$ | $1.79\pm0.12^{B,a}$ | $1.59\pm0.06^{AB,a}$ |
| Untreated control | $1.14\pm0.05^{A}$ | $3.77\pm0.02^{B,d}$ | $3.67\pm0.63^{B,d}$ | $3.99\pm0.02^{B,d}$ | $4.38\pm0.26^{B,d}$ |
| 150 mg/kg bw | $1.08\pm0.06^{A}$ | $1.83\pm0.66^{A,ab}$ | $1.83\pm0.49^{A,ab}$ | $1.96\pm0.09^{A,a}$ | $4.13\pm0.70^{B,cd}$ |
| 250mg/kg bw | $1.05\pm0.07^{A}$ | $1.71\pm0.14^{B,ab}$ | $2.80\pm0.06^{C,bcd}$ | $2.81\pm0.26^{C,b}$ | $3.03\pm0.18^{C,bc}$ |
| 300 mg/kg bw | $1.11\pm0.08^{A}$ | $2.06\pm0.07^{C,bc}$ | $1.51\pm0.20^{C,a}$ | $1.90\pm0.14^{B,a}$ | $2.04\pm0.11^{C,ab}$ |

Values±SE bearing different uppercase superscript vary significantly ($p<0.001$) between periods and lowercase superscript between groups.

**Table.3** Concentration of urea nitrogen (mg/dl) in blood of normal, urolithiatic and *Dolichos biflorus* extract treated rats

| Groups | Days post treatment | | | | |
|---|---|---|---|---|---|
| | 0th day | 7th day | 14th day | 21st day | 28th day |
| Healthy control | $11.90\pm0.35^{ab,A}$ | $8.22\pm0.86^{a,A}$ | $11.05\pm1.23^{a,A}$ | $11.87\pm0.36^{a,A}$ | $17.93\pm2.71^{a,B}$ |
| Untreated control | $10.57\pm0.38^{ab,A}$ | $24.38\pm2.27^{c,B}$ | $35.92\pm0.83^{d,C}$ | $42.47\pm0.47^{d,D}$ | $48.27\pm1.92^{d,E}$ |
| 150 mg/kg bw | $12.48\pm1.54^{ab,A}$ | $18.87\pm1.79^{b,A}$ | $27.96\pm3.98^{bc,B}$ | $32.21\pm2.91^{c,B}$ | $32.79\pm3.31^{c,B}$ |
| 250 mg/kg bw | $12.23\pm0.72^{ab,A}$ | $18.55\pm0.83b^{,B}$ | $26.88\pm1.64^{bc,C}$ | $27.75\pm1.80^{c,C}$ | $31.34\pm2.40^{c,C}$ |
| 300 mg/kg bw | $10.41\pm0.93^{ab,A}$ | $20.01\pm0.71^{bc,B}$ | $21.79\pm1.45^{b,BC}$ | $20.83\pm0.65^{b,BC}$ | $23.19\pm0.58^{ab,C}$ |

Values±SE bearing different uppercase superscript vary significantly ($p<0.001$) between periods and lowercase superscript between groups.

**Table.4** Kidney tissue calcium level in mg/g of wet weight

| Group | Calcium mg/g of tissue |
|---|---|
| Healthy control | 2.64 |
| Untreated control | 3.76 |
| 150 mg//kg | 3.37 |
| 250 mg/kg | 3.04 |
| 300 mg/kg | 2.94 |

**Fig.1** Microscopic changes in kidney parenchyma

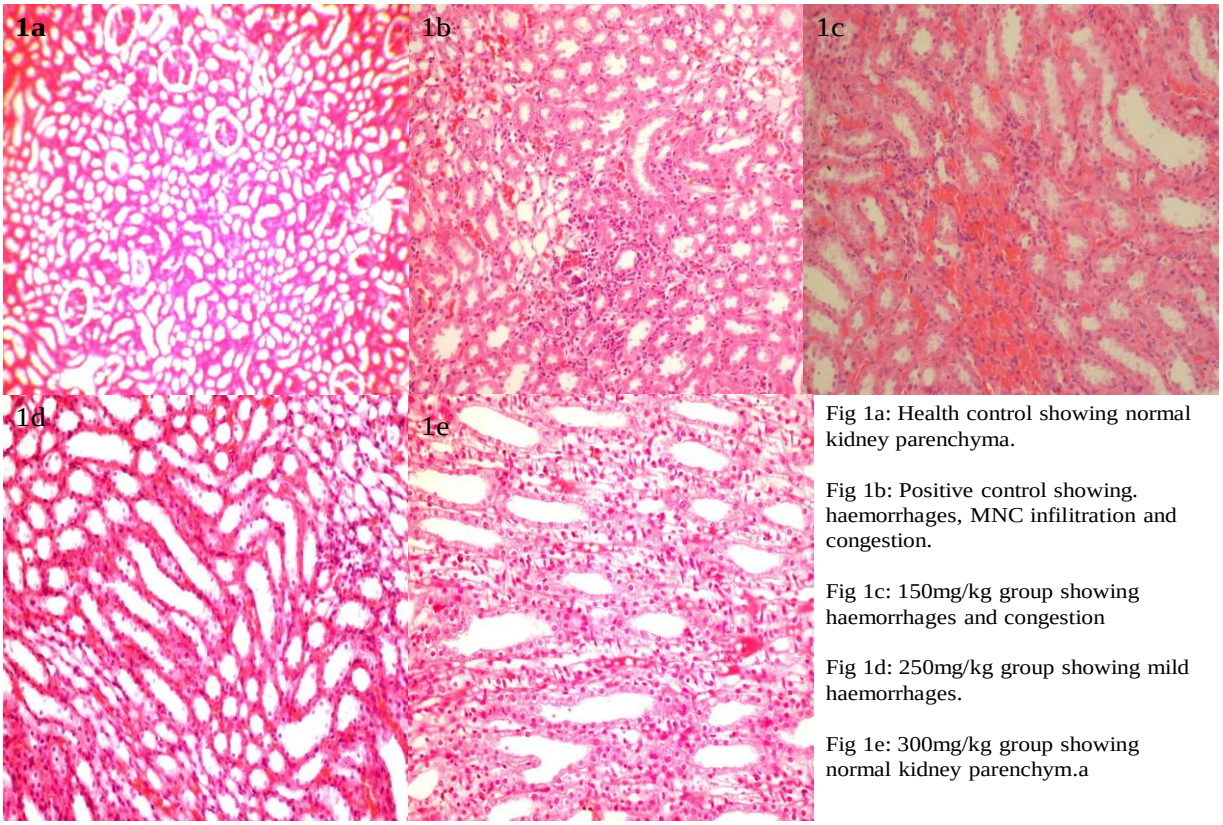


Fig 1a: Health control showing normal kidney parenchyma.

Fig 1b: Positive control showing. haemorrhages, MNC infilitration and congestion.

Fig 1c: 150mg/kg group showing haemorrhages and congestion

Fig 1d: 250mg/kg group showing mild haemorrhages.

Fig 1e: 300mg/kg group showing normal kidney parenchym.a

Administration of ethylene glycol (0.75% v/v) to young albino rats results in the formation of renal calculi this could be because of an increase in the urinary concentration of oxalate. Similar observations were also reported by other researchers[18, 19]. In urolithiasis, the glomerular filtration rate (GFR) decreases due to obstruction to outflow of urine by stones in urinary system.

Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid are accumulated in blood. Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet [20]. Oxalate has been shown to induce lipid peroxidation and cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane[21]. Scientific published report is available on *Dolichos biflorus* against urolithiasis however anti-urolithiatic effect of some other individual plant extract in ethylene glycol induced urolithiasis is 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 observed in rats by treating them with plant extract of *Solanum virginianum*[22]. In the present study rats were treated with two different doses of 200 mg/kg and 400 mg/kg and it was found that higher doses reduce the serum creatinine and BUN more compared to lower doses. Similar results were also observed in rats by feeding different doses of ethanolic extract of *Aspergillus racemosus* at doses rate of 200, 400, 800, 1600 mg/kg bw[23]. Higher dose has lowered serum creatinine and BUN values more than lower doses.

The stones formed in the kidneys were found to cause extensive damage and this was indicated

by elevated levels of blood urea nitrogen (BUN), creatinine in the rats fed with calculi inducing agents (untreated control group). The treatment with extract significantly ($P<0.001$) reduced the serum levels of creatinine, and BUN in all regimens. The reduction in concentration of serum urea nitrogen exhibited in rats received *Dolichos biflorus* extract 150 mg/kg bw was comparatively lower than rats received 300 mg/kg bw extract but 300 mg/kg bw produced a significant lowering of serum creatinine and serum urea nitrogen levels that was almost half to that found in untreated control group.

The calcium content in renal tissue was found to vary with the dose. The highest level of calcium was found in untreated rat group and rats received test extract 150 mg/kg bw and kidney calcium level was lowest in rats received test extract 300 mg/kg bw. This is because extract enhances the excretion of calcium in the kidney. The higher doses are having maximum effect and prevent deposition of calcium in the kidney.

Microscopic examination of kidney section derived from ethylene glycol induced urolithiatic rats showed polymorphic irregular crystal deposition inside the tubules which causes dilation of the proximal tubules along with interstitial inflammation, cell infiltration and haemorraghes that might be attributed to crystals[24].

Cotreatment with aqueous extract of *Dolichos biflorus* decreased the number and size of crystal deposition in different parts of renal tubule and also prevented damage to the tubules and calyces indicated anti-urolithiatic and renoprotective effect of seed extract of *Dolichos biflorus.* Histopathology of kidney revealed lesions in the renal parenchyma of untreated rats and kidney lesions were almost absent in 300 mg/kg body weight dose. Hence, it can be concluded that the aqueous extract of *Dolichos biflorus* seeds are effective against renal stone in experimental rats with $EC_{50}$ of 300 mg/kg body weight.

## Acknowledgements

Authors are thankful to the ICAR and Director, Indian Veterinary Research institute, Bareilly, U.P, for providing all the facilities to carry out this study.

## References

1. Radostits OM, Blood DC, Gay CC, Hinchcliff KW. Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horse. 10th ed. Bailliere Tindal, London: Saunders; 2000.
2. Hesse AT, Tiselius HG, Siener R. Urinary Stones, Diagnosis, Treatment and Prevention of Recurrence. 3rd ed. Basel, S. Karger AG; 2009.
3. Singh J, Singh K. 1990. Obstructive Urolithiasis and Uraemia in Cattle and Buffaloes. A Review. *Indian J Vet Surg* 11: 1-20.
4. Larson BL. 1996.Identifying, treating and preventing bovine urolithiasis. *Vet Med* 91:366- 377.
5. Van Metre DC, House JK, Smith BP, George LW. 1996. Treatment of urolithiasis in ruminants: Surgical management and prevention. *Comp Cont Ed Pract Vet* 18: 275-289.
6. Lipismita S, Ashok KP, Chinmoy M, Biswa RM, Laxmi NS, Rubina KB. 2011. Nutritional strategies to prevent Urolithiasis in Animals. *Vet World* 4(3):142-144
7. Garimella TS, Jolly C, Narayanan, S. 2001. In vitro studies on antilithiatic activity of seeds of *Dolichos biflorus* Linn. and rhizomes of *Bergenia ligulata* Wall. *Phytother Res*: 15: 351-355.
8. Laskar S, Bhattarcharyya UK, Sinhababu A, Basak BK. 1998. Antihepatotoxic activity of kulthi *Dolichos biflorus* seed in rats. *Fitoterapia* 69: 401-402.
9. Rao M, Rao P, Kamath R, Rao MN. 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.

10. Singh R, Singh MK, Chandra LR, Bhatt D, Aror MS, Nailwal TK. 2012. In vitro Antioxidant and free radical scavenging activity of *Dolichos biflorus* Gahat dal from Kumaun region. *Int J Fundamental App Sci* 1: 7-9.
11. Kanaka KD. 2012. Variability and divergence in horsegram *Dolichos uniflorus. J Arid Land* 4(1): 71-76.
12. Rao KN, Somasundarm G, Kumar DS, Manavalan R, Muthu AK. 2011. Antioxidant potential of various extracts from whole plants of *Dolichos biflorus* Linn evaluated by three *in vitro* methods. *Int Res J Pharm* 2: 252-256.
13. Wybenga DR, DiGiorgio J, Pileggi VJ. 1971. Manual and automated methods for urea nitrogen measurement in whole serum. *Clin Chem* 17(9): 891.
14. Frankel S, Reitman S, Sonnenwirth AC. 1970. *Gradwohl's Clinical Laboratory Methods and Diagnosis* 1(7): 340-343.
15. Economou C, Thomus J, Tombelem G, Arvis G. 1987. Predominance gauche de la lithiase renale. *Sem Hop Paris* 63: 277-80.
16. Kolmer JA, Spaudling EH, Robinson HW. 1951. Approved lab technique, 5th ed. Appleton century – crofts.Inc. New York.
17. Snedecor GW, Cochran WG. 1994. Statistical methods: Iowa: Iowa State University Press..
18. Selvam P, Kalaiselvi P, Govindaraj A, Murugan VB, Sathishkumar AS. 2001. Effect of Alanata leaf extract and Vediuppuchunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. *Pharmacol Res* 43: 89–93.
19. Huang HS, Ma MC, Chen J, Chen CF. 2002. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. *J Urol* 167: 258–259.
20. Sumathi R, Jyanthi S, Kalpnadevi V, Varalakshmi P. 1993. Effect of lipoic acid on tissue lipid peroxidation and antioxidant system in normal and glycollate treated rats. *Pharma Res* 27: 1-10.
21. Ernster L, Nordenbrand. 1967. Oxidation and phosphorylation. In: Ronald E, Maynard E, editors. Methods in Enzymology, Vol. X. New York: Academic press;. pp. 574-580.
22. Krishna MC, Srinivas S, Madhan ME. 2013. Antiurolithiatic activity of the plant extracts of *Solanum virginianum* on ethylene glycol induced urolithiasis in rats. *Int J Pharma Bio Sci* 3(4): 328-334.
23. Narumalla J, Somashekara S, Chikkannasetty, Damodaram G, Golla D. 2012. Study of antiurolithiatic activity of *Asparagus racemosus* on albino rats. *Ind J Pharm* l44(5): 576-579.
24. Saha S, Ramtej JV. 2012. Efficacy study of *Dolichos biflorus* in the management of nephrotoxicity. *Asian P J Trop Biomed.*, Pp. 1471-1476.

**How to cite this article:**

Azhar Shuaib Batoo, Sahadeb Dey, Mir Mudasir, Zamir Ali Ganaie, Afaq Amin Najar and Burhan Nabi. 2018. Determination of EC50 of Aqueous Extract of *Dolichos biflorus* Seeds against Ethylene Glycol Induced Renal Stone in Wistar Rats. *Int.J.Curr.Microbiol.App.Sci.* 7(06): 368-375. doi: https://doi.org/10.20546/ijcmas.2018.706.041