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Research Article

# ANTILITHIATIC ACTIVITY OF ALCOHOLIC EXTRACT OF *BOERHAAVIA DIFFUSA* ROOTS ON ETHYLENE GLYCOL INDUCED LITHIASIS IN RATS

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**ABSTRACT**

The alcoholic extract of roots of *Boerhaavia diffusa linn* (ABED) was evaluated for its antilithiatic activity in rats. Lithiasis was induced by oral administration of ethylene glycolated water (0.75%) in Adult male albino Wistar rats for 28days. The ionic levels of urine was altered by ethylene glycol (EG), Which elevated the urinary concentration of crucial ions, Viz Calcium, Phosphate, uric acid and oxalate there by contributing to renal stone formation. The AEBD, however significantly ( $P < 0.05$) reduced the elevated levels of these ions in urine. Also, it elevated concentration of urinary magnesium, which is considered as one of the inhibitor of crystallization. The histopathological studies confirmed the induction as degenerated glomeruli, necrotic tubule and inflammatory cells was observed in section of kidney from animals treated with ethylene glycol. This was reduced however after treatment with AEBD. These observations enable to conclude that AEBD is effective against ethylene glycol induced Lithiasis.

**Keywords**: Ethylene glycol, Calcium, Phosphate, Uric acid and Oxalates.

## INTRODUCTION

Urinary calculi are the third prevalent disorder in the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly costly and with these procedures recurrence is quite common. The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years . Various therapies including thiazide diuretics and alkali-citrate are being used in attempt to prevent recurrence but scientific evidence for their efficacy is less convincing . In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants.

These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects. As per the indigenous system of medicine, the roots of *Boerhaavia diffusa Linn* are reported to be useful in the treatment of a wide range of aliments including urinary stones. However, so far no scientific study has been reported regarding the antiurolithiatic property of the root extract of *Boerhaavia diffusa Linn* . In this study, we investigated protective effect of the alcoholic extract of *Boerhaavia diffusa Linn* roots against ethylene glycol induced urolithiasis and its possible underlying mechanisms using male Wistar albino rats.

## MATERIALS AND METHODS

### Drugs and chemicals

Cystone, Ethylene glycol

### Experimental protocol

All animals will be housed at ambient temperature (22±1°C), relative humidity (55±5%) and 12/12 h light/dark cycle. Animals had access to standard pellet diet and water given *ad libitum*. The experimental will be approved by our institutional ethical committee following the guide lines of CPCSEA. The proposal number submitted CPCSEA was IAEC/2010. The rats are allowed free access to food and water

Rats were divided into four groups each consisting of six animals .Group I served as negative control, Ethylene glycol (0.75%) in drinking water was fed to Groups II– IV for induction of renal calculi till 28th day. Groups II and Group III receives lower and higher doses of plant extract. Group IV receives standard antiurolithiatic drug, cystone (750 mg/kg body weight**.** The ionic level of urine was altered by Ethylene glycol which elevated the urinary concentration of crucial ions such as calcium, phosphate, uric acid and oxalate there by contributing to renal stone formation.

All animals are kept in a individual metabolic cages and urine samples of 24Hrs were collected on 28$^{th}$ day. Animals had free access to drinking water during the urine collection period. A drop of conc. HCl is added to urine before being stored at 4°c.And the urine is analyzed for magnesium, calcium, phosphate, oxalates and creatinine contents.

### Biological parameters

### Estimation Of Ionic Contents In Urine( calcium,phosphate, magnesium and creatinine)

All animals are kept in a individual metabolic cages and urine samples of 24Hrs were collected on 28$^{th}$ day. Animals had free access to drinking water during the urine collection period. A drop of conc. HCl is added to urine before being stored at 4°c.And the urine is analyzed for magnesium, calcium, phosphate, oxalates and creatinine contents by using kits.

### Serum Analysis

After the experimental period ,blood was collected from the retro orbital under anesthetic conditions and serum separated by centrifugation at 10000rpm for 10mins and analyzed for calcium,creatinine, BUN, uric acid by using kits.

### Estimation of blood Urea nitrogen

Photometric estimation of BUN in plasma on urease GLDH method as described in manufacturer's instructions manual (Merck Specialities Pvt Ltd)

### Estimation of creatinine

Creatinine forms a yellow orange compound in alkaline solution with picric acid. As a result of rapid reaction between creatinine and picric acid, the secondary reaction does not cause interferences.

### Estimation of uric acid

Uricase cleaves uric acid to form allantoin and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidatively couples with

4-amino antipyrine and DHBS to produce red quinoneimine dye. The intensity of the red colour formed is directly proportional to the concentration of uric acid in the specimen and is measured photometrically.

**Kidney Homogenate Analysis For (Calcium, Phosphate)**

The abdomen is cut open to remove both kidneys from each animal. Isolated kidneys are cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys are dried at 80°C in hot air oven.

A sample of 100mg of the dried kidney was boiled in 10ml of 1N hydrochloric acid for 30min and homogenized. The homogenate is centrifuged at 2000rpm for 10min and the supernatant is separated and the calcium, phosphate, oxalate contents in kidney homogenate are determined by using kit.

**Histopathological Studies**

Kidney samples were weighed and fixed rapidly with 10% neutralized formalin (pH 7.4) section of kidney in paraffin was prepared and stained with hematoxylin and eosin and observed for pathological changes.

**RESULTS**

**Histopathological Studies**

The section of kidney of Group I rats showing normal tubules and epithelial lining whereas the Group II rats showing dilated tubules and degenerated of epithelial lining. Group III animals showing comparatively similar to normal. Group IV animals showing reduction in features mentioned in Group II. Group V animals showing comparatively similar to normal.

**Total urinary volume**

| S.NO Groups | Urine output (ml/day/rat), Mean ± SEM |
|---|---|
| 1 Control | 5.5 ± 1.73** |
| 2 Lithiatic Control | 2.2 ± 0.05 |
| 3 Standard | 4.8 ± 1.50*** |
| 4 Test-I | 3.8 ± 0.035*** |
| 5 Test-II | 4.2 ± 2.14** |

**Change in the urinary excretion of stone forming constituent in control and AEBD treated animals.**

| Groups | Dose (mg/kg) | Urine parameters (mg/dL) Calcium | Phosphate |
|---|---|---|---|
| Control | Vehicle | 1.20 ± 0.07*** | 3.50 ± 0.03*** |
| Lithiatic Control | Ethylene glycol (0.75%). | 4.20 ± 0.03 | 6.90 ± 0.05 |
| Standard | Standard Cystone (750mg/kg, i.p) | 1.50 ± 0.05 | ** 3.50 ± 0.08 |
| Test-I | Extract of plant (250mg/kg,p.o) | 2.0 ± 0.05*** | 4.05 ± 0.09*** |
| Test-II | Extract of plant (500mg/kg, p.o) | 1.70 ± 0.06*** | 3.89 ± 0.05*** |

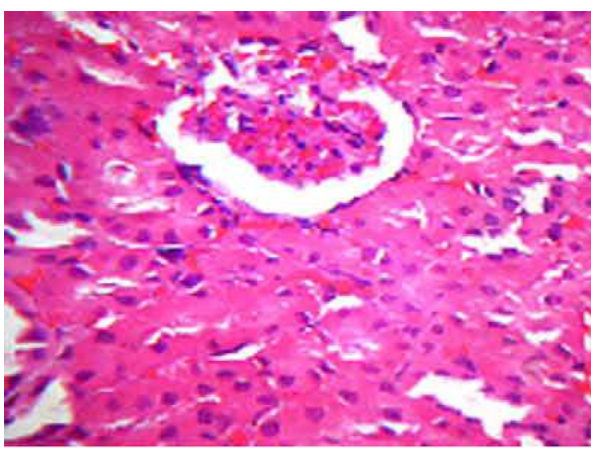
| Groups | Dose (mg/kg) | Urine parameters (mg/dL) Magnesium | Creatinine |
|---|---|---|---|
| Control | Vehicle | 1.20 ± 0.03*** | 6.20 ± 0.10*** |
| Lithiatic Control | Ethylene glycol (0.75%). | 0.520 ± 0.07 | 6.62 ± 0.19 |
| Standard | Cystone (750mg/kg, i.p) | 1.80 ± 0.07 | *** 6.30 ± 0.22*** |
| Test-I | Extract of plant (250mg/kg,p.o) | 0.90 ± 0.05*** | 4.50 ± 0.20 |
| Test-II | Extract of plant (500mg/kg, p.o) | 1.02 ± 0.19*** | 3.72 ± 0.05*** |

**Change in the Serum parameters in control and AEBD treated animals.**

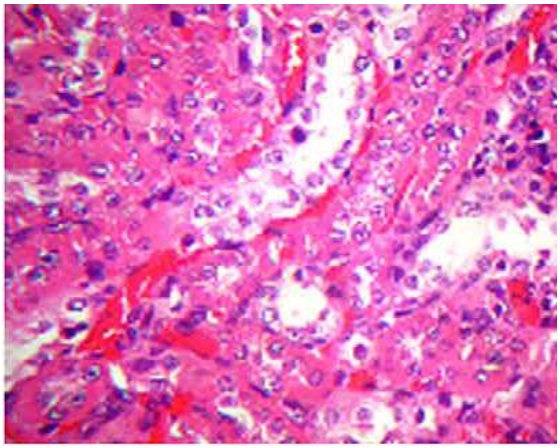
| Groups | Dose (mg/kg) | Serum parameters (mg/g) BUN | Creatinine | Uric acid |
|---|---|---|---|---|
| Control | Vehicle | 3.50 ± 0.014*** | 0.72 ± 0.01*** | 1.40 ± 0.07*** |
| Lithiatic Control | Ethylene glycol (0.75%). | 45.60 ± 0.69 | 0.90 ± 0.03 | 3.35 ± 0.01 |
| Standard | Cystone (750mg/kg, i.p) | 38.02 ± 0.46 | *** 0.81 ± 0.02** | 1.51 ± 0.04* |
| Test-I | Extract of plant (250mg/kg,p.o) | 43.56 ± 0.01*** | 0.86 ± 0.01*** | 2.01 ± 0.06*** |
| Test-II | Extract of plant (500mg/kg, p.o) | 41.76 ± 0.01*** | 0.83 ± 0.01*** | 1.85 ± 0.04*** |

**Change in the kidney retension of stone forming constituents in control and AEBD treated animals.**

| Groups | Dose (mg/kg) | Urine parameters (mg/g) Calcium | Phosphate |
|---|---|---|---|
| Control | Vehicle | 3.10 ± 0.04*** | 2.20 ± 0.03*** |
| Lithiatic Control | Ethylene glycol (0.75%). | 4.60 ± 0.15 | 3.70 ± 0.09 |
| Standard | Cystone (750mg/kg, i.p) | 3.14 ± 0.06 | * 2.50 ± 0.06*** |
| Test-I | Extract of plant (250mg/kg,p.o) | 4.40 ± 0.04*** | 3.05 ± 0.08*** |
| Test-II | Extract of plant (500mg/kg, p.o) | 3.72 ± 0.07*** | 2.70 ± 0.06*** |



**[A]**



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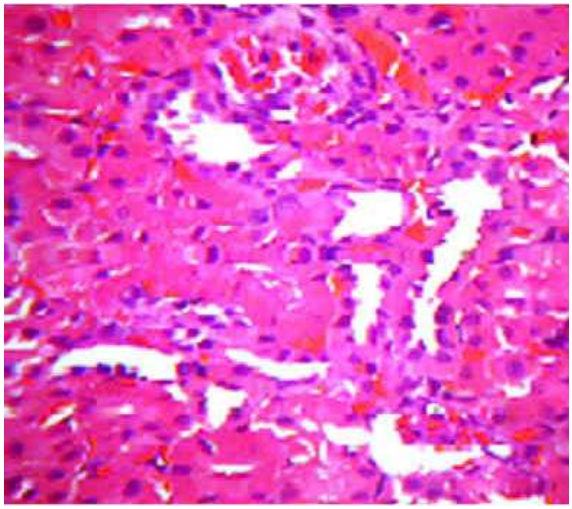

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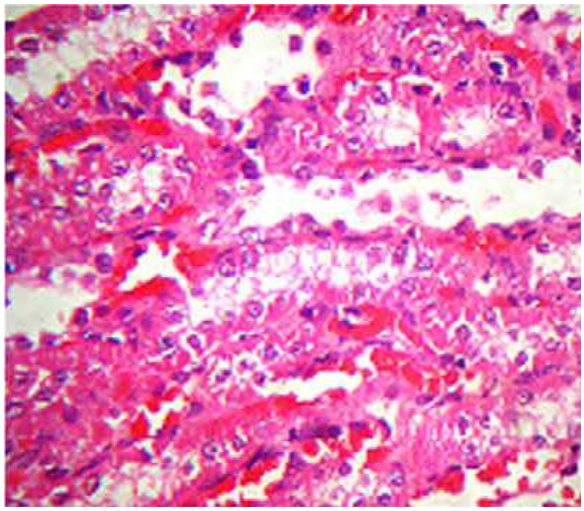

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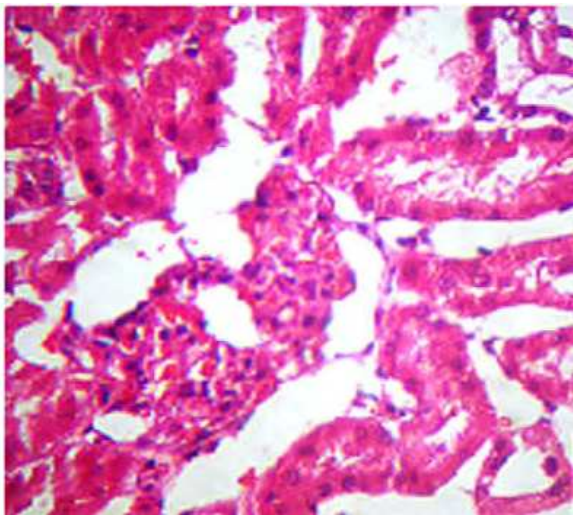

**[E]**

[A] Group I treated with vehicle

[B] Group II treated with ethylene glycol (0.75% v/v)

[C] Group III treated with standard Cystone

[D] Group IV treated with alcoholic extract of *Boerhaavia diffusa* (250mg/kg)

[E] Group V treated with alcoholic extract of *Boerhaavia diffusa* (500mg/kg)

**DISCUSSION**

As traditional medicines are usually taken by the oral route, same route of administration was used for evaluation of Anti-Lithiatic activity of the Alcoholic Extract Of *Boerhaavia diffusa* against ethylene glycol induced urolithiasis in rats.

Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies shown that the amount of stone deposition in female rats was significantly less.

Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Previous studies indicated that, upon 14 days administration of ethylene glycol to the young albino rats resulted into the formation of renal calculi composed mainly of calcium oxalate[19].

The biochemical mechanism for this process is related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate.

Renal calcium oxalate deposition by EG (ethylene glycol) and ammonium chloride in rats is frequently used to mimic the urinary stone formation in humans. Ammonium chloride reported to accelerate the lithiaisis.[20]

Therefore, this model was used to evaluate Anti-Lithiatic activity of the Alcoholic Extract Of *Boerhaavia diffusa* against urolithiasis.

Consistent with some previous reports, stone induction by EG caused an increase in oxalate and decrease in calcium urinary excretion in the Group II. The rate of decrease in calcium excretion was reduced by AEBD treatment in a dose-dependent manner.

As the volume of urine excreted by Group IV and Group V animals wre more when compared to that of volume excreted the Group II animals, this reinforces the plants having diuretic activities such as effect may be advantage in lithiatic condition. As increased urine output is recommended to reduce the possibility of stone formation.

It was observed that the amount of calcium excreted following administration of ethylene glycol in Group II animals increases. Previous study report states that more than 80% of the renal stones made up of with calcium oxalate and calcium phosphate. Increased urinary calcium is factor favouring the nucleation and precipitation of calcium oxalate. But on administration of AEBD to the animals, the amount of calcium in the urine were reduced in Group IV and V. Moreover the amount of phosphate excreted in urine was increased in Group II animals. An increased urinary phosphate excretion seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals. Following EG administration, after treatment with AEBD there is a decrease in phosphate level of the animals levels in group IV and V.

In Urolithiasis, the Glomerular filteration rate ( GFR ) decreases, due to the obstruction to the outflow of urine by stones in urinary system. Due to the waste products particularly nitrogenous substances such as urea, creatinine and as uric acid get accumulated in the blood in lithiatic control rats marked renal tissue damage was seen by the elevated serum levels of creatinine, uric acid and BUN. However the extract treated animals as a process of dissolving the performed stones and prevention of new stone formation in urinary system. In Group II the excretion of creatinine level is high where as in Group III , IV and V the levels are decreased. In serum of Group II animals where the BUN, creatinine and uric acid levels are increased where as in Group III , IV and V the levels are decreased.

In Group II the magnesium level in the urine was decreased which is the common feature in the stone formers. Where as in Group IV and V were increased thus reducing the intensity of crystallization.

**CONCLUSION**

The Alcoholic extract of Boerhaavia diffusa significantly reduced the elevated level of calcium oxalate and phosphate ions. The urinary concentration of magnesium was increased which is considered as one of the inhibitor of crystallization.The histopathological studies showed the sign of improvement with the plant extract of Boerhaavia diffusa linn. These effects could confirm the antiurolithiatic property of Boerhaavia diffusa. The oral administration of Boerhaavia diffusa linn did not induced signifiacant alterations in almost all biochemical, haematological and morphological parameters in Wistar rats. This investigation could be regarded as preliminary probes, requiring further studies to establish the mechanism of toxicity or pharmacological effects. Prospective studies should include among other investigations.

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