International Journal of Pharmacology and Clinical Research (IJPCR)

IJPCR |Volume 3 | Issue 2 | July - Dec - 2019
www.ijpcr.net

Research article *Clinical research*

ISSN: 2521-2206

Evaluation of antiurolithic activity of aqueous extracts of syzygium cumini bark

Karthik Raj Palanisamy*, Dr. Suresh Velayutham, G.Thamotharan

JKKM College of Pharmacy Komarapalayam, Tamilnad, India.

***Address for correspondence: Karthik Raj Palanisamy**

E-mail: rajkarthik.p@gmail.com

ABSTRACT

The bark of *Syzygium cumini* bark are beneficial in treating the various other ailments, which is also accountable for its action on renal calculi preferably at this juncture. Cold maceration technique was used for the extraction of coarse powder of the plant bark and a total of 200g of *Syzygium cumini* was used to make a coarse powder. The Adult Wistar rats Swiss mice weighing between (20-30 g) were used to calculate LD_{50}. In the present study, chronic induction of EG (0.75% v/v) to male wistar rats resulted in significant ($P<0.001$) increase in urinary excretion of calcium and phosphorous. Whereas the cystone-treated group III animals were shown significant reduction in calcium ($P<0.0001$) and phosphate ($P<0.001$) levels. Similarly treatment with plant extract significantly lowered the elevated levels of calcium ($P<0.0001$) and phosphate ($P<0.0001$) in 400 mg/kg as compared to EG induced group II animals. In conclusion, the aqueous extract of *Syzygium cumini* bark has both preventive as well as curative property in urolithiasis of rats.

Keywords: *Syzygium cumin,* Anti urolithic activity, Urolithiasis, Cold maceration technique.

INTRODUCTION

Urolithiasis and its Significance

Urolithiasis is defined as the formation of sediment anywhere within the urinary tract and consisting of one or more of the poorly soluble crystalloids of urine. It is the 3rd most common disorder of the urinary tract. Renal calculi are characterized clinically by colicky pain (renal colic) as they pass down along the ureter and manifest by hematuria. Major risk factors responsible for the nephrolithiasis are inadequate urinary drainage, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities i.e; deficiency of Vitamin-A, excess of vitamin D, metabolic diseases like hyperparathyroidism, cystinuria, gout, intestinal dysfunction and environmental factors related to regions with hot and dry climatic conditions [1-6].

The present medical management of urinary stone includes lithotripsy and surgical procedures which are prohibitively expensive for the common man and with these procedures recurrence is quite common and the patient has to be examined through careful follow up for several years. Various factors such as size of calculi, severity of symptoms, and degree of obstruction, kidney function, location of the stone and the presence or absence of associated infection, influence the choice of one type of intervention over the other.

Stones which are smaller than 5mm have a high probability of spontaneous passage which can take up to 40 days. During this watchful waiting period, patients can be treated with hydration and pain relieving medication. However, stones larger than 5 mm or stones that fail to pass through urine are treated by interventional procedure. Open surgical procedures for the treatment of ureteric stones have gradually disappeared in the last 30 years and have been replaced by minimal invasive techniques such as ESWL or ureteroscopy. ESWL is a noninvasive practice which uses shock waves to fragment calculi. This technique is the most extensively used method for managing renal and ureteral stones. However, treatment success rates depend on stone composition, size, properties and location of the stone as well as the instrumentation type and shock frequency. It also needs to be considered that the same forces that are directed at the stones have deleterious effects on surrounding tissues. Damage to almost every abdominal organ systems have been reported but by far the most common injury is acute renal hemorrhage although its true incidence is unclear and poorly defined. Most often renal hemorrhage can be managed predictably; however, in rare instances the complications are fatal. Reports of post-ESWL perirenal hematoma range from less than 1% to greater than 30%. Besides, ESWL has been associated with long-term medical effects such as diabetes mellitus and hypertension.

Preparation of extract

Cold maceration technique was used for the extraction of coarse powder of the plant bark and a total of 200g of *Syzygium cumini* was used to make a coarse powder. During the process, 100 g of the coarse powder was soaked in an Erlenmeyer flask with 1 L of 50% of Ethyl Acetate and then placed on a shaker (Bibby Scientific Limited Stone Staffo Reshire, UK) tuned to 120 rpm with occasional shaking for 72 h at room temperature. The extract was filtered first using a muslin cloth and then Whatman grade No-1 filter paper and the marc was re-macerated for a second and third time by adding another fresh solvent. The filtrates were left overnight in a deep freezer and then lyophilized using freeze dryer. The dried plant extract was reconstituted with distilled water for oral administration.

EXPERIMENTAL ANIMALS

The Adult Wistar rats *S*wiss mice weighing between (20-30 g) were used to calculate LD [7-10]. They were housed in clean polypropylene cages and maintained under standard conditions of light (12 hours with alternative day/night cycles), relative humidity (60-70%) and temperature (26 ± 1 °C). The animals were fed daily with rodent pellet diet and tap water *ad-libitum* under strict hygienic conditions.

Experimental procedure

Ethylene Glycol (EG) Induced Urolithiasis Model

EG induced hyperoxaluria method was used to assess the antiurolithic activity in male Wistar rats. Six animals were grouped and used for evaluating the effect of antiurolithic activity 400mg of aqueous extract of *Syzygium cumini*. The datas for control, cystone were collected from the previous reviewed journals. [11-15]. The 400mg aqueous extract of *Syzygium cumini* bark was fed with laboratory diet and water *ad labium*. All treatments were given once daily by the oral route.

Collection and Analysis of Urine

All animals were kept in metallic cage separately, and urine samples of 24 h were collected on the 28th day, and a drop of concentrated hydrochloric acid was added to the urine sample before being stored at 4°C. Animals had free admittance to drinking water during the urine collection period [16-18]. The collected urine sample was analyzed for urine volume, calcium, oxalate, phosphate, total protein, blood urea nitrogen (BUN), uric acid, and creatinine content using commercially available kit.

Reagents

1. Electrolytic zinc- Electrolytic zinc weighing approximately 250mg was taken and cleaned with 10 N nitric acid and later with distilled water before use.
2. Chromotropic acid – 1.0g of 4, 5—dihydroxy-naphthalene-2,7 disulphonic acid, disodium salt was dissolved in 100 ml water and stored at 4 degree celcius. The solution was prepared fresh, once a week.

3. Standard oxalate- solution containing 5 mg of oxalate in 1.0 ml was prepared using potassium oxalate monohydrate.

Procedure

The urine was acidified by adding concentrated hydrochloric acid (0.1 ml per ml of urine) to ensure the solubilization of any crystals of calcium oxalate. To 2.0 ml of urine was added 1.5 ml of urine was added 1.5 ml of water and a drop of bromothymol blue indicator. The PH was adjusted to 7.0 by the addition of diluted sodium hydroxide or diluted acetic acid and 2.0 ml of saturated aqueous solution of calcium sulphate followed by 14ml of ethanol, mixed well and allowed to stand at room temp for at least 3 hrs or preferably overnight. It was centrifuged at 2000 rpm for 10 mins. The supernatant was decanted. The precipitate was taken in 2.0 ml of 2N sulphuric acid and a piece of freshly cleaned zinc was added and heated in a boiling water bath for 30 mins. The tube was left unstopped to allow evaporation to occur and the final column was less than 0.5 ml to ensure full color development.

The zinc piece was then removed with a glass rod and 0.3 ml of chromotropic and was added. The tubes were kept in an ice bath and 5.0 ml of concentrated sulphuric acid was added with mixing, and heated in a boiling water bath for 30 mins. The tubes were cooled, diluted to 20 ml with 10N sulphuric acid and the colour was read at 570 nm. The colour was stable for several hrs. The oxalate in urine was expressed as mg per 24 hrs urine per rat.

Serum analysis

Blood was collected from tail vein method on the 28th day. Serum was separated by centrifugation at 6000 rpm for 15 minutes and was analysed for creatinine, urea,nitrogen, and uric acid using Automated Clinical Chemistry Analysis System.

Procedure

Hemolysis and lipid peroxidation of erythrocytes were carried out using hydrogen peroxide by a modification of the method. In this 0.02 ml of normal human erythrocyte were taken in 1.0 ml of 0.02 M phosphate buffered saline, PH 7.4 and was mixed with 0.5 ml of the test sample and 1.0 ml of hydrogen peroxide in phosphate buffered saline. The mixture was incubated for 1 hrs at 37 degree celcius and centrifuged at 1500 revolution/min for 10 mins. 1.5 ml of the red cell free supernatant solution from each tube was transferred to cuvettes and the optical density was read at nm in a spectrophotometer. Distilled water was used as a blank [19].

Lipid peroxidation was determined using 0.5ml supernatant. To this 1.0 ml of 5% trichloroacetic acid and 0.67% thiobarbituric acid were added and boiled in a water bath for 15 min. the tubes were cooled to room temperature and centrifuged. Absorbance at 535 nm was determined [20].

Inhibitory effects of the test compounds on hemolysis and lipid peroxidation were estimated by the following equation where O.D= Optical density:

$$\text{Inhibitory ratio} = \frac{\text{O. D with no addition of test compound} - \text{O. D with addition of test compound}}{\text{O. D with no addition of tested compound}} \times 100$$

Kidney homogenate analysis

The animals were sacrificed by cervical decapitation and the abdomen was cut open to remove the kidneys from each animal and perfused using phosphate buffer saline. The isolated kidneys were cleaned off and the left kidney was finely minced and 20% homogenate was prepared in Tris-HCl buffer (0.02 mol/L) of pH 7.4. Total kidney homogenate was used for assaying tissue phosphate, calcium, magnesium [21].

Histopathological studies

At the end of the experiment, all rats were sacrificed by euthanasia and remove the kidney then fixed in 10% formalin and tissue were then embedded in paraffin blocks for preparing sections (1-3 μm) which were then stained using hematoxylin and eosin dye.

RESULT AND DISCUSSION

The phytochemical screening results revealed that the after which it was observed whether the alkaloids were present by the indication of turbidity and/or precipitate formation. The colour changed from violet to blue or green in some samples indicated the presence of steroids. An interface with a reddish brown coloration was formed in the presence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was observed in the test tube, which indicated in the presence of tannins. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols. Below two observation indicated presence of Saponins Formation of stable foam confirmed the test the formation of a soluble emulsion confirmed the test. The formation of blue colour in acetic acid layer confirmed the test. The Formation of red color confirmed the test. Above two observations indicated presence of glycosides.

Table. No.1: Phyochemical Analysis

S.No	Phytochemicals	Inference
1	Alkaloids	+
2	Steroids	+
3	Terpenoids	+
4	Flavonoids	+
5	Tannins	+
6	Phytosterol	+
7	Saponin	+
8	Glycosides	+

+, Presence of the compound

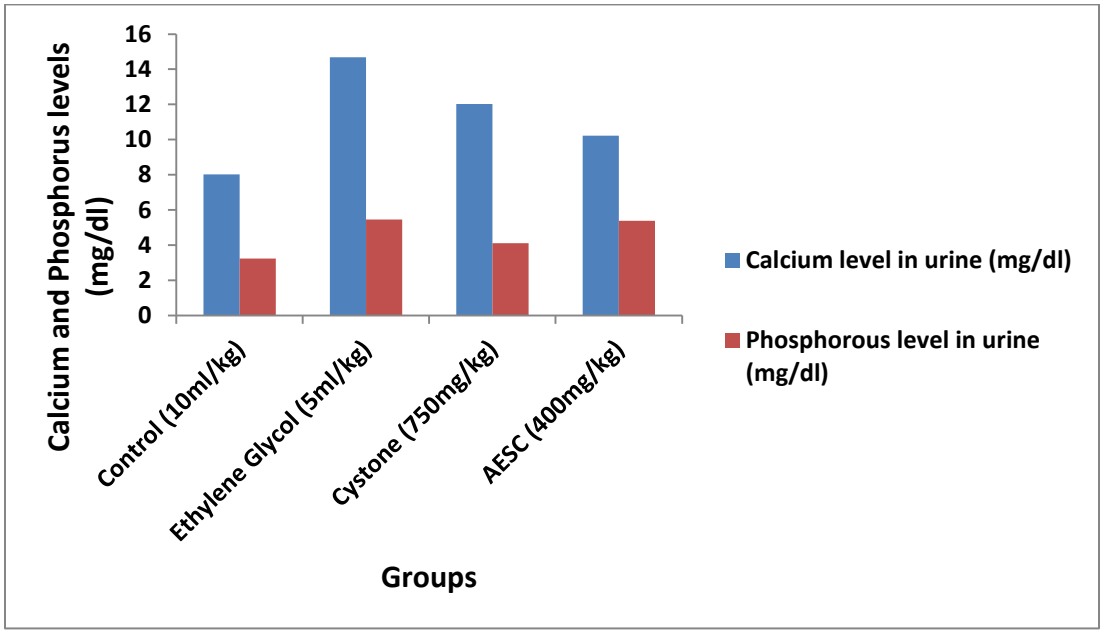
Ethylene Glycol (EG) Induced Urolithiasis Model

Urinary excretion of calcium and phosphorous

In the present study, chronic induction of EG (0.75% v/v) to male wistar rats resulted in significant (P<0.001) increase in urinary excretion of calcium and phosphorous. Whereas the cystone-treated group III animals were shown significant reduction in calcium (P<0.0001) and phosphate (P<0.001) levels. Similarly treatment with plant extract significantly lowered the elevated levels of calcium (P<0.0001) and phosphate (P<0.0001) in 400 mg/kg as compared to EG induced group II animals (Table 1).

Table No. 2: Effect ethylene glycol induced urolithiasis on the changes between calcium and phosphorous

S. No	Treatment group	Dose (mg/kg)	Calcium level in urine (mg/dl)	Phosphorous level in urine (mg/dl)
1	Control	10ml/kg	8.12±0.00	3.33±0.01
2	Ethylene Glycol	5ml/kg	14.78±0.03	5.56±0.01
3	Cystone	750mg	12.12±0.07	4.22±0.07
4	AESC	400mg	10.33±0.03	5.49±0.01



Blood Urea Nitrogen

The blood urea nitrogen (BUN), levels was significantly (P<0.0001) increased in EG-induced group II animals. While the BUN levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the BUN levels was significantly (P<0.0001) decreased the dose level 400 mg/kg group. The plant extract 400 mg/ kg treated group of animals blood urea nitrogen level significantly decreased when compared to EG induced group.

Table. No 3: Effect ethylene glycol induced urolithiasis on blood urea nitrogen

S. No	Treatment group	Dose (mg/kg)	Blood urea nitrogen (mg/dl)
1	Control	10ml/kg	36.19±0.01
2	Ethylene Glycol	5ml/kg	58.63±0.01
3	Cystone	750mg	34.61±0.00
4	AESC	400mg	39.97±0.03

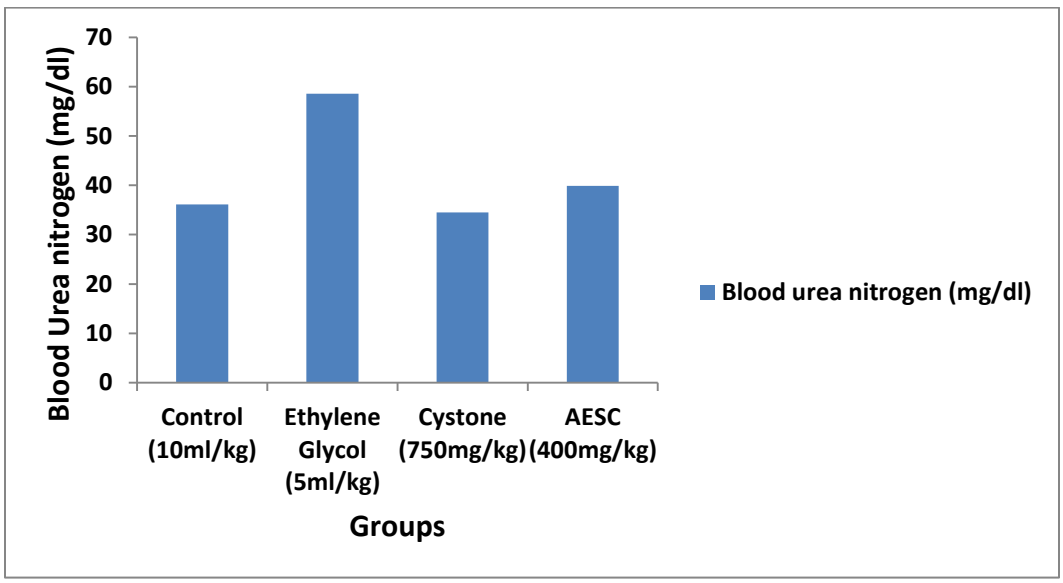


Figure No. 2 EG induced urolithiasis on Blood Urea Nitrogen

Serum creatinine

On collected and isolated Serum creatinine, levels were significantly (P<0.0001) increased in EG-induced group II animals. While the serum creatinine levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the serum creatinine levels was significantly (P<0.0001) decreased in 400 mg/kg group. The plant extract 400 mg/ kg treated group of animals serum creatinine level significantly decreased when compared to EG induced group.

Table. No.4: Effect ethylene glycol induced urolithiasis on serum creatinine

S. No	Treatment group	Dose (mg/kg)	Serum creatinine (mg/dl)
1	Control	10ml/kg	0.67±0.00
2	Ethylene Glycol	5ml/kg	0.98±0.00
3	Cystone	750mg	0.70±0.01
4	AESC	400mg	0.56±0.00

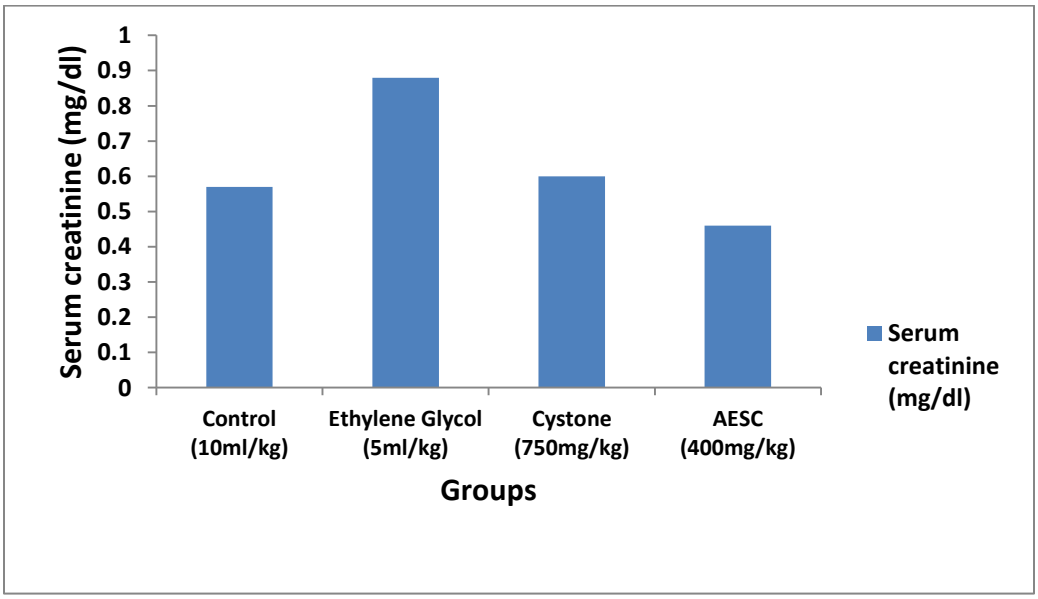


Figure No. 3 EG induced urolithiasis on Serum Creatinine

Serum uric acid

The levels of Serum uric acid on blood serum were indicated that the significantly (P<0.0001) increased in EG-induced group II animals. As the serum uric acid levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the serum uric acid levels was significantly (P<0.0001) decreased in 400 mg/kg group. The plant extract 400 mg/ kg treated group of animals serum uric acid level significantly decreased when compared to EG induced group.

Table. No.5: Effect ethylene glycol induced urolithiasis on uric acid

S. No	Treatment group	Dose (mg/kg)	Uric acid (mg/dl)
1	Control	10ml/kg	2.85±0.01
2	Ethylene Glycol	5ml/kg	5.91±0.00
3	Cystone	750mg	3.44±0.01
4	AESC	400mg	4.91±0.01

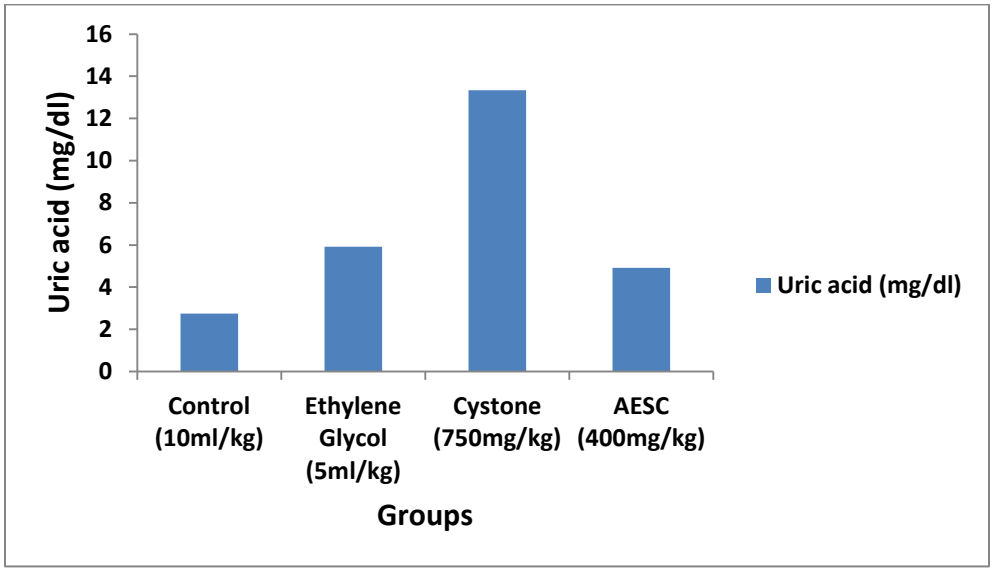


Figure No. 4 EG induced urolithiasis on Uric acid

Urine volume

Urine volume was significantly (P<0.0001) decreased in EG-induced group II animals. While the serum Urine volume levels was significantly (P<0.001) increased in cystone treated group III animals. However, the Urine volume levels was significantly (P<0.0001) decreased in 400 mg/kg group. The plant extract 400 mg/ kg treated group of animals serum uric volume level significantly decreased when compared to EG induced group.

Table. No.6: Effect ethylene glycol induced urolithiasis on urinary volume

S. No	Treatment group	Dose (mg/kg)	Urinary volume (ml)
1	Control	10ml/kg	17.93±0.00
2	Ethylene Glycol	5ml/kg	10.93±0.08
3	Cystone	750mg	16.50±0.00
4	AESC	400mg	21.95±0.08

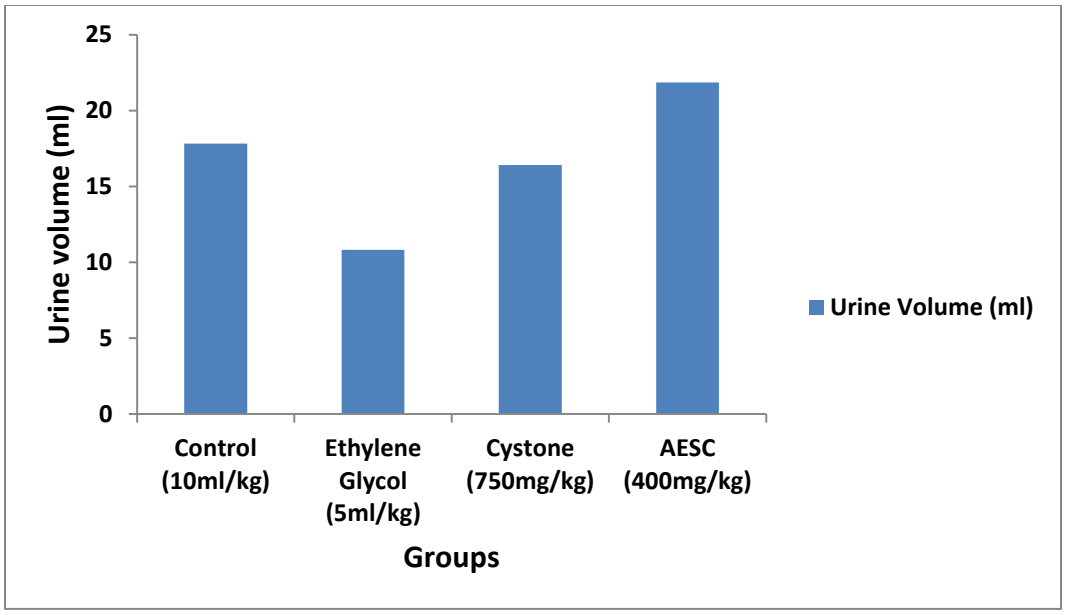


Figure No. 5: EG induced urolithiasis on Urine Volume

Kidney weight

Weight variation of kidney results were clarified that the significantly (P<0.0001) increased in EG-induced group II animals. While the Kidney weight levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the Kidney weight levels was significantly (P<0.0001) decreased 400 mg/kg group. The plant extract 400 mg/ kg treated group of animals kidney weight level significantly decreased when compared to EG induced group.

Table. No.7: Effect ethylene glycol induced urolithiasis on Kidney weight

S. No	Treatment	Dose (mg/kg)	Kidney weight (mg)
1	Control	10ml/kg	0.73±0.01
2	Ethylene Glycol	5ml/kg	1.16±0.01
3	Cystone	750mg	0.81±0.00
4	AESC	400mg	0.79±0.02

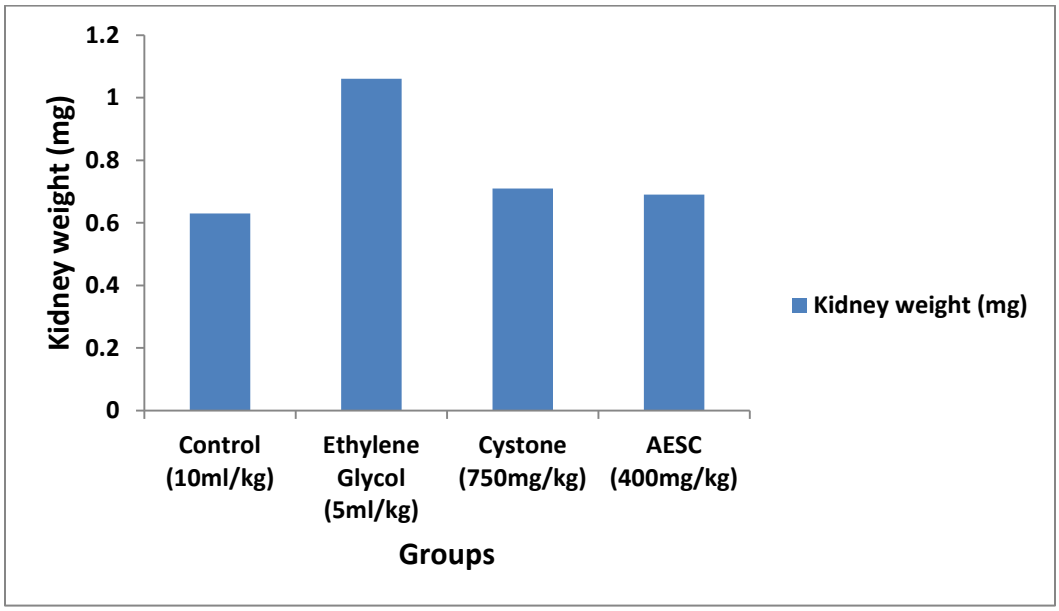


Figure No. 6: EG induced urolithiasis on Kidney weight

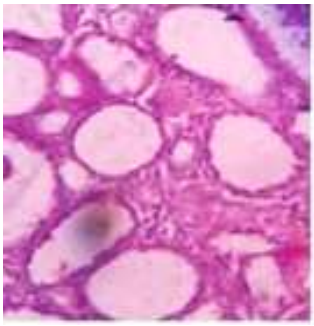


Figure No. 7: Group I - Control (10ml/kg)

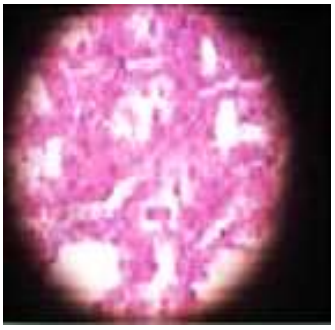


Figure No. 8: Group II – E.G. induced (5ml/kg)

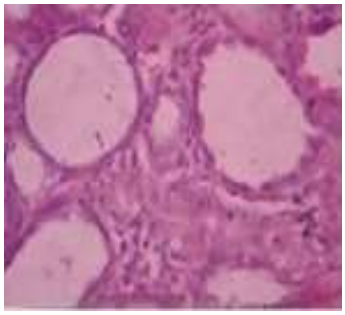


Figure No. 9: Group III- Cystone (750 mg/kg)

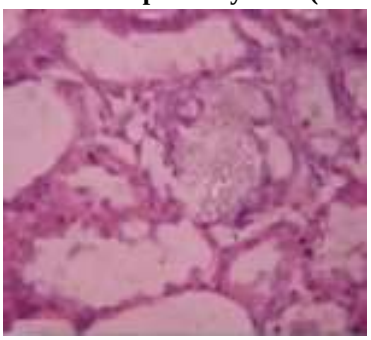


Figure No. 10: Group IV – A.E.S.C. barks (400 mg/kg Extract)

CONCLUSION

Urolithiasis can be produced in rats by induction of acute or chronic hyperoxaluria by using a variety of agents such as ethylene glycol, sodium oxalate, ammonium oxalate, hydroxyl-L-proline and glycolic acid. Kidney being the principal target for EG induced toxicity. EG is broken down in-vivo into four organic acids viz., glycolaldehyde, glycolic acid, glycooxalic acid and oxalic acid leading to hyperoxaluria which is the main initiative factor for lithiasis. Therefore in the present study, EG was preferred to induce lithiasis. Administration EG to the experimental animals for 28 days resulted in substantial elevation of oxalate and deposition of microcrystal's in kidney. In addition, oxalate precipitates as a calcium oxalate crystals in kidney since the oxalate metabolism is considered almost identical between rats and umans. Calcium and phosphate play a vital role in renal calculogenesis. In EG induced rats, the urinary excretion of calcium, phosphate was significantly increased. The increase in calcium and phosphate excretion could be due to defectivetubular reabsorption in the kidneys. While treatment with standard, curative and preventive regimens of Syzygium cumin markedly reduced the levels of these ions, suggested protective effect of Syzygium cumin urolithiasis. In urolithiasis, the calculi formed in the renal tissue leads to obstruction in the urinary system that decreases the glomerular filtration rate (GFR) and cause an accumulation of certain waste products like nitrogenous substances e.g., BUN, creatinine and uric acid in the blood. Marked renal damage was seen in EG induced rats indicated by decreased GFR, significant kidney weight gain and elevated serum level of BUN, creatinine, and uric acid. However treatment with ethanolic

extracts of S. virginianum plant extracts in both curative and preventive regimens caused diuresis along with loss of kidney weight and also decreased the elevated serum level of BUN, creatinine and urea. The findings of the histopathological studies suggested that no microcrystalline deposition and deposition and kidney damage in the Syzygium cumin extract treated groups all these observations enabled us to confirm the preventive curative potential of Syzygium cumin on ethylene glycol induced lithiasis. In conclusion, the aqueous extract of Syzygium cumini bark has both preventive as well as curative property in urolithiasis of rats. These finding, thus prompt thenecessity for further study to carry out the antilithiatic effect of Syzygium cumini bark by isolation of constituents and find out the actual constituent that active against stone formation. In this present research, the antiurolithic activity of Syzygium cumini bark has been planned to evaluate for its potential towards the action. The bark of Syzygium cumini bark are beneficial in treating the various other ailments, which is also accountable for its action on renal calculi preferably at this juncture.

REFERENCES

[1]. Ramachandran S, Kumar TM, Saisandeep V, Ramsai K, Dhanaraju MD *et al.,* Antilithiatic activity of poly-herbal extracts on ethylene glycol-induced lithiasis in rats. Eur J Biol Sci 3, 2011, 36-9.

[2]. Atmani F, Slimani Y, Mimouni M, Aziz M, Hacht B, Ziyyat A *et al.,* Effect of aqueous extract from *Herniaria hirusta* L. On experimentally nephrolithiasic rats. J Ethnopharmacol 95, 2004, 87-93.

[3]. Selvam R, Kalaiselvi P, Govindaraj A, Balamurugan V, Sathish AS *et al.,* Effect of *Aerva zanata* flowers extract and vediuppuchunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol Res 43, 2001, 89-93.

[4]. Fan J, Glass MA, Chandhoke PS *et al.,* Impact of ammonium chloride administration on a rat ethylene glycol urolithiasis model. Scanning Microsc 13, 1999, 299-306.

[5]. Onyenmechi JA, Orish EO, Chudi ED, Ejeatuluchukwu OB, Tobias E, Lasbery A, *et al*., Effect of rinbacin extract on rat kidney. Biol Pharm Bull 25, 2002, 1022-5.

[6]. Mohammed Touhami,Amine Laroubi, Khadija Elhabazi, Farouk Loubna, Ibtissam Zrara, younes Eljahiri et al. Lemon juice has protective activity in a rat urolithiasis model. BMC Urol 2007, 7-8.

[7]. Christina AJM, Ashok K, Packialakshmi M,Tobin GC,Preethi J Murugesh N. Antilithiatic effect of Asparagus racemosus wild on Ethylene Glycol-Indued Lithiasis in Male Wistar Rats. Meth Exp Clin Pharmacol, 27(9), 2005, 633-638.

[8]. Aroujo Viel T, Domingos CD, Da Silvamonteiro AP, Riggo Lima-Landman MT, Lapa AJ, Souccar C *et al.,* Eur Urol. 12, 1986, 308–313.

[9]. Vaidya Ratnam P S Varier's *et al.,* Indian Medicinal Plants. Orient Longman. 5, 164-166.

[10]. Crossland, J *et al.,* Lewis pharmacology, Churchill Livingstone, New York. 1980, 137.

[11]. Sathish R, Natarajan K, Mukesh MN *et al.,* Effect of Hygrophila spinosa T. Anders on ethylene glycol induced Urolithiasis in rats. Asia-Pac Pharm Clin Res 3, 2010, 61-63.9.

[12]. Mitra SK, Gopumadhan G, Venkataranganna MV and Sundaram R *et al.,* Effect of cystone, a herbal formulation on glycolic acid induced urolithiasis. Phytother Res 12, 1998, 372-374.

[13]. Ravindra VK, Navneet BG, Alagawadi KR, RudraprabuVS *et al.,* Ethylene glycol induced urolithiasis in effect of *Moringa oleifera* Lam. Root-wood on rats. J Ethnopharmacol 105(1), 2006, 306-311.

[14]. Onyenmechi JA, Orish EO, Chudi ED, Ejeatuluchukwu OBI, Tobias E, Lasbery A, Ukoha. Effect of *Rinbacin* extract on rat Kidney. Biol Pharm. Bull 25(8), 2002, 1022-1025.

[15]. Khan SR, Animal models of kidney stone formation: an analysis, World J Urol, 15, 1997, 236-243.

[16]. Schladt L, Ivens I, Karbe E, Ruhl-Fehlert C, Bomhard E *et al.,* Sub acute oral toxicity of tetra ethylene glycol and ethylene glycol administered to Wistar rats, Exp Toxicol Pathol, 50, 1998, 257-265.14.

[17]. Varalakshmi P, Shamila Y, Latha E *et al.,* Effect of *Crataeva nurvala* in experimental urolithiasis, J Elhnopharmacol, 28, 1990, 313-321.15.

[18]. Khatib N, Dhaval G, Hashilkar N, Rajesh KJ *et al.,* Antiurolithiatic potential of the fruit extracts of *Carica papaya* on ethylene glycol induced urolithiatic rats. J Pharm Res 3(11), 2010, 2772-2775.

[19]. Parekh BB, Jogiya BV, Vyas PM, Raut AA, Vaidya AB, Joshi MJ *et al.,* In vitro growth inhibition study of hydroxyapatite crystals in the presence of selected herbal extract solutions. Der Pharma Chemica 6, 2014, 128-135.

[20]. Patel PK, Patel MA, Vyas BA, Shah DR, Gandhi TR *et al.,* Antiurolithiatic activity of saponin rich fraction from the fruits of *Solanum xanthocarpum* Schrad. & Wendl. (Solanaceae) against ethylene glycol induced urolithiasis in rats. Ethnopharmacol 144, 2012, 160-170.

[21]. Periyanayagam K, Jagadeesan M, Kavimani S, Vetriselvan T *et al.,* Pharmacognostical and Phyto-physicochemical profile of the bark of *Piper betle* L. var Pachaikodi (Piperaceae) - Valuable assessment of its quality. Asian Pacific Trop Biomedi 2, 2012, S506-S510.