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Lithotriptic Activity of Siddha Drug Aavarai Vithaadhi Chooranam (AVC) Against Ethylene Glycol Induced Lithiatic Albino Rats

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ABSTRACT

One of the most essential organ in human is renal glands. Urolithiasis is the third most common disorder of the renal system. The present study was carried out to evaluate the effectiveness of Aavarai Vithaadhi Chooranam (AVC) against ethylene glycol induced urolithiasis in male Wistar albino rats. The Standard drug used was Cystone. Urolithiasis was induced in rats by administering 0.75% ethylene glycol in drinking water for 28 days and was manifested by high urinary calcium, phosphorus, creatinine, urea and BUN in serum. Simultaneous administration of AVC (100 and 200 mg/kg) and standard drug cystone for 28 days along with ethylene glycol (0.75%) restored all the elevated biochemical parameters (creatinine, blood urea nitrogen and uric acid). The results indicated that the administration of drug to rats with ethylene glycol-induced lithiasis significantly reduced and prevented the growth of urinary stones when compared to the model control drug.

INTRODUCTION

Urinary stone disease has affected humankind since antiquity and can persist, with serious medical consequences, throughout the patient's lifetime[1]. It is largely a recurrent disease with a relapse rate of 50% in 5-10years, and therefore a condition with substantial economic consequences and a great public health importance[2].

Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia. Etiology is multi-factorial and strongly related to dietary lifestyle habits or practices. Increased rates of hypertension and obesity, which are linked to urolithiasis, also contribute to an increase in stone formation[3]. Most calculi in the urinary system arise from a common component of urine, calcium oxalate (CaO), representing up to 80% of analyzed stones[1, 4, 5]. This 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[6]. Many remedies have been employed since ages to treat renal stones and most of them were from plants and proved to be useful[7-9].

The present day medical management of nephrolithiasis is either costly or not without side effects[10]. In traditional systems of medicine, most of the effective remedies found to be originating from medicinal plants[3]. The present study has been undertaken to evaluate the lithotriptic activity of AVC against ethylene glycol induced urolithiasis in rats.

MATERIALS AND METHODS

Materials

Cystone was procured from Himalaya herbal health care, Bangalore. The urea kit, creatinine mono reagent test kit and triglycerides test kit were procured from laboratory. AVC was prepared in Pharmacy, Government Siddha Medical College, Chennai.

Animals

Healthy Wistar albino rats of either sex weighing between 150-200 g (8 – 12 wks) were selected for the lithotriptic activity. The animals were acclimatized to standard laboratory conditions

(temperature: 25±2°C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water *ad libitum*. The animal care and experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) Reg no. KKCP/2013/004/CPCSEA.

Ethylene Glycol Induced Urolithiasis Model

Ethylene glycol-induced urolithiasis model was used to assess the effect of AVC. The study is designed to find out the effect of AVC against ethylene glycol induced urolithiasis. All rats were housed in metabolic cages for entire duration of the experiment. Animals were divided into five groups containing six animals in each. Group I served as control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to Groups II–V for induction of renal calculi for 28 days. Group III received standard antiurolithiatic drug, cystone (750 mg/kg body weight) from 15th till 28th day. Group IV and V received Unex at low dose (100 mg/kg) and high dose (200 mg/kg) (po) from 15th till 28th day[1, 11].

Group and Treatment

Group 1: Treated with regular food and drinking water
Group 2: Treated with ethylene glycol, drinking water
Group 3: Treated with standard (ethylene glycol + cystone)
Group 4: Treated with AVC (100 mg/kg) + ethylene glycol
Group 5: Treated with AVC (200 mg/kg) + ethylene glycol
All doses were given once daily by oral route.

Assessment of Lithotriptic Activity

Collection and analysis of urine

All the animals were kept in individual metabolic cages and urine samples for 24 h were collected on the 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium, phosphate, and oxalate content[10].

Serum analysis

After the experimental period, blood was collected from the retro-orbital under anesthetic condition and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10000 × g for 10 min and analyzed for creatinine, uric acid, and urea nitrogen[12].

Urine volume

Animals were placed in separate metabolic cages for 24 h and total urinary volume was measured using the measuring cylinder and reported in ml[10].

Kidney homogenate analysis

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80^{o}C in a hot air oven. A sample of 100mg of the dried kidney were boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 × g for 10 min, and the supernatant was separated. The calcium, phosphate, and oxalate content in kidney homogenate were determined[13].

Statistical Analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by the Bonferroni test. Statistical significant was set at $P < 0.05$. Results are presented as mean ± standard error of mean (SEM) [13].

RESULTS

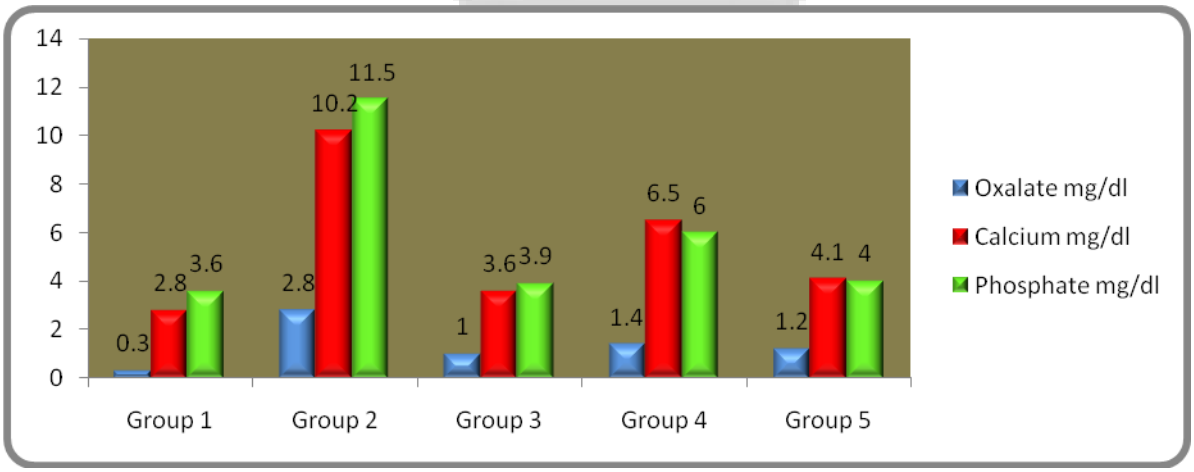
In this study, the administration of 0.75% (*v/v*) ethylene glycol aqueous solution to Wistar albino rats produced hyperoxaluria. Oxalate, calcium, and phosphate excretion were grossly increased in the calculi-induced animals however; supplementation with AVC significantly lowered the elevated levels of oxalate, calcium, and phosphate in urine when compared to the model control group (Table 1 & Picture1).

Table 1: Estimation of Urinary Electrolytes of Normal and Urolithiatic Rats.

Group & Drug Treatment	Group & Drug Treatment		
	Oxalate (mg/dl)	Calcium (mg/dl)	Phosphate (mg/dl)
Group 1: Treated with regular food and drinking water	0.38±0.012	2.86±0.45	3.65±0.751
Group 2: Treated with ethylene glycol, drinking water	2.89±0.714	10.25±1.35	11.54±2.001
Group 3: Treated with Standard (ethylene glycol + Cystone)	1.07±0.184	3.65±1.24	3.99±0.244
Group 4: Treated with AVC (100 mg/kg) + ethylene glycol	1.42±0.245	6.58±2.25	6.021±1.354
Group 5: Treated with AVC (200 mg/kg) + ethylene glycol	1.28±0.250	4.125±1.25	4.07±1.341

Values are expressed as mean ±S.E.M for six rats in each group. Statistic analysis was done by One-way ANOVA followed by Bonferroni test.

Picture-2: Estimation of Urinary Electrolytes of Normal and Urolithiatic Rats.



Group 1: Treated with regular food and drinking water

Group 2: Treated with ethylene glycol, drinking water

Group 3: Treated with standard (ethylene glycol + cystone)

Group 4: Treated with AVC (100 mg/kg) + ethylene glycol

Group 5: Treated with AVC (200 mg/kg) + ethylene glycol

Effect of AVC on urinary salts of experimental animals

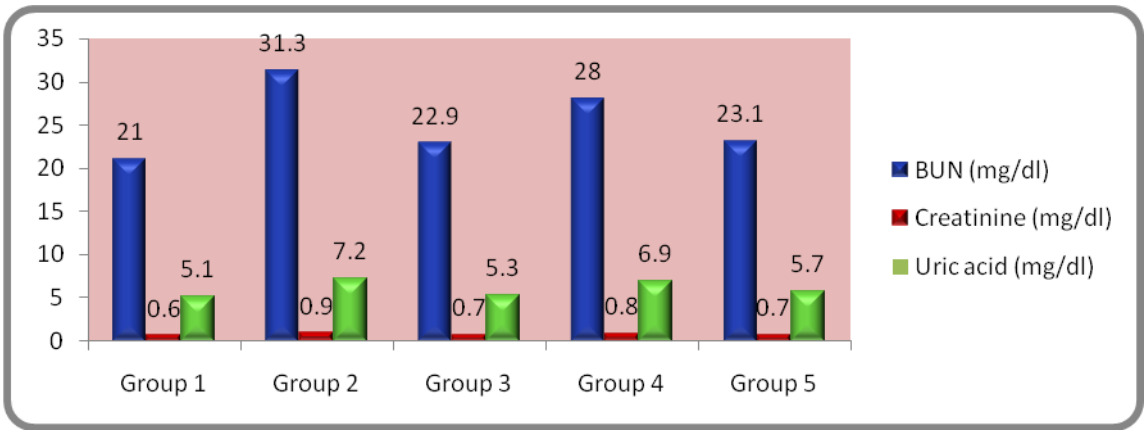
The level of serum uric acid, blood urea nitrogen (BUN), and creatinine were found to increase in the calculi-induced animal. In the case of AVC treated groups, the treatment significantly ($P < 0.001$) lowered the elevated level of creatinine, uric acid, and BUN. Urine volumes were increased by AVC and the standard drug cystone compared to the model control group. No significant differences were observed in the activity of AVC between low dose and high dose (Table 2 & Picture 2).

Table 2: Estimation of Serum Parameters of Normal and Urolithiatic Rats.

Group & Drug Treatment	Group & Drug Treatment		
	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group 1: Treated with regular food and drinking water	21.045±1.085	0.681±0.007	5.12±1.04
Group 2: Treated with ethylene glycol, drinking water	31.331±2.975	0.986±0.150	7.25±1.002
Group 3: Treated with Standard (ethylene glycol + Cystone)	22.970±2.047	0.758±0.34	5.327±1.004
Group 4: Treated with AVC (100 mg/kg) + ethylene glycol	28.012±4.521	0.896±0.001	6.921±1.008
Group 5: Treated with AVC (200 mg/kg) + ethylene glycol	23.12±1.951	0.794±0.127	5.751±0.95

Values are expressed as mean ±S.E.M for six rats in each group. Statistic analysis was done by One-way ANOVA followed by Bonferroni test.

Picture 2: Estimation of Serum Parameters of Normal and Urolithiatic Rats.



Group 1: Treated with regular food and drinking water

Group 2: Treated with ethylene glycol, drinking water

Group 3: Treated with standard (ethylene glycol + cystone)

Group 4: Treated with AVC (100 mg/kg) + ethylene glycol

Group 5: Treated with AVC (200 mg/kg) + ethylene glycol

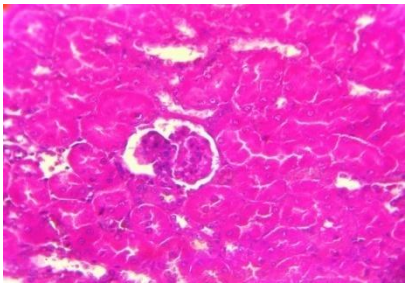
DISCUSSION

Kidney stone formation is a complex process that results from a succession of several physico-chemical events including supersaturation, nucleation, growth, aggregation, and retention within renal tubules[14]. The various systems for urolithiasis research are aimed at investigation of all or at least some of these partial events[15]. The supersaturation of urine with CaOx, the most common component of kidney stones[16], is an important factor in crystallization, with later factors being nucleation, growth and aggregation. Thus if supersaturation or later steps in crystallization can be prevented, then lithiasis should be avoided. Indeed, several measures are usually taken to reduce supersaturation, e.g. increasing fluid intake and medical therapy. Evidence in previous studies indicated that after 14 days period of ethylene glycol (0.75%, *v/v*) administration, renal calculi were formed in the young albino rat composed mainly of calcium oxalate[17]. Stone formation in ethylene glycol-fed rats is caused by hyperoxaluria, which cause increased renal retention and excessive excretion of oxalate in urine[10]. In this study, oxalate and calcium excretion were increased in calculi-induced animals (Group II). An increase in urinary phosphate is also observed in calculi induced rats (Group II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which induce calcium oxalate deposition.

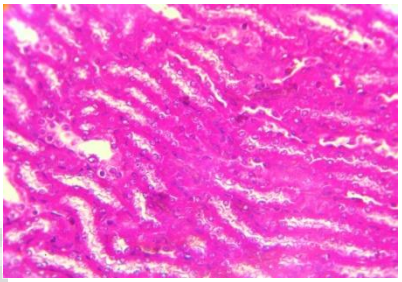
Treatment with the AVC restored the phosphate level, thus reducing the risk of stone formation[18]. In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid get 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. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane. In calculi-induced rats (Group

II), marked renal damage was seen by the elevated serum levels of creatinine and uric acid, and BUN[12]. However, the curative treatment with product AVC and cystone caused diuresis and hastened the process of dissolving the preformed stones and prevention of new stone formation in the urinary system.

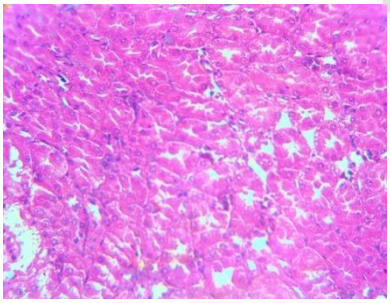
HISTOPATHOLOGY OF KIDNEY



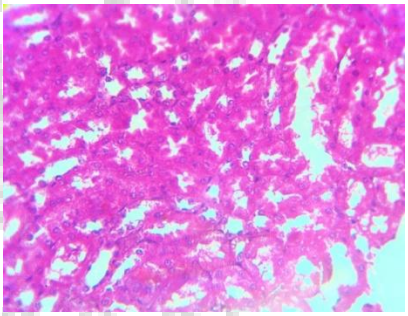
Group 1: Treated with regular food and drinking water



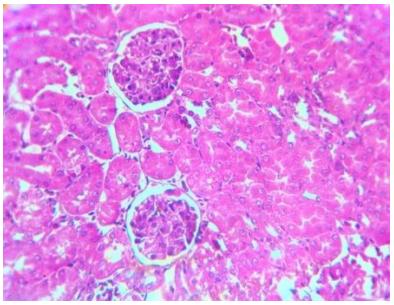
Group 2: Treated with ethylene glycol, drinking water



Group 3: Treated with Standard (ethylene glycol + Cystone)



Group 4: Treated with Aavarai Vithaadhi Choorana. (100 mg/kg) + ethylene glycol



Group 5: Treated with Aavarai Vithaadhi Choorana. (200 mg/kg) + ethylene glycol

CONCLUSION

In conclusion, this study indicated that the administration of AVC to rats with ethylene glycol-induced urolithiasis reduced the growth of the urinary stone and hastened the process of dissolving the formed stones. These effects could conclude the lithotriptic activity of AVC was less the effect of standard drug.

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