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# EFFECT OF *VEDIYUPPU CHEYANEER* IN ETHYLENE GLYCOL INDUCED HYPEROXALURIA MODEL IN RATS

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## ABSTRACT

*Vediyuppu cheyaneer* has been widely used in Siddha system of medicine for various diseases. The liquid form of *Vediyuppu cheyaneer* showed a significant inhibitory effect when screened at 500 mg/kg, for the *invivo* antiurolithiasis activity on ethylene glycol induced hyperoxaluria rats. The standard frusemide (20 mg/kg) and cystone (750 mg /kg) were used as comparison for diuretic and antiurolithiatic activity respectively.

**Keywords:** *Vediyuppu cheyaneer,* Urolithiasis, Frusemide, Hyperoxaluria, Cystone

## INTRODUCTION

The combination Siddha drug has the following constituents as described in the formulary of Siddha Medicines. The human dose prescribed traditionally by Siddha practitioners for diseases like urinary obstructions, inflammation of urogenital tract, gravel in urine and bladder is 1gm per day for an adult patient given daily in two fractions. Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones (Prien and Prien, 1968). Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of extracorporeal shockwave lithotripsy (ESWL), which has revolutionized urological practice and almost become and standard procedure for eliminating kidney stones. However, in addition to the traumatic effects of shock waves, persistent residual stone fragments, and the possibility of infection, suggest that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence. A number of vegetable drugs have been used in India and elsewhere, which claim efficient cure of urinary stones. In the Indian system of Siddha Medicine, the *Vediyuppu cheyaneer* is reported to be useful in the treatment of urinary stones. However, so far no systematic study has been reported regarding the antiurolithiatic property of *Vediyuppu cheyaneer*. In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of *Vediyuppu cheyaneer* using ethylene glycol induced hyperoxaluria model in rats.

## MATERIALS AND METHODS

***Preparation of stock solution***

The *Vediyuppu cheyaneer* was further diluted with distilled water so as to prepare 100mg/ml concentration at room temperature for oral administration by gastric intubation method.

***Pharmacological screening for antiurolithiatic activity***

***Animal selection***

For acute toxicity studies, Wistar albino mice of either sex weighing between 25-30g and for the antiurolithiatic activity albino rats of 200-240g were selected. 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 (Sai meera foods Pvt Ltd., Bangalore, India) and drinking water *ad libitum*. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC).

***Acute toxicity studies***

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD-425) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose ($LD_{50}$) was taken as an effective dose.

***Ethylene glycol induced urolithiasis model***

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats. Animals were divided into four groups containing six animals in each. Group I served as normal control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to Groups II-IV for induction of renal calculi till 28$^{th}$ day. Group IV received standard antiurolithiatic drug, Cystone (750mg/kg body weight) from 15$^{th}$ day till 28$^{th}$ day (Mitra et al., 1998). Groups II and III served as curative regimen received *Vediyuppu cheyaneer* (500mg/kg body weight) from 15$^{th}$ day till 28$^{th}$ day once daily by oral route.

***Assessment of antiurolithiatic activity***

***Collection and analysis of urine:***

All animals were kept in individual metabolic cages and urine samples of 24h were collected on 28$^{th}$ 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.

***Serum Analysis***

After the experimental period, blood was collected from the retro-orbital under anesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000x g for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

***Kidney homogenate analysis***

The abdomen was cut open to remove both kidneys form each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in a 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 was centrifuged at 2000x g for 10min and the supernatant was separated (Chow et al., 1975). The calcium, phosphate and oxalate content in kidney homogenate were determined.

***Diuretic study (Lipschitz method)***

Diuretic activity was carried out as per the method of Lipschitz et.al. In brief, *Vediyuppu cheyaneer* with 2% CMC (500mg/kg body weight) and *Vediyuppu cheyaneer* with adjuvant fresh lime-juice (500mg/kg body weight) were subjected to diuretic study. The screening was performed on healthy rats (160-200 gm). Frusemide (20 mg/kg), was used as reference standard and was dissolved in saline solution for administration while normal saline (25 ml/kg) was used as vehicle. The rats were divided in four groups each containing 6 rats (n = 6). Rats were kept for

fasting for 18 hrs before the study. The control group received normal saline and test groups received (500 mg/kg). The dose of test drug was decided on the basis of acute toxicity study. The doses were given by oral route and rats were kept in specially designed metabolic cages for the collection of urine for 6 hrs. The urine volume during 6 hrs is measured and urine electrolyte estimation was carried out for Na+, K+ using flame photometer and Cl- was estimated by titration.

***Statistical analysis***

Results were expressed as mean ± S.D. Differences among data were determined using one-way ANOVA followed by Dunnet 'T' test (INSTAT software for Windows, Version -V-3). Differences between the data were considered significant at $P<0.05$.

## RESULTS

From the acute toxicity study, the $LD_{50}$ cut-off dose was found to be 500mg/kg body weight. Hence, the therapeutic dose was taken as 500mg/kg body weight. In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to Wistar rats resulted in hyperoxaluria. Oxalate, calcium and phosphate excretion were grossly increased in calculi-induced animals (Table 1). However, supplementation with *Vediyuppu cheyaneer* significantly ($P<0.01$) lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney as compared to cystone-treated animals. The deposition of the crystalline components in the renal tissue, namely oxalate, phosphate and calcium, was increased in the stone forming rats. The *Vediyuppu cheyaneer* treatment significantly ($P<0.01$) reduced the renal content of these stone forming constituents.

The serum uric acid and BUN were remarkably increased in calculi-induced animals, while serum creatinine was elevated in Group II, indicating marked renal damage. However, *Vediyuppu cheyaneer* treatment significantly ($P<0.01$) lowered the elevated serum levels of Creatinine, Uric acid and BUN. Frusemide treated rats showed a significant increase in volume of urine and urinary excretion of sodium, potassium and chloride ($p< 0.01$) as compared to control while urea treated rats did not show any significant increase in urine volume but has high electrolyte excretion potential ($p< 0.01$).

**Table 1**
***Diuretic activity of Vediyuppu cheyaneer***

| Group | Treatment | Volume of Urine (ml/4hrs) | Sodium (mMol/l) | Potassium (mMol/l) | Chloride (mMol/l) |
|---|---|---|---|---|---|
| 1 | Normal saline (25 ml/ kg) | 4.7±0.40 | 51.33±2.33 | 45.83±1.72 | 1.120 |
| 2 | Calculi-induced control | 2.08±0.26 | 99.7±8.4 | 114.7±9.8* | 122±14.2* |
| 3 | Calculi-induced+ VC in lime juice (500mg/kg) | 3.05±0.18** | 68±2.0** | 59.83±2.48** | 1.136 |
| 4 | Calculi-induced+ VC with CMC (500mg/kg) | 3.6±0.20** | 53±2.31** | 45.66±2.25** | 1.097 |
| 5 | Frusemide (20 mg/ kg) | 6.24±0.32** | 87.16±3.31** | 72.66±2.44** | 1.251 |

***Values are mean ± SEM, * p< 0.01, ** p< 0.05 when compared to normal saline (control)***

The *Vediyuppu cheyaneer* with CMC was unable to produce significant actions in dose of 500 mg/kg but *Vediyuppu cheyaneer* with adjuvant lime showed significant increase in volume of urine and also urinary excretion of sodium, potassium and chloride. In general, *Vediyuppu cheyaneer* has shown diuretic activity ($p<0.01$) wherein significant increase in K+ but not in Na+ excretion when compared to control was observed.

**Table 2**
***Effect Of Vediyuppu cheyaneer On Urinary And Serum Parameters In Rats***

| Parameter (Unit) | Group I, Normal | Group II, Calculi-induced control | Group III, Calculi-induced+ VC in lime juice (500mg/kg) | Group IV, Calculi-induced+ VC with CMC (500mg/kg) | Group V, Cystone-treated |
|---|---|---|---|---|---|
| **Urine (mg/dl)** | | | | | |
| **Oxalate** | 0.37 ± 0.03 | 3.64 ± 0.11 | 1.60± 0.03 | 1.80± 0.05 | 0.53 ± 0.04 |
| **Calcium** | 1.27 ± 0.07 | 4.51 ± 0.10 | 2.21 ± 0.05 | 2.96 ± 0.06 | 1.50 ± 0.06 |
| **Phosphate** | 3.67 ± 0.04 | 7.29 ± 0.06 | 4.13± 0.05 | 4.87± 0.04 | 3.81 ± 0.09 |
| **Kidney (mg/g)** | | | | | |
| **Oxalate** | 1.41 ± 0.06 | 5.73 ± 0.06 | 1.95 ± 0.04 | 2.66 ± 0.08 | 1.61 ± 0.06 |
| **Calcium** | 3.23 ± 0.04 | 4.79 ± 0.16 | 3.68± 0.03 | 4.58± 0.07 | 3.42 ± 0.07 |
| **Phosphate** | 2.35 ± 0.03 | 3.74 ± 0.10 | 2.36± 0.04 | 3.16± 0.08 | 2.52 ± 0.07 |
| **Serum (mg/dl)** | | | | | |
| **BUN** | 37.61 ± 0.15 | 49.97 ± 0.48 | 32.14± 0.17 | 39.12± 0.22 | 39.30 ± 0.48 |
| **Creatinine** | 0.75 ± 0.01 | 0.94 ± 0.03 | 3.12± 0.05 | 1.08± 0.03 | 0.81 ± 0.02 |
| **Uric acid** | 1.49 ± 0.07 | 3.64 ± 0.11 | 1.95± 0.03 | 2.33± 0.05 | 1.71 ± 0.04 |

***All values are expressed as mean ± S.D. for six animals in each group. [a]Comparisons are made with Group I Vs Treatment group. [b]Comparisons are made with Group IV Vs Test group. Statistically significant at P < 0.05; P < 0.01.***

# DISCUSSION

In the present study, rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans. Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 days period of ethylene glycol (0.75%, v/v) administration, young albino rats form renal calculi composed mainly of calcium oxalate. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate (Selvam et al., 2001). Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate. In the present study, oxalate and calcium excretion and progressively increased in calculi-induced animals. Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria, the changes in urinary oxalate levels are relatively much more important than those of calcium (Robertson and Peacock, 1980). Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth. However, *Vediyuppu cheyaneer* lower the levels of oxalate as well as calcium excretion. An increase in urinary phosphate is observed in calculi-induced rats. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition (Roger et al., 1997). Treatment of *Vediyuppu cheyaneer* restores phosphate level, thus reducing the risk of stone formation. In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood. In calculi-induced rats, marked renal damage was

seen as indicated by the elevated serum levels of creatinine and uric acid and BUN. However, the curative treatment with *Vediyuppu cheyaneer* causes diuresis and hastens the process of dissolving the performed stones and prevention of new stone formation in urinary system. The significant lowering of serum levels of accumulated waste products is attributed to the enhanced GFR of *Vediyuppu cheyaneer*. Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart. This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure. Thus, diuretics play an important role in hypertensive patients. In present study, it was confirmed that *Vediyuppu cheyaneer* has diuretic effect by increasing the excretion of Na+, K+ and Cl -. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles. The regulation of Na+/ K+ balance is also intimately related to renal control of acid-base balance. The K+ loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended. In present study, ash and successive aqueous and ethanol extract showed elevated levels of K+ in urine, which may increase risk of hypokalemia, and hence its potassium sparing capacity has to be investigated. These active principles in *Vediyuppu cheyaneer* may be responsible for diuretic activity. Isolation of these active principles and study of their exact mechanism of action needs to be investigated. Results of present investigation showed that *Vediyuppu cheyaneer* with lime is most effective in increasing urinary electrolyte concentration of all the ions i.e. Na+, K+ and Cl –.

## CONCLUSION

In conclusion the presented data indicate that administration of the *Vediyuppu cheyaneer* to rats with ethylene glycol induced lithiasis reduced the growth of urinary stones. Further studies are required to understand the mechanism underlying this effect, but is apparently related to increased diuresis and lowering urinary concentrations of stone forming constituents.

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