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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of *Ficus carica* fruit Extract against Ethylene Glycol Induced Urolithiasis in Albino Wistar Rats

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ABSTRACT
Ficus carica is an edible fruit of Asian species used as medicine for number of centuries. The objective of the present study was to evaluate the effect of Ficus carica fruit on ethylene glycol induced urolithiasis in albino wistar rats. The preliminary phytochemical investigation was carried out with aqueous extract of Ficus carica (FCAE)for identification of phytochemical constituents. The male wistar rats were selected, divided into five groups and each group consists of 6 animals Group I(normal control), Group 2 (disease control), Group 3 (EG + Cystone 750mg/kg), Group 4 (FCAE-200mg/kg) and Group 5 (FCAE- 400mg/kg).Ethylene glycol 0.75% v/v was added with in drinking water for inducing renal calculi to all groups of animals except control group up to 28 days. After completion of treatment with drug and fruit extract, Serum and urine samples were collected analyzed to find out various parameters. The serum biochemical parameters like creatinine, urea, uric acid, and calcium were significantly reduced in animals treated with FCAE at the dose of 400mg/kg. Reduction of these parameters indicates that FCAE have potential anti-urolithiatic effect. The present study proved that Ficus carica has regulating effect on calculi formation, there by indicating its positive role in treating the Urolithiasis.
Key words: *Anti-urolithiatic activity, Ficus carica, ethylene glycol, ammonium chloride, cystone.*

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INTRODUCTION

Urolithiasis is a common clinical disorder. The stones or calculi are formed in anywhere in the urinary tract is called Urolithiasis. It differentiated into Nephrolithiasis (Calculi in kidney),Ureterolithiasis (Calculi in the ureters) and Cystolithiasis(Calculi in urinary bladder).Based on mineral composition, stones are of different types they are Struvite (magnesium ammonium phosphate), calcium oxalate, urate, cystine and silica.In most of the cases common component is either calcium oxalate or magnesium ammonium phosphate. The urinary stone disease is a common disorder estimated to occur in nearly 12% of the population with a recurrence rate of 70-80% in male, and 47-60% in female. The mechanism involved is crystal nucleation, aggregation and formation of insoluble particles. Urine supersaturated with common stone forming minerals but crystallization inhibiting capacity of urine does not allowed the formation of urinary calculi or urolithiasis whereas natural crystallization inhibiting capacity is deficient in stone formers.

The incidence of acute renal colic is about 1 to 2 cases per 1,000 people and the average life time risk is around 5-10%. When compared to women, men are more commonly affected and the male: female ratio is 3:1. This may due to lifestyle-associated factors, like obesity and Westerndiet. The peak age is between 30 and 50 for developing stones and its recurrence is common [1, 2].Risk of kidney stones was not associated with physical activity [3]. But risk factor was high with the copper, moderate with total manganese intake and no risk with the Zinc and iron intake [4].

Renal cells damage due to calcium oxalate crystals and which causes the generation of reactive oxygen species leads to further damage by lipid peroxidation (LPO)[5]. LPO mediated by free radicals and it not only damage the renal cells but also affects the retention[6]. It improved by the use of antioxidant rich herbal formulations as an antiurolithiatic drug[7]. Fig fruits (*Ficus carica*) used widely to treat health disorders like cancer, liver diseases, diabetes, tuberculosis, pyretic, leprosy, anemia and ulcers. *Ficus carica* is enriched with antioxidants, minerals, vitamins, dietary fibers, carbohydrates and high number of amino acids and it is free of fats and cholesterol. Leaves and fruits of fig were having excellent source of

volatile compounds, phenolic and organic acid there by, it might be decreased the risk of urolithiasis.This would throw light on the antiurolithiatic potential of the statistically optimized aqueous extract of *Ficus carica*[8, 9, 10].

MATERIAL AND METHODS

Preparation of Extract:

The *Ficus carica* fruits are powdered in a mechanical grinder. The collected powder was extracted with water by using Soxhlet apparatus. The extraction was carried out for 72 hrs at a temperature not exceeding the boiling point of the solvent. Solvent evaporation method was used to remove the excess solvent and obtained the dry weight of plant extract.

Preliminary Phytochemical Screening:

The preliminary phytochemical investigation was carried out with FCAE to identify the phytochemical constituents by standard methods.

Selection of Experimental Animals and Animal Care:

For this study Male Wister rats (200-300g) were selected, the animals were acclimatized for 7 days in the animal house of Chalapathi Institute of Pharmaceutical Sciences, maintained standard conditions like temperature (24 ± 10° C), relative humidity (45-55%) and 12:12 light: dark cycle. During this period animals were free access to water and *ad libitum* standard rat pellet diet.

Ethical Approval:

All the protocols were approved by Institutional Animal Ethical Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences and conducted according to Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA)registeredno:1048/PO/Re/S/07/CPCSEA, with (Approval no.05/IAEC /CLPT/2018-2019; Dt: 05/01/2019) Guntur.

Grouping of animals:

Male Wistar rats were divided into five groups of each containing six animals.

Group-I: Normal saline treated as normal control

Group II: Ethylene glycol (0.75%) + A.C (2%) as a disease control,

Group III: Ethylene glycol (0.75%) and Ammonium chloride (2%) along with Cystone-750mg/kg (Orally, standard).

Group IV: Ethylene glycol (0.75%) and Ammonium chloride (2%) along with FCAE (200mg/kg)

Group V: Ethylene glycol (0.75%) and Ammonium chloride (2%) along with FCAE (400mg/kg).

Pharmacological Screening for anti urolithiatic activity:

Ethylene glycol induced urolithiasis: Ethylene glycol is added with drinking water for inducing the urolithaisis at 0.75% v/v of was fed to all groups except control till the 28th day and test groups were received FCAE (once daily by oral route) from 15th day till 28th day, cystone was used as standard drug (750mg/kg body weight) to the group-III. During these study animals was free access to food.

Assessment of Anti-Urolithiatic Activity:

Collection and analysis of urine: Urine samples were collected by keeping the animals in individual propylene metabolic cages with free access to drinking water in metabolic cages. Microscopic examination of urine can also be done.

Serum analysis: Blood was withdrawn from the eyes by retro-orbital puncture under anesthetic conditions on the 28th day. Serum was separated by centrifugation at 10,000 RPM for 10 minutes and was analyzed for creatinine, urea, nitrogen, calcium and uric acid using spectrophotometric method.

Effect of plant extract on the kidney weight: The effect of FCAE was assessed by measuring the weight of the kidneys which were isolated from normal control, disease control, cystone and FCAE treated group animals. The FCAE treated group gained the least weight compared to the disease control and cystone treated groups.

Histopathology: All the animals were sacrificed at the end of 28th day, kidneys were isolated, cleaned off to remove the extraneous tissue and it transferred to 10° F neutralized formalin solution (pH 7.4)and subjected to the histopathological studies to confirm the incidence of renal calculi.

RESULTS AND DISCUSSION:

Phytochemical analysis:

FCAE was subjected for preliminary phytochemical analysis and the presence of following phytochemical constituents were observed.

Table-1:Phytochemical Constituents of FCAE

Phytochemicalconstituents	FCAE
Alkaloids	++
Carbohydrates	+
Flavonoids	++
Phenols	+
Saponins	+++
Terpenoids	+
Sterols	+
Tannins	-
Quinons	+++
Anthraquinons	-
Glycosides	++
Coumarins	-

+ indicates the compulsory present, **++**indicates the slightly present**, +++**indicates the moderately present**, -**indicates the absent

Qualitative analysis of FCAE:

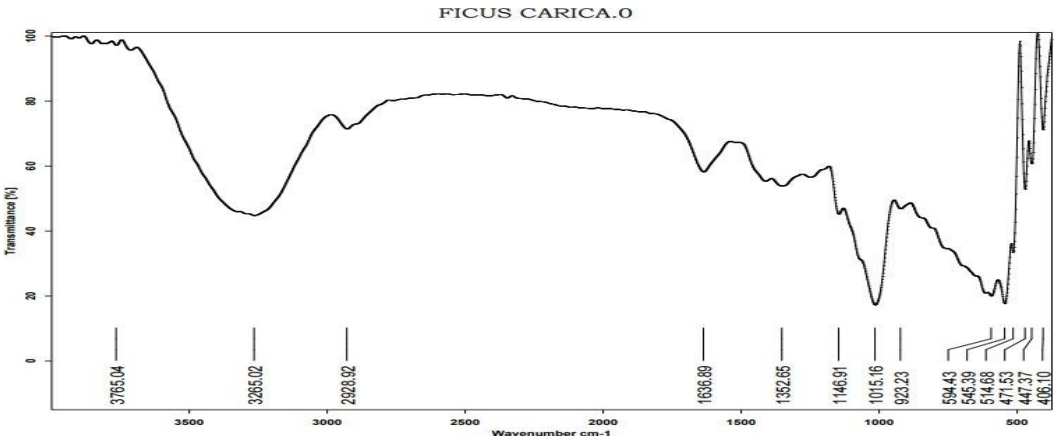


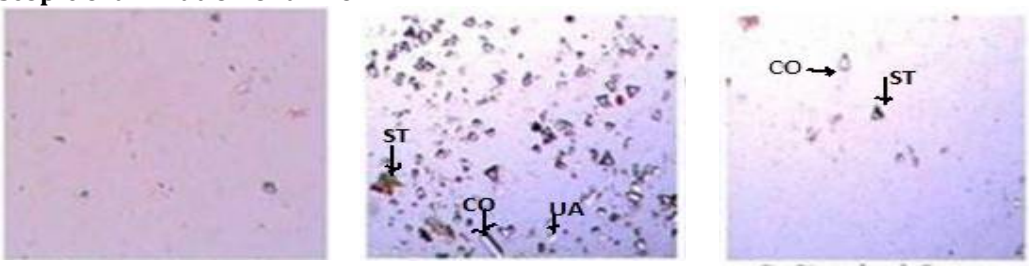
Figure-1: IR – Spectrum of aqueous extract of *Ficus carica*

Table-2: Qualitative analysis of FCAE

Functional groups		Wave number(cm^{-1})	
		Stretching	Bending
O-H	Alcohol	3265	-
C-H	Alkyl	2928	-
C=O	Carbonyl	1636	-
C-O	Ether	1015	-
C=C	Alkane	1352	-

From the IR spectral data, the presence of functional groups *viz.,* Alcohol (-OH), Alkyl (C-H), Carbonyl (C=O), Ether (C-O) and Alkane (C=C) were observed (Table-2).

Microscopic examination of urine:



A) Normal Control **B) Disease Control** **C) Cystone Treated**

D) FCAE (200 mg/kg) **E) FCAE (400 mg/kg)**

Figure-2: Microscopic examination of urine indicates presence of **ST-**Struvite crystals; **UA**- Uric acid crystals;**CO –** Calcium oxalate crystals in Normal control, disease control, Cystone treated, FCAE (200 mg/kg) and FCAE (400 mg/kg).

From microscopical examination, normal control group animals not shown remarkable crystals (Figure-2A), while disease control group animals contains the crystals of calcium oxalate, struvite and uric acid crystals (Figure-2B). In Cystone (Standard drug) treated group number crystal was debased (Figure-2C). In FCAE treated groups at least concentration has moderate recovery is observed (Figure-2D). In FCAE treated groups at high concentration has prominent recovery from renal calculi been observed in(Figure-2E).

Evaluation of urine parameters:

In present study ethylene glycol administered group (disease control) significantly elevated the urinary creatinine, calcium, urea, uric acid levels when compared to control group (Table-3; Figure-3).Evidence suggest that ethylene glycol fed rats developed renal stone due to hyperoxaluria; renal retention. Excessive excretion of oxalate was increased in urine in all animals received ethylene glycol [11].

Table-3: Effect of FCAEon urine parameters in urolithiatic rats

Groups	Creatinine (mg/dL)	Calcium (mg/dL)	Uricacid (mg/dL)	Urea (mg/dL)
Control	2.09±1.40	6.79±1.13	3.83±1.86	55.78±1.95
Disease control	5.36±2.07	18.93±1.45	7.97±0.90	71.51±2.13
Standard	2.32±0.59*	12.18±1.60***	4.56±0.91**	63.99±1.66***
FCAE-(200mg/kg)	2.35±1.06*	14.10±1.98***	2.50±1.46***	68.22±1.21*
FCAE-(400mg/kg)	2.87±1.22*	13.15±1.43***	1.99±1.34***	62.75±1.37***

Note: The values were expressed in mean ± SEM (n=6); (*p <0.05, **p < 0.01, ***p<0.001) significant while compare with disease control.

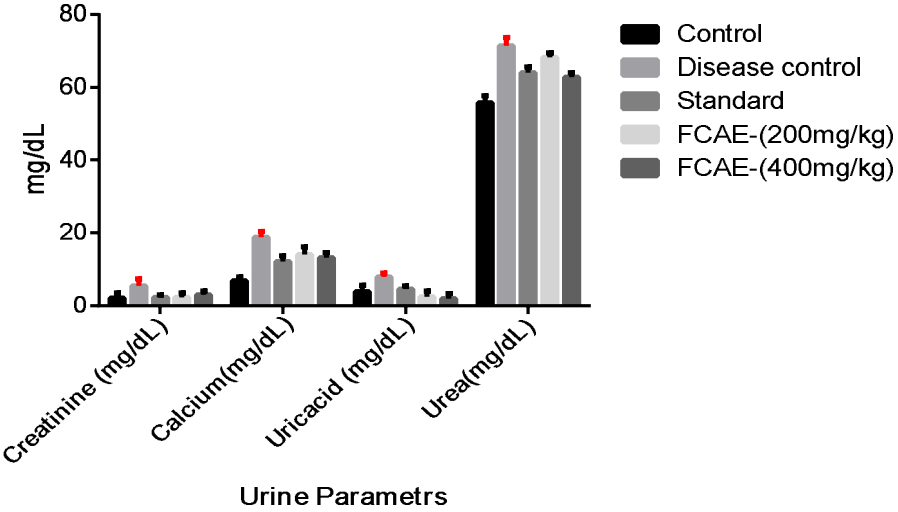


Figure-3: Effect of FCAE on urinary parameters in ethylene glycol induced urolithiatic rats

FCAE contains polyphenols and flavonoids which show the good antioxidant properties; Based on this evidence the fruit extract might reduce the uric acid, urea nitrogen and creatinine levels in diabetic rats while treated with *Ficus carica*[12]. In the present study the animals treated with FCAE400mg/kg and

FCAE200mg/kg, showed significantly reduces the creatinine, calcium, urea, and uric acid in urine when compared to the disease control (Table-3).FCAE at the 400mg/kg was significantly reduced the urinary parameters which is equally as compared to cystone treated(Table-3).Which is due to the presence of flavonoids and polyphenols with their antioxidant properties of *Ficus carica*.

Evaluation of serum parameters:

In present study Ethylene glycol administration showed a significantly elevation in the serum creatinine, calcium, urea, uric acid levels when compared to normal group (Table-4). In context, Obstruction to the outflow of urine by calculi in urinary system leads to decrease in glomerular filtration rate(GFR) has been reported the accumulation of urea, creatinine and uric acid in blood in urolithiasis.

Table-4: Effect FCAE on blood parameters in urolithiatic rats

Groups	Creatinine (mg/dL)	Calcium (mg/dL)	Uricacid (mg/dL)	Urea (mg/dL)
Control	1.0±0.68	3.86±1.61	4.05±1.47	31.15±1.98
Disease control	3.38±0.72	10.48±1.82	7.09±1.53	54±1.72
Standard	1.78±0.8*	4.12±1.63***	3.91±1.04**	44.96±1.73***
FCAE (200mg/kg)	0.55±0.16***	4.90±1.49***	1.80±1.47***	49.53±2.47**
FCAE (400mg/kg)	2.52±0.96	4.04±1.72***	2.00±1.64***	45.25±1.89***

Note: The values were expressed in mean ± SEM (n=6); (*p <0.05, **p < 0.01, ***p<0.001) significant while compare with disease control.

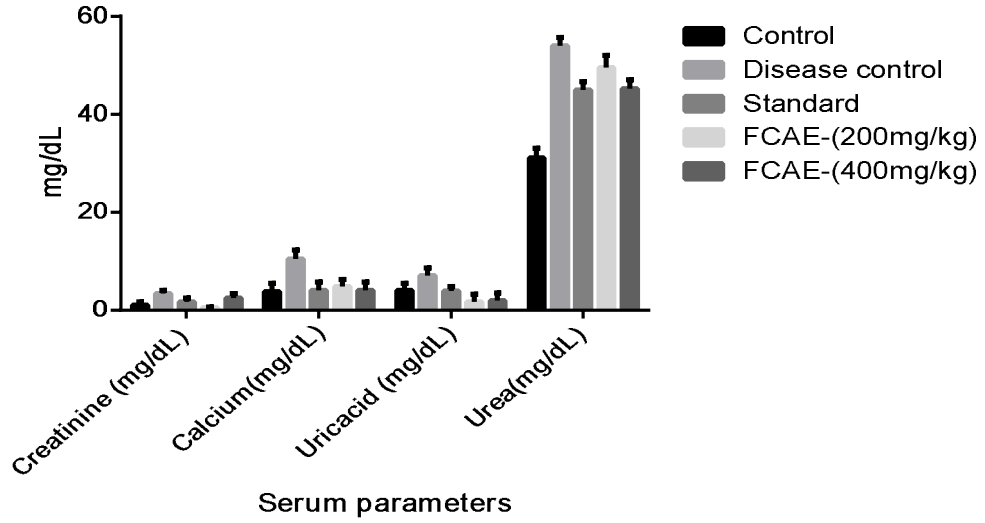
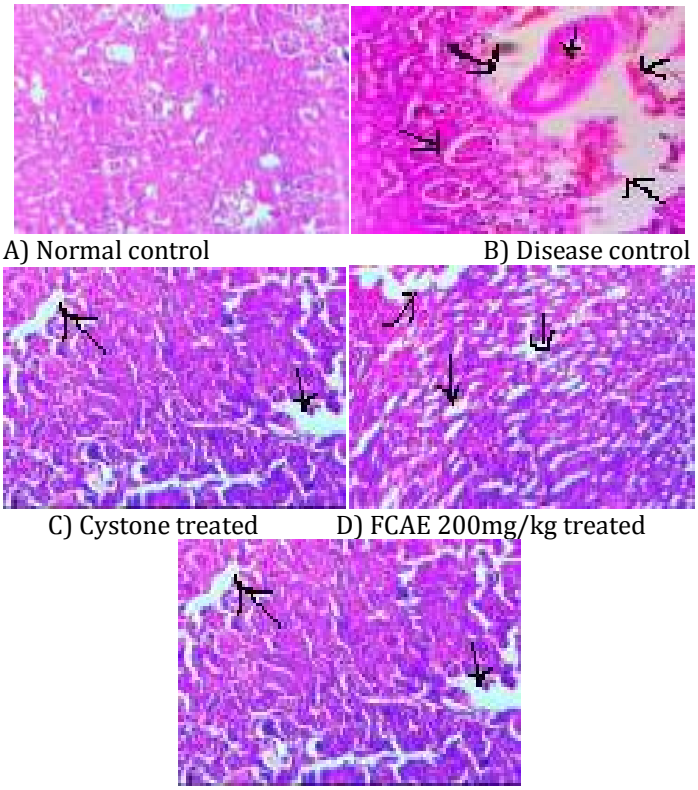


Figure-4: Effect of FCAE on serum parameters in ethylene glycol induced urolithiatic rats

Flavonoids have major role in the prevention of oxidative stress and disorders related to stress in human beings, evidence had that, reduced the uric acid, urea, nitrogen and creatinine in diabetic rats while treating with *Ficus carica* because of its antioxidant properties of polyphenoles and flavonoids. Based on this results obtained from the phytochemical studies of *Ficus carica* fruits and leaves, those having good antioxidant property; because of the presence of flavonoids, Vitamin C, Saponins, tannins, organic acids, and volatile compounds [12-16]. In present study, animals treated with FCAE400mg/kg and FCAE200mg/kg was significantly reduced the serum parameters like creatinine, calcium, uric acid, urea which is similar to that of standard and normal control. Specifically FACE at the dose of 400mg/kg was showed significant reduction in serum parameters. This was because of flavonoids, quinines, saponins and alkaloids in the FCAE(Table.1.)

Histopathological profile of normal control group rat kidney showed normal Bowman's capsule (Fig-5A).Ethylene glycol treated animals, dilatation of proximal tubules, focal basophilic tubules with thickened basement membrane and mild inflammatory cell infiltration was observed (Fig-5B). Evidence, calcium oxalate lithiasis patients have chronic pro- inflammatory intestinal dysbiosis followed by total number of bacteria and immunoregulating microbe's reduction and prominent reduction observed in muconutritive microbes [17].



A) Normal control B) Disease control

C) Cystone treated D) FCAE 200mg/kg treated

E) FCAE 400mg/kg treated

Figure-5:(A)-Histology of normal control; (B) Histology of the diseased animal; (C) Histology of the cystone (750 mg/kg) treated animal; (D) - Histology of the FCAE (200 mg/kg) treated animal; (E) Histology of the FCAE (400 mg/kg) treated animal**.**

Histology of cystone (750 mg/kg) treated group rat kidney showed mild colloidal cast inside tubules, cloudy changes and congestion of these glomeruli, evident by increased urine volume (Figure-2C/5C). Whereas the FCAE at the dose range of 200mg/kg treated animals showed mild dilated distal tubules in the renal cortex as well the congestion of blood vessels and the renal epithelial cell recovery, reduced cast density, improved renal architecture(Figure-5D) which is evidenced by the biochemical parameters (Table – 3 and 4). However, the FCAE at the dose range of 400mg/kg, animals was found to be less significant compared to the cystone treated group, the normalizing proximal and distal tubules in the renal cortex, normal glomeruli as well the congestion of blood vessels producing renal tubular epithelial cell recovery (Figure-5E).

CONCLUSION

The anti-urolithiatic effect of aqueous extract of *Ficus carica* have been investigated in the present study. Preliminary phytochemical investigation supported the presence of carbohydrates, flavonoids, glycosides, saponins, carbohydrates, phenolic compounds, terpenoids and sterols. The presented data indicates that FCAE found to improve the renal functions by reducing the elevated levels of creatinine, uric acid, calcium, which was evidenced by reversing the dilation of distal and proximal tubules in renal cortex supported by histopathological studies. FCAE treated groups significantly reduced the elevated serum biochemical as well urine parameters thereby improving the renal functioning. The possible protective action of FCAE could be the counter action againstrenal calculi caused by the reactive oxygen species or by attenuating the inflammatory infilatration induced by ethylene glycol. It was observed that FCAE having more potent anti-urolithiatic activity in ethylene glycol induced urolithiasis rat model.

CONFLICT OF INTEREST: No

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