Antiurolithiatic activity of *Coffea arabica* seed extract

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ABSTRACT

Evaluated the antiurolithiatic activity of Coffea arabica seed extract in validated experimental animal models. The antiurolithiatic activity of Coffea arabica seed extract in Ethylene Glycol and Sodium Oxalate induced animals. Preliminary phytochemical screening was carried out for Coffea arabica seed extract. The parameters like body weight, kidney weight, urine analysis, serum analysis and histopathology of kidney were studied. Ethylene Glycol and Sodium Oxalate administration resulted in hyperoxaluria as well as increased excretion of calcium, oxalate, phosphate and potassium. Treatment with Coffea arabica seed extract produced significant reduction of excretion of calcium oxalate in ethylene glycol and sodium oxalate induced animals. Treatment with Cystone 750mg/kg and Coffea arabica (low dose 100mg/kg, high dose 250mg/kg) increases the level of magnesium in ethylene glycol and sodium oxalate animals. Magnesium level which helps in prevent the formation of calcium oxalate crystals in ethylene glycol and sodium oxalate induced urolithiatic animals. Coffea arabica seed extract (low dose 100mg/kg, high dose 250mg/kg) significantly reduces the activity of calcium oxalate in the kidney indicates the reduction of stone formation. The present study concluded that Coffea arabica seed extract was found to be effective against reduction of stone formation induced by ethylene glycol and sodium oxalate.

Keywords:- *Hyperoxaluria, Ethylene Glycol, Sodium Oxalate, Coffea Arabica*

1. INTRODUCTION

Urolithiasis is the process of forming stones in the kidney, bladder, and/or urethra. Urolithiasis, which is referred to as the process of formation of calculi (singular calculus) in the urinary system includes nephrolithiasis (renal calculi or kidney stones), ureterolithiasis (ureter calculi) and cystolithiasis bladder calculi). These calculi (stones) create problems by blocking the flow of urine and cause severe pain termed as renal colic when they move along the ureter. Urolithiasis can also be associated with morbidity and renal damage. The disease affects all age groups from less than 1 year old to more than 70 years. After their initial stone episode the recurrence rate of stone is approximately 10% within one year, 35% within five years, and 50% within 10 years [1]. The medical management of urolithiasis involves drug treatment and extracorporeal shock wave lithotripsy (ESWL). In present day management of urolithiasis with open renal surgery is an unusual and rarely used one since the introduction of ESWL, which has almost become the standard procedure for eliminating the kidney stone [2].

Kidney stones are mainly lodged in the kidney(s) [3]. Mankind has been afflicted by urinary stones since centuries dating back to 4000 B.C. and it is the most common disease of the urinary tract. The prevention of renal stone recurrence remains to be a serious problem in human health. The prevention of stone recurrence requires better understanding of the mechanisms involved in stone formation [4]. Kidney

stones have been associated with an increased risk of chronic kidney diseases [5], end-stage renal failure, cardiovascular diseases, diabetes, and hypertension [6]. It has been suggested that kidney stone may be a systemic disorder linked to the metabolic syndrome. Nephrolithiasis is responsible for 2 to 3% of end-stage renal cases if it is associated with nephrocalcinosis [7].

The symptoms of kidney stone are related to their location whether it is in the kidney, ureter, or urinary bladder [8]. Initially, stone formation does not cause any symptom. Later, signs and symptoms of the stone disease consist of renal colic (intense cramping pain), flank pain (pain in the back side), hematuria (bloody urine), obstructive uropathy (urinary tract disease), urinary tract infections, blockage of urine flow, and hydronephrosis (dilation of the kidney). These conditions may result in nausea and vomiting with associated suffering from the stone event [9]. Thus, the treatment and time lost from work involves substantial cost imposing an impact on the quality of life and nation's economy.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant material:

The fresh *Coffea arabica* seeds used for the present studies was collected from local market at Mangalore. It was authenticated.

2.2 Preparation of *Coffea arabica* seed extract

Coarsely powdered unbaked beans of *Coffea arabica* 250g were subjected to successive extraction by cold maceration for 7 days. The extraction was done with ethanol. The extract was concentrated by rotary vacuum evaporator and evaporated to dryness.

2.3 Preliminary qualitative phytochemical analysis

Preliminary phytochemical analysis of extract was conducted to check the presence of various phytoconstituents.

3. EXPERIMENTAL ANIMALS

Healthy Male Wistar Albino rats (150 to 200 g) were used for this study. They were maintained under standard conditions (temperature 25 ± 2°C, relative humidity 60±5% and 12 h light/dark cycle) and had free access to standard pellet diet and water *ad libitum*. The animals were housed in sanitized polypropylene cages with stainless steel top grill having facilities for pelleted food. The Institutional Animal Ethics Committee reviewed and approved the experimental protocol. All the procedures was performed in accordance with Institutional Animal ethics committee constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

4. PHARMACOLOGICAL EVALUATION

Antiurolithiatic activity of *Coffea arabica* was evaluated by following animal models.

1) Ethylene glycol (0.75%) induced hyperoxaluria model

2) Sodium oxalate induced hyperoxaluria model

5. EXPERIMENTAL DESIGN

5.1 Ethylene glycol induced urolithiatic model [10]

The Wistar albino rats (150g-200g) of either sex was randomly divided into four groups (n=6) as follows.

Group I: Control rats (normal diet)

Group II: Urolithiasis induced rats (Ethylene glycol 0.75% v/v in drinking water)

Group III: Urolithiasis induced rats (Ethylene glycol 0.75% v/v + Cystone 750mg/kg)

Group IV: Urolithiasis induced rats (Ethylene glycol 0.75% v/v + *Coffea arabica* 100mg/kg)

Group V: Urolithiasis induced rats (Ethylene glycol 0.75% v/v + *Coffea arabica* 250mg/kg)

Group I animals served as normal control, maintained on regular laboratory diet and water *ad libitum*. Group II to IV animals was fed with 0.75% Ethylene Glycol (EG) in water for induction of renal calculi till 28th day. Group III was received reference standard antiurolithiatic drug Cystone (750mg/kg body weight) from 15th to 28th day. Group IV was received *Coffea arabica* (100mg/kg body weight) from 15th day to 28th day respectively. and group v was received *Coffea arabica* (250mg/kg body weight) All the treatment was done orally for 28 days.

A) Collection and analysis of urine

All animals was kept in individual metabolic cages and urine samples of 24h were collected on 28th day. A drop of concentrated hydrochloric acid would be added to the urine before being stored at 4°C and it is analyzed for calcium, magnesium, sodium and oxalate by standard procedure.

B) Serum Analysis

Blood was collected from the retro orbital under anesthetic condition and animals was sacrificed by decapitation. Blood samples was separated by centrifugation at 10,000rpm for 10 minutes and analyzed for creatinine and uric acid.

5.2 Sodium oxalate induced urolithiatic model [11]

The Wistar albino rats (150g-180g) of either sex was randomly divided into four groups (n=6) as follows.

Group I: Control rats (vehicle control)

Group II: Urolithiasis induced rats (Sodium oxalate 70 mg/kg)

Group III: Urolithiasis induced rats (Cystone 750mg/kg + Sodium oxalate 70 mg/kg)

Group IV: Urolithiasis induced rats (*Coffea arabica* 100mg/kg + Sodium oxalate 70 mg/kg)

Group V: Urolithiasis induced rats (*Coffea arabica* 250mg/kg + Sodium oxalate 70 mg/kg)

Group I animals served as normal control, maintained on regular laboratory diet and water ad libitum. In Group II to IV animal's urolithiasis was induced by intra peritoneal injection of sodium oxalate (70 mg/kg). Group III was received reference standard Antiurolithiatic drug Cystone (750mg/kg body weight). Group IV was received Coffea arabica(250mg/kg body weight). All the treatment was done orally for 7 days.

A) Collection and Analysis of Urine samples

On 7th day all animals were kept in individual metabolic cages and urine samples was collected for 24hr. 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 and it was analyzed for calcium, magnesium, creatinine and oxalate by standard procedure.

B) Serum analysis

Blood was collected from the retro orbital under anesthetic condition and animals were sacrificed by decapitation. Blood samples was separated by centrifugation at 10,000rpm for 10 minutes and analyzed for creatinine and uric acid.

6. HISTOPATHOLOGICAL STUDIES

At the end of the experiment animals were sacrificed, kidneys were excised, washed with normal saline and fixed with formalin for histology studies using hematoxyline eosin.

7. STATISTICAL ANALYSIS

All data were expressed as mean ± SEM. The statistical significance between groups was compared using one-way ANOVA, followed by Tukey's (Multiple comparison test) and student t test.

8. RESULTS

8.1 DETERMINATION OF ANTIUROLITHIATIC ACTIVITY

In the present study, Ethylene Glycol and Sodium Oxalate induced urolithiatic model was used to screen the antiurolithiatic activity of *Coffea arabica* in experimental animals.

8.2 PRELIMINARY PHYTOCHEMICAL SCREENING

Results of the preliminary phytochemical investigation *of Coffea arabica* seed extract is shown in table.

Table -1: Preliminary phytochemical screening of *Coffea arabica* seed extract

TEST	RESULTS
Alkaloids	+ve
Flavonoids	+ve
Proteins	+ve
Polyphenols	+ve

9. EFFECT OF *COFFEA ARABICA* SEED EXTRACT ON ETHYLENE GLYCOL INDUCED UROLITHIATIC ANIMALS

Table -2: Effect of *coffea arabica* seed extract on ethylene glycol induced urolithiatic animals

Urine parameters	**Group 1 Normal control**	**Group 2 (urolithiatic control)**	**Group 3 Cystone (750 mg/kg)**	**Group 4 (Coffea arabica low dose 100mg/kg**	**Group 5 (Coffea arabica high dose 250mg/kg**
Calcium	1.27±0.01	4.49±0.02	1.48±0.02***	2.27±0.02**	1.98±0.01**

Oxalate	0.34±0.01	3.61±0.04	0.54±0.01***	1.30±0.01***	0.83±0.01***
Phosphate	3.63±0.05	7.18±0.02	3.81±0.01***	5.26±0.02**	4.10±0.01**
Magnesium	1.39±0.01	0.92±0.01	1.37±0.01***	0.62±0.01***	0.79±0.01**
Creatinine	0.31±0.01	9.48±0.01	3.72±0.01***	7.51±0.02**	4.10±0.02**

All values are expressed as mean ± SEM n=6, One way analysis variance (ANOVA) followed by tukeys multiple comparison test *P<0.05 **P<0.01 ***P<0.001 compared with urolitiatic control groups.

Table -3: Effect of *Coffea arabica* seed extract on serum parameters is Ethylene Glycol induced Urolithiatic animals

Serum parameters	**Group 1 Normal control**	**Group 2 (urolithiatic control)**	**Group 3 Cystone (750mg/kg)**	**Group 4 (Coffea arabica low dose 100mg7kg)**	**Group 5 (coffes Arabica high dose 250mg/kg)**
Creatinine	0.74±0.01	2.08±0.01	0.82±0.01***	1.20±0.01**	1.09±0.01**
Uric acid	1.40±0.01	3.49±0.01	1.71±0.01***	2.93±0.01**	1.54±0.01**

All values are expressed as mean ± SEM n=6, One way analysis variance (ANOVA) followed by tukeys multiple comparison test *P<0.05 **P<0.01 ***P<0.001 compared with urolitiatic control groups

Table -4: Effect of *Coffea arabica* seed extract on body weight and kidney weight in Ethylene Glycol induced urolithiatic animals

Parameters	**Group 1 Normal control**	**Group 2 (urolithiatic control)**	**Group 3 Cystone (750mg/kg)**	**Group 4 (Coffea arabica low dose 100mg/kg)**	**Group 5 (Coffea arabica high dose 250 mg/kg)**
Body weight,gain(g)	57.78±0.21	24.32±0.20	40.53±0.18***	26.98±0.20**	32.05±0.16**
Kidney weight (g/100g b.w.)	1.21±0.01	2.23±0.01	1.87±0.11**	2.12±0.01*	1.71±0.01*

Values are expressed as mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 as compared with urolithiatic control followed by Student's t-test.

10. HISTOPATHOLOGICAL STUDIES

10.1 Ethylene glycol induced urolithiasis

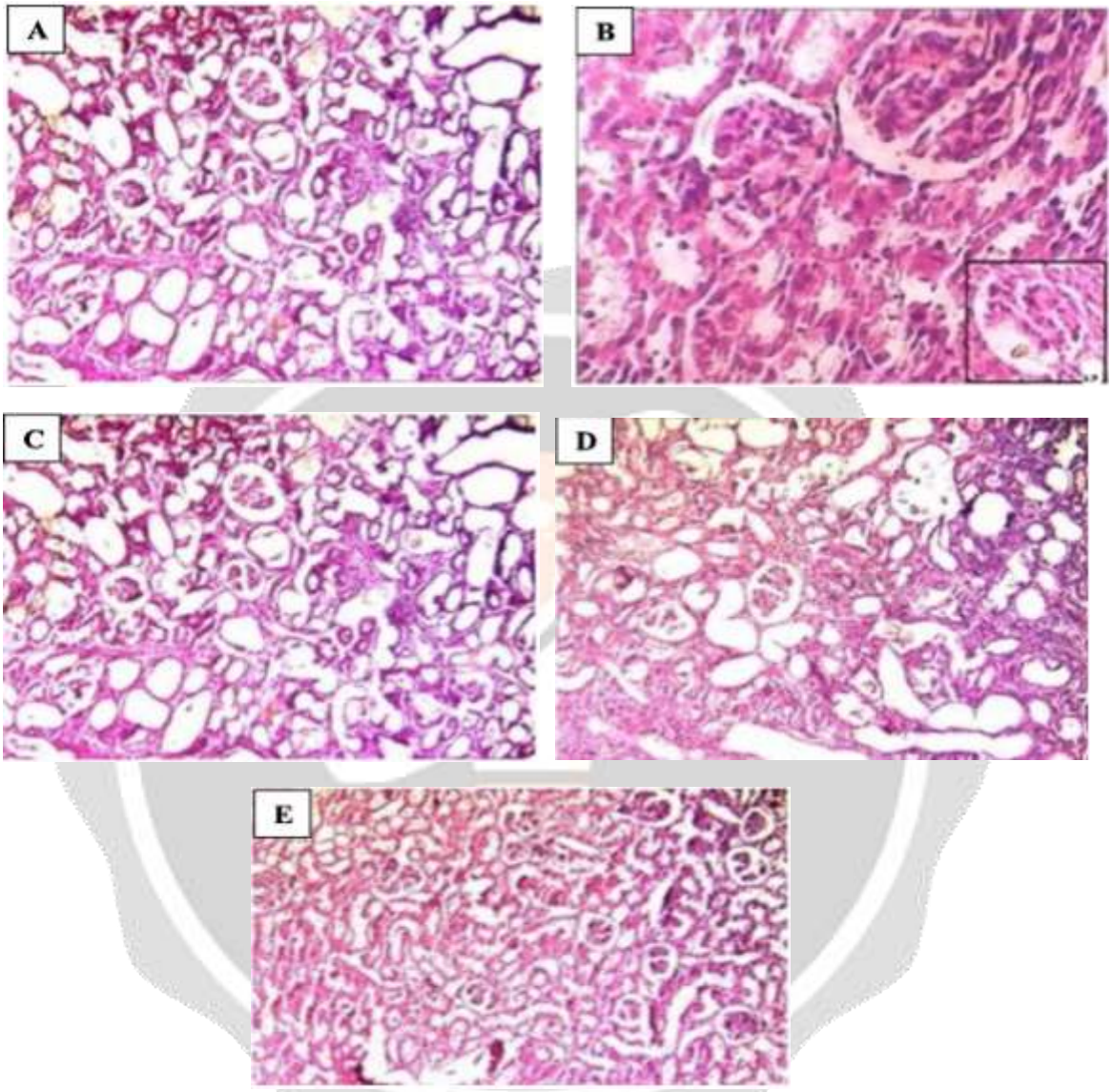


Figure -1: Histopathology of Kidney in Ethylene Glycol induced Urolithiatic models

Kidney histopathological analysis revealed no CaOx crystal deposit or other abnormalities in the kidney of the control group (Group I) as shown in [Figure 1]A. On the other hand, many CaOx crystal deposits in the renal tubules and congestion and dilation of the parenchymal blood vessels were seen in the renal tissue of the stone-induced group (Group II) as shown in [Figure 1]B. In the standard group (Group III), the kidney showed normal architecture with dilation of tubules in the cortico-medullary junction, minima interstitial inflammation, and occasional renal tubules showed CaOx crystal deposits [figure 1]C. In the treatment(Group IV,V), the kidney showed normal architecture and few renal tubules that revealed vacuolar degeneration with no CaOx crystal deposits [Figure 1]D,E

10.2 Sodium oxalate induced urolithiasis

On 8th day all animals was kept in individual metabolic cages and urine samples were collected for 24hr. Animals had free access for drinking water during the urine collection period. The animals were weighed and urine samples

were collected and analysed for biochemical parameters. Blood samples was separated by centrifugation at 10,000rpm for 10 minutes and analysed for serum biochemical parameters.

10.3 Effect of *Coffea arabica* seed extract on urinary parameters (calcium, oxalate, potassium, and creatinine):

Seven days administration of sodium oxalate led to elevation of urinary oxalate levels compared to the control animals. Treatment with cystone significantly reduced the oxalate levels. Treatment with both the low dose and high dose also reduced the oxalate level which was comparable with Cystone. Urinary excretion of calcium, potassium, creatinine was also significantly increased in sodium oxalate induced groups compared to normal group. Treatment with Cystone and *Coffea arabica* reduced the urinary excretion of calcium, potassium, and creatinine. Sodium oxalate administered animals shown reduction in the excretion of magnesium whereas treatment with both Cystone and *Coffea arabica* seed extract normalizes the level of magnesium excretion.

10.4 Effect of *Coffea arabica* seed extract on serum parameters

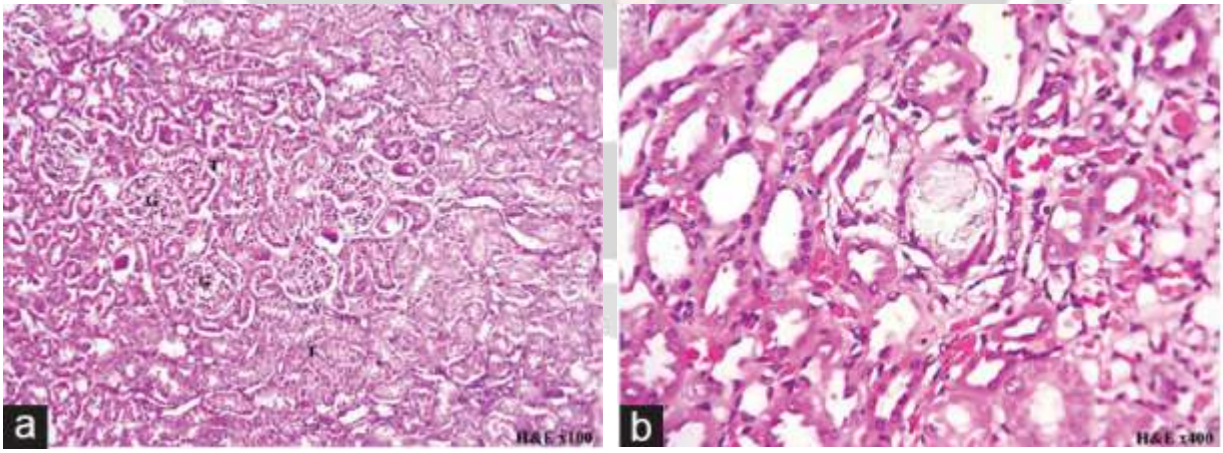
Administered of sodium oxalate led to elevation of serum creatinine and uric acid levels compared to the control animals. Treatment with Cystone and *Coffea arabica* extract significantly reduced the serum creatinine and uric acid.

10.5 Effect of *Coffea arabica* seed extract on body weight and kidney weight

Sodium oxalate administered urolithiatic animals gained the least body weight as compared to normal control whereas treatment made the animals to gain normal body weight during experimental period. In addition, there was a significant increase in the kidney weight of urolithiatic animals, which was normalized in the treated groups.

11. HISTOPATHOLOGICAL STUDIES

11.1 Sodium oxalate induced urolithiasis



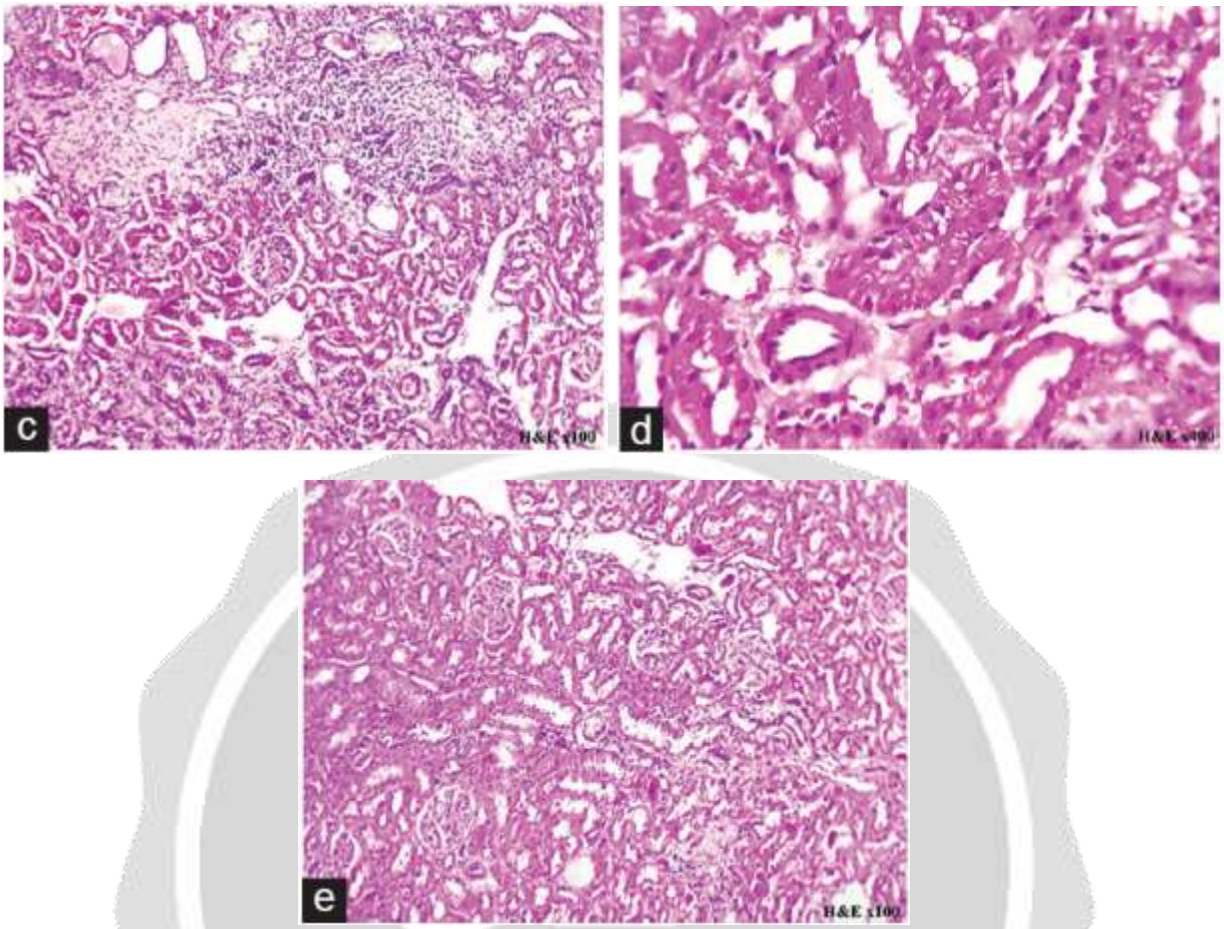


Figure -2: Histopathology of kidney in Sodium Oxalate induced urolithiatic models

12. DISCUSSION

Kidney stone disease is a crystal concretion formed usually within the kidneys. The etiology of kidney stone is multifactorial. *Coffea arabica* has been used for many medicinal purposes. The main constituent present in *Coffea arabica* or coffee is caffeine. other than that *Coffea arabica* consists of other constituents such as chlorogenic acid which is an alkaloid, trigonelline, amino acids , polyphenols and flavonoids . Among these the main constituents responsible for antiurolithiatic effect is flavonoids and polyphenols. Hence the plant is selected for the present study. In the present study, albino rats were selected to induce urolithiasis because the similarity between the urinary system in rats and the humans. Urolithiasis can be produced in rats by induction of acute or chronic hyperoxaluria by using variety of agents such as ethylene glycol, sodium oxalate, ammonium oxalate, and glycolic acid[12]. Administration of EG to the experimental animals for 28 days resulted in substantial elevation of oxalate and deposition of microcrystals in kidney. The level of calcium in urine was increased significantly after administration of ethylene glycol, inducing the nucleation and precipitation of calcium oxalate. Increased excretion of phosphate has been reported in stone formers. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals. Low level of magnesium are also encountered in stone formers as well as in stone forming rats. The magnesium levels return to normal on the drug treatment.

In urolithiasis, there is a decrease in the glomerular filtration due to the obstruction of urine flow by stone in urinary system. This causes the impairment of renal function resulting in decreased excretion of waste product, particularly nitrogenous substances such as urea, creatinine and uric acid with concurrent accumulation in blood.

Sodium oxalate administration causes an increase in the severity of microscopic calcium oxalate crystals deposition along with high crystals concentration in the kidney. The waste products, particularly creatinine and uric acid, accumulate in the blood, leading to marked renal damage.

13. CONCLUSION

The result indicated that the administration of Coffea arabica seed extract significantly reduces the excretion of calcium oxalate in ethylene glycol and sodium oxalate urolithiatic animals. Antiurolithiatic activity of Coffea arabica seed extract was confirmed by measuring the serum marker (creatinine and uric acid), urinary parameter (calcium, oxalate, phosphate, potassium, uric acid and magnesium) and kidney weight and body weight and thus it significantly reduced and prevented the growth of urinary stones.

The preliminary phytochemical screening of the Coffea arabica seed extract revealed the presence of alkaloids, flavonoids, polyphenols and protein. The underlying biochemical mechanism involved in antiurolithiatic activity of Coffea arabica may be due its antioxidant properties. These studies supported the folk information regarding anti-urolithiatic activity of the coffee. Thus present study showed that Coffea arabica seed extract possess antiurolithiatic effect.

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