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Assessment of *Ceiba pentandra* on Calcium Oxalate Urolithiasis in Rats

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ABSTRACT

The effect of oral administration of aqueous and alcohol extracts of bark of Ceiba pentandra on calcium oxalate urolithiasis has been studied in male albino wistar rats. Ethylene glycol and ammonium chloride feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Ethylene glycol is a metabolic precursor of oxalate. Regular administration of ethylene glycol caused hyperoxaluria in ethylene glycol fed animals and causes increased renal retention and excretion of oxalate, calcium and phosphate. Ammonium chloride used in conjunction with ethylene glycol to promote the deposition of Calcium oxalate crystals in rat kidneys. Supplementation with aqueous and alcohol extracts of bark of Ceiba pentandra significantly reduced the elevated urinary oxalate showing a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was significantly lowered by preventive treatment using aqueous and alcohol extracts. The Result of the present study confirmed that Ceiba pentandra can be used as curative agents for urolithiasis.

Keywords: Ceiba pentandra, Ethylene glycol, Ammonium chloride, Hyperoxaluria, Calcium oxalate crystals, Urolithiasis.

INTRODUCTION

Kidney stone formation or urolithiasis is a multifarious process that is the result of an imbalance between inhibitors and promoters in the kidneys [1]. The repetition of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another. Today, many standard pharmaceutical drugs are available to prevent urolithiasis in all patients, but some of the drugs many have adverse effects that compromise their long-term use [2].

In the global countries, numbers of people are increasing which are suffering from urinary stone problem. Approximately 50% of patients with previous urinary calculi have reappearance within 10 years. Stone disease is 2-3 times more common in males than in females. Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a

recurrence rate of 70–81% in males, and 47–60% in females [3]. Not only the humans but animals and birds also suffer from the urinary stone problem. The occurrence in some areas is so disquieting that they are known as ‘Stone Belts’ [4].

Urolithiasis occurs in patients at the age between 20–49 years. Urinary tract calculi are far less common in Native Americans as compared, to the prevalence in Asian and Whites, Africans, African Americans and some natives of Mediterranean regions. Urolithiasis occurs more frequently in hot, arid than in temperate regions [5]. The area of high incidence of urinary calculi include Mediterranean countries, Scandinavian countries, Central Europe, British islands, Northern Australia, Northern India, Pakistan, [4]. In India, 15% of the population of northern India suffers from kidney stones. Nearly, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage. Few cases of urinary calculi are found in southern India, which may be due to regular dietary intake of tamarind [6].

Plants used in traditional medicine to treat kidney stones represent a valuable alternative for the control of this disease. *Ceiba pentandra* (L) Gaertner known as silk cotton tree and locally as “dum” belongs to the Bombacaceae family. Various parts of this plant are widely reputed in African traditional medicine. The plant has been reported to be a useful diuretic and effective remedy against diabetes, hypertension, headache, dizziness, constipation, mental trouble, fever, peptic ulcer, rheumatism, leprosy. [7-10]

This is the first report of assessment of Bioactivity of *Ceiba pentandra* on Calcium Oxalate Urolithiasis in Rats.

MATERIALS AND METHODS

Plant material

The whole plant of *Ceiba pentandra* were identified and collected in the month of November from the local market of the Bhopal. Voucher samples were preserved for reference in the Safia College of science, (135/bot/safia/2010). The bark of the whole plant is selected for the study.

Extraction

Successive Solvent extraction

The bark were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned barks were coarsely powdered in hand grinder. Powder barks were weighed (75 gm) and packed in soxhlet apparatus. The drug was defatted with petroleum ether (40⁰-60⁰) for about 72 hrs. Complete defatting was censured by placing a drop from the thimble on the filter paper which did not exhibit any oily spot.

The defatted material was removed from the soxhlet apparatus and air dried to remove last traces of petroleum ether, the defatted materials was subjected to extraction by chloroform, ethanol and water as solvent. The process was carried out for about different timings for different solvents. The liquid extract was collected in a tarred conical flask. The solvent removed by distillation. Last races of solvent being removed under vaccum. The extract obtained with each solvents was weighed to a constant weight and percentage w/w basis was calculated.

Qualitative analysis

The Aqueous, Ethanol, extracts of *Ceiba pentandra* were screened for the presence of secondary metabolites. Two (2) milliliters of each extract was measured into a test tube for each of the tests

and concentrated by evaporating the extractant in a water bath. Tests were carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes.

***In vivo* experimental design**

Animals

Swiss albino rats were obtained from animal house VNS institute of Pharmacy with due permission from Institutional animal ethical committee (Registration Number. 778/03/c/cpsa).

Acute toxicity studies were conducted by using albino mice of either sex weighing between 20 and 25 gms and healthy adult male albino rats weighing between 150 and 200 gms were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25±20C) and maintained on 12-h light: 12-dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water ad libitum.

Acute toxicity

Female albino rats were used for studies. The animal were kept fasting over night providing only water. Dose was administered orally at 250 mg/kg, 500 mg/kg and the animal were observed for 14 days. If the mortality was observed in two out of three animals, than the dose administered was assigned as toxic dose. If the mortality was observed in one animal, than the same dose was repeated again to confirm the toxic dose if the mortality was not observed, the procedure was repeated for the higher dose, i.e. up to 2000 mg/kg.

Induction of Renal calculi in rats

There are two methods for induced calcium oxalate crystals in rats. Method .I Ethylene glycol induced Urolithiasis model, Method .2 Ethylene glycol and Ammonium chloride induced urolithiasis

Method .I Ethylene glycol induced Urolithiasis model

Ethylene glycol induced Urolithiasis model was used to assess the antiurolithiatic activity in albino rats. Animals were divided into seven 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 to VII for induction of renal calculi till 28th day. Group III received standard anti urolithiatic drug, Cystone (750mg/kg body weight) from 15th day till 28th day. Group IV received aqueous extract (250mg/kg body weight) from 15th day till 28th day, Group V received ethanolic extract (250mg/kg body weight) from 15th day till 28th day, Group VI received aqueous extract (500mg/kg body weight) and Group VII received ethanolic extract (500mg/kg body weight) from 15th day till 28th day, All extracts were given once daily by oral route [12].

Assessment of Anti Urolithiatic Activity

1. Collection and analysis of urine

On 28th day all animals which were kept in metabolic cages are taken and urine samples were collected. 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 40C. Urine was analyzed for calcium, phosphate and oxalate content [12].

2. Serum analysis

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

3. 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°C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2,000×g for 10 min and the supernatant was separated. The calcium phosphate and oxalate content in kidney homogenate were determined [12].

4. Histological Examination

Single kidney of each animal was preserved in 10% neutral formalin except those kidneys which were used in homogenate. All kidneys were subsequently embedded in paraffin and sections were cut by microtome. The sections were stained by haematoxylin and eosin to study the calcium oxalate crystal deposition [13].

Method. II Ethylene glycol and Ammonium chloride induced Urolithiasis model

Ethylene glycol and ammonium chloride induced urolithiasis model was used to assess the anti urolithiatic activity in albino rats. Animals were divided into seven groups containing six animals in each. Each group underwent a different treatment protocol for 10 days. Group I served as normal and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) and Ammonium chloride (2% w/v) in drinking water was fed to Groups II to VII for induction of renal calculi. Group III received standard anti urolithiatic drug, Cystone (750mg/kg body weight). Group IV received aqueous extract (250mg/kg body weight) from, Group V received ethanolic extract (250mg/kg body weight), Group VI received aqueous extract (500mg/kg body weight) and Group VII received ethanolic extract (500mg/kg body weight), all extracts were given once daily by oral route [14].

Assessment of Anti Urolithiatic activity

1. Serum analysis

After the 10th days of experimental period, blood was collected from the retro-orbital puncture under anesthetic conditions. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for calcium, phosphorus, urea and creatinine [12].

2. Kidney homogenate analysis

After the blood collection, animals were sacrificed by cervical decapitation. 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. Single kidney of each animal was used for homogenate. The kidneys were dried at 80 °C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2,000 rpm for 10 min and the supernatant was separated. The calcium content of the mixture was determined using flame spectroscopy [12]

3. Histological Examination

Single kidney of each animal was preserved in 10% neutral formalin except those kidneys which were used in homogenate. All kidneys were subsequently embedded in paraffin and sections

were cut by microtome. The sections were stained by haematoxylin and eosin to study the calcium oxalate crystal deposition [12].

Statistical Analysis

Results are presented as mean \pm standard error (S.E.). A one-way ANOVA was used to determine the significance of differences among groups. Student's *t*-test was used to assess differences between means. Conventional Windows software was used for statistical computations. A *P* value < 0.05 was considered to indicate a significant difference.

RESULTS AND DISCUSSION

Qualitative analysis

Preliminary Phytochemical screening was performed for each extract. It was noted that Aqueous extract contain alkaloids, glycosides carbohydrates, flavonoids and tannins, petroleum ether extract contain alkaloids, flavonoids, tannins, while in ethanolic extract showed the presence of alkaloids, glycosides carbohydrates, flavonoids and tannins.

In vivo studies

From the acute toxicity study, the LD50 cut-off dose was found to be 2500mg/kg body weight for both aqueous and alcoholic extracts. Hence, the therapeutic dose was taken as 250 & 500 mg/kg body weight for both aqueous and ethanolic extracts of *Ceiba pentandra*.

Method.1 Ethylene glycol induced Urolithiasis

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male albino rats resulted in hyperoxaluria. Calcium, Oxalate, and phosphate excretion were grossly increased in calculi-induced animals [Table 1.1, 1.2 Group-II]. However, supplementation with 250 mg/kg & 500 mg/kg aqueous and ethanolic extracts of *Ceiba pentandra* significantly ($P < 0.001$) lowered the elevated levels of Calcium, Oxalate, and Phosphate in Group IV- VII as compared to Cystone-treated animals (Group III). The serum uric acid was remarkably increased in calculi-induced animals [Table 1.3, Group II] while serum creatinine and blood urea nitrogen was only slightly elevated in Group II indicating marked renal damage. However, 250 mg/kg & 500 mg/kg aqueous and ethanolic extracts of *Ceiba pentandra* significantly ($P < 0.001$) lowered the elevated serum levels of creatinine, uric acid and Blood urea nitrogen [Table 1.3, Group IV-VII] as compared to Cystone-treated animals (Group III).

Method.2 Ethylene glycol and Ammonium chloride induced Urolithiasis

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male albino rats resulted in hyperoxaluria. Calcium, phosphorus, urea and creatinine were grossly increased in calculi-induced animals [Table.1.4,1.5. Group-II]. However, supplementation with 250 mg/kg & 500 mg/kg aqueous and ethanolic extracts of *Ceiba pentandra* significantly ($P < 0.001$) lowered the elevated levels of calcium, phosphorus, urea and creatinine in blood serum Group IV- VII as compared to Cystone-treated animals (Group III). The deposition of the crystalline components in the renal tissues, namely calcium, was increased in the stone forming rats [Table 1.5, Group II]. The aqueous and ethanolic extracts of *Ceiba pentandra* treatment significantly ($P < 0.001$) reduced the renal content of these stone forming constituents in both 250 & 500 mg/kg doses [Table 1.5, Group IV-VII]. Calcium level in kidney homogenate was remarkably increased in calculi-induced animals [Table 1.5, Group II] as compared to Cystone-treated animals (Group III).

Histological Examination

On Histopathological examination both the Ethylene glycol & Ethylene glycol and Ammonium chloride induced Urolithiasis group showed presence of polymorphic irregular calcium oxalate crystals in Lumina of tubules accompanied by edema and cast formation which causes dilation of proximal tubules along with interstitial inflammation this might be attributed to oxalate formation and also causes extensive intertubular hemorrhages and congestion of blood vessels. (Figure. 1.1, 1.2). These histological observations support the presence and growth of renal calculi in renal medulla region as observed in human urolithiasis. On administration of aqueous extract of *Ceiba pentandra* moderate to few crystals are observed along the mild appearance of edema and dilation in tubules and crystals are present focally indicating the ability of aqueous extract of *Ceiba pentandra* to dissolve the pre-formed stones to an greater extent. Similarly on administration of ethanolic extract of *Ceiba pentandra* some crystals were observed indicating the ability of ethanolic extract of *Ceiba pentandra* to dissolve pre-formed stones to some extent. These histological studies support the calcium and oxalate deposition data in kidney by ethylene glycol and Ammonium chloride induction. Thus, Aqueous extract of *Ceiba pentandra* showed significant antiurolithiatic activity than ethanolic extract of *Ceiba pentandra*.

Method .1 Ethylene glycol induced Urolithiasis

Table 1.1 Effect of *Ceiba pentandra* extract on Urinary Parameters in Control and experimental

S.no	Treatment (mg/kg)	Urinary Parameters (mg/dl)		
		Calcium	Oxalate	Phosphate
1	Normal	2.09±0.15	0.70±0.03	3.50±0.65
2	Renal -Calculi induced	3.70±0.25*	3.25±0.01*	7.55±0.06*
3	Standard. (Cystone) (750 mg/kg body weight)	2.55±0.18***	1.00±0.15***	3.91±0.01***
4	Aqueous extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.70±0.25***	1.29±0.15***	4.09±0.09***
5	Ethanolic extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.80±0.25***	1.28±0.66***	4.19±0.15***
6	Aqueous extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	2.80±0.12***	1.39±0.01***	4.29±0.33***
7	Ethanolic extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	3.19±0.25**	1.86±0.15**	4.28±0.24***

Values are in mean ± S.E.M; No of observations = 6; *P<0.05, **P<0.01, ***P<0.001, significance versus control (ANOVA followed by student's t- Test)

Table 1.2 Effect of *Ceiba pentandra* on Kidney Parameters in Control and experimental animals

S. no	Treatment (mg/kg)	Kidney Parameters (mg/g)		
		Calcium	Oxalate	Phosphate
1	Normal	2.54±0.06	1.79±0.6	2.48±0.03
2	Renal -Calculi induced	3.76±0.05*	3.79±0.14*	3.69±0.16*
3	Standard. (Cystone) (750 mg/kg body weight)	2.80±0.18***	2.15±0.016***	2.55±0.30***
4	Aqueous extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.85±0.12***	2.25±0.40***	2.75±0.15***
5	Ethanolic extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.90±0.18***	2.25±0.01***	2.95±0.14***
6	Aqueous extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	3.10±0.13***	2.35±0.18***	3.15±0.17**
7	Ethanolic extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	2.89±0.01***	2.98±0.14**	2.75±0.32***

Values are in mean ± S.E.M; No of observations = 6; *P<0.05, **P<0.01, ***P<0.001, significance versus control (ANOVA followed by student's t- Test)

Table 1.3 Effect of *Ceiba pentandra* extract on Serum Parameters in Control and experimental animals

S.no	Treatment (mg/kg)	Serum Parameter (mg/dl)		
		Creatinine	Uric acid	Blood Urea Nitrogen
1	Normal	0.49±0.02	1.50±0.03	41.11±0.15
2	Renal -Calculi induced	1.09±0.10*	3.52±0.45*	56.25±0.49*
3	Standard. (Cystone) (750 mg/kg body weight)	0.72±0.15***	2.07±0.02***	43.44±0.16***
4	Aqueous extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	0.78±0.61***	1.80±0.12***	44.53±0.34***
5	Ethanolic extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	0.84±0.60***	1.86±0.01***	45.88±0.38***
6	Aqueous extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	0.83±0.14***	2.46±0.06**	43.15±0.15***
7	Ethanolic extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	0.90±0.12**	2.55±0.012**	46.18±0.17***

Values are in mean ± S.E.M: No of observations = 6; *P<0.05, **P<0.01, ***P<0.001, significance versus control (ANOVA followed by student's t- Test)

Method .2 Ethylene- Glycol and Ammonium Chloride induced Urolithiasis

Table 1.4 Effects of *Ceiba pentandra* extracts on serum parameters in control and experimental animals

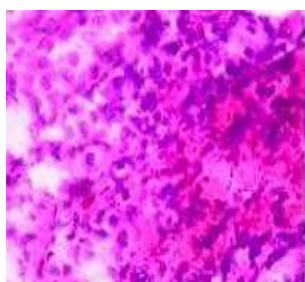
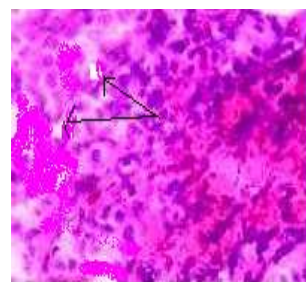
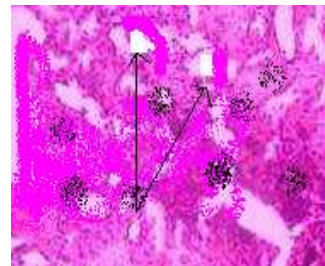
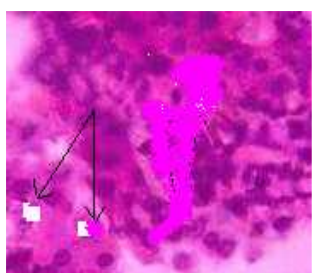
S.no	Treatment (mg/kg)	Serum Parameters (mg/dl)			
		Calcium	Urea	Phosphorous	Creatinine
1	Normal	2.09±0.15	3.70±0.4	3.69± 0.04	0.55±0.02
2	Renal -Calculi induced	3.79±0.25*	7.75±0.12*	7.27±0.06*	1.29±0.10*
3	Standard. (Cystone) (750 mg/kg body weight)	2.52±0.18***	4.0±0.10***	3.8 ±0.09***	0.75±0.15***
4	Aqueous extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.76±0.25***	4.25±0.22***	4.03±0.08***	0.80±0.61***
5	Ethanolic extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.84±0.25***	4.3±0.15***	4.10 ±0.10**	0.88s±0.60***
6	Aqueous extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	2.82±0.12***	4.25±0.18***	3.95±0.06***	0.83±0.14***
7	Ethanolic extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	3.12±0.25**	4.50±0.15***	4.20± 0.09**	0.95±0.12**

Values are in mean ± S.E.M: No of observations = 6; *P<0.05, **P<0.01, ***P<0.001, significance versus control (ANOVA followed by student's t- Test)

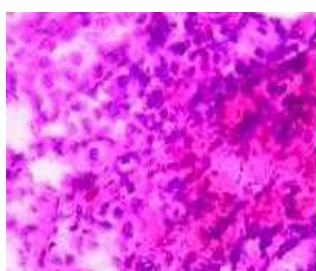
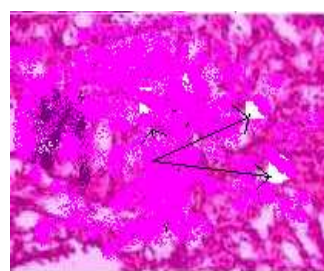
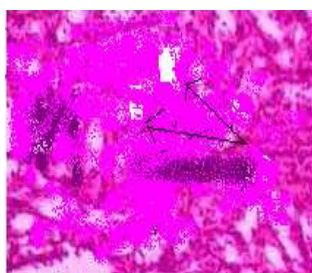
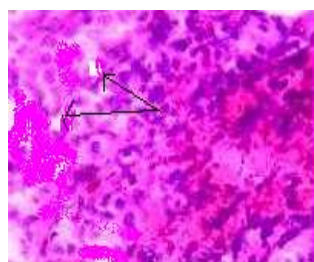
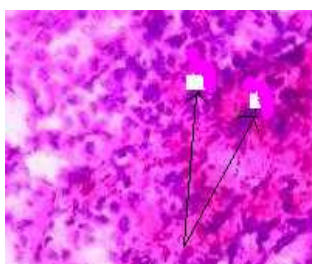
Table 1.5 Effect of *Ceiba pentandra* extract on kidney Parameter in control and experimental animal.

S.no	Treatment (mg/kg)	Kidney Parameter (mg/g)
		Calcium
1	Normal	2.49±0.06
2	Renal -Calculi induced	3.96±0.05*
3	Standard. (Cystone) (750 mg/kg body weight)	2.69±0.18***
4	Aqueous extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.79±0.12***
5	Ethanolic extract of <i>Ceiba pentandra</i> (250 mg/kg body weight)	2.90±0.18***
6	Aqueous extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	3.19±0.13***
7	Ethanolic extract of <i>Ceiba pentandra</i> (500 mg/kg body weight)	2.88 ±0.01 ***

Values are in mean ± S.E.M: No of observations = 6; *P<0.05, **P<0.01, ***P<0.001, significance versus control (ANOVA followed by student's t- Test)

Histological Examination**Method.1 Ethylene glycol and induced Urolithiasis method****Fig. 1.1 (a)****Fig. 1.1 (b)****Fig. 1.1 (c)****Fig. 1.1 (d)****Fig. 1.1 (e)****Fig. 1.1 (f)****Fig. 1.1 (h)**

Photomicrographs of calcium oxalate crystals in kidney homogenate, crystalline formation in the renal parenchyma. Sections were viewed using a BX41 optical microscope and polarized light Fig. 1.1 (a) Normal rats, Fig. (b) Rat receive ethylene glycol (0.75 % v/v), Fig. (c) Rat receive ethylene glycol (0.75 % v/v) and Standard drugs (Cystone). Fig. (d) Rat receive ethylene glycol (0.75 % v/v), 250 mg/kg of aqueous extract of Allium sativum, Fig. (e) Rat receive ethylene glycol (0.75 % v/v), 250 mg/kg of ethanolic extract of Allium sativum, Fig. (f) Rat receive ethylene glycol (0.75 % v/v), 500 mg/kg of aqueous extract of Allium sativum Fig. (g) Rat receive ethylene glycol (0.75 % v/v), 500 mg/kg of ethanolic extract of Allium sativum.

Method .2 Ethylene glycol and Ammonium chloride induced Urolithiasis**Fig 1.2 (a)****Fig 1.2 (b)****Fig 1.2 (c)****Fig 1.2 (d)****Fig 1.2 (e)****Fig 1.2 (f)****Fig 1.2 (g)**

Photomicrographs of calcium oxalate crystals in kidney homogenate, crystalline formation in the renal parenchyma. Sections were viewed using a BX41 optical microscope and polarized light
Fig 1.2 (a) Normal rats. **Fig. (b)** Rat receive ethylene glycol (0.75 % v/v), Ammonium chloride (2% w/v) **Fig. (c)** Rat receive ethylene glycol (0.75 % v/v), Ammonium chloride (2% w/v) and Standard drugs (Cystone), **Fig. (d)** Rat receives ethylene glycol (0.75 % v/v), Ammonium chloride (2% w/v), 250 mg/kg of aqueous extract of *Allium sativum*, **Fig. (e)** Rat receives ethylene glycol (0.75 % v/v), Ammonium chloride (2% w/v), 250 mg/kg of ethanolic extract of *Allium sativum*, **Fig. (f)** Rat receives ethylene glycol (0.75 % v/v), Ammonium chloride (2% w/v), and 500 mg/kg of aqueous extract of *Allium sativum* **Fig. (g)** Rat receive ethylene glycol (0.75 % v/v), Ammonium chloride (2% w/v). 500 mg/kg of ethanolic extract of *Allium sativum*.

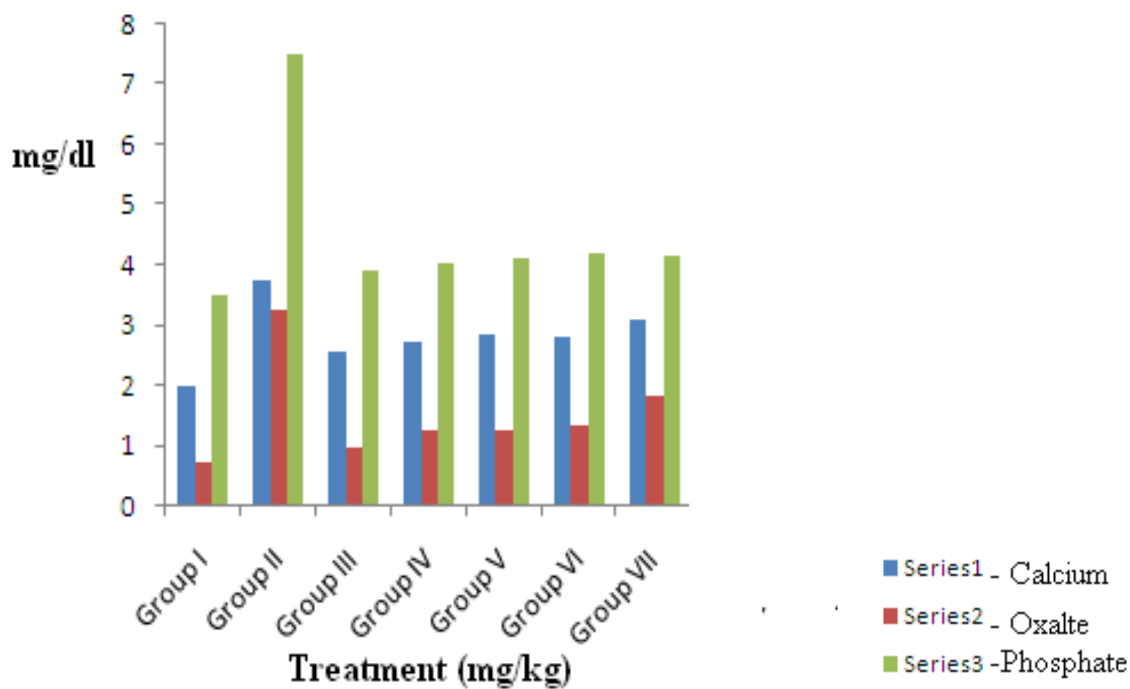


Figure 2.1 Effect of *Ceiba pentandra* extract on Urinary Parameter (Calcium, Oxalate, Phosphate) in Control and experimental animals (n=6)

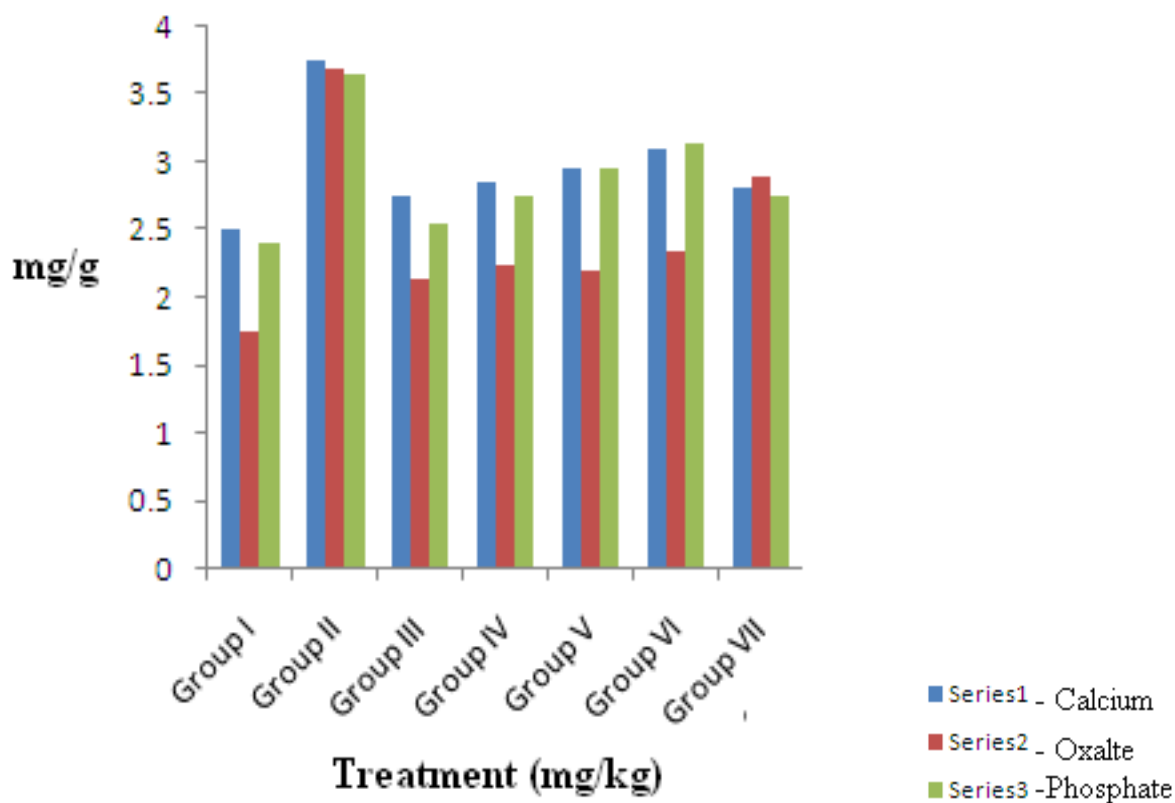


Figure 2.2 Effect of *Ceiba pentandra* extract on Kidney Parameter (Calcium, Oxalate, Phosphate) in Control and experimental animals (n=6)

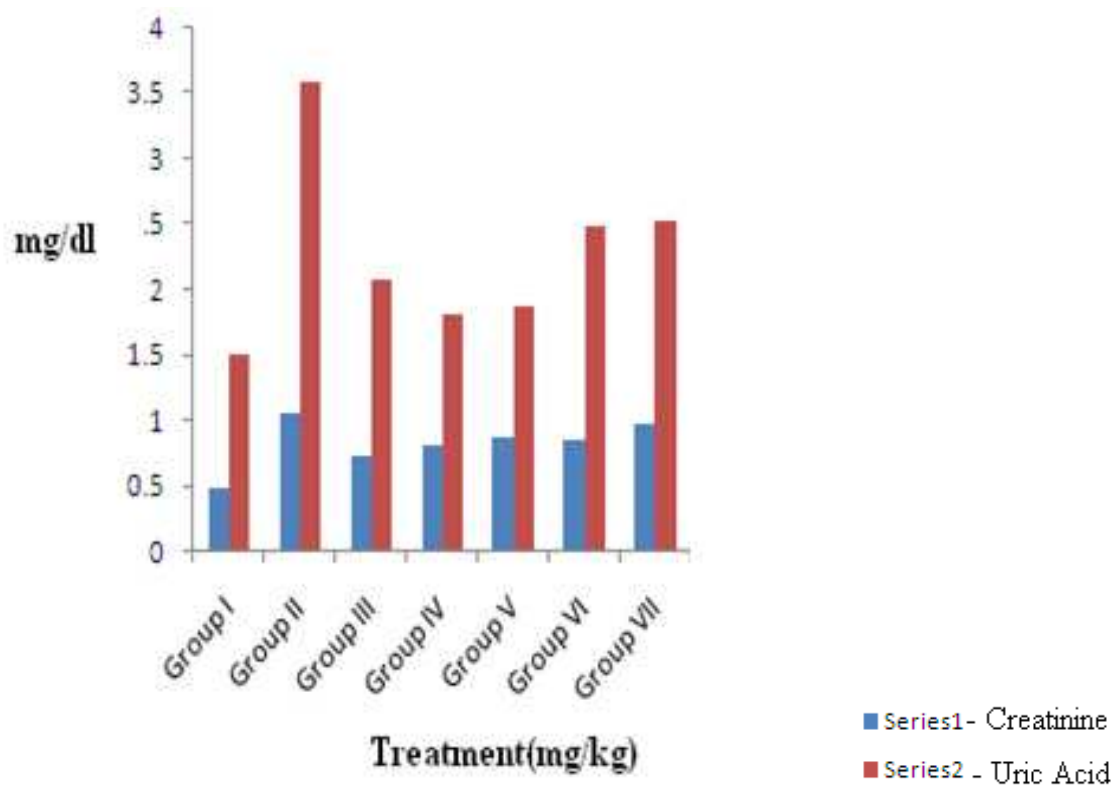


Figure 2.3 Effect of *Ceiba pentandra* extract on Serum Parameter (Creatinine, Uric Acid) in Control and experimental animal (n=6)

Method .2 Ethylene- Glycol and Ammonium Chloride induced Urolithiasis\

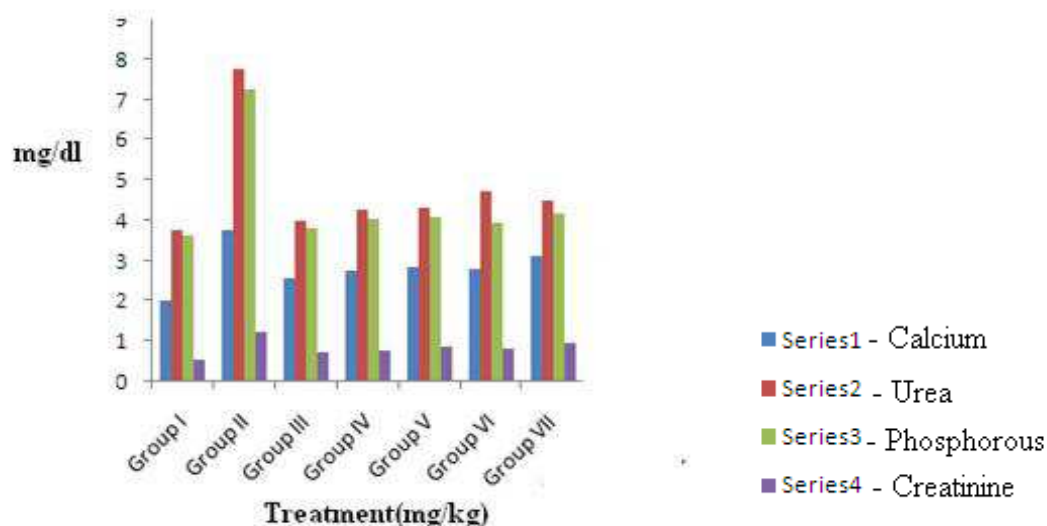


Figure 2.4 Effect of *Ceiba pentandra* extract on Serum Parameter (Calcium, Urea, Phosphorous Creatinine) in Control and experimental animals (n=6)

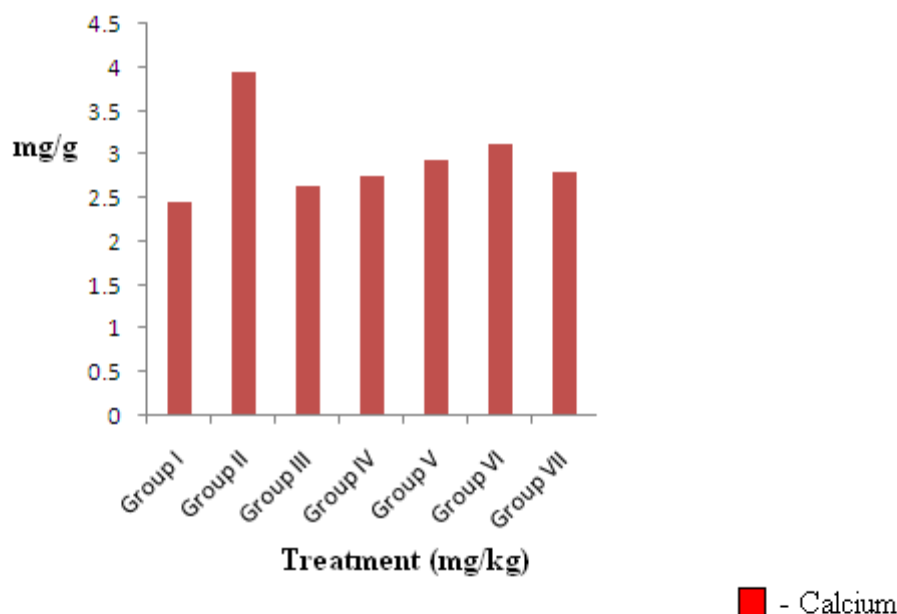


Figure 2.5 Effect of *Ceiba pentandra* extract on Calcium (Kidney Parameter) in Control and experimental animal (n=6)

CONCLUSION

The present study was done to see the Assessment of bio activity of *Ceiba pentandra* of different extract on calcium oxalate urolithiasis in rats. Ethylene glycol & Ethylene glycol and Ammonium chloride induced urolithiasis; the finding suggested that 250 mg/kg & 500 mg/kg aqueous and alcohol extracts of *Ceiba pentandra* have more significant $P < 0.001$. The presented data indicate that administration of the aqueous and ethanolic extracts of *Ceiba pentandra* to rats with Ethylene glycol & Ethylene glycol and Ammonium chloride induced urolithiasis reduced and prevented the growth of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the antiurolithiatic property of *Ceiba pentandra*

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