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IN-VIVO STUDY OF POLYHERBAL FORMULATION IN CALCIUM OXALATE INDUCED KIDNEY STONES IN WISTAR RATS

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Abstract
The goal of the current study is to determine how a polyherbal formulation (PHF) affects rats with experimentally produced kidney stones. Experimentally, male rats were given 0.76 percent v/v ethylene glycol in drinking water along with 1 percent w/v ammonium chloride for three days, then 0.76 percent v/v ethylene glycol alone for 25 days to generate oxalate kidney stones. Rats from the kidney stone-induced test group received three doses of the PHF over the course of 28 days: 250, 350, and 450 mg/kg, respectively. After 28 days, kidney stone control group rats showed significantly more calcium oxalate deposition in the kidneys than normal group rats and also increases other parameters also. The serum analysis of the rats in the kidney stone control group demonstrated a significantly greater level of blood urea, serum creatinine, and serum uric acid.. The amount of calcium oxalate that was formed in the kidneys was greatly reduced with daily oral administration of PHF at dosages of 350 and 450 mg/kg, and all of the biochemical alterations brought on by calcium oxalate kidney stones were reversed, corroborating the traditional claim of the drug. However, it was discovered that the 250 mg/kg dose of PHF was not relevant in this regard.

Keywords: Calcium oxalate, ethylene glycol, kidney stone, Polyherbal formulation.

1. INTRODUCTION
Kidney stones are one of the prevalent and excruciating symptoms of a urinary tract problem that have been plaguing people for millennia. The prevalence and incidence rates are rising globally, especially as industrialization progresses.[1,2] Additionally, the age of onset is getting younger, raising biomedical concerns.[3,4] Furthermore, it has been found that frequent recurrence is linked to risk during relapse and a shorter time between relapses.[5] Young age of onset, family history, genetic make-up, concurrent infection, stone size, and underlying medical conditions are traits connected to significant causes of these recurrences.[6,7] Additionally, epidemiological research showed that males (12%) were more likely than women to experience it (6 percent).[8,9] Kidney stones develop as a result of the kidneys' ability to excrete substances with low solubility and conserve water failing to function properly.

During adaptation to nutrition, climate, and other activities, these two opposing requirements must be balanced. Crystals develop, expand, and consolidate to form stones if the urine gets oversaturated with insoluble material due to high excretion rates and/or decreased water conservation. The impact of these stones is multiplied when additional conditions like hypertension, obesity, hepatic dysfunction, etc. are present.[10,11]

1.2 KIDNEY STONE
The majority of kidney stones are located there. Additionally, it is the most dangerous UTI. Renal stone prevention continues to be a significant issue for human health.[12,13]

TYPES OF KIDNEY STONES

Calcium stones: Oxalate of calcium About 80% of all urinary calculi are calcium stones, which are the most common kidney stones. Crystalline calcium oxalate monohydrate, calcium oxalate dihydrate, and calcium oxalate trihydrate are the components of calcium oxalate stones.

Calcium phosphate: Apatite carbonate, are among the crystalline components of calcium phosphate stone. There are rocks made of calcium phosphate and calcium oxalate.

Uric acid stones or urate: Uric acid stone, a crystalline form of uric acid anhydrous and uric acid dehydrate, typically affects 5–10% of the population of renal stones under study. Low urine volume, hyperuricosuria, and an acidic urine pH are the key contributors to this form of stone (pH 5.05).

Cystine stone: Due to excessive quantities of necessary amino acids, cystine in the urine is what causes cystine stones. One to three percent of kidney stone patients have cystine stones, a rare inherited metabolic condition that typically manifests in childhood.

Struvite stone: Phosphate is an intriguing inorganic mineral that is strongly linked to persistent urinary tract infections because of particular microbes including bacteria that produce urease. Together with magnesium and phosphate, the bacterium converts urea into ammonium.

Drug –induced stone: Drugs including xypurinol are among the excessively high doses of medications that cause renal calculus, which in turn causes renal stones. Patients who use the HIV medication indinavir sulphate protease inhibitor run the risk of getting kidney stones. Such lithogenic medications or their metabolites may have previously formed renal calculus or may have been deposited to produce a nidus.[14,15,16]

2. MATERIAL AND METHODS

2.1 Plant Materials

The leaves of *Phyllanthus niruri, Tribulus terrestris, and Abutilon indicum* were gathered from local farmers in various regions of Rajasthan, India. Before extraction, plant materials were shade-dried and coarsely pulverized.

2.2 Polyherbal Formulation

S.No.	Ingredient	PHF-1	PHF-2	PHF-3	PHF-4	PHF-5
1.	Powder of EEAI	75	75	75	75	75
2.	Powder of EETT	75	75	75	75	75
3.	Powder of EEPN	75	75	75	75	75
4.	Lactose	75	70	65	60	55
5.	Talc	15	15	15	10	10
6.	Starch	40	45	50	55	60
7.	Sodium Benzoate	5	5	5	5	5
8.	Gelatin	5	5	5	5	5
9.	Microcrystalline cellulose	10	10	10	15	15

2.3 Animals:

For this experiment, male Wistar albino rats weighing 120–150g were employed. The relative humidity was kept between 45 and 55 percent, while the temperature was kept at 25 ± 2 °C.

2.3.1 Acute Toxicity Study

According to the OECD's recommendations, an acute toxicity study was conducted. Animals were acclimated to the testing environment five days before the experiment. The fixed dose approach was

used to measure animal body weight and perform individual identification. For the test method, a starting dose of 5 mg/kg was administered. Various extract doses up to 5000 mg/kg were chosen. Three animals were given the first dose of extract after p.o. Route and animals are watched for changes in behavior and mortality.

2.3.2 Pharmacological evaluation

2.3.2.1 Calcium oxalate induced kidney stone:

Experimental animals were separated into five groups, each with six animals, and given an oral treatment regimen according to the information listed below, as well as the addition of ethylene glycol (0.76 percent v/v) to drinking water for a total of 28 days.

Group	Treatment	Dose and route
I (Normal Control)	Distilled water	10 ml/kg
II (kidney stone induction control)	DMSO+(0.76% v/v) ethylene glycol	10 ml/kg
III PHF	PHF+(0.76% v/v) ethylene glycol	250 mg/kg
IV PHF	PHF+(0.76% v/v) ethylene glycol	350 mg/kg
V PHF	PHF+(0.76% v/v) ethylene glycol	450 mg/kg

2.4 Collection and analysis of urine:

On the 28th day of the calculi induction treatment, all the mice were housed in separate metabolic cages, and 24-hour urine samples were collected. The following parameters were assessed in the collected urine samples.

2.4.1 Urine volume:

For 24 hours, the animals were kept in separate metabolic cages. Using a measuring cylinder, the total volume of urine was recorded in milliliters (ml).

2.4.2 Urine pH:

The concentrated acid urine was observed to deposit uric acid crystals more frequently. Consequently, the pH metre was used to assess the urine's acidity.

2.4.3 Urinary oxalate:

In order to dissolve crystals, 1 ml of concentrated HNO_3-acidified urine was then brought to pH 7 with sodium hydroxide in the presence of the colour indicator bromothymol blue. Oxalate was precipitated overnight using 14 ml of pure ethanol and around 2 ml of saturated CaSO4. The samples were spun at 450g for 10 minutes before being filtered via filter paper.

In 10 ml of water that had been acidified with 2 ml of strong sulfuric acid, the precipitate that had resulted was dissolved. In order to titrate the samples, a $KMnO_4$ solution was used.

2.4.4 Urine calcium:

It was calculated using an o-cresolphthalein complex one technique standard kit that is commercially available from Biolab Diagnostics Pvt. Ltd. in India. Using a autoanalyzer, urine calcium was determined.

2.4.5 Urine magnesium:

It was calculated using a standard kit that is available for purchase and was provided by Biolab Diagnostics Pvt. Ltd. Utilizing an autoanalyzer, urine magnesium levels were determined.

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2.5 Collection and Serum analysis:

On the 28th day of the calculi induction treatment, all the mice were housed in separate metabolic cages, and 24-hour urine samples were collected. urine samples that were measured and gathered.

2.5.1 Serum uric acid:

It was calculated using a standard kit that is commercially available from Biolab Diagnostics Pvt. Ltd., according to the Colorimetric Enzymatic Method, and was then examined by an autoanalyzer.

2.5.2 Serum creatinine:

Utilizing a commercially available standard kit and the urease/salicylate technique, it was approximated. Utilizing a biochemistry autoanalyzer, serum creatinine was determined.

2.6 Blood urea:

It was calculated using the diacetymonoxime colorimetric end-point method and a commercially available standard diagnostic kit from Biolab Diagnostics-India. Utilizing an autoanalyzer, blood urea was determined.

2.7 Histopathological Examination:

The kidneys of the animals used in the aforementioned sacrifices were fixed in paraffin, embedded in 10 percent buffered formalin, sectioned into 5 m sections for slides, and stained with hematoxylin and eosin. With the use of a polarising light microscope, the slides were examined. Each from the cortex, medulla, and papilla, six low power fields (100x) were used to count the number of crystal deposits.

3. RESULTS AND DISCUSSIONS

3.1 Acute toxicity study

Up to a dose of 2000 mg/kg, none of the mice displayed any toxicity within the permitted range specified by the OECD recommendations. Three distinct doses of the polyherbal formulation—250, 350, and 450 mg/kg—were chosen for additional research based on this information, the outcomes of individual extracts, and the results of the pilot study.

Test animal sequence	Dose (mg/ml)	Result	
		Short term	Long term
01	175	0	0
02	550	0	0
03	2000	0	0
04	2000	0	0
05	2000	0	0

3.2 Calcium oxalate induced kidney stone:

The oxalate-induced kidney stone resulted in considerable changes in the urine parameters as compared to the healthy control group. The urinary volume increased, as did the amounts of oxalate, calcium, and magnesium in the urine. When compared to the control group, these changes were much less pronounced in the groups treated with the polyherbal formulation (350 and 450 mg/kg). Both the 350 mg/kg and 450 mg/kg dosages had an equal impact (p0.01) in lowering urolithiasis-related increases in urinary oxalate and volume.

In the reduction of urine magnesium, the dose of 450 mg/kg was more significant (P 0.01) than the dose of 350 mg/kg, whereas the opposite was shown in the reduction of urinary calcium. It was discovered that the dose of 250 mg/kg was essentially irrelevant in this matter.

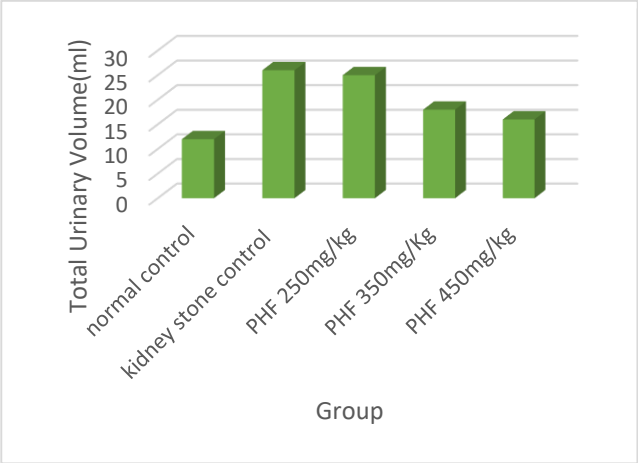

Figure 1. Total urinary volume

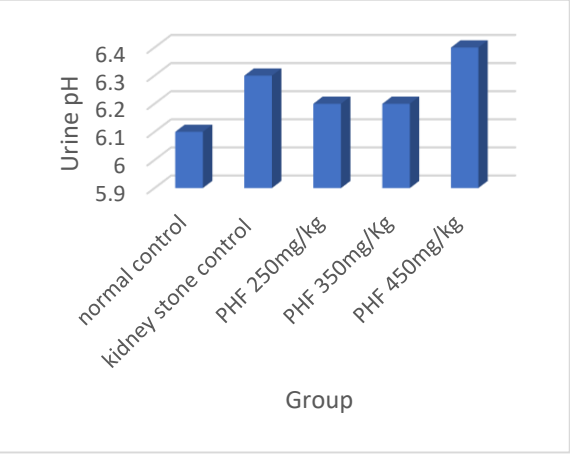

Figure 2. Urinary pH

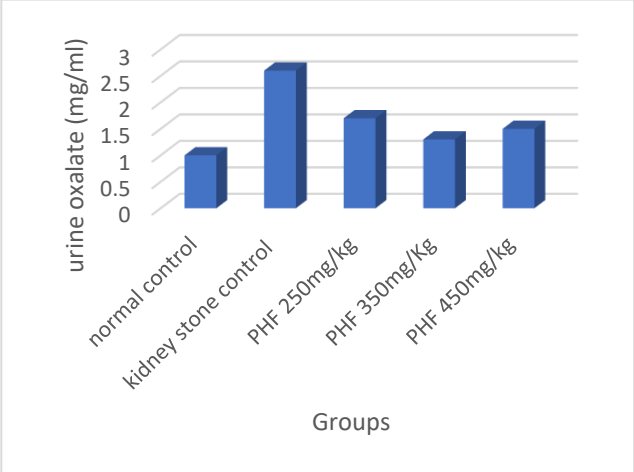

Figure 3. oxalate in urine

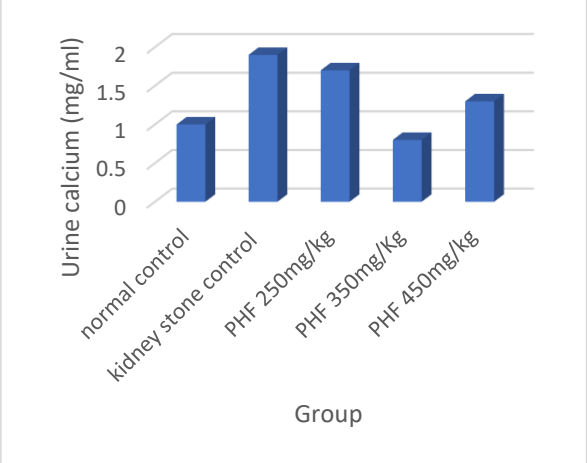

Figure 4. calcium in urine

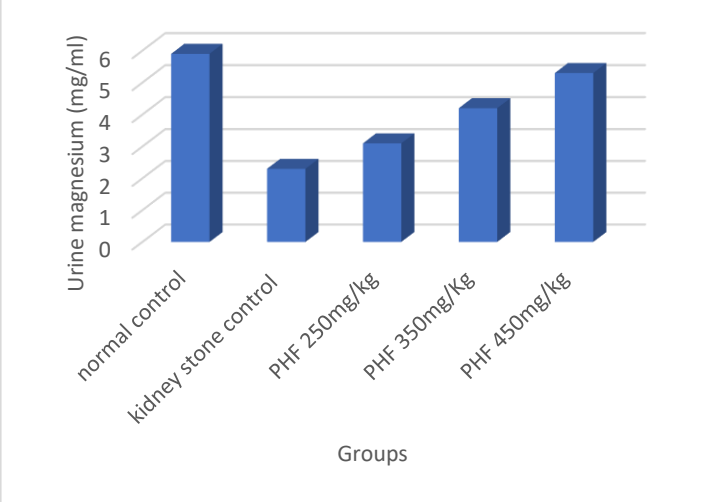

Figure 5. magnesium in urine

3.3 Collection and Serum analysis:

When compared to the normal control group, the induction control group had considerably higher levels of blood urea, serum creatinine, and uric acid. These levels were dramatically reduced by PHF at dosages of 350 and 450 mg/kg, and both doses had about equal activity.

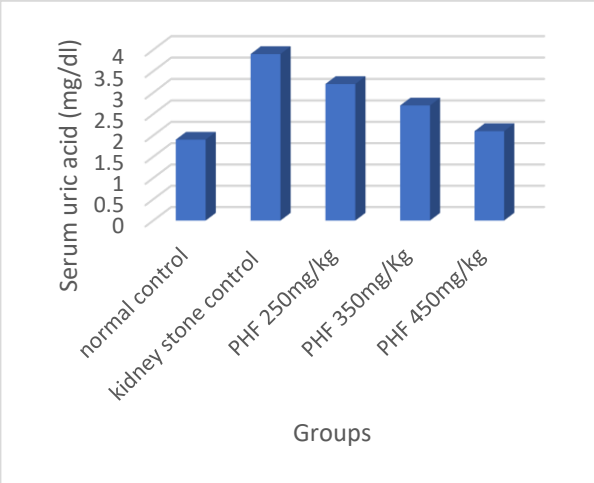

Figure 6. serum uric acid

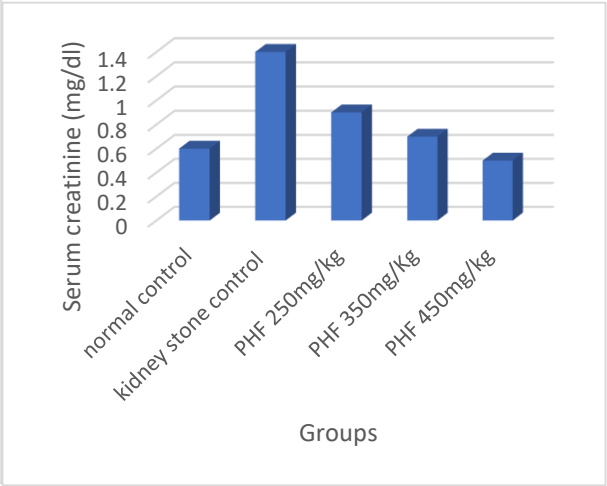

Figure 7. serum creatinine

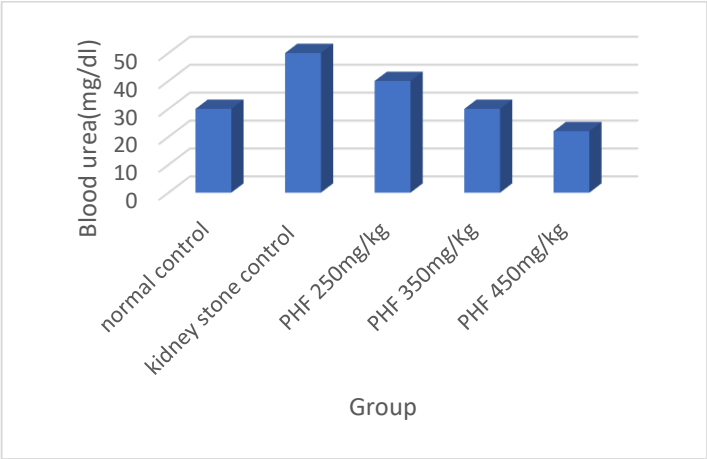

Figure 8. blood urea

3.4 Histopathology:

Significant alterations to the kidney's normal architecture were seen after exposure to ethylene glycol, most notably interstitial fibrosis, severe interstitial inflammation, and hazy abnormalities in tubular epithelial cells.

The aforesaid alterations were significantly restored by the PHF 350 and 450 mg/kg, demonstrating considerable action.

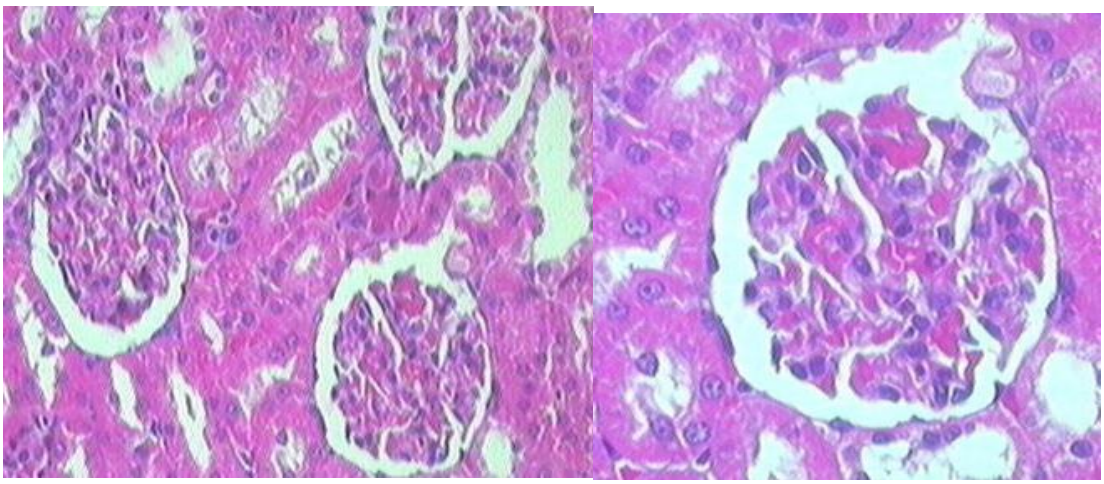

Figure 9. Kidney section of normal rats, (H and E × 400)

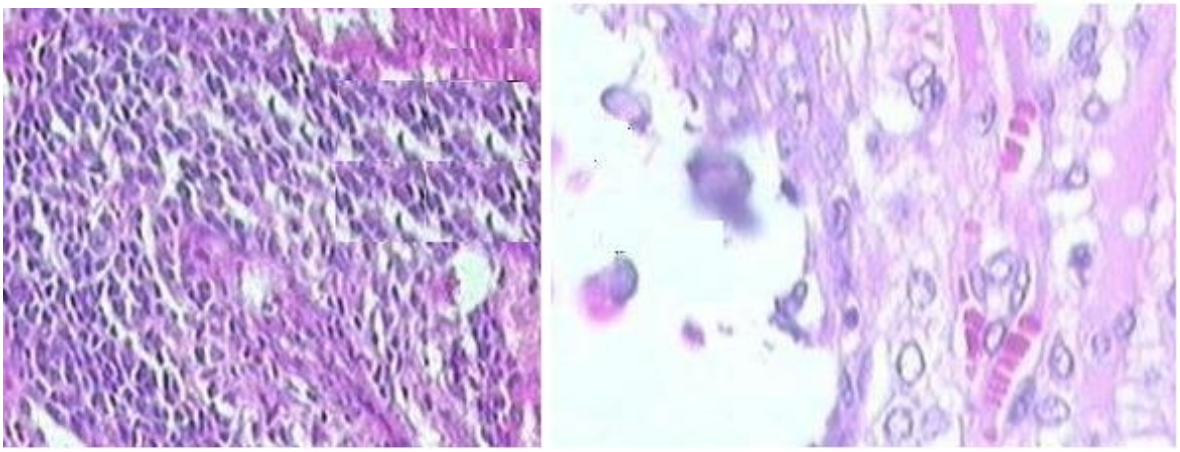

Figure 10. Kidney section of ethylene glycol treated rats

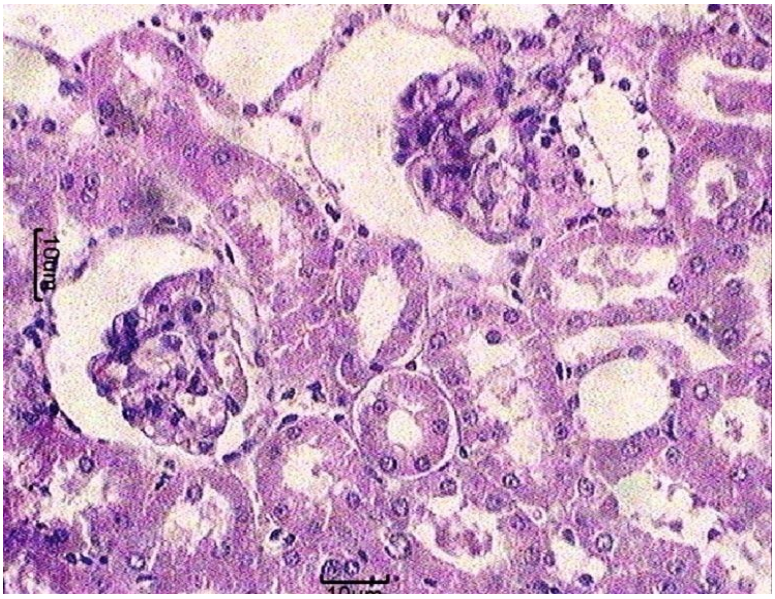

Figure 11. Kidney section of PHF 350 mg/kg rats, showing marked congestion and haemorrhage. Acute tubular necrosis.

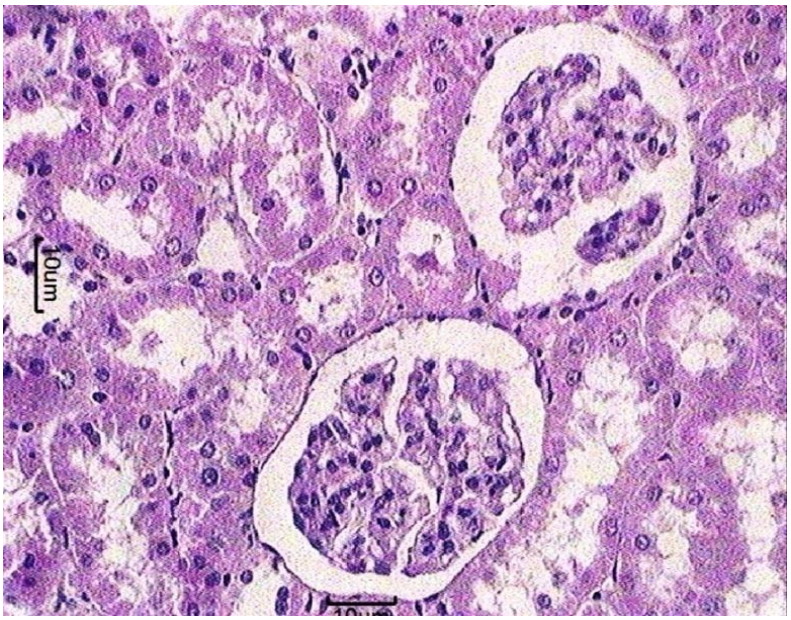

Figure 12. Kidney section of PHF 450 mg/kg rats

4. CONCLUSION

PHF demonstrated considerable anti-urolithiatic action in the current investigation against calcium oxalate-induced urolithiasis at dosages of 350 and 450 mg/kg. In order to identify the phytochemical components of the Polyherbal formulation that are responsible for the anti-urolithiatic activity, further investigation and separation of these extracts are required.

5. REFERENCE

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