IJBPAS, July, 2024, 13(7): 3496-3511

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF POLYHERBAL FORMULATION OF AQUEOUS LEAVES EXTRACTS OF *MORINGA OLEIFERA* LAM AND *PELTOPHORUM PTEROCARPUM* DC ON ETHYLENE GLYCOL-INDUCED UROLITHIATIC EXPERIMENTAL ANIMALS

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Received 15th March 2023; Revised 8th July 2023; Accepted 23rd Oct. 2023; Available online 1st July 2024

https://doi.org/10.31032/IJBPAS/2024/13.7.8186

ABSTRACT

Urolithiasis is the condition where urinary calculi are formed or located anywhere in the urinary system, or the process of formation of stones in the kidney, bladder or ureters. The plants *Moringa oleifera Lam* (family: Moringceae) *and Peltophorum pterocarpum* DC (family: Fabaceae / Leguminosae) aqueous leaves extracts were investigated for its antiurolithiatic activity. Ethylene glycol (0.75% in water) induced resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Aqueous extracts of *Moringa oleifera* Lam and *Peltophorum pterocarpum* DC were given orally in (Groups III and IV) over a period of 15 to 28 days. The levels of urea, uric acid, creatinine, and protein in serum and urine were significantly ($P<0.05$) reduced by treatment with plant extract when compared to disease control (Group II). Histopathological examination showed that the treatment of plant extracts decreased the size of calcium oxalate deposits in different parts of the renal tubules and also prevent damage to the tubules and calyxes. The reduction of stone-forming constituents in urine and their decreased kidney retention reduces the solubility products of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the antiurolithiatic property of the extracts. Finally, the results suggested that the polyherbal formulation of aqueous leaves extracts of *Moringa oleifera Lam* and *Peltophorum pterocarpum* DC leaves have potent antiurolithiatic activity against ethylene glycol–induced calcium oxalate Urolithiasis in male albino rats.

Keywords: Antiurolithiatic, *Moringa oleifera* Lam, *Peltophorum pterocarpum* DC, Ethylene glycol, calcium oxalate, kidney, cystone, Hyperoxaluria

INTRODUCTION

Excretion is the process by which unwanted substances and metabolic wastes are eliminated from the body. A large amount of waste materials and carbon dioxide are produced in the tissues during the metabolic process. All these substances must be removed to keep the body in a healthy condition. It plays a major role of homeostasis. Urolithiasis (from Greek ouron, “urine” and lithos, “stone”) is a condition where urinary stones are formed or located anywhere in the urinary system **[1]**.

Urinary stones are typically classified by their location or by their chemical composition. In humans, calcium oxalate is a major constituent of most urinary stones. About 80% of those with kidney stones are men. Men most commonly experience their first episode between 20-30 years of age **[2]**. The disorder is estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females **[3]**. Annual incidences of kidney stones are about 0.1-0.4% of the population and lifetime prevalence in the USA and Europe ranges between 8 and 15%. In the present study treatment with 0.75% ethylene glycol in drinking water resulted in hyperoxaluria and subsequent development of stones. Rats are the most commonly used animals in urolithiasis studies because they mimic the etiology of the formation of stones in humans **[4]**. Furthermore, male rats have been selected for our study because various researchers have suggested that the incidence of urolithiasis is more in males than in their counterparts **[5]**. Calcium oxalate crystals are the most common constituent of kidney stones **[6]**. The present study evaluated the Effect of polyherbal formulation of aqueous leaf extracts of *Moringa oleifera* Lam and *Peltophorum pterocarpum* DC on ethylene glycol-induced urolithiasis experimental animals.

MATERIAL AND METHODS:

COLLECTION AND IDENTIFICATION

Plant sources selected for the present study are *Peltophorum pterocarpum* DC and *Moringa* *oleifera* Lam. The plants are collected from Trichy District. Identified with the help of Flora of the Presidency of Madras and confirmed by comparing with the Herbarium specimen deposited at Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

PREPARATION OF PLANT EXTRACT

The plant materials were shade dried and coarsely powdered with an electrical blender plant powder was mixed with 6 times its volume of water and boiled until it was reduced to one-third and filtered. The filtrate was evaporated to dryness. The paste form of the extract was stored. The aqueous extract of the plant was mixed in one part of *Peltophorum pterocarpum* DC and two parts of *Moringa oleifera* Lam (Ratio 1:2) and was used as the formulation for the pre-clinical screening.

EXPERIMENTAL ANIMAL

Healthy adult Wistar strains of albino rats of both sexes between two to three months old and weighing 150-200g were obtained from Tamil Nadu Veterinary and animal sciences university, Chennai. Rats were housed in standard polypropylene cages. Six rats were housed per cage, to provide them with sufficient space, and to avoid unnecessary morbidity and mortality. Animals were maintained under standard conditions of a 12:12 hour light /dark cycle and at an ambient temperature at 23±1°C, with 65± 5% humidity. Animals were fed with standard rat chow pellets obtained from saidurga foods and Feeds, Bangalore, India, and water ad libitum. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committees (Approval No: 790/03/AC/CPCSEA).

EXPERIMENTAL DESIGN

Rats were divided into five groups each group containing six rats. Ethylene glycol (0.75% v/v) in drinking water was fed to all groups except the control for induction of renal calculi for 15 days. After induction the animals in group III and IV were treated with plant extract of *Moringa oleifera* Lam and *Peltophorum pterocarphoum* DC and group V were treated with cystone is used as a standard drug (750 mg/kg body weight) from 14 th day to 28th days. During the study, animals were allowed free access to a normal pellet diet and water ad libitum.

Group I: Normal animals received water

Group II: Disease control animals received Ethylene glycol (0.75% v/v) using water for 28 days.

Group III: Treatment of ethylene glycol (0.75% v/v) induced urolithiasis rat treated with PHAE at doses 150mg/kg bw from the 15th day to the 28th day.

Group IV: Treatment of ethylene glycol (0.75% v/v) induced urolithiasis rat treated with PHAE at doses 300mg/kg bw from 15 th day to the 28th day.

Group V: Treatment of ethylene glycol (0.75% v/v) induced urolithiasis rat treated with the standard drug for cystone (750 mg /kg bw) from 15 th to 28th day.

At the end of the experimental period, the animals were sacrificed by cervical dislocation. Blood was collected and used for various biochemical estimations. The kidneys were dissected out and washed in ice-cold 0.15 M KCl and their weight was recorded. After the kidney was used for histopathological studies and kidney homogenate was used for various biochemical analyses.

ANALYSIS OF URINE pH

Urine was collected on 28 th day keeping the animals in polypropylene cages. Animals are free to access to drinking water during urine collection. After the acidity of urine pH was measuring pH meter. (Baheti DG and Kadam SS, 2013)

ANALYSIS OF SERUM

The blood was collected from the retro-orbital sinus under anesthetic conditions and serum was separated by centrifugation at 10,000 rpm for 10 minutes and analyzed for calcium, phosphate, uric acid, urea, oxalate, and creatinine using (Lark and Collip´s method, Alkaline picrate, caraway method, Fisk and Subbarow, Hodgkinson, and Williams) methods.

ANALYSIS OF KIDNEY HOMOGENATE

The animals were sacrificed under anesthesia and after dissection; both kidneys were removed and washed with cold 0.15 M KCl. The right kidney was minced with scissors and then homogenized in 0.15 M KCl, using a homogenizer. The homogenate was centrifuged at 1500 rpm for 10 minutes using a refrigerated research centrifuge, to remove the cell debris. The supernatant was used for estimation of calcium, phosphate, uric acid, urea, oxalate, and creatinine using (Lark and Collip´s method, Alkaline picrate, caraway method, Fisk and Subbarow, Hodgkinson, and Williams) methods.

PARAMETERS

ESTIMATION OF PROTEIN IN SERUM AND URINE [7]

Reagents:

1. Alkaline Copper Sulphate Reagent:

Solution A: 2% Sodium carbonate in 0.1 N sodium hydroxide

Solution B: 0.5%Coppersulphate, 1 %Sodium potassium tartrate in water. Solution A was mixed with B in a 50:1 ratio.

2. Folin’s phenol Reagent: Into a 1500ml round bottom flask, 100mg sodium tungstate,

25gm of sodium molybdate, 100ml water, 50 ml of a-phosphoric acid, and 100ml of concentrated hydrochloric acid were added and refluxed for 10 hrs. Then 150 gm of lithium sulfate, 50 ml of distilled water and a few drops of bromine were added. The mixture was boiled to remove excess bromine. The reagent was diluted 1:2 with distilled water just before use.

3. Standard Bovine Serum Albumin: 10mg of crystalline Bovine Serum Albumin was dissolved in 100 ml of distilled water.

4. Working Standard solution: 1 in 5 Dilutions

Procedure: A series of test tubes with various concentrations of standard protein solution were taken and the volume was made up to 1ml with distilled water. For the test 0.1ml of the sample (0.1ml serum or 0.1 ml tissue homogenate was diluted to 20ml) was taken. This was made up to 1ml with distilled water. Added 4.5ml of Lowry's reagent to all tubes and allowed to stand for 10minutes. After incubation added 0.5ml of Folin's reagent to all the tubes and again incubated for 20 minutes at room temperature. The blue color developed was read colorimetrically at 620nm.

ESTIMATION OF PHOSPHORUS [8]

Reagents required:

1. Ammonium molybdate
2. ANSA reagent
3. Standard phosphorus: 35.1 mg of Potassium dihydrogen phosphate was dissolved in 100ml of double distilled water.
4. Working Standard: 1 ml of stock solution was made upto 10ml to give a concentrationof80 µg phosphorus/ml.

Procedure: The test sample was made up to 4.3 ml with water and 0.5 ml of ammonium molybdate reagent was added and incubated at room temperature. Then added 0.2 ml of ANSA and the color developed was read at 640 nm after 20 minutes.

ESTIMATION OF SERUM UREA [9]

Reagents:

1.10% Sodium Tungstate

2. 2/3N H2SO4

3. Diacetyl Monoxime (DAM) reagent 2g of DAM was dissolved in 60 ml of distilled water and 2 ml of 2%acetic acid was added to it. The total volume was made up to 100 ml with distilled water.

4. Sulphuric acid –Phosphoric acid reagent 85% of phosphoric acid was added to 140 ml of distilled water. To this 50ml of H2SO4 was added.

5. Stock standard glucose solution: 100 mg of urea dissolved in 100 ml of distilled water. 6. Working standard solution: 1 in 10 dilutions.

Procedure: To 0.1 ml of blood, 2 ml of 10% sodium tungstate and 2 ml of 2/3 N H_2SO_4 were added and centrifuged for a few min at

3000rpm. To 2ml of the supernatant 2ml of distilled water, 0.6ml of DAM reagent, and 0.6 ml of acid reagent were added. Various concentrations of standards (20-100µg/ml) were added with 0.4ml of DAM and 1.6ml of acid reagent. The tubes were incubated a boiling water bath for 20 min. Urea reacts with acidic Diacetyl monoxime in the presence of thiosemicarbazide acid resulting in a rose-purple complex, which was measured colorimetrically at 700nm. The amount of urea was expressed as mg/dl.

ESTIMATION OF SERUM URIC ACID [10]

Reagents:

1. 10% Sodium tungstate.
2. 2/3 N Sulphuric acid.
3. 10% Sodium Carbonate.
4. Phosphotungstic acid reagent: 5g of sodium tungstate dissolved in 20 ml of water. To this added 4ml of 85%phosphoric acid and deflexed gently for 2hrs, cooled and transferred into a flask and made up to 50ml with distilled water and kept in a brown bottle. This was diluted 10 times before use. 5. Standard uric acid solution: 100mg of uric acid was dissolved in a few ml of water. 60 g of lithium carbonate dissolved in 20 ml and heated to 60°C and poured onto uric acid. Mixed the contents and transferred them to a standard flask and made up to 100ml and was used as stock. This was diluted 10 times before use. Procedure: To 0.5ml of serum added 2.0ml of 10% sodium tungstate and 2.0ml of 2/3 N sulphuric acid. Mixed well and centrifuged the contents. 3.0ml of filtrate was taken and added 0.6ml of sodium bicarbonate and 0.6ml of phosphotungstate reagent. A blank was taken consisting of 3ml of water and treated similarly. Various concentrations of standards were also added with 0.6ml sodium carbonate and 0.6ml of Phosphotungstate reagent. The tubes were kept in room temperature for 30 min. Uric acid in the filtrate reduces phosphotungstic acid in an alkaline medium into phosphotungstic acid, (tungsten blue) a blue color compound, which was read at 700nm. The values were expressed as mg/dl.

ESTIMATION OF SERUM CREATININE [11]

Reagents: 1. Picric acid: Dissolve 10.5g of picric acid (hydrated) or 3g of anhydrous picric acid in 500ml of hot water and cool to room temperature and then diluted to 1000ml in a volumetric flask.

2. 0.75 sodium hydroxide: 3gm of sodium hydroxide was dissolved in 100ml distilled water.
3. 10% Sodium tungstate
4. 2/3 N Sulphuric acid

5. Stock solution: 100mg creatinine in 0.1N hydrochloric acid in drops and made up to 100ml with 0.1N hydrochloric acid.

6. Working standard: 1 in 20 dilutions.

Procedure: Into a series of test tubes various concentration of standard solution was taken and a volume was made up to 3ml with distilled water. For the test 0.5ml of serum was added then 2ml of 2/3 N sulphuric acid and 2ml of sodium tungstate and centrifuge. From that 3ml was taken. Then added 1ml of picric acid and 1ml of sodium hydroxide to all tubes are heated in a boiling water bath for 15 minutes. The color developed was read at 500nm

ESTIMATION OF CALCIUM [12]

Reagents:

1. 4% Ammonium oxalate solution
2. Diluted ammonia (2% solution)
3. 2N sulphuric acid
4. 0.01N potassium permanganate
5. Distilled water

Procedure: 2 ml of serum 2 ml of distilled water and 1 ml of 4% of ammonium oxalate solution are added and mixed well and allowed to stand overnight. Tapered centrifuge tubes are used. After precipitating the calcium, it is centrifuged, and the supernatant is removed. 5ml of 2 % ammonium is added to the precipitate and mixed well. It is centrifuged again for a few mins. The supernatant is discarded and washing is replaced for the second time. The precipitate is dissolved in 2ml of 1N sulphuric acid, using the glass rod, warm by placing the beaker in boiling water to dissolve oxalate. Then it is titrated against 0.01N potassium permanganate at 70 to 90 °C. the endpoint is the appearance 0.01N potassium permanganate. The difference gives the volume of 0.01N potassium permanganate required to titrate calcium oxalate. (1 ml of 0.01 N potassium permanganate denotes 0.02 mg of calcium).

ESTIMATION OF OXALATE [13]

Reagents:

1. Conc Nitric acid
2. Bromothymol blue
3. Calcium sulfate
4. Ethanol
5. Sulphuric acid
6. Potassium permanganate

Procedure: 1 ml of the sample was acidified beforehand by concentrated nitric acid to solubilize crystals and then adjusted to pH 7 by NaOH in the presence of a color indicator, the bromothymol blue. About 2 ml of saturated calcium sulfate and 5 ml of ethanol were added to precipitate oxalate overnight. The sample was centrifuged at 4000rpm for 10 mins. Then filtered on filter paper. The precipitate obtained was solubilized in 10 ml of water acidified by 1 ml of conc sulphuric acid. The sample was titrated by a solution of KMnO4.

ESTIMATION OF CITRATE [14]

Reagents:

1. 25% Ammonium hydroxide solution
2. 0.2M Magnesium chloride solution
3. 10M HCl solution
4. Citric acid trisodium salt was used as standard
5. 18mM Ferric chloride solution was prepared freshly, in deionized water instead of HCl solution Procedure: To 0.1mL of ammonium hydroxide (25%), 1mL of the sample was added and was mixed well by the vortex. To this 0.45mL of magnesium chloride solution was added and mixed on a vortex mixer and the mixture was centrifuged at 400rpm (RCF) for 10 min to obtain a phosphate-free sample. The supernatant was then transferred to clear tubes. After adjusting the pH of the supernatant to 2 with 0.1mL of 10M HCl, the supernatant was again mixed by the vortex. A to the mixture 0.15mL of ferric chloride (18mM) was added and mixed on a vortex mixer and absorbance was immediately read against the deionized water at 390nm.

HISTOLOGICAL STUDIES [15]

Introduction: Histopathology is the microscopic study of the tissues affected by disease. The technique deals with the preparation of tissues for microscopic examination. The various steps involved in the preparation of tissue for histological examination are

1. Fixation
2. Dehydration
3. Clearing
4. Impregnation
5. Embedding
6. Cutting
7. Staining
8. Mounting Fixation: Preparation of 10% Buffered Neutral Formalin NaH2PO4 3.5 g Na_2HPO_4 6.5 g Formalin 100 ml Distilled water 900 ml Tissue up to 4 mm thick was adequately fixed in buffered neutral formalin for about 8hrsat room temperature.

Tissue Processing: Selection of tissue: A small block of tissue that is representative of the whole specimen with a surface area of 3x2 cm and thickness of 4 mm was taken. The block of tissue was then wrapped in a piece of filter paper and placed in a metal capsule with perforations to allow free access of the tissue to further processing.

Dehydration: The tissues were dehydrated so that they may be filled with wax. Alcohol was used as the dehydrating agent. Dehydration was performed using graded series of alcohol beginning with 70% and ending up with absolute alcohol. Dehydration becomes diluted due to the absorption of water from the tissues. Hence the solution was changed; 70% solution was discarded and the tissue was placed in 80%, 90% and finally the tissue was placed in absolute alcohol.

Clearing: Chloroform was used as the clearing agent. Chloroform is preferred because it causes minimal shrinkage or hardening of tissues. It takes about 6-24 hrs for complete clearing in chloroform. Wax Impregnation: Preparation of Paraffin wax Hard wax or White wax 700g Bees wax 35g White wax and Bees wax were melted together in about 100 ml of water. Boil the water for another half an hour after it had reached its boiling point. Removed from heat and cooled until the dirt in wax settles in the water layer. The wax was then poured into molds. The average time taken for complete impregnation of a piece of tissue was about 3 hrs. Automated Tissue processing was carried out with the help of an automated tissue processor. The machine consists of 10 glass beakers and two or three thermostatically controlled wax baths. A16 hr schedule was followed for the accomplishment of the processing. 1. 10% buffered neutral formalin 1 hr 2. 10% buffered neutral formalin 1 hr 3. 70% alcohol 2 hrs 4. 80% alcohol 2 hrs 5. 95% alcohol 1 hr 6. 100% alcohol 3 hrs

7. 100% alcohol 1 hr

8. 100 % alcohol 1 hr

9. Chloroform 1 hr

10. Chloroform 1 hr

11. Paraffin Wax 1 hr

12. Paraffin Wax 1 hr

Embedding: Leuckhards L molds were used for embedding. The mold was filled with paraffin wax. The paraffin was hardened by rubbing ice cubes at the bottom of the tray. The specimen was pressed gently to achieve a flat surface. The specimen was pressed with forceps such that all the tissues were flat. The mold was placed in cold water for hardening for 20-30 mins. The excess wax surrounding the tissues was trimmed properly and the blocks were subjected to microtomy. Microtomy: The rear end of the block was placed on a hot plate and quickly attached to a wooden block holder. The microtome knife was inserted in position and fixed securely in its place. The thickness was set at 15 µm and few sections were cut. Further trimming was performed to remove the excess wax. The thickness was again reset at 3-5µm and sections were cut. The sections were allowed to float on warm water to remove the folds that developed during sectioning. Staining: The sections were transferred to clean glass slides and placed in an incubator at 110ºCfor1 hr to ensure the attachment of the section to the slide. Sections placed in slides were treated with xylene for 20 min. The slides were then immersed in alcohol to remove the xylene. The slides were passed through descending concentrations of alcohol for deparaffinization. This results in the hydration of the tissues. The sections were also placed in running tap water for 5-10 min. Haematoxylin is the stain that is used in the present work.

1. Preparation of Harris's haematoxylin.
Haematoxylin 2.5 g
2. Absolute alcohol 25 ml
3. Potassium alum 50 g
4. Distilled Water 500 ml;
5. Mercuric Oxide 1.25 g
6. Glacial Acetic Acid 20 ml Haematoxylin was dissolved in alcohol. Potassium alum was dissolved in warm water. Both solutions were mixed and boiled. The mercuric oxide was then added. Cooled rapidly and added glacial acetic acid. 2. Preparation of Eosin 1. Eosin Y 10 g 2. Distilled water 50 ml 3. Glacial Acetic Acid 10 ml 4. 95% Alcohol 950ml Eosin was dissolved in distilled water and stirred continuously. Add alcohol and finally Glacial Acetic Acid. Procedure: After deparaffinization, the slides were stained with Harris's hematoxylin for 10-15min. Washed in running tap water for 5 min. The sections were blotted and dried. The slides were placed in Eosin stain for 1 min. The slides were dipped in 95% alcohol 2-4 times. The sections were finally cleared with xylene and dried. Mounting: After staining the slides were made permanent for microscopic examinations using DPX mountant. Preparation of DPX 1. Destrene 80 10 g 2. Phthalate (dibutyl phthalate) 5 ml 3. Xylene 35 ml.

Procedure: After staining a drop of mountant was added to one edge of the section. A cover slip was placed slowly and carefully on top of the section, allowing the mountant to flow across the section without trapping air bubbles.

STATISTICAL ANALYSIS

All the statistical comparisons between the groups were made by means of One-Way Analysis of Variance (ANOVA) and followed by Dunnett's Multiple Comparison tests. The values were considered significantly different at $P < 0.05$. The data expressed were Mean ± standard error of the mean (SEM).

Table 1: Effect of PHF of AEMO and AEPP on Urine pH in Urolithiasis Experimental Animals

Groups	P^H
I (Normal)	5.5 ±0.10
II (Disease control)	7.7±0.12
III (Treatment Group 150mg/kg bw)	6.2±0.11
IV (TreatmentGroup 300mg/kg bw)	5.7±0.25
V (Standard drug 750mg/kg bw)	5.3±0.16

Values are expressed as mean ±SEM, n=5; P <0.05 indicates significance when Groups II compared with Group I
P<0.05 indicates significant when Groups III and IV compared with Group II compare

Table 2: Effect of PHF of AEMO and AEPP on serum analysis of ethylene glycol induced Urolithiatic Experimental Animal

Group	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Calcium mg/dl	Oxalate mg/dl	Phosphate mg/dl
I	11.77±0.28	5.35±0.27	0.77±0.06	8.22±0.04	1.75±0.016	3.42±0.06
II	22.27±0.08	11±0.35	6.22±0.29	15.07±0.24	4.91±0.03	7.22±0.07
III	17.25±0.81	7.67±0.13	3.93±0.08	9.55±0.07	2.13±0.006	5.7±0.09
IV	15.78±0.77	6.67±0.136	2.81±0.04	6.52±0.11	1.76±0.002	5.17±0.05
V	16.05±0.30	6.17±0.136	0.71±0.04	8.77±0.12	1.81±0.005	4.35±0.16

Table 3: Effect of PHF of AEMO and AEPP on kidney homogenate analysis of ethylene glycol-induced Urolithiatic Experimental Animals

Group	Calcium mg/dl	Oxalate mg/dl	Phosphate mg/dl
I	3.15±0.05	1.41±0.002	1.7 ±0.10
II	7.3±0.08	5.21±0.06	4.3 ±0.07
III	6.4±0.08	2.12±0.004	2.7±0.09
IV	5.52±0.11	2.07±0.04	2.05±0.05
V	6.85±0.06	1.65±0.018	3.2±0.16

Histopathological studies:

The present Histopathological studies showed EG-exposed urolithiasis rats showed deposition of calcium oxalate crystals in the lumen of the Proximal convoluted tubule. Tubules were highly dilated and cystic sloughing off the tubular epithelial cells. While in plant extract-treated animals, it apparently retained normal morphological and microscopical anatomical architecture of the nephrons. However, mild degeneration of cells and cyst formation was observed.

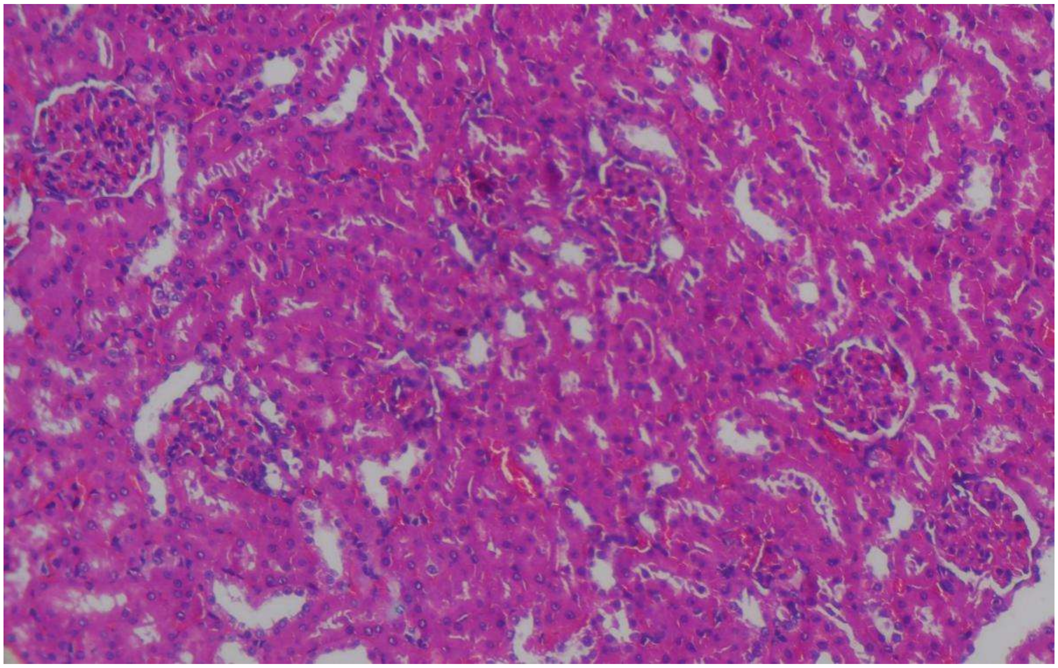

Plate I: Photomicrograph of kidney section of normal wistar albino rats

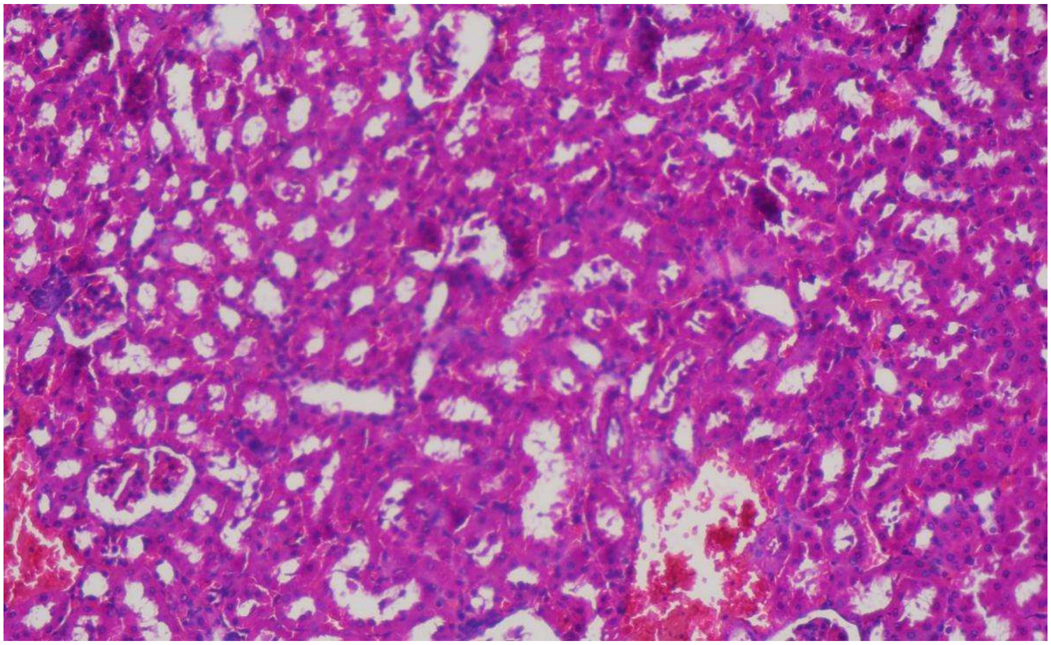

Plate II- Photomicrograph of urolithiatic induced kidney section of experimental animals

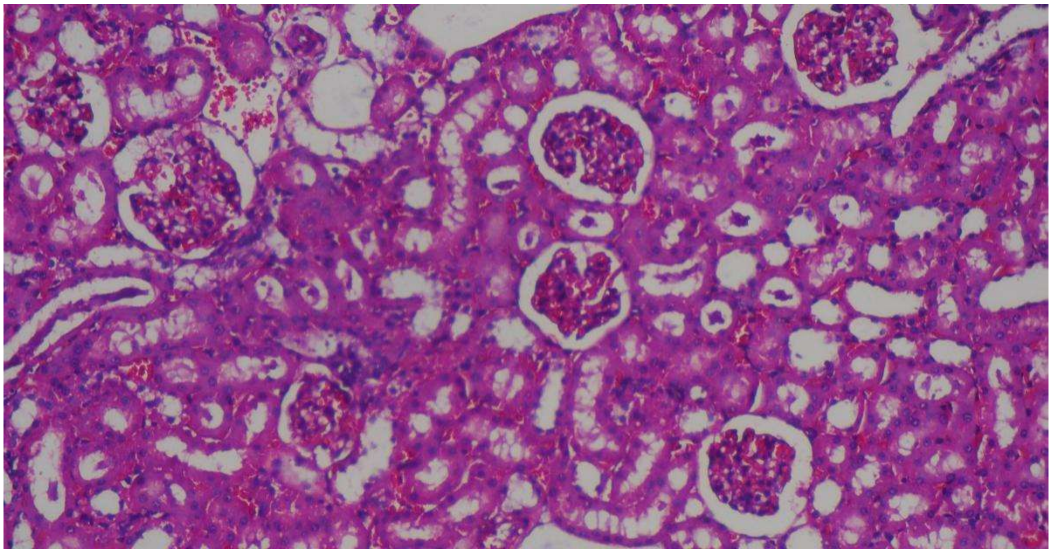

Plate III-Photomicrograph of kidney section of urolithiatic induced experimental animals treated with PHAE (150mg/kg Body weight)

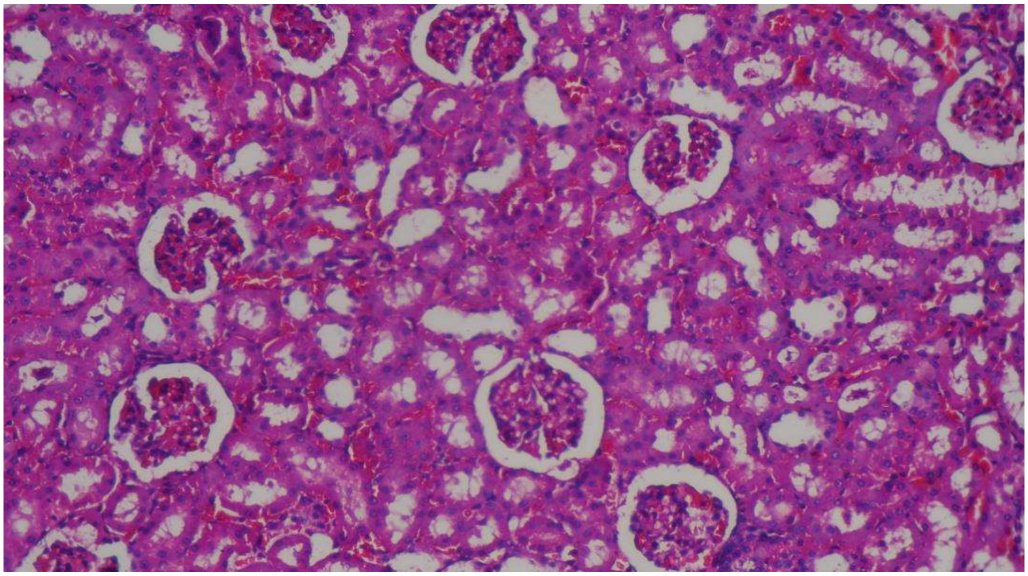

Plate IV: Photomicrograph of kidney section of urolithiatic induced experimental animals treated with PHAE (300mg/kg Body weight)

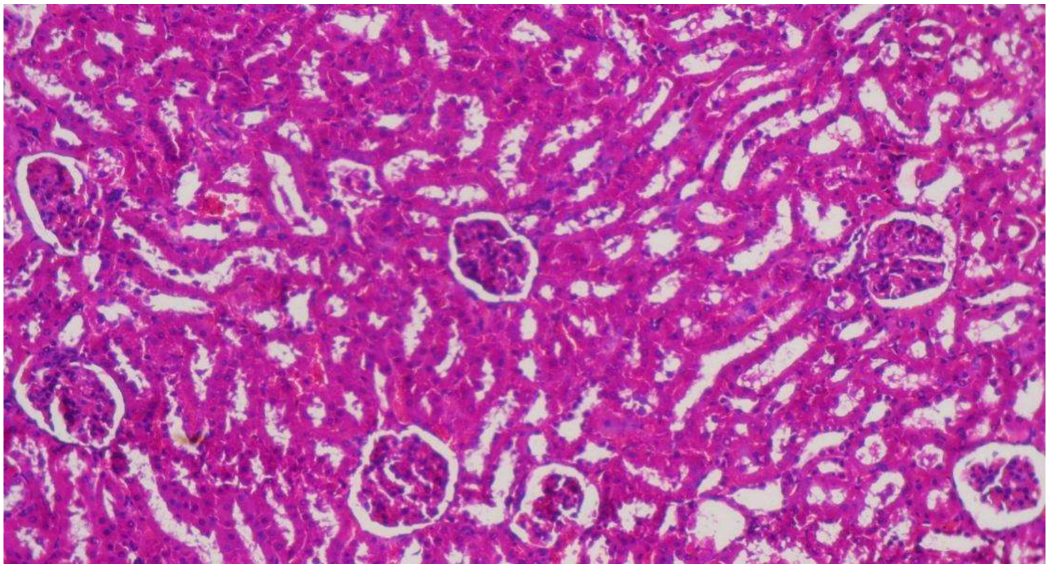

Plate V: Photomicrograph of kidney section of urolithiatic induced experimental animals treated with standard drug (cystone 750mg/kg Body weight)

The figures (Plates 1-5) represented to antiurolithiatic effect that was further confirmed by kidney histopathological studies. Indeed, kidney sections of untreated rats showed abundant crystal depositions. Furthermore, renal epithelial cells had more tubular dilatation and damage shown by large spaces in the tissue. In plant-treated rats, fewer crystal depositions were seen compared to disease control, and the necrosis as well as the tubule dilatation was very limited. Renal stone deposition damages the renal tissue and deteriorates renal function. Lithogenic treatment caused impairment of renal functions of the untreated rats as evidenced by the markers of glomerular and tubular damage: raised BUN, uric acid, urea, and serum creatinine that was lowered in animals receiving plant extract.

RESULTS AND 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 carcinogenesis. The present study 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.

Ethylene glycol disturbs oxalate metabolism by way of an increase in substrate availability that increases the activity of oxalate in the synthesis enzyme of glycolic acid oxidase in the rat. Decreased urine volume, increased urine pH, protein, urea, uric acid, creatinine, phosphate, calcium, and oxalate level. Decreased level of citrate was observed. These alterations were significantly attenuated with these plant extract treatment groups III and IV.

In serum, kidney, and urine analysis disease control showed a significant increase in the protein, urea, uric acid, creatinine, phosphate, calcium, and oxalate levels the plant-treated groups showed significantly and dose-dependently decreased by the polyherbal formulation of aqueous extracts of *Moringa oleifera* Lam and *Peltophorum pterocarpum* DC leaves. Normal urine contains many calculi inhibitors and citrate is one such inhibitor which is present in low level of citrate is observed in stone forming in the rat. The citrate forms a complex with oxalate and reduces the supersaturation of calcium oxalate and as a consequence reduces the growth and nucleation rate of calcium oxalate crystals.

After the treatment significant increase in the urinary citrate and reduce the risk of calcium oxalate stone formation. The examination of histological slides also supported the results of biochemical change in the urine and serum the disease control showed the deposition of oxalate crystals in renal tubules, shrinkage of glomeruli, and altered renal architecture.

The oxalate crystals are usually associated with injury of renal epithelial cells that facilitate the adhesion and tubular retention of crystals. The plant extracts significantly restored the normal cellular, organization renal architecture, and renal functions no crystal deposition was observed, and cellular integrity was recovered. Hyperoxaluria is the most significant risk factor in the pathogenesis of renal stones. It has been reported that oxalate plays an important role in stone formation and has about 15-fold greater effect than urinary calcium. Increased oxalate concentration is responsible for the precipitation and deposition of CaOx crystals. In urolithiasis, the Glomerular Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid get accumulated in the blood.

CONCLUSION

Herbal therapy improves the renal function by increasing the excretion of urea and creatinine. The herbal drugs exert their antipathogenic effect by altering the ionic composition of urine, e.g., decreasing the calcium and oxalate ion concentration or increasing magnesium and citrate excretion. The antiurolithiatic activity may be apparently due to flavonoids, saponins and phenolic compounds present in plants. the polyherbal formulation of aqueous leaves extracts of *Moringa oleifera Lam* and *Peltophorum pterocarpum* DC leaves have potent antiurolithiatic activity against ethylene glycol–induced calcium oxalate Urolithiasis in male albino rats.

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