



## Research Article

### EVALUATION OF ANTI-NEPHROLITHIATIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *MORUS ALBA* L. IN ANIMAL MODELS

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#### ABSTRACT

Kidney stone is a common painful condition of multifactorial etiopathogenesis, and calcium oxalate (CaOx) is the commonest component of stone. The effect of the ethanolic leaf extract of *Morus alba* L. against Calculi-Producing Diet induced nephrolithiasis in *Wistar* rats is summarized in the present study. Nephrolithiasis was induced by fed with Calculi-Producing Diet (CPD: Rat pellet mixed with 5 % sodium oxalate) and it was manifested by high calcium, oxalate, serum creatinine, Blood Urea Nitrogen, reduction in urine volume and creatinine clearance. The extraction was done by successive cold maceration and evaporation by distillation using 95 % ethanol. After the extraction the crude extract were subjected to phytochemical screening. The preliminary phytochemical screening showed the presence of alkaloids, glycosides, flavonoids, phenols, tannins, carbohydrates, proteins, saponins and sterols. The extract of *Morus alba* L. showed a potent percentage inhibition in different *in vitro* models. The treatment with plant extract (500 and 1000 mg/kg/day), exhibited a significant effect in preventing calcium oxalate formation and also in dissolving the preformed stones. Significant effect is exhibited by the treatment groups with 1000 mg/kg/day. Histopathological evaluation also supports the protective effect of *Morus alba* L.

**Keywords:** *M. alba* L., Nephrolithiasis, Calcium oxalate, Calculi-Producing Diet, Anti-nephrolithiatic activity.

#### INTRODUCTION

Kidney Stone is one of the painful urologic disorders that occur in approximately 12 % of the global population and its re-occurrence rate in males is 70-81 % and 47-60 % in female with a relapse rate of 50 % in 5-10 years<sup>1</sup>. Here the term mentioned nephrolithiasis is the formation of stones within the kidney or nephrons. Kidney stone formation is a complex process and it results as a cascade of events, including nucleation, crystal growth, aggregation and crystal retention. The most common type of stone contains calcium in combination with either oxalate or phosphate. These chemicals are part of the normal diet and which are essential for the formation of bones and muscles. Once afflicted, the subsequent relapse rate is increased and the recurrence interval is shortened<sup>2</sup>. The pharmacological treatment includes Non-Steroidal Anti Inflammatory Drugs, diuretics,  $\alpha_1$ - antagonists and alkalizing agents. Extra Corporeal Shock Wave Lithotripsy, Percutaneous nephrolithotomy and Uteroscopy are the surgical treatment used to eliminate kidney stones. The treatment are costly, produce adverse effects include long term infertility and renal damage. Also, there is chance of recurrence is more. As there are no satisfactory standard drugs in modern medicine, herbal remedies are proved to exert their effectiveness at kidney stone formation; the plant based therapy is used as adjunct therapy for better relief<sup>3</sup>. *Morus alba* L. (Moraceae) is a potent herb, commonly known as White Mulberry. It has been used since ancient times in folk medicine for its many medicinal properties. Recent evidence shows that the leaves and shoots from mulberry possess several medicinal properties including diuretics and kidney protective activity. But there is no report is available for its antilithiatic activity. Hence the present study is undertaken to investigate the anti-nephrolithiatic activity of ethanolic leaf extract of *Morus alba* L. in Calculi Producing Diet induced nephrolithiasis animal model.

#### MATERIALS AND METHODS

##### Collection and extraction

Plant material used in this study was collected from Chalakkudy, Thrissur district, India. The plant was authenticated by Dr. Jomy Augustine, Head of the department Botany, St. Thomas College, Pala. A specimen was deposited with voucher number 2265. The shade dried leaves was powdered mechanically and extracted using 95 % ethanol. Extraction was done by successive cold maceration and evaporation by distillation. The crude extract was further used to perform phytochemical screening.

##### Animals

Male *Wistar* albino rats (160-240) were used in the present study to evaluate anti-nephrolithiatic activity. They were housed under controlled conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity (45- 55 %) and light controlled room under 12 h light and 12 h dark cycles. The rats were fed with rat pellet and water *ad libitum* for several days before the beginning of experiment. The experimental protocol was approved by the IAEC (No: 007/MPH/UCP/CVR/13).

##### Extraction

The extraction was done by successive extraction by maceration using 95 % ethanol. Finally the solvent evaporation from the extract was done by distillation.

##### Preliminary phytochemical screening

The ethanolic extract of *Morus alba* (Linn.) leaves were subjected to qualitative tests for the identification of various phytoconstituents<sup>4</sup>.

##### *In vitro* Studies

##### Nucleation assay

Solution of calcium chloride and sodium oxalate were prepared at a final concentration of 3 mmol/L and 0.5 mmol/L respectively, in a buffer containing tris 0.05 mol/L

and sodium chloride 0.15 mol/L at pH 6.5. Both solutions were filtered three times through a 0.22 µm filter. 950 µL of calcium chloride solution was mixed with 100 µL of the herb extract at different concentrations. Crystallization was started by adding 950 µL of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm using Ply Tetra Fluro Ethylene (PTFE) coated stirring bar. The temperature was maintained at 37°C. The absorbance of the solution was measured at 620 nm. The rate of the nucleation was estimated by comparing the induction time in the presence of the extract with that of the control<sup>5-9</sup>.

#### Crystallization assay in whole urine

The undiluted urine samples (collected over 24 h) from healthy subjects were accumulated in the Polypropylene bottle containing Sodium Azide as an anti-bacterial agent. The urine sample was refrigerated after collection. Aliquots of 2 ml of urine were transferred to tubes and allowed to warm to 37°C; 50 µL of herb extract solutions at different concentrations were added to the tubes. Tubes with no extract added were used as controls. Finally, 50 µL of 0.1 mmol/L sodium oxalate solution was added and the tubes incubated at 37°C for 30 minutes and the OD was read at 620 nm. Finally the percentage inhibitions were calculated<sup>5,6</sup>.

#### Aggregation assay

Calcium oxalate monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60°C in a water bath for 1 hour and then cooled to 37°C. COM crystals were used at a final concentration of 0.8 mg/mL, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37°C in the absence or presence of plant extract. The rate of aggregation was estimated by comparing the slope of the turbidity in the presence of the extract with that obtained in the control<sup>5,7-9</sup>.

#### In vivo Study

The original protocol was modified and used for *in vivo* study<sup>10</sup>. Modified version often used to evaluate effect of ethanolic leaf extract of *Morus alba* L. on Calcium oxalate stones. The experimental animal *Wistar* albino rats were divided in to seven groups each group consists of 6 animals. Group I: Received only vehicle (Distilled water) orally for 30 days, serve as normal animals (NC). Group II: Received CPD (from day 1- 8) and vehicle (from day 1 to 15). This group is served as preventive control (PC). Group III: Received CPD (from day 1- 8) and extract (low dose 500 mg/kg from day 1 to 15). This group is served as Preventive Treatment Group1 (PTG1). Group IV: Received CPD (from day 1- 8) and extract (High dose 1000 mg/kg from day 1 to 15). This group is served as Preventive Treatment Group 2 (PTG2). Group V: Received CPD (from day 1- 15) and vehicle (from day 16 to 30). This group is served as Curative control (CC). Group VI: Received CPD (from day 1- 15) and extract (low dose 500 mg/kg from day 16 to 30). This group is served as Curative Treatment Group1 (CTG1). Group VII: Received CPD (from day 1- 15) and extract (High dose 1000 mg/kg from day 16 to 30). This group is served as Curative Treatment Group 2 (CTG2).

#### Assessment of Urinary parameters

The rats were hydrated with distilled water (20 ml/animal), housed in separate metabolic cages and urine samples were

collected for 24 hours, at the 8<sup>th</sup> and 15<sup>th</sup> day (II, III, and IV) and 15<sup>th</sup> and 30<sup>th</sup> day ( I, V, VI, and VII). The urinary volume and pH were determined. The samples were centrifuged at 2500rpm for 5min and the supernatant was used to estimate urinary calcium, oxalate, and creatinine.

#### Assessment of haematological parameters

At the end of experiment blood was collected from retro orbital sinus and transfer in to a centrifuge tube without anti-coagulating agent. Then the samples were centrifuged at 2000 rpm for 20 minutes and the supernatant was used for the estimation of BUN, creatinine, and creatinine clearance.

#### Assessment of kidney parameters

At the end of the experiment, the rats were sacrificed by cervical dislocation. The kidneys were removed and washed in ice cold 0.15M KCl. The right kidney was taken for homogenization and the left kidney was weighed and taken for histopathological studies. The kidney was homogenized in 10 % HCl. The centrifuged at 2500 rpm for 3 minutes and the supernatant was used for the estimation of kidney calcium and oxalate<sup>10</sup>.

#### Histopathological examination

For microscopic evaluation, the kidney was fixed in 10 % formalin solution. The tissues were cleared in xylene and embedded in paraffin<sup>12</sup>. Tissue section was stained with hematoxylin-eosin. Each kidney slide was examined for renal tubular necrosis, lymphocyte infiltration, and tubular dilation.

## RESULTS

#### Nucleation assay

It is the initial step in the renal stone formation. The Figure 1 shows the effect of the different concentrations of the ethanolic leaf extract of *Morus alba* L. on the nucleation of CaOx crystals.

With respect to control the absorbance get reduces with increasing concentration as a dose dependent manner. The values are depicted as Mean ± SD. The percentage of inhibition was found to be 41.07 % with 20 µg/ml of plant extract, which is increased to 83.94 % with increase in concentration of *Morus alba* L. extract to 100 µg/ml. The IC<sub>50</sub> value was found to be 25.85 µg/ml.

#### Crystallization assay in whole urine

It is a critical step in the urinary stone formation due to agglomeration of the particles. The results of aggregation assay are shown below in the Figure 2. The result displayed is the effect of the different concentrations of the ethanolic leaf extract of *Morus alba* L. on the Crystal growth of CaOx crystals.

The leaf extract prevented the crystallization drastically. The values are depicted as Mean ± SD. The percentage of inhibition was at 25 % with 20 µg/ml of plant extract, which is increased to 52.41 % with increase in the concentration of *Morus alba* L. extract to 100 µg/ml. The IC<sub>50</sub> value was found to be 97.53 µg/ml.

#### Aggregation assay

It is the third step in the calculus formation. That constitutes the most effective mechanism to increase the size of the particle, composition and structure of urinary crystals. The Figure 3 showed below depicts the effect of the different concentrations of the ethanolic leaf extract of *Morus alba* L. on the aggregation of CaOx crystals.

When compared with control the absorbance get reduces with increasing concentration. The values are depicted as Mean  $\pm$  SD. The percentage of inhibition was found to be 6.66 % with 20  $\mu$ g/ml of plant extract, which is increased to 26.66 % with increase in the concentration of *Morus alba* L. extract to 100  $\mu$ g/ml.

#### **In vivo study: CPD- induced urolithiasis**

The CPD induced urolithiasis showed a marked changes in animal body weight, urinary volume, urine pH, urinary parameters (calcium, oxalate, and creatinine), haematological parameters (BUN, creatinine, and creatinine clearance), kidney parameters (calcium and oxalate), and in the histopathology of kidney.

#### **Effect of *M. alba* L. extract on animal body weight (g)**

The preventive control groups on 15<sup>th</sup> day and curative control groups on 30<sup>th</sup> day showed an increase in body weight when compared to negative controls. There was a decreasing body weight in case of treatment groups (preventive and curative treatment groups) with 500 and 1000 mg/kg of ethanolic leaf extract of *Morus alba* L. when compared to preventive and curative controls.

#### **Urinary parameters**

##### **Effect of ethanolic leaf extract of *M. alba* L. on Urine volume (ml)**

At the end of experiment the urine output in the ethanolic leaf extract of *Morus alba* L. treated groups was increased in comparison to preventive and curative controls. When the treatment groups 2 compared with treatment groups 1 (both preventive and curative) the results showed that there was an increase in urine output in case of treatment groups 2 (1000 mg/kg). The results were recorded in the following Figure 4.

##### **Effect of ethanolic leaf extract of *M. alba* L. on Urinary pH**

The urinary pH in negative control was 8. On induction of calcium oxalate stones, the pH reduced to 6.0-7.0 in the preventive control and curative control groups when compared to negative control. At the end of the study, preventive (PTG1 and PTG2) and curative (CTG1 and CTG2) treatment groups showed an increase in urinary pH (7.0-8.0) when compared to respective control groups.

##### **Effect of ethanolic leaf extract of *M. alba* L. on Urinary calcium (mg/dl)**

Urinary excretion of calcium was significantly increased in case of both preventive and curative controls when compared with negative control. On treatment with extract showed that there is a reduction in urinary excretion of calcium in treatment groups. When curative treatment group 2 compared with preventive treatment group 2, there is a significant reduction of excretion of calcium was observed in preventive treatment group 2 (1000 mg/kg/day). The Table 1 shows the effect of extract on urinary calcium.

##### **Effect of ethanolic leaf extract of *M. alba* L. on Urinary oxalate (mg/dl)**

Urinary excretion of Oxalate was significantly increased in case of both preventive and curative controls when compared with negative control. On treatment with extract showed that there is a reduction in the urinary excretion of Oxalate in treatment groups (both preventive and curative). When curative treatment group 2 compared with preventive

treatment group 2, there is a significant reduction of excretion of calcium was observed in preventive treatment group 2 (1000 mg/kg/day). The extract is more effective at high concentration. The Table 1 depicts the effect of extract on urinary oxalate.

#### **Haematological parameters**

##### **Effect of ethanolic leaf extract of *M.alba*L. on Blood Urea Nitrogen (BUN)**

There is a significant increase in BUN was observed in both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in excretion of BUN in treatment groups (both preventive and curative). When curative treatment group 2 compared with preventive treatment group 2, there is a significant reduction of excretion of BUN was observed in preventive treatment group 2 (1000 mg/kg/day). The extract is more effective at high concentration. The Table 2 depicts the effect of extract on BUN.

##### **Effect of ethanolic leaf extract of *M. alba* L. on Serum creatinine (mg/dl)**

There is a significant increase in serum creatinine was observed in case of both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in Serum Creatinine in treatment groups (both preventive and curative). When curative treatment group 2 compared with preventive treatment group 2, there is a significant reduction in serum Creatinine was observed in preventive treatment group 2 (1000 mg/kg/day). The extract is more effective at high concentration. The Table 2 given below shows effect of extract on serum creatinine.

##### **Effect of ethanolic leaf extract of *M. alba* L. on Creatinine Clearance (ml/min)**

The Creatinine Clearance was significantly reduced in both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in urinary excretion of Oxalate in treatment groups (both preventive and curative). When curative treatment group 2 compared with preventive treatment group 2, there is a significant reduction of excretion of calcium was observed in preventive treatment group 2 (1000 mg/kg/day). The extract is more effective at high concentration. The Table 2 depicts the effect of extract on creatinine clearance.

#### **Kidney parameters**

##### **Effect of ethanolic leaf extract of *M. alba* L. on Kidney Calcium (mg/g)**

Kidney calcium was significantly increased in case of both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in Kidney calcium in treatment groups. When curative treatment group 2 compared with preventive treatment group 2, there is a significant reduction of calcium was observed in preventive treatment group 2 (1000 mg/kg/day). The Table 1 and reports the effect of extract on kidney calcium.

##### **Effect of ethanolic leaf extract of *Morus alba* L. on Kidney Oxalate (mg/g)**

Kidney oxalate was significantly increased in case of both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in Kidney oxalate in treatment groups (both preventive and curative). When curative treatment group 2 compared with

preventive treatment group 2, there is a significant reduction of oxalate was observed in preventive treatment group 2 (1000 mg/kg/day). The extract is more effective at higher concentration. The Table 1 and depicts the effect of extract on kidney oxalate.

#### **Effect of ethanolic leaf extract of *Morus alba* L. on Kidney Weight (g/100g wt.)**

Kidney weight seems to be increased in case of both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in Kidney weight in treatment groups (both preventive and curative). When curative treatment group 2 compared with preventive treatment group 2, the effect is more in preventive treatment group 2 (1000 mg/kg/day). The extract is more effective at higher concentration.

#### **Histopathological evaluation**

Administration of CPD caused glomerular destruction, tubular dilation and lymphocyte infiltration in both preventive and curative controls. Treatment with extract reduced renal tubular damage, tubular dilation and lymphocyte infiltration when compared to control kidney sections.

The effect was more observed in preventive treatment groups when compared to curative treatment groups. The Preventive treatment group 2 was almost similar to that of normal control. Hence more effective was seemed to be higher concentration (1000 mg/kg). Figures 5-11 depict the histopathological variations in normal, induced and treatment groups.

#### **DISCUSSION**

Hyperoxaluria can provoke calcium oxalate nephrolithiasis in both humans and rats. Oxalate metabolism is considered to be almost identical between rats and humans. Thus, a rat model of calcium oxalate urolithiasis can be used to investigate the mechanism involved in human kidney stone formation and also for screening new agents with anti-nephrolithiatic activity. Phytochemical screening revealed the presence of alkaloids, tannins, phenols, flavonoids, sterols, saponins, and glycosides. Different activities observed in the crude extract might be due to the presence of these phytochemicals. For example, flavonoids and phenols are known to possess antispasmodic and  $Ca^{++}$  channel blocking, antioxidant and diuretic activities. Renal cellular exposure to oxalate (Ox) or CaOx crystals leads to the production of Reactive Oxygen Species (ROS), development of oxidative stress followed by injury and inflammation. Renal injury and inflammation appear to play a significant role in stone formation. An overproduction of ROS and a reduction in cellular antioxidant capacities are due to the down-regulation expression of the antioxidant enzymes<sup>13</sup>. The presence of natural antioxidants ameliorates hyperoxaluria induced renal cell injury in urolithiasis. This might be a possible mechanism in preventing nephrolithiasis. Saponins are known to possess anti-crystallization property by disaggregating the suspension of mucoproteins, promoters of crystallization<sup>7</sup>. Presence of these phytochemicals might be responsible for the anti-nephrolithiatic activities of plant *M. alba* L. Urinary super saturation is generally considered to be one of the causative factors in calculogenesis. The supersaturation of urine with CaOx may be an important factor in crystallization. COM crystals are considered to be more harmful than COD because of their tendency to attach

with the membrane to form aggregates and are more likely to attach with the kidney epithelial cells than CaOx dehydrate, resulting in the formation of kidney stones<sup>7</sup>. The mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty of treatment. Thus if supersaturation or later steps in crystallization can be prevented, then lithiasis should be avoided. In this present study the induction of crystals by using different methods like nucleation, crystallization and aggregation shows turbidity and there is an increase in absorbance seen in case of preventive and curative controls. In the presence of various concentrations of extraction showed that a decrease in absorbance indicates low density of crystals. The results showed a very good inhibitory effect against crystal nucleation and crystallization. The limiting factors in stone formation could be those processes that affect the size of the particles formed, because particles may become large enough too occluded in the urinary tract, leading to stone formation. The extract of the plant causes fewer numbers of crystals in solution, thereby reduced supersaturation and the size of the particles. These results are of much great interest since supersaturation may be lowered and small crystals can be easily flushed out through urinary tract. This property of the extract is therefore, advantageous, preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of retention in the urinary tract. Administration of CPD to *Wistar* albino rats in a particular group upto 8 (preventive) and 15 (curative) days significantly shows the renal stones which were mainly composed of calcium oxalate in renal calculi. The Ammonium oxalate accelerates lithiasis through urinary acidification and also disturb oxalate metabolism by increasing the substrate availability thus lead to hyperoxaluria. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Oxalate plays an important role in stone formation and has about 15 fold greater effect than urinary calcium. Stone formation is also caused by hyperoxaluria, which leads to increased renal retention and excretion of oxalate<sup>10</sup>. In the present study, observed hypercalciuria in CPD-induced nephrolithiatic rats favouring the nucleation and precipitation of calcium oxalate from urine and finally leads to subsequent crystal growth. In the present study we can see that an increase in body weight in preventive and curative controls may be due to edema. On treatment with extract showed a decrease in animal body weight. It might be due to reduction in edema and recovery from urolithiasis. The result supports the protective activity of plant extract. In the study there is an increase in urine output and urinary pH. Dissolution of stones can be achieved by alteration in the urinary pH. If the pH is 5-7 the stones likely to form are calcium oxalate<sup>33</sup>. In the study the treatment with extract improved urinary pH slightly and also urinary volume. This increase in pH might be responsible for dissolving the complexes of calcium oxalate. An increase was observed in the 24 hour urine volume in the rats treated with extract when compared with lithiasis induced groups. This improvement in the urinary volume and pH may contribute to their anti-nephrolithiatic activity. The results showed that a significance reduction in urinary excretion of calcium and oxalate. Thus reduces the supersaturation and acidification of urine. This might be responsible for preventing the stone formation. The increase in BUN and serum creatinine and reduction in urinary creatinine is due to the disturbance in the out flow of urine.

Table 1: Effect of Ethanolic Leaf Extract of *M. alba* L. on urinary and kidney calcium and oxalate at the end of the experiment

Groups	Urinary Parameters(mg/dl)		Kidney parameters (mg/g)	
	calcium	oxalate	Calcium	oxalate
NC	1.850 ± 0.016	0.865 ± 0.017	3.230 ± 0.026	1.410 ± 0.010
PC	4.478 ± 0.155	3.330 ± 0.241	5.533 ± 0.309	3.710 ± 0.293
PTG1	2.208 ± 0.154	1.022 ± 0.067	3.752 ± 0.155	1.840 ± 0.076
PTG2	1.922 ± 0.055	0.931 ± 0.065	3.422 ± 0.059	1.627 ± 0.071
CC	5.827 ± 0.404	4.727 ± 0.271	6.348 ± 0.264	4.605 ± 0.371
CTG1	4.393 ± 0.307	3.747 ± 0.298	4.607 ± 0.206	3.365 ± 0.248
CTG2	3.190 ± 0.258	2.693 ± 0.242	4.060 ± 0.156	2.647 ± 0.212

Table 2: Effect of Ethanolic Leaf Extract of *M. alba* L. on Hematological Parameters at the End of the Experiment

Groups	BUN (mg/dl)	Creatinine (mg/dl)	Creatinine Clearance (ml/min)
NC	20.83 ± 0.600	0.915 ± 0.447	0.880 ± 0.074
PC	50.73 ± 3.492	1.967 ± 0.115	0.138 ± 0.009
PTG1	33.33 ± 2.462	1.113 ± 0.050	0.548 ± 0.055
PTG2	24.82 ± 1.684	0.955 ± 0.049	0.815 ± 0.074
CC	64.85 ± 3.790	2.940 ± 0.109	0.053 ± 0.006
CTG1	52.00 ± 2.027	2.232 ± 0.104	0.185 ± 0.028
CTG2	41.20 ± 2.824	1.940 ± 0.061	0.265 ± 0.043

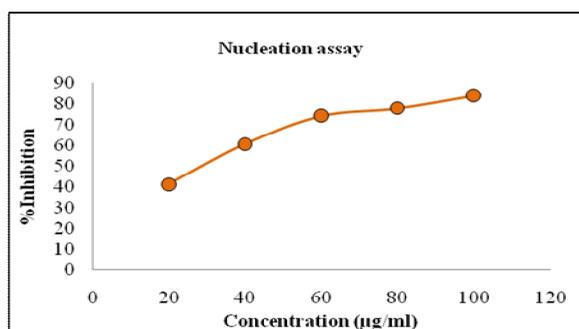


Figure 1: Nucleation assay, the percentage inhibition was increased with increasing concentration

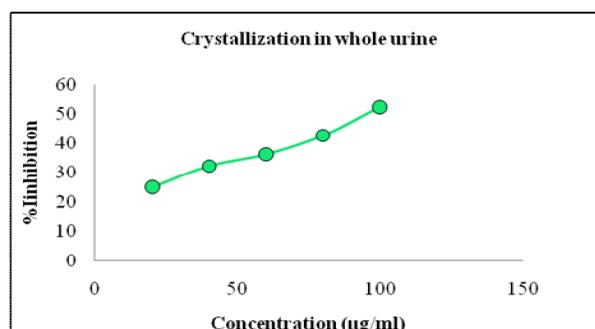


Figure 2: Crystallization assay in whole urine, the percentage inhibition was increased with increasing concentration

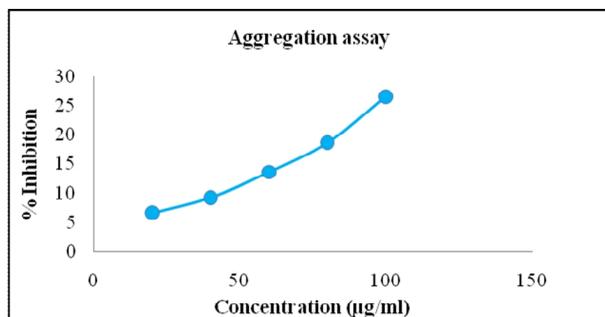


Figure 3: Aggregation assay, the percentage inhibition was increased with increasing concentration

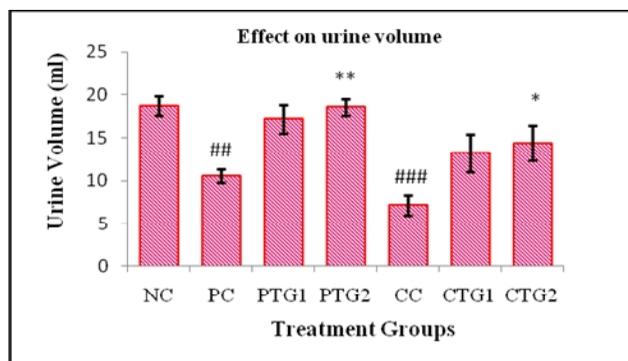


Figure 4: Effect of *M. Alba* L. extract on urine volume at the end of the experiment

All values are shown as Mean ± SEM and n = 6, ##P < 0.01 and ###P < 0.001 when preventive and curative controls compared to negative control; \*\*P < 0.01 when Preventive Treatment Group 2 compared to Preventive Control; \*P < 0.05 when Curative treatment Group 2 compared to Curative Control. There is no significant difference is observed when Preventive Treatment Group 2 compared with Curative Treatment Group 2

## Histopathological Evaluation

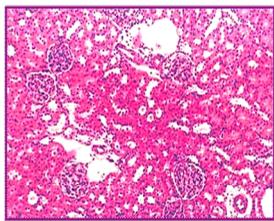


Figure 5: Normal control

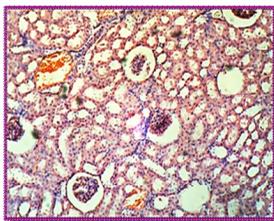


Figure 6: Preventive control

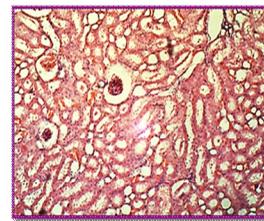


Figure 7: Preventive Treatment Group 1

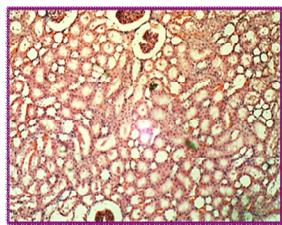


Figure 8: Preventive Treatment Group 2

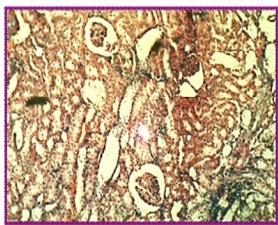


Figure 9: Curative Control



Figure 10: Curative Treatment Group 1

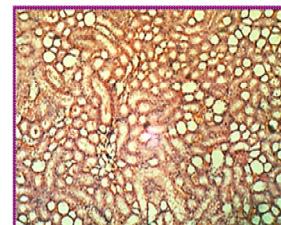


Figure 11: Curative Treatment Group 2

The result impaired GFR<sup>14</sup>. Treatment with extract significantly decreased the plasma creatinine, BUN and increased the urine creatinine levels. The significant increase in urine creatinine and the significant decrease in plasma creatinine in treatment groups is a strong indication of the positive impact on treatment with extract on the glomerular filtration rate. Thus these results support its anti-nephrolithiatic activity. Creatinine levels in blood and urine are usually used to calculate the creatinine clearance which reflects the glomerular filtration rate. GFR is clinically important because it is a marker of the renal function. Renal dysfunction diminishes the ability to filter creatinine, thus decreases creatinine clearance<sup>10</sup>. The present study showed that the creatinine clearance was restored by using ethanolic leaf extract. Results of histopathological slides and kidney weight support the results of deposition of calcium oxalate in kidney and excretion of calcium and oxalate. On histopathological studies both curative preventive control groups showed glomerular necrosis, renal tubular dilation and lymphocyte infiltration. Treatment with extract showed recovery and it indicates the ability of extract in dissolving stones. On treatment with extract also showed a reduction in kidney weight when compared with induction groups. It supports the anti-nephrolithiatic activity of *Morus alba* L. ethanolic leaf extract. All the results obtained were support the anti-nephrolithiatic activity of ethanolic leaf extract of *Morus alba* L.

## CONCLUSION

On preliminary phytochemical screening of ethanolic leaf extract of *Morus alba* L. showed the presence of carbohydrates, proteins, alkaloids, phenols, flavonoids, sterols, saponins, glycosides, and tannins. The presence of these compounds offers nephroprotection and anti-nephrolithiatic activity. Ethanolic leaf extract of *Morus alba* L. exhibited significant percentage inhibition on nucleation and crystallization. Also, extract exhibited less effect in aggregation of crystals when compared with nucleation and crystallization. This indicates that the extract has *in vitro* inhibition capability in different models. In the present study, ethanolic leaf extract of *Morus alba* L. reduced animal body

weight and significantly reduced the elevated levels of urinary, haematological, and other kidney parameters. Also, significantly increases urinary volume, pH, urinary creatinine and creatinine clearance. Significant effect was observed with higher concentration (1000 mg/kg). In CPD- induced animals, there will be a presence of glomerular necrosis, renal tubular dilation, and large amount of lymphocyte infiltration were observed. In this study, ethanolic leaf extract of *Morus alba* L. treated groups showed a reduction in such changes in kidney histology. The extract treated groups were seems to be almost similar to that of normal. The result exhibited by the ethanolic leaf extract of *Morus alba* L. (1000 mg/kg/day) showed significant anti-nephrolithiatic activity in preventing treatment groups when compared with curative treatment groups. The results of the present study have shown that the urinary stone formation could be prevented with ethanolic extract of *Morus alba* L. Based on the improvement in biochemical markers, histopathological studies, *in vitro* studies and the presence of phytochemical constituents, concluded that the ethanolic leaf extract of *Morus alba* L. possess anti-nephrolithiatic activity. The antilithiatic activity of this plant can be attributed to its ability to reduce the supersaturation of urine with calculogenic ions, diuretic activity and nephroprotective activity.

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