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Research Article

Antiurolithiatic and Antioxidant Activity of *Abies webbiana* Leaves on Ethylene Glycol-Induced Urolithiasis in Rats

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ABSTRACT

One major issue is the recurrence of urolithiasis. The majority of the treatments were derived from plants and worked well. Many important active phytochemicals are found in *Abies webbiana*. This study evaluates the protective potential of *A. webbiana* in Wistar albino rats with ethylene glycol-induced urolithiasis. 0.75% ethylene glycol (v/v) for a period of 28 days was given to induce the formation of renal calculi. Cystone (750 mg/kg) is used as a standard drug and leave extract (200 mg/kg and 400 mg/kg) test drug. The standard and test drug was administered on days 15 through 28. Parameters such as oxalate, uric acid, blood urea nitrogen, potassium, creatinine, calcium, urea and histopathology of the kidney were assessed. The present study showed significant antiurolithiatic and antioxidant properties of the test drug at 400 mg/kg. The extract's flavonoids, terpenoids, and alkaloids may be the cause of these effects. In addition to maintaining dose-dependent levels of oxalate (p <0.0001), urea (p <0.05), uric acid (p <0.001), creatinine (p <0.05), and lipid peroxidation (p <0.01), the extract can lessen the buildup of chemicals that cause stones. The extract was quite successful in preserving the histological integrity. Documentary proof of *A. webbiana's* antiurolithiatic properties has been presented in this study.

INTRODUCTION

An imbalance between the kidneys' promoters and inhibitors causes the complex disorder known as urolithiasis. The development and progression of renal calculi continue to be major concerns despite improvements in contemporary medical treatment. Although several kinds of stones have been found, calcium stones are the most common in rats and humans.[1] Solid, hardened deposits called kidney stones can form in the urinary tract. These stones are frequently small enough to pass through the body without posing any problems. However, even a small stone can cause excruciating discomfort and necessitate prompt medical attention if it blocks the passage of urine. Recurrent stone development is a persistent problem for many people. The most common kinds are those that contain calcium, specifically calcium oxalate monohydrate, calcium oxalate dihydrate, and basic calcium phosphate.[2] Even though there are contemporary methods for treating this condition, like percutaneous nephrolithotomy and extracorporeal shock wave lithotripsy (ESWL), some people may have negative side effects. About 12% of people worldwide suffer from urolithiasis, a common condition with recurrence rates that range from 70 to 81% for men and 47 to 60% for women.[1] Not all patients respond well to synthetic medications intended to prevent urolithiasis, and many of them have side effects that restrict their long-term usage. A disturbance in the solubility and precipitation balance of salts in the kidneys and urinary system is the first step in the development of renal calculi. Severe

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abdominal pain may result from a kidney stone that blocks the passage of urine. In severe circumstances, if the crystals are too big to pass down the tract, blood may show up in the urine. In other cases, the disease may be accompanied by nausea and vomiting.[3] Kidney stone illness has been successfully treated and eradicated using a variety of herbal-based natural medicines in addition to contemporary medical interventions. This is due to the fact that organic molecules like terpenoids, steroids, alkaloids, and other phytochemicals that have positive effects on the human body are frequently found in medicinal plants.[4] Many important active phytochemicals are found in *Abies webbiana* under the 'Pinaceae' family. These phytochemicals have various therapeutic properties, such as antibacterial as nanoparticles, antitumor, mast cell stabilizing, anti-inflammatory, anxiolytic, female antifertility, antitussive, antidepressant, antispasmodic etc.[5] This plant has several uses in the Siddha medical tradition, such as a tonic, stomachic, carminative, and expectorant. The scalp can be treated with a pungent mixture consisting of pulverized ingredients and vinegar, which can help alleviate headaches and discomfort.[5-7] Traditional medicine takes a holistic and personalized approach, focusing on addressing the root cause of health issues rather than merely managing symptoms. Herbal remedies help restore balance and strengthen immunity, contributing to overall health and well-being. The use of medicinal plants like *A. webbiana* can be a more cost-effective alternative to expensive pharmaceutical drugs, particularly in low-income regions where access to conventional medical care is limited. Identifying the chemical compounds responsible for the antiurolithiatic properties of *A. webbiana* could aid researchers in developing new pharmaceutical treatments that harness these natural compounds, offering innovative therapy options for urolithiasis. The goal of this study was to identify the pharmacological basis for the plant's traditional use in treating urolithiasis, even though there is currently no research on the plant's anti-ulcer benefits.

Table 1: Results of preliminary phytochemical screening of *A. webbiana*

S. No	*Constituents*	*Observation*
1	Glycosides	+
3	Alkaloids	+
4	Flavonoids	+
5	Tannins	+
6	Phenols	+
7	Resin	-
9	Saponins	+
10	Terpenoids	+

(-) indicates the absence of compound (+) indicates the presence of compound

Based on this, an effort has been undertaken to use the phytoconstituents of *A. webbiana* leaves to counteract the urolithiatic and antioxidant effects of ethylene glycol on rats.

Materials And Methods

Plant Collection and Authentication

The leaves of *A. webbiana* were collected from Assam State Zoo cum Botanical Garden, RG Baruah Rd, Guwahati, Assam, 781005 on March 2023. They were authenticated by Dr Noorunnisa Begum, Curator of FRLHT, Jarakababande Kaval, Post Attur, Bangalore-560064.

Preparation of Plant Extract

A. webbiana had its needles washed in clean water to make it seem as good as new. After 20 days of drying in the shade, the leaves were ready to be used. The methanolic extract was made using a coarse powder made from dried leaves. Methanolic extraction was performed using the Soxhlet apparatus. Two 6-hour rounds of soxhleting coarsely powdered leaves with methanol (Temperature-30^{o}). The concentrate was strained using Whatman filter paper. As a first step in preparing the extract for phytochemical and pharmacological analysis, it was measured and then sealed in airtight containers. The percentage yield of methanolic extract of leaves of *A. webbiana* was found to be 12.8% w/w.

Phytochemical Screening

Concentrated extracts were preliminarily analyzed to identify phytoconstituents such as flavonoids, tannins, resins, phenols, alkaloids, glycosides, terpenoids, and saponins. The detection of these compounds was performed following standard methods described in established protocols (Table 1).[7]

Treatment of Animals

Female Wistar albino rats weighing 150–200 g were used in this experiment. The source of these rats was Bangalore's Sri Venkateshwara Enterprises. In accordance with the CPCSEA's ethical rules, the animals were always given access to standard feed and water, and they were given 15 days to adapt before the study began. IAEC's approval was obtained for the experiments' procedures. (Reg. No. KCP/IAEC/11/22-23/10/22/12/22).

Experimental Design

The antiurolithiatic activity was investigated using albino Wistar rats as subjects in a rat model of urolithiasis brought on by exposure to ethylene glycol.[6] O Prakash *et. al*, find that the acute oral toxicity of *A. webbiana* is 2000 mg/kg according to the OECD guidelines-423.[8] Based on that, I selected the high dose (400 mg/kg) and Low dose (200 mg/kg). The animals were distributed evenly among five separate groups of six individuals each (n = 6). The rats in group I, which served as the control,

Table 2: Effect of MEAW on urinary parameters

Groups	*Oxalate (mg/d)*
Normal control	18.90 ± 0.72
Disease control	36.50 ± 0.68$^{\#\#\#\#}$
Standard	28.70 ± 0.37****
Low dose	32.10 ± 0.45**
High dose	29.40 ± 0.63****

The data are expressed as Mean ± SEM (n = 6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as $p^{\#\#\#\#} < 0.0001$ vs. the Normal control, $p^{**} < 0.01$, and $p^{****} < 0.0001$ *vs.* the Disease control.

were not restricted in any way in terms of their access to either food or water. The members of group II were given drinking water containing 0.75% ethylene glycol (v/v) for 28 days to induce the formation of renal calculi. Cystone (750 mg/kg; p.o.) was given to group III as a typical antiurolithiatic medication. Curative regimen (CR) groups IV and V were given MEAW at 200 and 400 mg/kg; p.o of body weight on days 15 through 28. Oral administration was used once a day for all extracts, including the gold standard.[6,8,9]

The following parameters were estimated from serum that was extracted from blood samples taken from the retro-orbital venous plexus at the end of day 28 by centrifugation at 1500 rpm for 15 minutes: Uric Acid, BUN (Blood Urea Nitrogen), creatinine, calcium, potassium, chloride, urea.[1] Urine collects with the help of a metabolic cage. This urine was used for the estimation of oxalate.[3,10]

Immediately after blood collection, animals were given a lethal dosage of pentobarbitone (100 mg/kg) to end their lives. Each rat's kidneys were removed surgically, and one of them was given a quick rinsing in ice-cold normal saline. Using a homogenizer and 10 mL of 0.1M tris HCl homogenizing buffer at pH 7.5, 1 g of minced kidney tissue was reduced to a 10% homogenate. These enzyme activities were measured by testing the homogenate. Lipid peroxidation (LPO), glutathione stimulating hormone (GSH). The animals were killed and their kidneys were removed for histological analysis in order to verify the presence of urolithiasis. After meticulously removing any excess tissue, the kidneys were kept in a 10% neutralized formalin solution (pH 7.4). Following paraffin embedding, hematoxylin and eosin staining, and histological analysis, kidney slices were examined.[11]

Statistical Analysis

The data from each group of rats (n = 6) is presented as a Mean SEM. Graph Pad Prism version 10 was used for the statistical analysis. To evaluate whether or if there were statistically significant differences between the groups, a one-way analysis of variance and Turkey multiple comparisons were carried out. A statistically significant value was determined to be one with $p < 0.05$.

Table 3: Effect of MEAW on serum biochemical parameters

Groups	*Urea (mg/dL)*	*Uric Acid (mg/dL)*	*Creatinine (mg/dL)*	*Potassium (mole/L)*	*Calcium (mg/dL)*	*Sodium (mole/L)*	*Chloride (mole/L)*	*BUN (mg/dL)*
Normal control	22.27 ± 0.23	5.48 ± 0.41	0.97 ± 0.015	3.83 ± 0.18	9.52 ± 0.26	140.0 ± 1.00	100.6 ± 1.54	10.56 ± 0.09
Disease control	27.59 ± 0.34	7.615 ± 0.12$^{\#\#}$	1.055 ± 0.06	5.600 ± 0.32$^{\#}$	14.32± 0.63$^{\#\#}$	143.5 ± 1.50	110.7 ± 1.45	13.74 ± 0.80
Standard	24.41± 0.45	5.305 ± 0.15**	0.7300 ± 0.05*	4.670 ± 0.23	10.28 ± 0.32**	139.5 ±1.50	99.87 ± 1.40	11.95 ± 1.22
Low dose	26.03 ± 0.12	5.66 ± 0.20**	0.91± 0.045	5.68±0.18	13.39 ± 0.41*	139.5 ± 2.50	102.4 ± 3.15	15.27 ± 0.23
High dose	21.64 ±1.89*	4.210 ± 0.13***	0.7050 ± 0.055*	4.530 ± 0.07	10.86 ± 0.4050*	135.5 ± 0.50	97.50 ± 0.50*	11.26 ± 0.13

The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, $p\#<0.05$, $p\#\#<0.01$ *vs.* the Normal control, $p^{*}<0.05$, $p^{**}<0.01$, $p^{***}<0.001$ *vs.* disease control.

Result and Discussion

When it comes to stones formed from calcium, calcium oxalate is much more common than calcium phosphate. One of the most important contributors to the development of calcium nephrolithiasis is hypercalciuria. Magnesium, ammonium, and phosphate combine to form struvite stones, the second most frequent kind of urinary stone. These stones are frequently called infection stones due to their association with certain illnesses of the urinary system. Hyperuricosuria, an acidic urine pH, or both may lead to uric acid stones, the third most prevalent kind of urinary stone. Rare cysteine stones are made of cysteine and are brought on by genetic renal transport issues. Also, children have lately been shown to suffer from kidney stones due to exposure to melamine.[1] The four most common types of kidney stones are calcium (75–85%), struvite (2–15%), uric acid (6–10%), and cystine (1%) (1–2%).[12]

In the last several decades, many treatment options for urinary stone diseases have emerged. Most of these therapies require surgery, which is both expensive and not widely accessible. Consequently, many patients either prefer or are limited to using traditional herbal remedies like Ayurveda for the management of urinary stones. A few studies have been conducted on the effectiveness of various herbal and industrial medications marketed to minimize stone formation.[8,9] The goal of this study was to determine whether *A. webbiana* extract might prevent calcium stones in female Albino rats after exposure to ethylene glycol. Depositions of calcium oxalate crystals in the kidney are reduced in my study's extract-receiving groups, and this effect is most pronounced in the preventative group getting a large dosage of extract. More crystals were seen in the illness group despite the prophylaxis group receiving a higher amount of extract.

According to our findings, phenolic components like flavonoids make up the bulk of *A. webbiana* extract; as a result, we speculate that flavonoids, terpenoids, and tannin may be responsible for the extract's anti-kidney-stone activity.

Extract significantly reduced urine oxalate (Fig. 1), urea (Fig. 2), serum uric acid (Fig. 3), creatinine (Fig. 4), calcium (Fig. 5), chloride (Fig. 6) and LPO (Fig. 7) in both the low and high dosage groups compared to DC, demonstrating its efficacy. After receiving ethylene glycol, the treatment group had significantly decreased urinary oxalate concentrations compared to the DC group. These findings confirm the hypothesis that increased citrate excretion plays a role in the formation of calcium oxalate (Tables 2 & 3).

Because of its ability to generate reactive oxygen species like superoxide and hydrogen peroxide, ethylene glycol may induce oxidative damage, which in turn can cause inflammation and the precipitation of calcium oxalate. It was previously believed that the major component in the pathophysiology of oxalate stones was the interaction of calcium oxalate crystals with renal tubular epithelial cells. Crystallization modulator production is tightly regulated by the rate at which reactive oxygen species (ROS) are produced under normal circumstances. Damage, inflammation, and oxidative stress may all result from an excess of ROS or a deficiency of antioxidant components. As a result of ROS, calcium oxalate causes inflammation. Oxalate-induced membrane damage is mediated by ROS produced by lipid peroxidation.[13] These studies have demonstrated that MEAW has a significant antioxidant effect to help prevent the development of kidney stones (Table 4).

The histopathological study shows that a healthy glomerulus (Blue Arrow) with a tuft of capillaries encircled by Bowman's capsule (Green Arrow), tubules surrounded by columnar epithelial cells, and a healthy architecture. No abnormalities, such as interstitial inflammation and proximal tubules dilatation (PCT, Black Arrow) and distal convoluted tubule (DCT, Orange Arrow) inside the renal tissue in the normal control animals (Fig. 8 A). Ethylene glycol induces disease control group's histopathology image shows bowman's capsule around glomerular

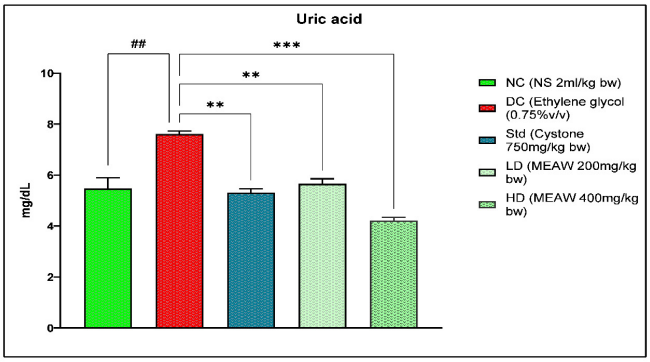

Fig. 1: Effect of MEAW on Urinary oxalate level. The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, $p^{\#\#\#\#}<0.0001$ vs. the Normal control, $p^{****}<0.0001$ vs. disease control.

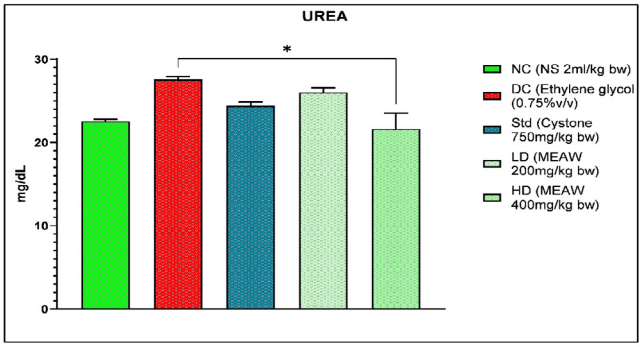

Fig. 2: Effect of MEAW on serum Urea level. The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, $p^{*}<0.05$ vs. disease control

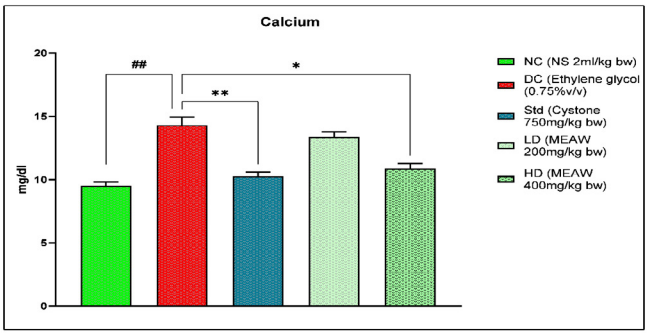

Fig. 3: Effect of MEAW on serum Uric acid. The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, *p*##<0.01 vs. the Normal control, *p***<0.01, *p****<0.001 *vs.* disease control

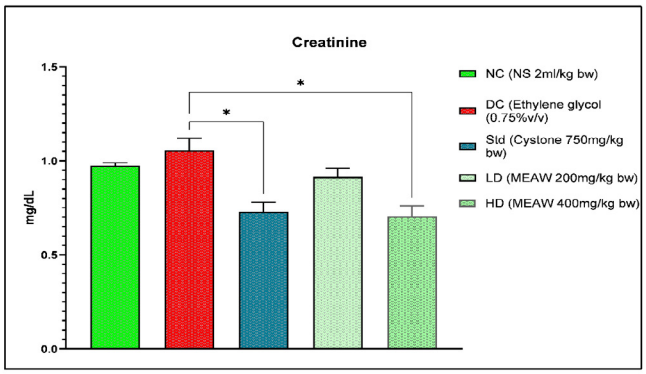

Fig. 4: Effect of MEAW on serum Creatinine level. The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, *p*<0.05 vs. disease control

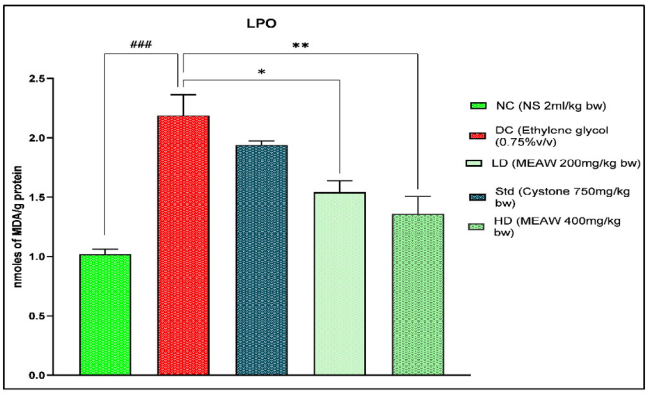

Fig. 5: Effect of MEAW on serum Calcium level. The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, *p*##<0.01 vs. the Normal control, *p**<0.05, *p***<0.01 *vs.* disease control

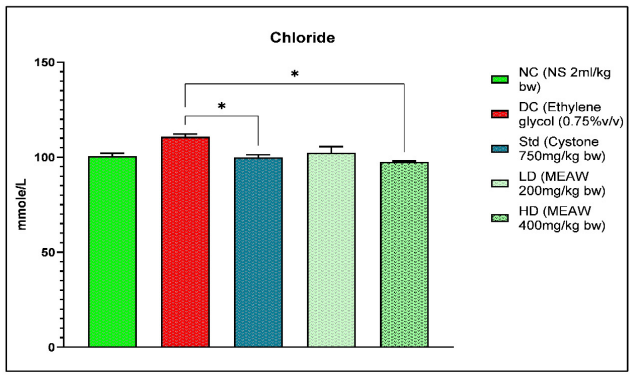

Fig. 6: Effect of MEAW on serum Chloride level. The data are expressed as Mean ± SEM (n = 6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, *p*<0.05 vs. disease control

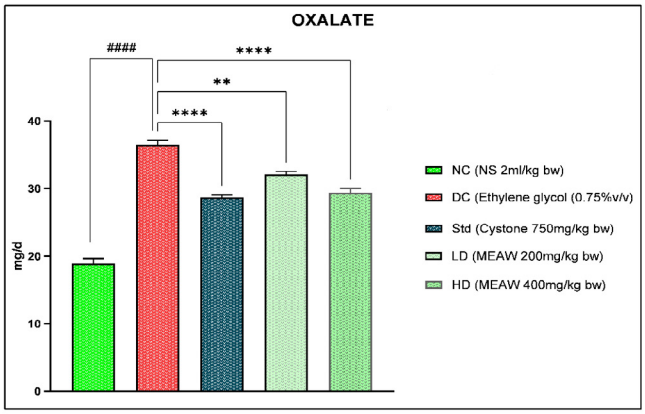

Fig. 7: Effect of the MEAW on antioxidant LPO level. The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, *p*###<0.001 vs. the Normal control, *p**<0.05, *p***<0.01 *vs.* disease control

Table 4: Effect of MEAW on Antioxidant parameters

Groups	*LPO (nmoles/min/mg of protein)*	*GSH (nmoles of MDA/g protein)*
Normal control	1.02 ± 0.04	1.53 ± 0.11*
Disease control	2.19 ± 0.17###	2.85 ± 0.12
Standard	1.94 ± 0.034	1.48 ± 0.11
Low dose	1.54 ± 0.098*	1.56 ± 0.11
High dose	1.36 ± 0.14**	1.52 ± 0.11

The data are expressed as Mean ± SEM (n=6) and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance is indicated as, *p*###<0.001 vs. the Normal control, *p**<0.05, *p***<0.01 vs. disease control.

degeneration with capillary loss. The proximal tubules, DCT, and interstitial renal tissue of the animal revealed prominent dilatation and inflammation (Fig. 8 B). The standard group showing the tubules appear to have a normal design and are recovering from toxicity. There are no interstitial inflammation cells. No abnormalities, such as proximal tubule dilatation and distal convoluted tubule inside the renal tissue (Fig. 8 C). The LD group restoration of renal corpuscles and the glomerulus' normal but dilated architecture. Treatment with MEAW

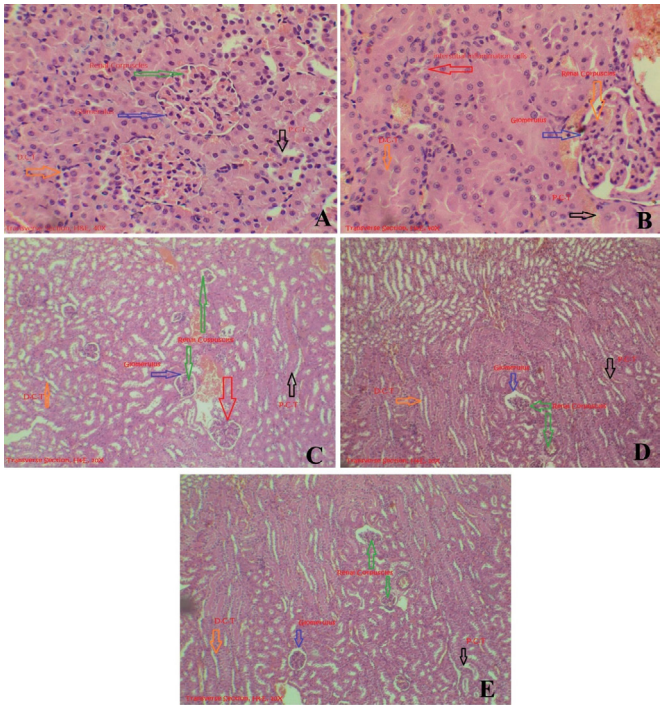

Fig. 8: Effect of MEAW on histopathological evaluation of Kidney. A. The Kidney of Normal control animal, B. The kidney of Disease control animal C. The Kidney of Standard control animal, D. The Kidney of Low Dose (LD) animal E. The Kidney of High Dose (HD) animal

(200 mg/kg) attenuated kidney tissue degeneration, including inflammatory cell infiltration into the interstitium, epithelial cell dissociation, and dilated collecting ducts and proximal tubules (Fig. 8 D). The HD group Demonstrated, restoration and normal glomerular architecture with tufts of capillaries encircled by Bowman's capsule. Most tubules exhibit typical morphology and healing. However, only a small number of tubules displayed moderate degeneration, which was visible as the accumulation of inflammation cells in the tubule center. No abnormalities in the proximal tubule DCT dilatation were reduced as a result of the MEAW (400 mg/kg) treatment (Fig. 8 E).

Flavonoids,[14] saponin,[15] and alkaloids[16] are reported for their anti-urolithiatic activity. All the above-mentioned phytoconstituents are present in *A. webbiana* and hence might have contributed to anti-urolithiatic activity.

The antiurolithiatic action of the MEAW may be mediated, in part, by their antioxidant nature and their propensity to reduce stone-forming components. *A. webbiana* has been shown to have antiurolithiatic activity, and more research into isolating the active principles responsible for this activity and establishing its mechanisms of action is warranted.

Conclusion

Based on the findings of this study, the MEAW, administered at a dose of 400 mg/kg, exhibited significant anti-urolithiasis activity in animal models. The extract effectively reduced serum levels of nitrogenous waste products, including uric acid and creatinine. Additionally, the extract also demonstrated potent antioxidant activity across various assays, further supporting its therapeutic potential. These effects are likely attributed to the presence of bioactive compounds such as flavonoids, polyphenols, and terpenoids.

Overall, this study highlights the promising anti-urolithiasis and antioxidant properties of MEAW at 400 mg/kg. Further research is warranted to elucidate the precise molecular mechanisms underlying these effects, which could contribute to the development of novel therapeutic strategies for urolithiasis and related disorders.

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