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Original Article

Effect of *Wedelia chinensis* Leaf Extracts against Ethylene Glycol Induced Urolithiasis in Rats

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ABSTRACT

Background: Finding a hyperoxaluria treatment that especially targets lowering oxalate excretion is essential due to the drawbacks of present urolithiasis treatments. It's interesting to note that *Wedelia chinensis* (Family: Asteraceae) leaves are used traditionally by several Indian tribes for treating renal disease. **Objectives:** The current study aimed to assess the effectiveness of *W. chinensis* leaf extracts as therapeutic agents in rats with experimentally induced calcium oxalate urolithiasis. **Materials and Methods:** Rats were given 0.75% ethylene glycol orally to induce calcium oxalate lithiasis for fourteen days in succession. *W. chinensis* leaf extracts, 400 mg/kg body weight in aqueous and ethanolic forms, were administered in the same way for a further 14 days in succession. The standard antiurolithiatic medicine used was cystone, administered at a dose of 750 mg/kg body weight. The study focused on the measurement of serum biochemical indicators and the excretion of salt components in the urine and renal deposits of the elements which were shown to be challenging. **Results:** Ethylene glycol administered orally caused hyperoxaluria and increased calcium and phosphate renal excretion. Nevertheless, *W. chinensis* leaf extracts significantly reduced the elevated oxalate levels in the urine, indicating an inhibitory action on internal oxalate synthesis. The renal accumulation of components that cause stone formation in rats with calculogenic conditions decreased considerably after the administration of curative treatments with aqueous and ethanolic extracts. **Discussion and Conclusion:** In accordance with the results, *W. chinensis* leaves possess lithontriptic action that justifies more research towards a possible treatment for urolithiasis. Further study is required to isolate and clarify the active phytoconstituents found in leaves responsible for their antiurolithiatic effects.

Keywords: Antiurolithiatic activity, Herbal medicine, Phytochemical analysis, Phytotherapy, Renal calculi.

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INTRODUCTION

The presence of calculi in the kidney or any other part of the urinary system is known as urolithiasis or nephrolithiasis and it is the third most common renal illness that has affected humans since antiquity. It threatened public health all over the world, not just in a single geographic region. Over 80% of kidney stones are made of calcium oxalate, which may be found in both mono and di-hydrate forms.[1] A series of physiochemical steps, beginning with the supersaturation of urine and continuing through the nucleation, development, aggregation and subsequent retention of crystals, lead to the formation of urinary stones.[2] In spite of the fact that urolithiasis has been recognised since the stone age and the recent advances in urology, there is currently no clinically effective medication that can dissolve and/or prevent the formation or recurrence of urinary calculi, with the exception of a small number of alkalizers and diuretics.[1] Typically, surgery and interventional procedures such as Percutaneous Nephrolithotomy, Ureteroscopy and Extracorporeal Shock Wave Lithotripsy are used for the treatment of this condition. These procedures do not impede, but rather enhance the likelihood of stone recurrence and are associated with significant adverse consequences such as haemorrhage, renal fibrosis and infections.[3] Because of this, patients who get these treatments have to endure a lengthy period of attentive follow-up and the expense of such treatment is unaffordable for everyone else. Therefore, there is a continuing need to search for more therapeutically beneficial antiurolithiatic treatments that are inexpensive for the average person alter the rate of recurrences and have few or no adverse effects. There are several herbal treatments for urolithiasis that are extremely effective and have relatively few adverse effects in alternative health care systems. Several ancient herbal remedies for renal calculi are also employed as ingredients in herbal formulations given for patients with urolithiasis. However,

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the rationale for such traditional herbal remedies has not been substantiated by current scientific research.

Worldwide, urolithiasis is a prevalent and severe condition that affects around 12% of people. In Asian nations, this illness is a recurring and potentially fatal condition, with a recurrence rate of up to 50% during a span of 5-10 years. The average rate for urolithiasis in Pakistan is around 16%. Men have a larger chance of developing urolithiasis compared to women, with a ratio of 3:1. This increased risk may be attributed to many predisposing variables, such as testosterone levels and dietary disparities. An increased consumption of animal protein in one's diet has been associated with an elevated risk of urolithiasis. Consuming a substantial amount of water has the potential to mitigate the risk by inducing urine dilution and decreasing the duration of presence of unbound stone particles in urine. Kidney stones are more likely to develop in the summer due to perspiration that causes precipitation of urine components. Urolithiasis risk may increase with low magnesium and citrate levels and high calcium, oxalate and uric acid levels in the urine.[4]

The WHO claims that 75% of people worldwide rely on natural remedies because of their lower risk of side effects. Plants are valuable and economical sources that are used to treat a wide range of illnesses. Scientific research has discovered several plant-based remedies through investigating the traditional use of medicinal plants for treating various diseases.[5]

Wedelia chinensis (Osbeck) Merill commonly known as Pilabhamgara or Bhringraj, a member of Asteraceae family.[6] Its historical use spans across treating alopecia, colds, amenorrhea, hair colouring, skin diseases and renal failure. In the tribal communities of Kolli Hills, Namakkal, Tamil Nadu, India, the decoction of *W. chinensis* has been utilized to induce sleep, alleviate mental tension and reduce anxiety.[7-9] Phytochemical studies have indicated that the plant contains triterpenoids, diterpenoids, sesquiterpenes, flavonoids, tannins, organic acids and steroids.[10] It is reported to have antioxidant, anticancer androgen suppressing, wound healing, adaptogenic and antistress, sedative, analgesic and anti-inflammatory, hepatoprotective and anticonvulsant activities.[11,12]

Currently, there is a lack of evidence addressing the effectiveness of *W. chinensis* leaves for treating urolithiasis. However, despite the extensive exploration of its therapeutic potential, scientific research regarding the antiurolithiatic activity of *W. chinensis* remains scarce. Thus, the intent of the research was to investigate the antiurolithiatic activity of *Wedelia chinensis* leaf extracts on rats that had urolithiasis caused by Ethylene Glycol (EG).

MATERIALS AND METHODS

Collection of *Wedelia chinensis* leaves

The leaves of *W. chinensis* were procured from the Solapur region of Maharashtra in February 2021. Dr. D. L. Shirodkar, a research scientist at the Botanical Survey of India, Pune, Maharashtra, led the process of authentication (voucher specimen: MSSSC2).

Chemicals

The Ethylene Glycol (EG) was bought from the Merck Laboratories located in Mumbai. The standard antiurolithiatic drug used in this study was Cystone tablets, that are a commercially available pharmaceutical manufactured by Himalaya Healthcare Company in Bangalore, India. The urine test strips-UroColor 10 were procured from Abbott Diagnostics Korea Inc (Lot. No. U100G068A; MFG DATE: 26/11/2021; EXP DATE: 25/11/2023).

Extract preparation

250 g of coarse powdered leaves of *W. chinensis* was extracted with absolute ethanol using a continuous hot extraction process to obtain the ethanolic extract. Another batch of 250 g of coarsely powdered leaves underwent a cold maceration technique in water for 7 days to prepare the aqueous extract.

The 2 extracts were concentrated individually under reduced pressure employing a rotary flash evaporator. Subsequently, the residue was dried over sodium sulfate using a desiccator. The percentage yield of the ethanolic and aqueous extracts was determined to be 13.83 and 11.75, respectively. The aqueous and ethanolic extracts were suspended in aqueous sodium carboxymethylcellulose (0.5% w/v) solution for oral administration in rats.

Qualitative analysis of phytochemicals

Each extract underwent phytochemical analysis to identify the presence of several metabolites, including terpenoids, carbohydrates, steroids, coumarins, alkaloids, tannins, glycosides, saponins, proteins, flavonoids and amino acids.[13]

Antiurolithiatic activity

Animal selection

In the context of acute toxicity experiments, albino Wistar rats, regardless of gender, with weights ranging from 150 to 200 g was chosen. Wistar albino rats weighing 150-200 g were utilized to test the antiurolithiatic effect. Rats were acclimated to laboratory settings and then placed on a 12:12 hr light-dark cycle. The rats were given unrestricted access to standard rat food as well as water to drink. The handling and care of the animals during experiments was conducted in line with NIH standards. The Ethics Committee of Biocyte Institute of Research and Development, Sangli, Maharashtra approved the research protocol (CPCSEA Registration no.: IAEC/Sangli.2020-21/19).

Acute toxicity studies

In accordance with Organisation for Economic Cooperation and Development Guidelines No. 423, a study on acute oral toxicity was done.[14] The effective therapeutic dose was calculated based on a threshold value of median fatal Dose (LD_{50}).

Ethylene glycol induced urolithiasis model

The antiurolithiatic activity was evaluated using the Atmani *et al* approach with the necessary modifications.[15] Rats were split up into 5 groups, each with 6 rats. Group I acted as the normal control. In groups II to V, oral 0.75% Ethylene Glycol (EG) in drinking water caused rats' hyperoxaluric till 14th day. Rats in Group II acted as negative control; they were not exposed to any kind of treatment from day 15 to day 28. In groups III, IV and V, curative treatments were given using cystone at a dosage of 750 mg/kg body weight, AEWC at a dosage of 400 mg/kg body weight and EEWC at a dosage of 400 mg/kg body weight, respectively. Each of the dosage was administered orally once a day via the gastric intubation technique.

Urine collection and analysis

All rats were housed in separate metabolic cages after the experiment and 24 hr urine were collected. The UroColor test strips were used to carry out routine urinalysis from urine samples in rats. The test comprised measurement of pH and specific gravity, in addition to the detection of proteins, glucose, bilirubin, urobilinogen, nitrite, occult blood, leucocytes and ketone bodies. A little quantity of strong hydrochloric acid was introduced into the urine prior to its storage at a temperature of 4°C. The urine was examined to analyse the level of calcium, phosphate and oxalate.[16-18]

Microscopic examination of urine

On day 28, fresh collected urine samples were inspected under a microscopy at magnification of 50x to check for the existence of distinctive CaOx and CaPh crystals. A digital camera, compatible with Avercap program was used to capture their photomicrographs.

Serum analysis

After the experimental duration, blood was taken from the orbital vein retrogradely of the rats after they had been slightly anaesthetized with ether. Finally, greater doses of ether anaesthesia were used for sacrificing the rats. The blood sample was centrifuged at 10,000 g for 10 min to extract the serum, which was then tested for blood urea nitrogen, uric acid and creatinine.[19,20]

Kidney homogenate analysis

Both kidneys from every animal were extracted by cutting the abdomen. Once superfluous tissue was removed from isolated kidneys, they were stored in 10% neutral formalin. A hot air oven was used to dry (at 80°C) each of the extracted kidneys. 100 mg sample of dry kidney was homogenized after 30 min of boiling in 10 mL of 1 N HCl solution. The homogenate was separated from the supernatant after centrifuging it for 10 min at 2000 g. The levels of calcium, phosphate and oxalate in the kidney homogenates were analysed.[18,21]

Histopathological analysis of the kidney

Using conventional methods, another extracted kidney was paraffin embedded, sectioned into 5 μm thick pieces, stained with haematoxylin and eosin dye and then mounted using diphenyl xylene. Each piece of sections was examined for the histopathological alterations in kidney architecture using a compound microscope set to 50x magnification. A digital camera was used to capture photomicrographs of the sections. A general scoring system was used to visualize several fields to monitor the degree of nephritic injury and the progression of recovery. At least ten fields per kidney slide were examined and scores on a Scale of None (NS), mild damage (+), moderate damage (++) and significant damage (+++) were used to indicate the severity of the alterations.[22]

Statistical analysis

The results are expressed in mean±SEM, one way ANOVA followed by Tukey's post hock test. A data difference was regarded as significant when the *p*-value was below 0.05.

RESULTS

The qualitative chemical investigation of *W. chinensis* leaf extracts (AEWC and EEWC) showed the presence of tannins, anthraquinones, flavonoids, volatile oils, steroids, terpenoids and carbohydrates (Table 1). The therapeutic dose of 400 mg/kg body weight for AEWC and EEWC were derived based on acute oral toxicity experiments in rats.

With our current study, oral delivery of Ethylene Glycol (EG) for a continuous period of 14 days led to the occurrence of hyperoxaluria (Figure 1). Rats with negative control i.e. calculi-induced (Group II) had substantially higher excretion of oxalate ($p<0.01$) in their urine, in comparison with control group (Table 2, Group I). The excretion of phosphate and calcium was also elevated in Group II. The kidney tissue of rats with calculi revealed a higher level of phosphate, calcium and oxalate. The deposition of these components that form stones in the renal tissue was considerably ($p<0.05$) decreasing within this group. The therapeutic use of aqueous and ethanolic extracts of *W. chinensis* leaf (AEWC and EEWC) was also shown to be beneficial in reducing the increased levels of harmful salts in the urine and kidneys. The elevated urine levels of phosphate, calcium and oxalate were considerably ($p<0.05$) normalised after administering cystone (Table 2, Group III). The administration of aqueous and ethanolic extracts of *W. chinensis* leaf (AEWC and EEWC) resulted in a statistically significant reduction in the output of calcium ($p<0.01$) and oxalate ($p<0.05$) from urine as compared to the standard medicine, cystone (Table 2, Group IV and V). Elevated blood levels of BUN, creatinine and uric acid were indicative of significant damage to the renal architecture caused by kidney stones (Table 2, Group II). On the contrary, it was observed that both extracts of *W. chinensis* leaf (AEWC and EEWC) exhibited a noteworthy reduction (p<0.01, p<0.05) at the higher echelon of serum creatinine and uric acid. Aqueous Extract of *W. chinensis* leaf (AEWC) showed a substantial decrease in BUN (p<0.05). The administration of aqueous extract was found to be substantial (p<0.01) and equivalent to that of cystone (Table 2, Group V), notwithstanding the non-significant decrease in these amassed waste materials seen in the intergroup comparison between the treatments of aqueous and ethanolic extracts.

The average pH of the calculi-induced rats was 7.60, according to the findings of routine urinalysis (Table 3, Group II). The raised urine pH was, however, found to drop to almost normal levels with the administration of *W. chinensis* leaf extracts (AEWC and EEWC). Glucosuria was not detected and all experimental groups showed non-significant differences in the specific gravity of their urine as well as in the levels of another five parameters excreted in the urine (Table 3). Additionally, the calculi-induced group showed proteinuria, indicating a severe cellular injury to the nephritic kidney. In contrast, the administration of aqueous and ethanolic extracts of *W. chinensis* leaf (AEWC and EEWC) resulted significant reduction in protein excretion in the urinary system, like the rats treated with cystone (Table 3, group III).

The urine from the control group showed no signs of crystals or other comparable structures when examined under a microscope (Figure 2A). The urine sample from rats with calculi-induced conditions exhibited a significant presence of sizable crystals showing the distinctive morphology of calcium oxalate and triple phosphate (Figure 2B). By contrast, urine sample collected from rats treated with cystone (Figure 2C), aqueous extract and ethanolic extract (Figures 2D and E, respectively) exhibited a lower prevalence of crystal fragments, which were notably smaller in size. The disintegration pattern of crystals shown by *W. chinensis* leaf extracts was observed to be identical as compared to cystone. Nevertheless, there were solitary and smaller crystal pieces that were observed within this group.

The histopathological analysis of the kidney provided further corroboration for the findings obtained from the urine microscopy and serum biochemical studies. Significant impairment was found in the last segment of the nephron, collecting system and peritubular interstitium across all the rats forming stones (Figure 3B), compared to the normal rat's kidney anatomy (Figure 3A). The tubules had an inflammatory infiltrate around them and were focally ectasic (Figure 3B). The tubules were surrounded by flattened epithelium with localised vacuolar degeneration and necrosis of individual cells. These tubules contained hyaline casts in certain areas. Mature lymphocytes invading the tubular epithelium constituted the majority of the inflammatory infiltration. Irregular crystals appeared along the nephron, at the papillary level and in the tubules and peritubular interstitium. However, the aqueous and ethanolic extracts treated groups (Figures 3D and E) revealed normal glomeruli and a modest recuperation of renal histology, analogous to the group receiving cystone treatment (Figure 3C and Table 4).

DISCUSSION

The types of crystals that form and the molecules attached to their surfaces are largely determined by urinary chemistry. The main risk factors for stone formers are significantly reduced volume of urine, pH, moderate hyperoxaluria, elevated calcium levels, hypocitraturia, hyperuricosuria and hypomagnesuria.[23] An additional indicator for identifying the kind of calculi is the pH of the urine (less than 5.5 for uric acid stones and more than 6 for calcium stones). Phosphate and oxalate nucleate with calcium when urine is found to be basic, more than pH 7.2 in calcium oxalate urolithiasis.[24] In this study, young albino male rats had elevated pH levels (7.79) and formed kidney stones primarily made of calcium oxalate after administering Ethylene Glycol (EG) orally for 14 days. Evidence from earlier research suggests that the molecular processes behind this are caused by an increase

Table 1: Qualitative chemical analysis of *W. chinensis* leaf extracts.

Metabolites	AEWC	EEWC
Carbohydrates	+	-
Steroids	-	+
Terpenoids	-	+
Volatile oils	-	+
Anthraquinones	+	+
Flavonoids	+	+
Tannins	+	+

'+' Present; '-' Absent.

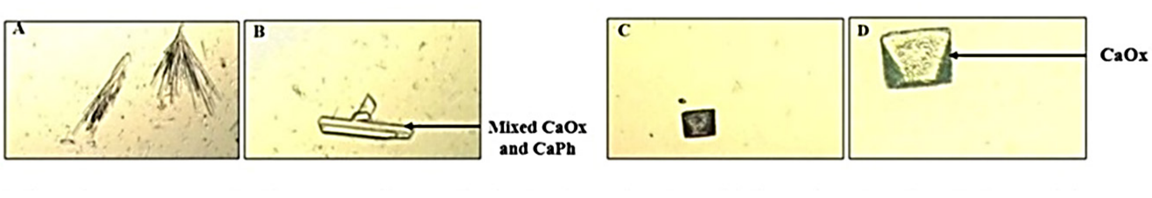

Figure 1: Photomicrographs of urine samples collected on the fourteenth day showing by (A) crystal aggregation pattern at magnification of 50x, (B) distinct CaOx and CaPh crystals at magnification of 100x, (C) distinct Calcium Oxalate (CaOx) crystals at magnification of 100x and (D) distinct Calcium Oxalate (CaOx) crystals at magnification of 450x.

Table 2: Effect of *W. chinensis* extracts of leaves on kidney homogenate, serum and urine parameters in both experimental and control groups of animals.

Parameters (Unit)	Group I Normal Control	Group II Disease Induced	Group III Standard Drug	Group IV AEWC Treated	Group V EEWC Treated
Dose (mg/kg, p.o.)	-	-	750	400	400
Urine (mg/dL)					
Calcium	3.01±0.49	5.51±0.79^{**a}	3.49±0.61^{*b}	3.00±0.16^{**b}	3.18±0.20^{**b}
Phosphorous	140.70±47.29	349.70±51.83^{**a}	194.10±54.67	165.80±23.36^{*b}	229.61±19.34
Oxalate	1.44±0.19	4.65±1.11^{**a}	1.76±0.29^{*b}	2.01±0.59^{*b}	2.60±0.69
Serum (mg/dL)					
Blood Urea Nitrogen	21.80±0.81	34.21±4.20^{**a}	25.41±0.81	24.05±2.59^{*b}	28.54±4.11
Creatinine	0.60±0.04	0.81±0.03^{**a}	0.59±0.02^{**b}	0.59±0.04^{**b}	0.64±0.02^{*b}
Uric acid	1.69±0.19	5.07±0.29^{**a}	1.97±0.49^{**b}	1.70±0.19^{**b}	2.98±0.24^{**b}
Kidney homogenate (mg/g)					
Calcium	5.99±1.39	12.12±2.67^{*a}	6.23±1.09^{*b}	9.16±1.09	11.03±4.69
Phosphorous	1.20±0.19	4.15±1.29^{**a}	1.60±0.28^{*b}	1.76±0.49^{*b}	2.18±0.70
Oxalate	2.99±0.55	8.38±0.79^{**a}	3.42±0.49^{**b}	4.45±0.89^{**b}	5.03±0.69

Urinary parameters were measured in 24-hr urine samples, with mean±SEM values from 6 animals per group; $^{*}p<0.05$, $^{**}p<0.01$; ^{a}Comparisons are made with Group I (Normal control); ^{b}Comparisons are made with the Group II (Disease induced).

Table 3: Effect of leaf extracts of *W. chinensis* on various urine parameters in both experimental and control groups of animals.

Parameters	Units	Group I Normal Control	Group II Disease Induced	Group III Standard Drug	Group IV AEWC Treated	Group V EEWC Treated
pH	-	6.89±0.13	7.60±0.17	7.04±0.22	7.01±0.19	7.11±0.23
Specific Gravity	-	1.01±0.00	1.02±0.00	1.01±0.00	1.01±0.00	1.01±0.00
Glucose	mg/dL	-	-	-	-	-
Protein		1.66±1.70	23.49±4.19	6.59±2.08	8.57±1.71	13.21±5.49
Nitrite		0.32±0.16	0.68±0.20	0.43±0.14	0.43±0.19	0.43±0.21
Ketone		0.80±0.79	4.99±1.19	3.31±1.06	5.02±1.79	0.85±0.79
Bilirubin		-	0.32±0.12	0.16±0.12	0.24±0.13	0.15±0.12
Urobilinogen		0.10±0.00	1.21±0.61	0.10±0.00	0.10±0.00	0.10±0.00
Leucocyte †	WBC/µL	-	++	+	+	+
Blood††	RBC/µL	-	++	+	+	+

†Leucocyte (WBC/µL) +25, ++ 75; ††Blood (RBC/µL) +10, ++50, +++ 250; '-' no change in color after qualitative test.

in the urinary concentration of oxalate.[15] Rats given Ethylene Glycol (EG) undergo hyperoxaluria due to the quick conversion of glycolate to oxalate.[25] Rats treated Ethylene Glycol (EG) has shown comparable results.[26]

In rats with calculi-induced conditions (Group II), there is a gradual rise in the excretion of calcium in the urine, possibly due to impaired tubular reabsorption in the kidneys. The differences in oxalate levels in the urine are, in comparison, significantly more substantial compared to those in calcium levels,[27] because hyperoxaluria is thought to be a most important risk factor for the formation of kidney stones than hypercalciuria.[28] Higher levels of calcium in the urine promote the formation and development of calcium oxalate or apatite crystals.[29] However, in this study the curative treatment of *Wedelia chinensis* leaf extracts decreases the excretion levels of calcium and oxalate.

In addition to oxalate load, a gradual rise in urinary phosphorus as seen in calculi-induced rats appears to foster the formation of calculi through the epitaxial accumulation of calcium oxalate and the formation of a nidus composed of calcium and triple phosphate crystals.[30] However, the risk of stone formation is reduced by supplementing with extracts of *Wedelia chinensis* leaf, which replenish the phosphate level.

Table 4: Microscopic examination of effects of *W. chinensis* leaf extracts on renal histology.

Parameter	Group I Normal Control	Group II Disease Induced	Group III Standard Drug	Group IV AEWC Treated	Group V EEWC Treated
Tubular congestion	+	+++	++	+	++
Loss of brush border	NS	+++	++	++	+
Tubular cast	NS	++	+	NS	NS
Tubular degeneration	NS	+++	+	NS	NS
Tubular desquamation	NS	++	++	+	++
Glomerular congestion	+	++	++	++	++
Widening of Bowmen's capsule	NS	++	NS	+	++
Interstitial oedema	NS	++	+	NS	NS
Interstitial inflammatory cell infiltration	NS	++	+	+	+
Interstitial haemorrhage	NS	+	NS	NS	NS
Intravascular haemolysis	NS	NS	NS	NS	NS
Total extent of damage	Normal	Significant damage	Moderate damage	Mild damage	Mild damage

Grades were determined by averaging observations of kidney injury that are shown in at least ten distinct fields per slide, + Mild damage (=<3), ++ Moderate damage (=4 to 6), +++ Significant damage (=>7) findings; 'NS' Not seen.

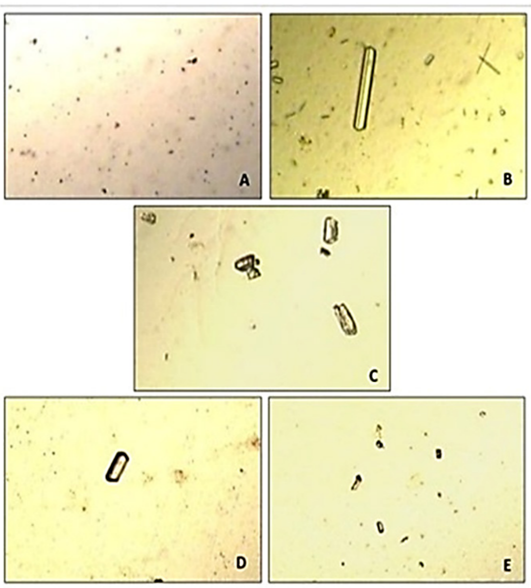

Figure 2: Microscopic investigation of urine sample excreted on twenty-eighth day at magnification of 50x by (A) normal control rats, (B) rats administered just 0.75% ethylene glycol, (C) rats were administered 0.75% ethylene glycol and then treated with Cystone, (D) rats were administered 0.75% ethylene glycol and then treated with AEWC leaf and (E) rats were administered 0.75% ethylene glycol and then treated with EEWC leaf.

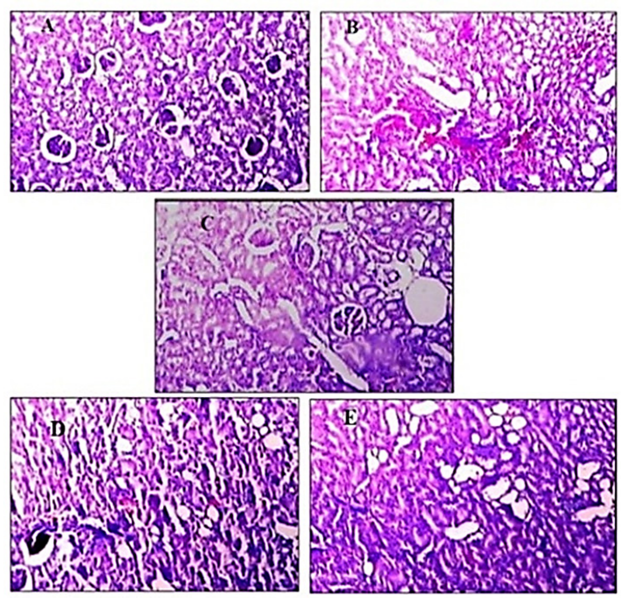

Figure 3: Microscopic examination of kidney section at magnification of 50x by (A) normal control rats, (B) rats administered just 0.75% ethylene glycol, (C) rats were administered 0.75% ethylene glycol and then treated with Cystone, (D) rats were administered 0.75% ethylene glycol and then treated with AEWC leaf and (E) rats were administered 0.75% ethylene glycol and then treated with EEWC leaf.

Urolithiasis affects kidney function by reducing the glomerular filtration rate as a result of urinary flow obstruction caused by calculi in the urinary system. As a result, waste materials accumulate in the blood, especially nitrogenous compounds like urea, creatinine and uric acid.[31] Glycolate feeding is known to induce elevated lipid peroxidation and reduce antioxidant potential levels in the kidneys.[32] The compound oxalate, which is the starting point for lipid peroxidation, interacts with polyunsaturated fatty acids in the cell membrane to further damage renal tissue.[33] In the present scenario, the higher serum level of creatinine, uric acid and BUN (Group II, Table 2) in the calculi-induced rats are attributed to extensive kidney damage (Table 4, Group II). Substantial proteinuria and haematuria confirm the severity of nephritic injury (Group II Table 3). The antioxidant effects of *Wedelia chinensis* and its increased GFR may be responsible for the significant reduction in serum levels of deposited waste materials.[11]

CONCLUSION

Finally, the findings show that *W. chinensis* leaf extracts decreased and reversed the formation of urinary stones in rats with experimentally induced urolithiasis, confirming traditional beliefs about the plant's antiurolithiatic activity. Although the precise mechanism of the effect is unknown, it seems to be connected to both increased urine output and decreased levels of components that cause stone to develop in the urine. Renal injury may heal in part due to the preventive action against oxalate-induced peroxidation of lipids. It is possible that the observed antiurolithiatic activity might be due to the presence of tannins, flavonoids and triterpenoids.[34] These outcomes could support the antiurolithiatic activity of *W. chinensis* leaf.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; **EG:** Ethylene Glycol; **NIH:** National Institutes of Health; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethics Committee; **AEWC:** Aqueous Extract of *Wedelia chinensis*; **EEWC:** Ethanolic Extract of *Wedelia chinensis*; **CaOx:** Calcium Oxalate; **CaPh:** Calcium Phosphate; **BUN:** Blood Urea Nitrogen; **GFR:** Glomerular Filtration Rate.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study conducted in experimental animals, and the protocol was approved by Ethics Committee of Biocyte Institute of Research and Development, Sangli, Maharashtra (CPCSEA Registration No.: IAEC/Sangli.2020-21/19).

SUMMARY

The purpose of the present research was to evaluate the efficacy of *W. chinensis* leaf extracts as therapeutic agents in rats with experimentally produced calcium oxalate urolithiasis. Rats were given an oral dose of 0.75% Ethylene Glycol (EG) to induce calcium oxalate urolithiasis. A commonly used antiurolithiatic drug was cystone, which was administered at a dosage of 750 mg/kg of body weight. The elevated oxalate levels in the urine were considerably lowered by *W. chinensis* leaf extracts, suggesting an inhibitory effect on internal oxalate synthesis. The renal accumulation of components that cause stone formation in rats with calculogenic conditions decreased considerably after the administration of curative treatments with aqueous and ethanolic extracts of *W. chinensis* leaf. The qualitative analysis of *W. chinensis* leaf extracts revealed the presence of tannins, anthraquinones, flavonoids, volatile oils, steroids, terpenoids and carbohydrates. It is possible that the observed antiurolithiatic activity might be due to presence of tannins, flavonoids and triterpenoids. These outcomes could support the antiurolithiatic activity of *W. chinensis* leaf.

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