Anti-Urolithiatic Activity of Polyherbal Formulation of Medicinal Plants: An *In-Vitro*, *In-Vivo* ActivitySupported by Molecular Docking Studies

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Abstract:

Aim of the study: The current study's objective was to assess the *invitro*, *invivo*, and *insilico* anti-urolithiatic activity of few selected medicinal plants, such as *Kalanchoe pinnata*, *Acalypha indica*, *Tribulus terrestris*, *Areva lanata*, and *Boerhaavia diffusa*, which are used as anti-urolithiatics in traditional medicine.

Materials and methods: *Invitro* calcium oxalate crystallization was evaluated utilizing nucleation, aggregation, and oxalate depletion assay. Based on percent inhibition values of *invitro* experiments, two doselevels of three different methanolic extracts of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* were made into polyherbal formulation in the ratio of 3:2:1 and *invivo* activity was assessed by delivering two experimental dosages (200 and 400 mg/kg body weight) of this polyherbal formulation for 10 days, in ethyleneglycol (0.75%) induced urolithiasis model in male wistar albino rats. *Insilico* studies were performed by docking selected protein targets and chemical constituents of given medicinal plants.

Results: The nucleation, aggregation of calcium oxalate crystallization were hindered and density of crystals was lowered by polyherbal methanolic extract of *Kalanchoe pinnata*, *Acalypha indica*, and *Areva lanata* (3:2:1). At a dosage of 200 mg/kg b.w, this polyherbal methanolic extract has reduced uric acid buildup in urine, along with serum concentration of uric acid, creatinine and urea. Reduced tissue damage was found in kidneys of treatment group after histopathological investigation. The findings were pertinent to healthy untreated control group. Molecular docking studies show that chemical constituents like acalyphin, kaempherol and quercetin have good binding interactions with protein targets involved in urolithiasis formation, thus a better understanding of the mechanism of urolith formation is achieved.

Conclusion: This research shows that the polyherbal methanolic extract of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* (3:2:1) may demonstrate a synergistic effect in decreasing the formation of kidney stones,supporting the conventional notion.

Keywords: Anti-urolithiatic; *invitro*; *invivo*; polyherbal; synergistic.

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1. Introduction:

Urolithiasis refers to the formation of hard, solid substances in the urinary system. In numerous cases, the uroliths are quite diminutive and steam through the body with ease. If a urolith (even a tiny one) obstructs thepassage of urine, it can cause severe pain and demand medical intervention. Calcium-containing uroliths such as calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD), and typical calcium phosphate uroliths are prevailing. Urolithiasis is a persistent condition in almost every country. Men are more prone than women to acquire urolithiasis, with a risk ratio of 3:1, though this gap appears to be reducing[1].

The risk of getting stones is highest in those between the ages of 20 and 40. Urolithiasis is increased byany factor that produces urine stasis due to a reduction or restriction of urinary flow. Men, for example, excrete less citrate and more calcium than women, which could explain why men are more likely to develop urolithiasis. In addition to sex, a person's ethnic background can be a risk factor, as people of Native American, African, or Israeli ethnicity are prone to this disease[2,3].

The genesis of uroliths commences with the propagation of crystals in supersaturated urine adhering to the urothelium, providing seminary for succeeding urolith development. Scientists are conducting experimentsto find the biological processes by which the crystals bind to the urothelium. Randall's plaques, which are formed from calcium phosphate (hydroxyapatite) crystals, generate innumerable calcium oxalate stones. The urothelium has degenerated, and a calcium oxalate accumulation nucleus is developed. The function of cell surface chemicals in crystal adhesion favoring or hindering has been the subject of more recent hypotheses. Urothelial injury and repair following a stone occurrence may increase the expression of these molecules on the surface, permitting for extensive crystal attachment[4].

The objective of kidney stone prevention is to identify and reduce the variables that cause crystal formation. In traditional medicine, several plants are used to treat urinary problems as a diuretic and anti-urolithiatic, which are more effective than allopathic medicines. This study is aimed to see if varying amounts of methanolic extracts from the leaves of *Kalanchoe pinnata*, *Acalypha indica*, *Tribulus terrestris*, *Areva lanata*, *Boerhavia diffusa*, and Cystone, a positive control, could dissolve artificial kidney stones *invitro* and based on the percentage inhibition values of *invitro* experiments, a polyherbal methanolic extract will be formulated to test its synergistic antiurolithiatic activity *invivo*, and perform insilico studies to strengthen our work.

2. Materials and methods:

2.1 Plant Material:

In November-December 2020, we collected *Aerva lanata*, *Acalypha indica*, *Kalanchoe pinnata*, *Boerhaavia diffusa* and *Tribulus terrestris* from our surroundings in the fields of our college "Raghavendra Institute of Pharmaceutical Education and Research" and Dr. J. Ravindra Reddy identified and certified the plant materials.

2.2 Chemicals:

Our college laboratory provided Ammonium chloride, Calcium oxalate (CaOx), Sodium chloride (NaCl), Calcium chloride ($CaCl_2$) solution, Cysteine, Dihydrogen sulfate (H_2SO_4), Hydrogen chloride (HCl), Sodium oxalate ($Na_2C_2O_4$), Potassium permanganate ($KMNO_4$), Paraffin, Formalin solution, Creatinine, Uric acid, Ethylene glycol (0.75%). Diagnostic implements for calcium, creatinine, and uric acid were provided by our laboratory. Additional chemicals, solvents, and reagents of analytical grade were procured from reliable chemical suppliers.

2.3 Instruments:

Metabolic cages, Centrifugation, UV absorbance, Soxhlet apparatus, Erba Chem semi-automatic analyzer.

2.4 Extraction:

The plant material was cleaned, dried in the shade, and pulverized. About 50 g of the powder was extracted with methanol in a Soxhlet apparatus. Using a Rota Evaporator, the extract was condensed[5]. All of the extracts phytochemical characterization has been previously published. Additional pharmacological testing was performed using these methanolic extracts of *Aerva lanata*, *Acalypha indica*, *Kalanchoe pinnata*, *Boerhaaviadiffusa* and *Tribulus terrestris* respectively.

3. Evaluation of anti-urolithiatic activity *invitro*:

Experimental urolithiasis (CaOx stones) is triggered homogenously. The 50 mM solutions of Calcium chloride ($CaCl_2$) and Sodium oxalate ($Na_2C_2O_4$) were combined. Calcium and oxygen were combined to form the crystals of Calcium oxalate (CaOx). The mixture was heated to 60°C for about an hour in a water bath. Then, it is incubated over the night at 37°C in an oven. The oxalate depletion assay, aggregation assay, and nucleationassay were carried out according to standard procedures[6,7].

3.1 Preparation of Polyherbal Formulation:

Based on percent inhibition values of *invitro* experiments, two dose levels of three different methanolic extracts of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* were made into the polyherbal formulation in the ratio of 3:2:1.

4. Evaluation of Antiurolithiatic activity *invivo*:

Rats were made to develop urolithiasis using the ethylene glycol (0.75 percent) and ammonium chloride-instigated hyperoxaluria paradigm.

4.1 Animals:

For the duration of the study, 30 healthy male wistar albino rats (weighing 120–220 g) were kept in five groups of six rats each in clean polypropylene cages with a 12 h light-dark cycle and controlled temperature

(25±2°C). Throughout the experiment, they were given a standard pellet meal and unrestricted water access. However, no food was provided during the collection of 24-hour urine samples inside metabolic cages[8,9]. The experimental procedure outlined in this research was approved by the Institutional Animal Ethical Committeeof CPCSEA, Govt. of India (IAEC/XV/08/RIPER/2020).

4.2 Experimental Design:

Thirty animals in all were divided into five groups of six, each receiving the following care throughout the treatment for 10 days. (Table-1).

Table 1. Experimental Design

Group	Treatment	Induction of Urolithiasis
1	Normal control	Plain drinking water
2	Urolithiasis control	EG (0.75%) + AC (2%)
3	Standard - Cystone (20mg/kg b.w, P.O)	EG (0.75%) + AC (2%)
4	Polyherbal formulation (200mg/kg b.w, P.O)	EG (0.75%) + AC (2%)
5	Polyherbal formulation (400mg/kg b.w, P.O)	EG (0.75%) + AC (2%)

Urolithiasis was assessed by observing the following parameters: **Urine** –Urea, Uric acid, Calcium and **Serum**-Creatinine, Urea, Uric acid.

4.3. Collection and Analysis of Urine and Serum:

On the tenth day, 24-hour urine samples were gathered from all of the animals, who were kept in discrete metabolic cages. Throughout the period of urine collection, the animals were permitted to drink water. After the 10-day experiment, rats were anesthetized, and blood was collected from the aortic vein. The blood was centrifuged for 10 minutes at 3000 rpm to collect serum. Using the Erba diagnostic kits, the levels of calcium, creatinine, and uric acid in the blood were determined with the aid of an "Erba Chem Semi-automatic analyzer".Creatinine clearance was also assessed[10].

4.4. Kidney Section Histopathological Analysis:

A sample of the kidney was obtained from rats and preserved in a 10% neutral formalin solution. Employing standard procedures, one of the detached kidneys was conserved in paraffin and sliced into tenuous pieces of roughly 5 mm using a cryostat microtome. Each slice was then examined and captured under a light microscope with polarized light (magnification 4x and 10x) to look for variations in kidney anatomy and calcium oxalate sediments. Hematoxylin and eosin were used for the terminal examination[5].

5. Statistical Analysis:

The findings were examined and displayed as Mean ± SEM using the statistical software GraphPad Prism Version (8.4.3). The probability of $p < 0.001$ was studied significantly.

6. Docking Verification and Analysis:

From the analysis of *invitro* and *invivo* data, it was found that the polyherbal formulation of three plants showedgood antiurolithiatic activity results. The constituents from these three plants namely *Aerva lanata*, *kalanchoe pinnata*, *Acalypha indica* were identified, and to find the best natural molecule that can inhibit the protein targets implicated in the formation of renal calculi, the SDF data of these plant constituents were gathered fromPubchem and screened using docking. The ligand molecules were docked with various protein targets involvedin the formation of urolith such as 2ETE, 4WRN, 1JRP, 2RDU and 5FBH. The ligand molecules are docked using Schrodinger (Maestro) software. The optimized model with the lowest binding energy was selected andresearched for different bonding distances and associations with the binding site residues[11,12].

7. Results:

7.1 *Invitro* Antiurolithiatic Activity:

The addition of Sodium Oxalate ($Na_2C_2O_4$) solution to the reaction blend containing Calcium Chloride ($CaCl_2$) ensued in the fabrication of numerous Calcium Oxalate (CaOx) crystals in the nucleation test. The methanolicextracts of *Kalanchoe pinnata*, *Acalypha indica*, *Tribulus terrestris*, *Areva lanata*, and *Boerhaavia diffusa* andCystone diminished the proportions and quantity of Calcium Oxalate (CaOx) crystals respectively. The methanolic extract reduced the size of CaOx crystals by 62.97 percent, which was superior to the results obtained with Cystone (60.57 percent). The above-mentioned methanolic extracts considerably reduced the agglomeration of prepared CaOx crystals in the aggregation assay (P 0.05). The CaOx crystal growth hampering effect of the methanolic extracts was indistinguishable to Cystone at all concentrations and is exhibited in Fig. 1, Fig. 2, and Fig. 3. In the figures, ME represents methanolic extract.

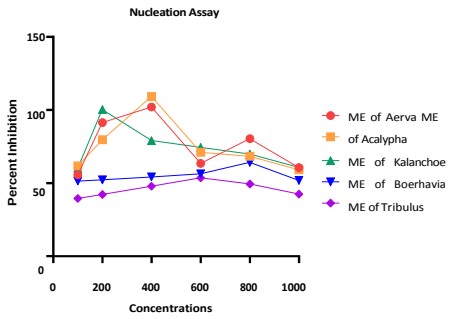

Fig. 1. Nucleation assay

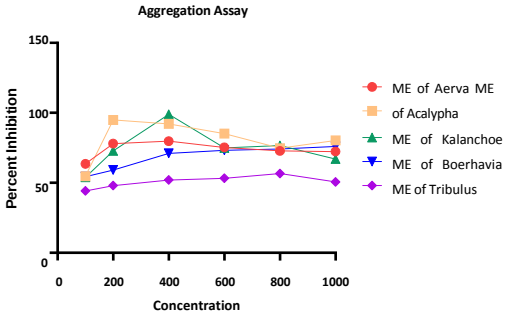

Fig. 2. Aggregation assay

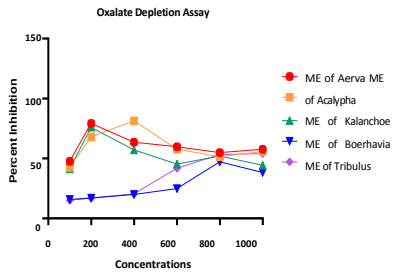

Fig. 3. Oxalate depletion assay

7.2 Histopathology:

Histopathological analysis of kidney sections showed changes in the morphology of the kidney with dilatationof the tubules and degeneration of the epithelium for the urolithiasis control group (Fig. 4).

Group I: Kidney tissue-looking renal structure with glomerulus and tubules.
Group II: kidney showing shrunken glomerulus, degenerating epithelial cells of both proximal and distal tubules.
Group III: Histoarchitecture of kidney looking normal
Group IV: kidney observed with fewer structural changes.
Group-V: Kidney has shown normal tubular epithelial cells and glomeruli, with hardly any crystals andminor edema and dilatation.

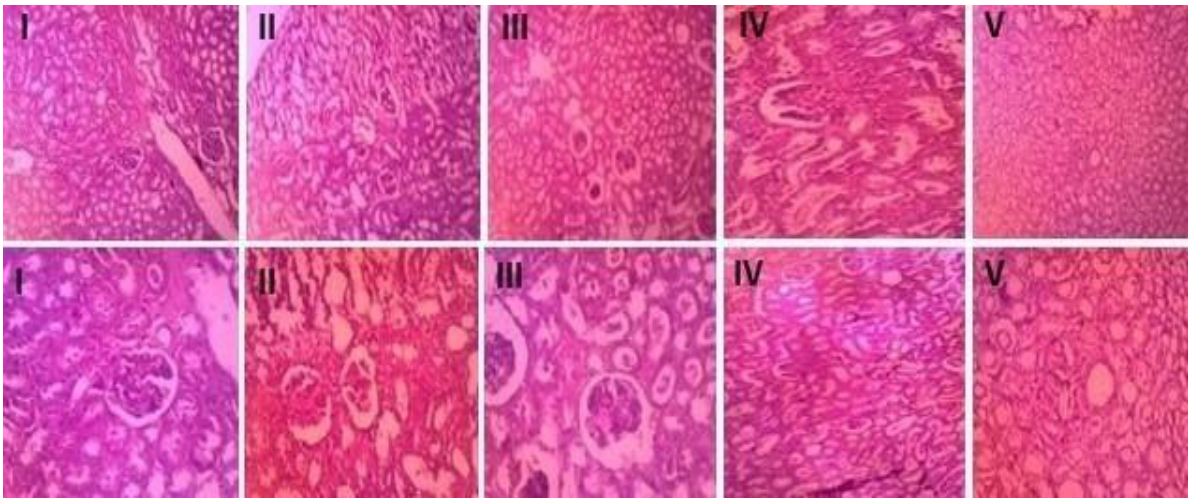

Fig. 4: Histopathological analysis of kidney stones. (I) healthy control, (II) urolithiasis control, (III) group treated with cystone, (IV) group treated with polyherbal formulation 200 mg/kg b.w, (V) grouptreatedwith polyherbal formulation 400 mg/kg b.w (magnification 10X and 40X).

7.3 *Invivo* Antiurolithiatic Activity:
7.3.1 Serum and Urine Kidney Function Parameters:
The antilithiatic effect of polyherbal methanolic extracts from *Aerva lanata*, *Acalypha indica* and *Kalanchoe pinnata* (3:2:1) was scrutinized in a rat model of artificially produced urolithiasis at two experimental dosages(200 mg/kg b.w and 400 mg/kg b.w). The groups treated with the polyherbal methanolic extract of *Aerva lanata*, *Acalypha indica* and *Kalanchoe pinnata* (3:2:1) at two dosages (200 mg/kg b.w and 400 mg/kg b.w) revealed a reduction in excretion of compounds or ions like calcium, uric acid, urea (Table 2) when comparedto the diseased control group. Furthermore, in untreated rats, lower creatinine clearance values (Table 3) suggested deterioration of renal function. Damage to the glomerulus and tubules is another sign of urolithiasis.

Table 2. Effect of polyherbal formulation on urine kidney function parameters

S.No.	Group	Calcium(mg/dl)	Uric acid(mg/dl)	Urea(mg/dl)
1	I	0.48 ± 0.07	0.30 ± 0.05	45.05 ± 0.54
2	II	14.59 ± 0.70	0.63 ± 0.07	102.72 ± 1.05
3	III	4.01 ± 0.37***	0.32 ± 0.01***	61.39 ± 3.49***
4	IV	8.82 ± 0.36***	0.37 ± 0.02***	59.11 ± 2.17***
5	V	9.14 ± 0.43***	0.47 ± 0.01***	68.48 ± 3.29***

Values are expressed as mean ± SEM and compared with diseased: *p<0.05, **p<0.01, ***p<0.001

Table 3. Effect of polyherbal formulation on serum kidney function parameters

S.No.	Group	Uric acid(mg/dl)	Creatinine(mg/dl)	Urea(mg/dl)
1	I	10.4±0.08	0.2±0.09	1.8±4.01
2	II	15.8±0.40	0.6±0.02***	10.5±1.54 ***
3	III	7.9±0.42***	0.2±0.08***	1.2±2.00***
4	IV	10.5±0.17**	0.4±0.05*	1.5±1.92***
5	V	19.1±0.64	0.5±0.03	2.8±3.43***

Values are expressed as mean ± SEM and compared with diseased: *p<0.05, **p<0.01, ***p<0.001.

7.4 Docking verification and analysis:

Molecular docking is a technique for predicting the ligand's primary binding modes with a protein with a familiar three-dimensional construction. Binding mode research is necessary for elucidating crucial structural property interactions and providing useful information for building effectual inhibitors. The ligand's docking score, which identifies molecules based on their potential to interact, was enumerated. The docking score withthe lowest energy was the most effective[11,12].

The principal constituents of *Acalypha indica*, *Aerva lanata*, *Kalanchoe pinnata* were selected from literature source, and their structures were downloaded from PubChem. The molecular docking studies show that the constituents of the plants *Acalypha indica*, *Aerva lanata*, *Kalanchoe pinnata* have good interaction with a protein associated with enzymes involved in the process of urolithiasis. Docking results of different chemical constituents of *Acalypha indica*, *Aerva lanata*, *Kalanchoe pinnata* with proteins like 2ETE, 1JRP, 4WRN, 2RDU and 5FBH are compared in Table 4. The best docking scores were found to be -8.27, -8.19, -

9.32 Kcal/mol respectively for the constituent's quercetin with protein 1JRP, acalyphin and kaempherol with protein 2RDU.

Table 4. Docking result

Plant	Constituent	Protein ID				
		2ETE (Kcal/mol)	1JRP (Kcal/mol)	4WRN (Kcal/mol)	2RDU (Kcal/mol)	5FBH (Kcal/mol)
Areva lanata	Quercetin	-6.299	**-8.272**	-7.828	-7.618	-7.287
	D-Glucoside	-5.088	-	**-7.774**	0.424	-5.733
Acalypha indica	2-Methyl anthrquinone	-5.355	**-7.640**	-7.385	-7.385	-7.022
	Acalyphin	-5.550	-6.150	-5.932	**-8.191**	-6.191
	Clitorin	-7.309	**-8.460**	-8.238	-4.728	-6.762
	Nicotiflorin	-6.522	-6.571	**-6.818**	-4.427	-5.058
	Acalyphamide	-2.626	-	**-7.006**	-	-2.874
	Aurantiamide	-5.962	**-8.642**	-5.173	-4.224	-7.123
	Flindersine	-6.091	**-7.593**	-6.568	-6.698	-7.200
Kalanchoe pinnata	Kaemferol	-6.309	-7.685	-7.076	**-9.321**	-6.878
	Rutin	-4.441	-6.058	**-7.026**	-4.887	-5.485

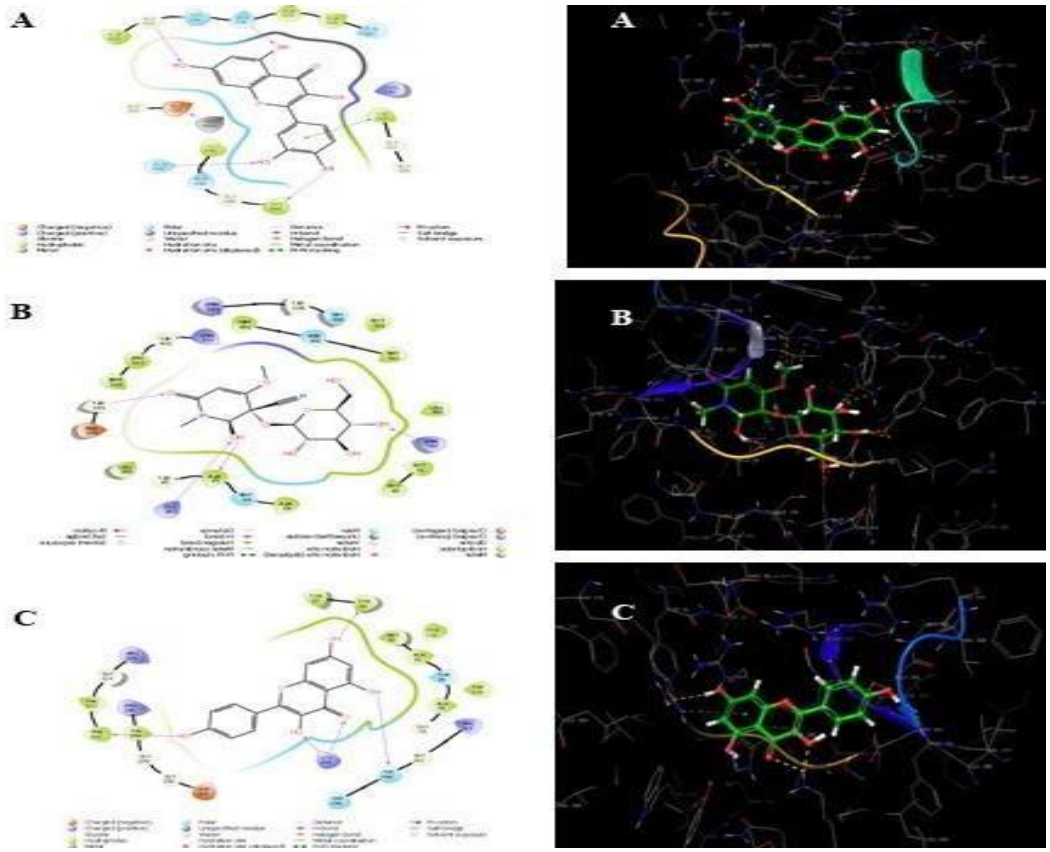

Fig. 5: Molecular docking analysis of constituents with proteins. (A) Quercetin docked with 1JRP (B)Acalyphin docked with 2RDU (C) Kaempherol docked with 2RDU

8. Discussion:

Urolithiasis is an extensive pathological condition around the world, which leads to renal failure if untreated. Treatment options are limited and are overpriced. Natural herbal formulations are the best alternative to treat kidney stones with fewer side effects. This research investigated the anti-urolithiatic effectiveness of some commonly used medicinal plants like *Kalanchoe pinnata*, *Acalypha indica*, *Tribulus terrestris*, *Areva lanata,* and *Boerhaavia diffusa*. The methanolic extracts of these plants are evaluated *invitro* and ones which exhibitedgood percentage inhibition of nucleation and aggregation of calcium oxalate (CaOx) crystals were compounded into a polyherbal formulation and screened for *invivo* effectiveness. *Insilico* studies were accomplished by docking the principal constituents of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* with proteins like2ETE, 1JRP, 4WRN, 2RDU, 5FBH obtained from PDB to reinforce the anti-urolithiatic activity.

The purpose of the *invitro* study was to explore the efficacy of methanolic extracts of *Kalanchoe pinnata*, *Acalypha indica*, *Tribulus terrestris*, *Areva lanata,* and *Boerhaavia diffusa* as anti-urolithiatics. Nucleation is obligatory for the pathophysiology of urolithiasis[5]. The nucleation of CaOx crystals was significantly inhibited, as evidenced by the smaller crystals that formed in the presence of these methanolic extracts, which was superior than in the presence of cystone. The CaOx crystallization assessment demonstratesthat these methanolic extracts disclose anti-crystallization activity.

In experimentally generated urolithiasis in rats, the anti-urolithiasis action of polyherbal methanolic extracts of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* (3:2:1) was investigated at two different doses (200 mg/kg b.w and 400 mg/kg b.w). The pathogenesis of urolithiasis is typically evaluated in rats withcalcium oxalate urolithiasis instigated by ethylene glycol (EG) alone or in combination with ammonium chloride (AC). This study used an accelerated model in which rats were fed 0.75 percent EG and 2 percent AC for 10 days.

On the other hand, biochemical parameters are demonstrated at the end of the experiment. The results are submitted as mean standard deviation (SEM) and contrasted to a healthy control group as follows: (n=6)

*p 0.05, **p 0.01, ***p 0.001 are all statistically significant. In comparison to the control groups, the groups treated with the polyherbal methanolic extract (200 mg/kg b.w and 400 mg/kg b.w) manifested a significant reduction in the quantity and size of calcium oxalate crystals.

Group II evidenced an eloquent elevation in calcium, uric acid, and urea excretion in urine as comparedto the healthy control group. Groups III and IV saw a modest rise, with group IV having the lowest numbers. The polyherbal methanolic extract prevents the production of stones or dissolves the crystals that have already formed, at this dose. The Kruskal-Walli's test divulged significant variance between the treatment and controlgroups (p 0.05), as well as a dose-dependent effect.

The presence of uric acid crystals in calcium oxalate stones, as well as the fact that uric acid-binding proteins may bind calcium oxalate and govern its crystallization, indicate that uric acid is involved in stone formation. The reference values for serum uric acid, creatinine, and urea in male Wistar rats are between 0.20and 0.91 mg/dL (12–54 molL-1), 0.35 and 0.54 mg/dL (31.0–48.0 molL-1), and 24.02 and 55.86 mg/dL (4.0–
9.30 mmolL-1). Taking these reference values into consideration, renal damage was demonstrated in the treatment of urolithiasis (Group II) by an increase in serum creatinine and uric acid levels.

The rise of these parameters was substantially lower in the Cystone group than in the groups given the polyherbal methanolic extract at doses of 200 mg/kg b.w and 400 mg/kg b.w, with the best outcomes coming at 200 mg/kg b.w. Urolithiasis causes a decrease in glomerular filtration in rats due to obstruction of urine flow caused by stones accumulating in the urinary system and harm to the renal parenchyma. As a result, the taints,particularly nitrogenous molecules like urea, creatinine, and uric acid, build in the blood. The decrease in serum levels of these urea, creatinine, and uric acid is due to the anti-urolithiatic effect of the polyherbal methanolic extract of *Aerva lanata*, *Acalypha indica*, *Kalanchoe pinnata* (3:2:1). Renal insufficiency impairs the body's ability to filter creatinine, resulting in elevated blood creatinine levels. When compared to hyperoxaluric animals, the normalization of this parameter in animals treated with *Aerva lanata, Acalypha indica*, *Kalanchoe pinnata* polyherbal methanolic extract (3:2:1) suggests that this species protects to avoid renal function decline,minimizes tubular injury and crystal deposition.

The kidneys of the rats who did not receive any medicine appeared normal, with normal glomeruli, proximal and distal convoluted tubules, normal blood arteries, and no calcium oxalate deposits. Microscopic investigation of urolithiasis kidney slices under polarized illumination revealed intratubular and interstitial crystal deposits. Kidney sections were histopathologically inspected for healthy controls (I), urolithiasis controls (II), cystone treatment group (III), polyherbal methanolic extract treatment group (IV), (200 mg/kg b.w), and polyherbal methanolic extract treatment group (V), (400 mg/kg b.w) (magnification 10X and 40X).

Rats given a polyherbal methanolic extract of *Aerva lanata*, *Acalypha indica*, *Kalanchoe pinnata* (3:2:1) had much less calcification in their kidneys than those given a placebo. On histological examination, the healthy control exhibited normal-sized tubules with a single epithelial lining at the edge. In the urolithiasiscontrol rats, the crystals caused tubular dilation and destruction of the epithelial lining, as well as infiltration of inflammatory cells into the interstitial space.

At all doses, animals treated with Cystone, as well as those treated with polyherbal methanolic extracts of *Aerva lanata*, *Acalypha indica* and *Kalanchoe pinnata* (3:2:1) at doses 200 mg/kg b.w and 400 mg/kg b.w seemed normal in tubular epithelial cells and glomeruli, with few crystals and minor edema and dilatation. Hyperoxaluria causes oxidative stress and kills renal epithelial cells, providing a nidus for crystals to attach, resulting in crystal aggregation retention and deposition in the kidney. As a result, a decrease in oxalate could explain the decrease in oxidative stress and renal crystal deposition. Polyherbal methanolic extracts *Aerva lanata*, *Acalypha indica* and *Kalanchoe pinnata* (3:2:1) have been shown to interfere with oxalate metabolism in an animal model of urolithiasis. At a dose of 200 mg/kg b.w, this polyherbal methanolic extract diminishedthe concentration of uric acid in the urine, as well as the serum concentration of uric acid, creatinine and this dose is found to be the best.

The molecular docking studies reveal that the constituents of the plants *Acalypha indica*, *Aerva lanata*, *Kalanchoe pinnata* have good interaction with proteins of the enzymes (2ETE, 1JRP, 4WRN, 2RDU, 5FBH) involved in the process of urolithiasis. Among all, the best docking scores were found to be -8.27, -8.19, and -
9.32 Kcal/mol. for the constituent's quercetin with protein 1JRP, acalyphin and kaempherol with protein 2RDU respectively. The least docking score indicates a more stable interaction of the chemical constituent with a particular protein and shows desired anti-urolithiatic activity[11,12].

9. Conclusion:

The results of this study show that the polyherbal methanolic extract of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* (3:2:1) at doses of 200 mg/kg b.w and 400 mg/kg b.w have anti-urolithiatic effectiveness against CaOx-induced urolithiasis *invitro*. All steps of CaOx stone formation (nucleation, growth, and aggregation) were inhibited by the methanolic extracts of above-mentioned plants, favoring the development of more accessible CaOx crystals. Furthermore, in a rat model of urolithiasis, administration of the polyherbal methanolic extracts reduced the development of urolithiasis. Based on the findings of this study, we may conclude that taking this supplement prevents urolithiasis. Molecular docking studies of chemical constituents of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* showed good interactions with protein part of enzymes involved in urolith formation, i.e., the chemical constituents are capable to interact and inhibit the enzymes involved in urolithiasis process, thus achieving antiurolithiatic activity, and this strongly supports the *invitro* and *invivo* experimental data. This study indicates that the polyherbal methanolic extract of *Aerva lanata*, *Acalypha indica*, and *Kalanchoe pinnata* (3:2:1), may be effective in the prevention of urinary stone formation, and substantiates the traditional claim.

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Conflict of interest:

The authors declare no conflict of interest.

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