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ANTIUROLITHIATIC STUDY OF LEAVES OF SIDA VERONICAEFOLIA (LAM) ON RATS

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ABSTRACT

The point of the current exploration is to assess the antiurolithiatic impact of ethanolic extract ready from the leaves of *Sida Veronicaefolia (Lam),* in regard to its ethnobotanical record. Urolithiasis is actuated by Ethylene glycol (0.75%v/v)- Ammonium chloride (2%w/v) induce urolithiasis in exploratory wistar rats. Experimental urolithiatic rats received the prepared *Sida veronicaefolia (Lam)* leaves extract for 14 days. Histopathological and biochemical examination of a kidney section were carried out. Leaves extricate *Sida Veronicaefolia (Lam)* (500 mg/kg) applied potential antiurolithiatic movement on urolithiatic test rodents.

KEYWORDS: *Sida Veronicaefolia (Lam),* Urolithiasis, Eethylene-glycol-Ammonium chloride, wistar rats etc.

INTRODUCTION

Urolithiasis is the third most common kidney or urinary tract condition. Kidney problems are one of the most common problems that affect people all over the world due to the fact that the kidney is the primary organ that excretes waste in both animals and humans. Roughly 12% of individuals overall will encounter urolithiasis sooner or later in their lives. "Stone belts" are geographical areas with such a high and concerning rate of kidney stone formation. The "Stone Belt" refers to India. Urolithiasis is a common disease that affects people in Goa, South Coast Maharashtra, and Northwest Karnataka. Men are more likely than women to experience it between the ages of 20 and 40, despite the fact that it affects people of all ages, genders, and races.[1]

It is difficult to predict urolithiasis because it is a complicated condition with multiple causes. It involves the formation of stones from the urination of minerals like calcium, oxalate, phosphate, and uric acid. Calcium oxalate is the most prevalent component of these stones in India. The course of stone arrangement is the consequence of different substance and actual occasions like supersaturation, nucleation, gem development, and total as pee travels through the nephron tubules.[2]

Herbal medicine is now widely used because it is thought to be more effective and has fewer side effects. "pashanbheda" are plants in Ayurveda that are known for breaking down and dissolving stones.[3] *Sida Veronicaefolia (Lam)* is used in Indian customary medication like Ayurveda and Siddha for its restorative purposes. The plant is prized for its diuretic properties, which are helpful in uropathy treatment. Additionally, leucorrhea, gonorrhea, and dysentery are all treated with this plant's bark and root. It is widely acknowledged in the Ayurvedic medical system for its antioxidant, hepatoprotective, anti-inflammatory, and antitumor effects.[4]

MATERIALS AND METHODS

Plants collection and authentication

The fresh *Sida Veronicaefolia (Lam)* plants were obtained from Nanded, Maharashtra, India, when they were still in the vegetative stage. The Department of Botany at the Yashwantrao Chavan Institute of Sciences, Satara, verified the authenticity of the plants.

Preparation of Extract

The powdered leaves of *Sida Veronicaefolia (Lam)* were extracted through a series of Soxhlet extractions using solvents of increasing polarity. The ethanolic extract was specifically chosen for this study. A rotary flash evaporator was used to get rid of the solvent after the extraction, and the concentrated extract was kept at room temperature.

Animals

Male Wistar rats weighing between 200 and 250 grams were obtained from the Animal House at the Yashoda Technical Campus, Satara, of YSPM. They were kept in standard conditions with a 12-hour light-dark cycle. They approached standard pellet diet and water not indispensable. The guidelines in the Guide for the Care and Use of Laboratory Animals were followed in the experiments, taking into account the rights to biodiversity and animal welfare. The Dr. Babasaheb Ambedkar Technological University, Lonere**,** University Experimental

Animal Ethics Committee (IAEC) approved the experimental protocol. Six rats were in each experimental group at the very least.

Method used for investigating the antiurolithiatic activity

Ethylene glycol (EG-0.75%v/v) – Ammonium Chloride (AC-2%w/v) Induce Urolithiasis

Ethylene glycol Ammonium chloride instigated urolithiasis model will be utilized for this examination. 30 male wistar rats were devide in to 5 groups 6 animals in each. The treatment plan for each took 28 days. These are the groups:

Group I had a commercial diet and was the control group.

Group II: regular food and water with 0.75 % (v/v) ethylene glycol (EG) and 2 % (w/v) ammonium chloride (AC): CPD

Group III: 250 mg/kg b.w. of extract of *Sida veronicaefolia (lam)* managed orally.

Group IV: CPD + 500 mg/kg b.w. of extract of *Sida veronicaefolia (lam)* managed orally.

Group V: CPD + standard drug of urolithiatic, Cystone (500 mg/kg b.w.; p.o.)

Requirements

Animal- 30 Male wistar rats Body weight-200-250 gm

Chemicals & others: Saline Solution, extract of leaves of *Sida veronicaefolia (lam),* Ethylene glycol, Ammonium chloride, Cystone, Chloroform, Formaline Digital weighing balance, metabolic cages etc.

Urine Parameters

A measuring cylinder was used to measure the total volume of the urine, which was then reported in milliliters (mL) after the animals were individually housed in metabolic cages for 24 hours. The degrees of creatinine (estimated by Jaffe's technique), uric corrosive (estimated by the Uricase strategy), magnesium, and oxalate were broken down in the gathered pee tests.

Serum Parameters

At the conclusion of the experiment, a cardiac puncture was used to collect blood from each rat. Using a centrifuge machine from Satara, Maharashtra, the blood samples were centrifuged for 15 minutes at 3000 rpm at 4°C to examine the parameters of the serum. Serum levels of calcium, blood urea nitrogen, uric corrosive, and creatinine were surveyed utilizing autoanalyzer indicative packs with a spectrometer.

Histopathological Analyse

The experiment came to an end when the animals were killed by dislocating their cervical spines. One kidney from each rat was preserved in a solution of 10% formalin for histopathological analysis after both kidneys were removed. Using a light microscope (Nicon Eclipse Ci equipped with both polarizing attachment and Kameram Digital Image Analysis System), tissue samples were embedded in paraffin, stained with haematoxylin and eosin (HE).

RESULTS

Evaluation of urine parameters and serum parameters of experimental animals treated with Ethylene glycol (Eg-0.75%v/v)- Ammonium chloride (Ac-2%w/v) induce urolithiasis.

1) Evaluation of urine parameters

Table 1: Evaluation of urine parameters of EG-AC induce urolithiasis.

Group	Urine volume (mL)	Urine pH	Creatinine (mg/dl)	Oxalate (mg/dl)	Uric acid (mg/dl)	Magnesium (mg/l)
Normal Control	16.76±0.22	7.23±0.08	2.25±0.06	1.41±0.04	0.41±0.02	0.17±0.0006
Negative Control	10.18±0.28	8.36±0.2	2.81±0.11	2.36±0.05	1.003±0.05	0.08±0.0014
Test 1 group	12.66±0.31	7.65±0.07	2.5±0.05	2.22±0.04	0.83±0.03	0.12±0.001
Test 2 group	14.11±0.34	7.31±0.07	2.4±0.05	1.92±0.06	0.63±0.02	0.14±0.0003
Standard control	15.93±0.12	7.16±0.05	2.35±0.07	1.65±0.02	0.50±0.03	0.16±0.±0004

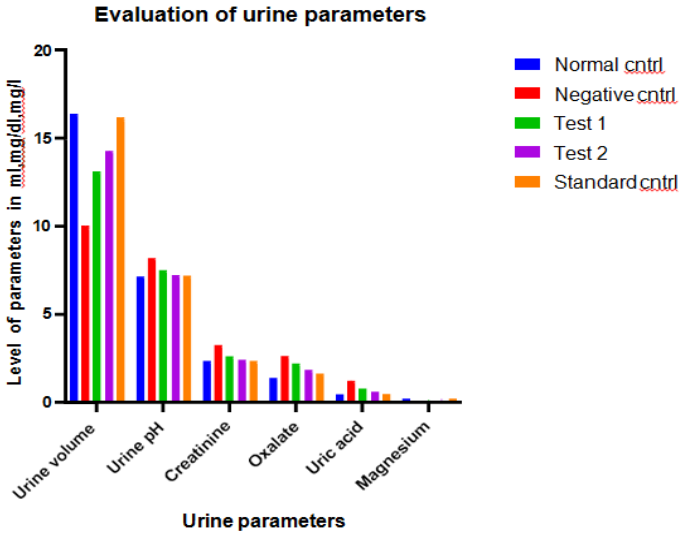
On day 14, rats treated with EG-AC produced 10.010.20 mL/24 hours per rat. On the other hand, rats given *Sida veronicaefolia* (lam) extract at a dose of 250 mg/kg (test 1 group) produced 13.130.16 mL/rat over the course of 24 hours. The outputs for the Cystone group and the test 2 group, which received 500 mg/kg of the extract, were 14.25 mL/24 h/rat and 16.15 mL/24 h/rat, respectively. The pH of the urine was lower in the EG-AC group than in the control group. On the other hand, when compared to the control group, *Sida veronicaefolia (lam)* extract at doses of 250 mg/kg, 500 mg/kg, and Cystone significantly raised the pH of the urine (Table 1).

More than a 14-day time frame, regulating EG 0.75% v/v-ac 2% w/v orally brought about expanded discharge levels of oxalate (2.61±0.06 mg/dL), uric corrosive (1.21±0.16 mg/dL), and creatinine (3.23±0.14 mg/dL) in the EG-AC group. On the other hand, oxalate (2.20.05 mg/dL), uric acid (0.050.03 mg/dL), and creatinine (2.60.06 mg/dL) levels decreased in the test

1 group of rats given *Sida veronicaefolia (lam)* extract at a dose of 250 mg/kg. Oxalate (1.810.07 mg/dL), uric acid (0.580.03 mg/dL), and creatinine (2.310.04 mg/dL) levels were also lower in the Cystone group and the Lam extract *(Sida veronicaefolia*) at 500 mg/kg (test 2 group). Magnesium excretion was significantly reduced to 0.070.004 mg/L when EG-AC was administered. On the other hand, treatment with *Sida veronicaefolia (lam)* extricate at portions of 250 mg/kg and 500 mg/kg, as well as Cystone, forestalled urinary magnesium misfortune and reestablished it to ordinary levels (Table 1).

Graphical analysis of urine parameters of EG-AC induces urolithiasis model

Evaluation of urine parameters



Graph 1: Analysis of urine parameters of EG-AC induces urolithiasis model.

1) Evaluation of serum parameters

Table 2: Evaluation of serum parameters of EG-AC induce urolithiasis.

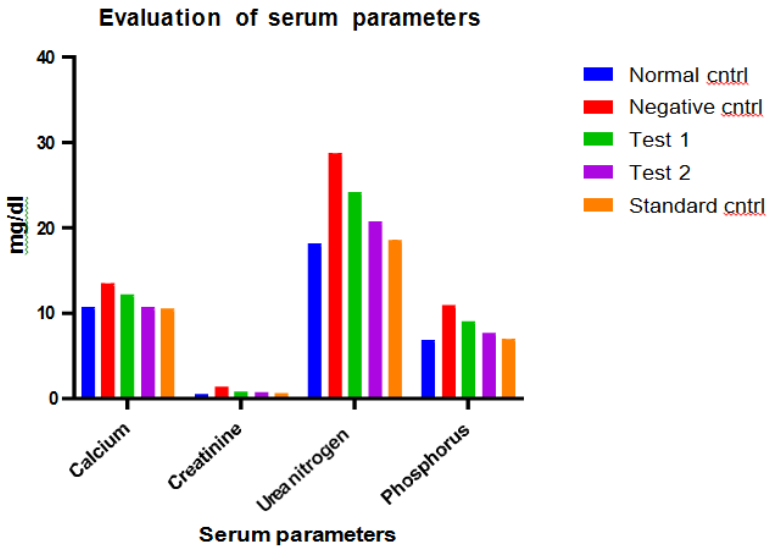
Group	Calcium (mg/dl)	Creatinine (mg/dl)	Urea nitrogen (mg/dl)	Phosphorus (mg/dl)
Normal control	10.76±0.21	0.56±0.04	18.16±0.79	6.91±0.20
Negative control	13.55±0.18	1.41±0.07	28.83±0.94	11.01±0.16
Test 1 group	12.21±0.11	0.85±0.02	24.16±1.4	9.05±0.17
Test 2 group	10.78±0.17	0.78±0.03	20.83±0.94	7.78±0.22
Standard control	10.61±0.15	0.65±0.02	18.66±1.08	7.03±0.17

Serum calcium, urea, and phosphorus levels were elevated in the EG-AC group, indicating impaired renal function. This group also had higher levels of creatinine (1.410.07 mg/dL). On the other hand, the rats in the Cystone group and those that were given 500 mg/kg of *Sida veronicaefolia (lam)* extract had lower creatinine levels, which were 0.780.03 mg/dL and 0.650.02 mg/dL, respectively. Besides, the *Sida veronicaefolia (lam)* extricate at portions of

250 mg/kg and 500 mg/kg, as well as Cystone, fundamentally decreased serum calcium and phosphorus levels contrasted with the EG-AC group. Blood urea nitrogen levels were raised in the EG-AC group however were altogether brought down by treatment with *Sida veronicaefolia* (lam) extricate at dosages of 250 mg/kg and 500 mg/kg, or Cystone (Table 2).

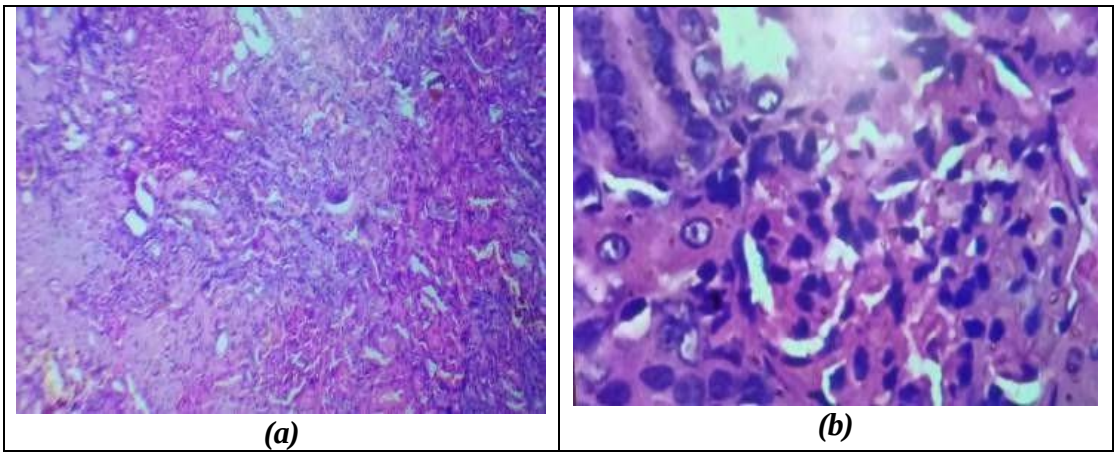
Graphical analysis of serum parameters of EG-AC induces urolithiasis model

Evaluation of serum parameters



Graph 2. Analysis of serum parameters of EG-AC induces urolithiasis model.

3) Histopathological study of kidney section of animals treated with EG-AC induce urolithiasis model.



(a) ***(b)***

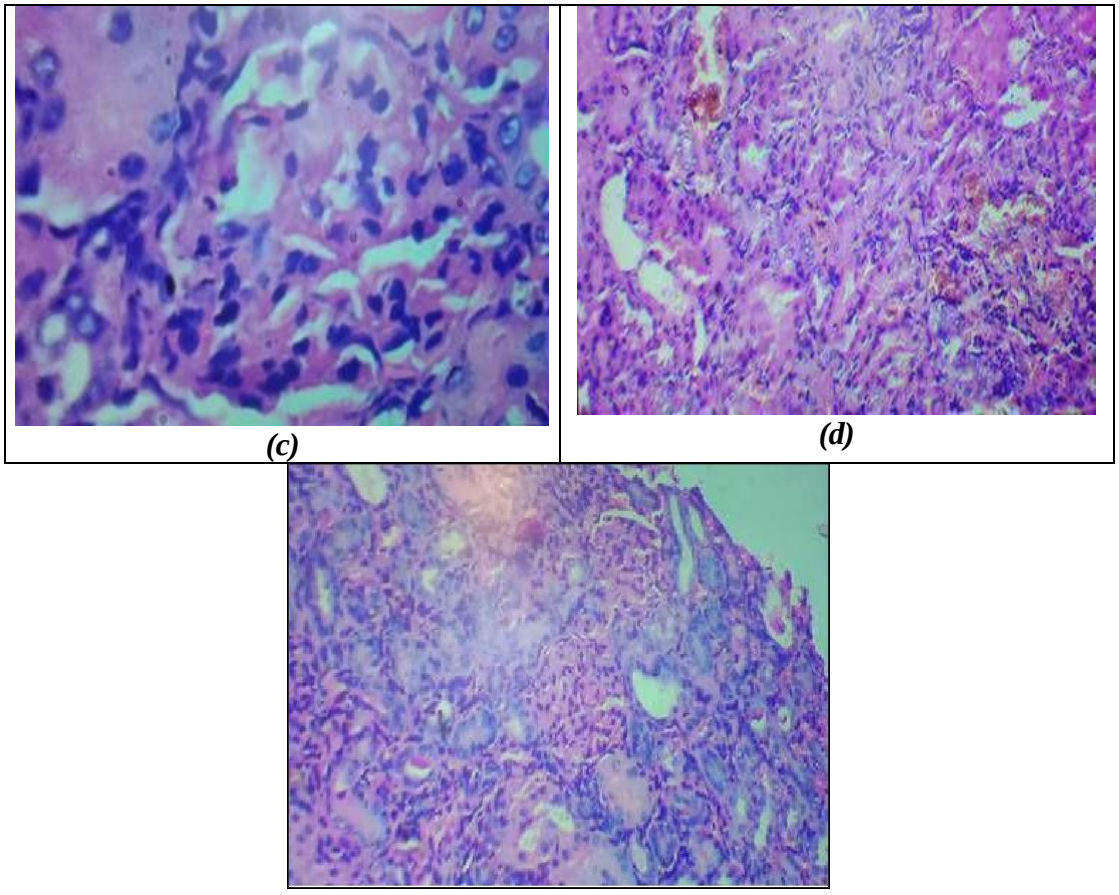


Figure 1: View of the experimental groups through histopathology. The kidney, stained with hematoxylin and eosin (HE), is shown in sections. Polarized light and a Nicon Eclipse Ci optical microscope were used to examine the sections. Renal calculus is indicated by arrows.

The scale bars in (a)–(e) represent 150 millimeters, and the initial magnification was 100 millimeters.

(a) Normal control group (b) Negative control group (c) Test I group (d) Test II group (e) Standard control group

The kidneys' histopathology revealed additional evidence of the antiurolithiatic effect. In the untreated rats, microscopy revealed deposits of polymorphic, irregular crystals, and numerous crystal deposits were observed in kidney sections. Additionally, these rats displayed greater tubular dilatation as well as tissue damage, including substantial voids in the renal epithelial cells. On the other hand, the treated rodents had significantly fewer tubule dilatation, damage, and crystal deposits. Markers of glomerular and tubular damage showed that untreated rats' deposition of renal stones caused significant tissue damage and impaired renal function. The

histopathological study recommends that cystone treatment, alongside the organization of ethanolic concentrate of Sida veronicaefolia (Lam) to rodents with EG 0.75%v/v-AC 2%w/v-initiated urolithiasis, really decreased and forestalled new stone development, instigated diuresis, and kept a harmony between stone advertisers, in this manner supporting the plant's antiurolithiatic action.

DISCUSSION

EG-AC organization causes an expansion in the seriousness of minuscule CaOx precious stones affidavit alongside high stone focus in the kidney.[5] In the current review, the movement of ethanolic concentrate of *Sida veronicaefolia (lam)* separates was explored by utilizing EG-AC-prompted urolithiasis model. Additionally, one of the symptoms of urolithiasis is an increase in the pH of the urine caused by the accumulation of CaOx crystals in the kidney.[6] The current study found that while Cystone treatment decreased urine pH, NaOx administration raised it. The co-application of the ethanolic extract of *Sida veronicaefolia (lam)* with EG-AC may decrease the urine pH that was raised by the application of EG-AC. Thusly, the concentrate treated bunches didn't influence the pH of the pee. Due to their acidifying properties, *Sida veronicaefolia (lam)* species' components like organic acid and phenolic acid [7] might be able to balance the pH of urine.

Based on the findings presented here, *Sida veronicaefolia (lam)* might be thought to have diuretic properties. For sure, *Sida veronicaefolia (lam)* species were demonstrated to have diuretic impact in the past reports.[7] The sodium oxalate induced urolithiasis model was used in previous studies, and excessive uric acid and creatinine excretions were found in the urine.[6] Similar to this, the EG-AC group excreted more uric acid and creatinine than the ethanolic extract of *Sida veronicaefolia (lam*) or the reference group.

Low degree of urinary magnesium is likewise a sign of the kidney stone.[6] The EG-AC group had lower magnesium levels in their urine, but the ethanolic extract of *Sida veronicaefolia (lam*) and the Cystone treatment increased magnesium excretion in this study.

Due to kidney obstruction, blood levels of urea, creatinine, calcium, and phosphorus were found to rise in urolithiasis, as was urine output and glomerular filtration rate.[6,8] In this setting, the signs of damage to the kidney were the elevated serum levels of urea, creatinine, calcium, and phosphorus.[8] Compared to the ethanolic extract of *Sida veronicaefolia (lam)* and the Cystone-treated groups, the EG-AC group's serum parameters were significantly higher.

As per the histopathological discoveries, basically renal analytics and degeneration, cystic dilatations of tubules, intratubular hyaline chambers, discharge, and aggravation, urolithiatic injuries were recorded from feeble to serious as reference, ethanolic remove *Sida veronicaefolia (lam),* vehicle, and EG-AC gatherings, separately. By reacting with unsaturated fatty acids in cell membranes, oxalate causes stone formation, lipid peroxidation, and liver damage.[9] Notwithstanding, rise in lipid peroxidation was restrained by the organization of the ethanolic extricate *Sida veronicaefolia (lam)*as well as Cystone.

It was discovered that *Sida veronicaefolia (lam)'s* phenolic components have a high antioxidant capacity.[10] As a result, the present study's antioxidant activity findings were found to be consistent with previous data. *Sida* species contain triterpenoids, coumarins, flavones, tannins, phenolic acids, and organic acids, according to phytochemical research.[11] It is common knowledge that phenolic content and antioxidant activity of plant extracts typically correlate. Lipid peroxidation and CaOx crystal deposition in the kidney may be prevented by phenolic compounds in *Sida veronicaefolia (lam).*[12] *Sida veronicaefolia (lam)'s* antiurolithiatic activity may be linked to its diuretic and inhibition of oxalate levels and free radical production, likely due to its phenolic content.

CONCLUSION

The current research study showed the antiurolithiatic action of the leaves extract of *Sida veronicaefolia (lam).* The current study's promising findings have provided scientific support for the ethnopharmacological data on *Sida veronicaefolia (lam).*

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