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Evaluating the Anti-Ulolithiatic Potential of A Methyl Extract from the whole Hygrophila Salicifolia plant using the in Vitro method of Urolithiasis Induction by Ethylene Glycol

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Abstract - Utilising an in vivo approach to induce urolithiasis with ethylene glycol, the research examines the anti-urolithiatic potential of a methanolic extract derived from the whole Hygrophila salicifolia plant. Over the course of 28 days, five groups of male Wistar albino rats were randomly assigned to different treatment groups. The first group was used as a control, the second group got a lithiatic agent, the third group had Cystone, and the fourth and fifth groups got two doses of the methanolic extract. From the urine samples, many parameters were analysed, including the amounts of calcium, creatinine, phosphate, magnesium, and oxalate. The development of calcium oxalate monohydrate (COM) crystals was significantly inhibited by the methanolic extract in comparison to the control and other extracts. A significance threshold of P<0.001 was used in the statistical study to validate the effectiveness.

Keywords: Vivo, Lithiatic Agent, Hygrophila Salicifolia, Methanolic, Urolithiasis.

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INTRODUCTION

There are about 80,000 species of higher plants on Earth, and more than 80,000 of those species have medical use. It is estimated that India is home to more than 45,000 different plant species, making it one of the 12 most biodiverse areas in the world. Due to the presence of sixteen different agro-climatic zones, ten vegetation zones, twenty-five biotic provinces, and four hundred and twenty-six biomes (habitats of unique species), India has an unprecedented level of biodiversity. It is estimated that between 15,000 and 20,000 of these plants have medicinal use today. [1]

Traditional civilizations, on the other hand, make use of the curative powers of around 7,500 different species. Traditional Indian medical practices such as Ayurveda and Unani have a long history of using herbal medicines in their treatment of various ailments. In its therapeutic system, Unani makes use of around thirty species, in addition to the seven hundred species that are used by Siddha and Amchi practices. Ayurveda makes use of seven hundred different species. [2] Numerous components of the plant are used to produce medicines. These components might include the whole plant or particular portions of the plant, such as the stem, bark, roots, flowers, seeds, and so on. Plant excretory products such as gum, resins, and latex are examples of materials that are used in the manufacturing of certain pharmaceuticals. Even within the allopathic medical system, a significant number of the medications that are now in use are derived from plants. [3] In addition, plants provide a source of many essential chemical intermediates that are used in the manufacturing of modern medications. These intermediates include diosgenin, solasodine, and α-ionone. Furthermore, plants continue to be an important source for the development of innovative medications, and the market for drugs that are derived from plants is rather consistent throughout the whole world. [4]

Among the ancient civilizations, India was renowned for being a country that had a wealth of medicinal herbs. There are a great number of medicinal and aromatic plants that are mostly utilized as raw materials in the manufacturing of pharmaceuticals and perfumes, and the forest in India is the primary source of these plants. Regarding 8,000 different herbal treatments, Ayurveda has codified them. Ayurvedic medicine continues to make use of the 67 medicinal plant species that are listed in the Rigveda (5000 BC), 81 in the Yajurveda (400–250 BC), 290

in the Atharvaveda (4500–2500 BC), and 1100 and 1270 species in the Charak Samhita (700 BC) and the Sushrut Samhita (200 BC), respectively, who are responsible for the compounding of drugs. [5]

Regrettably, there is a troubling rate of extinction for both significant flora and a significant portion of the historical information. Due to the rapid depletion of forests, which is hurting the availability of raw medicines, [6] Ayurveda has reached a particularly vital phase, much like other systems of herbal treatment. This is because of the increasing demand for raw pharmaceuticals. Approximately fifty percent of the world's tropical forests, which are home to an astounding array of flora and animals, have already been devastated by human activity. [7]

The remaining space is a pitiful 8%, which is far less than the minimum 33%.A great number of valuable medicinal plants are on the verge of extinction on the planet. In the Red Data Book of India, there are a total of 427 entries for endangered species. Of these, 28 are thought to be extinct, 124 are considered endangered, 81 are vulnerable, 100 are rare, and 34 are not well known.[8]

The most notable kinds of traditional Indian medicine include Ayurveda, Siddha, Unani, and Folk remedies. There are many more forms of traditional Indian medicine as well. Within the realm of alternative medicine in India, Ayurveda stands out as the most contemporary and widely practiced approach. Ayurveda's history, which spans from 1500 to 800 B.C., is widely regarded as an essential component of Indian civilization. The Sanskrit word "Au" translates to "life," while the word "Veda" means "knowledge." Not only does it contain the science of treating sickness, but it also spans the whole spectrum of human flourishing, which includes the material, immaterial, and spiritual elements. This is what the term signifies. Ayurveda recognizes that in order to achieve optimum health, it is essential to have a balanced state of mind, sense organs, and consciousness in addition to having a balanced condition of the components of the body. [9]

People have depended on plants and the derivatives of plants for medicinal purposes for close to three thousand five hundred years. There are a great number of medications that are found in nature that have their roots in the ethnobotanical knowledge of "native doctors" who practice traditional medicine in natural settings that are rich in biodiversity. In 2010, a total of 156 clinical studies were conducted to investigate the pharmacological activity and therapeutic applications of one thousand different plants. The results of these research were found to be positive. As a consequence of this, there has been a resurgence in the pursuit of discovering medications and nutritional supplements that are derived from plants. [10]

RESEARCH METHODOLOGY

- **Hardware and tools**

x A rat operating table made by INCORP in India x A weighing scale for animals x A RO water system from Merck Life Science Pvt. Ltd. in India

- **Materials, chemicals, and reagents**

Cystone was procured from Bangalore's Himalaya Herbal Health Care. Emerck Ltd. of Mumbai supplied the solvents, which were of laboratory quality. [11] The standard reagent kits were acquired from Coral Clinical Systems in Goa and used for all the biochemical assays. [12]

Table 1: Product catalogue

Sr. No	Name of the kits	Name of dealer
1	Calcium	Coral
2	Creatinine	Coral
3	Phosphate	Coral
4	Magnesium	Coral
5	Oxalate	Coral

- **Extract and plant matter**

After being shade dried, the plant was ground into a coarse powder by using a grinder. The next step was to use methanol and Soxhlet's equipment to get an extract. [13] A vacuum evaporator was used for the purpose of extract concentration. Desiccators were used to dry it. [14]

- **Animal**

The study's rats were selected from male Wistar albino rats ranging in weight from 160 to 210 grammes. [15] All animals were provided with water ad libitum and kept in regular circumstances with standard rat diet. After receiving approval from the IAEC, all procedures involving experimental animals were carried out in accordance with CPCSEA rules. [16]

- **Principle**

A solution of 0.75 percent ethylene glycol in water was used to cause urolithiasis. [17] The fast absorption and hepatic metabolism of ethylene glycol to glycollic acid by alcohol dehydrogenase/aldehydedehydrogenase makes it an attractive and easy-to-deliver chemical in drinking water. [18] Lactate dehydrogenase promotes hyperoxaluria by oxidising glycollic acid to glyoxylic acid, which is further oxidised to oxalic acid. [19]

- **Assessment of pharmacological agents for potential anti-urolithiatic effects**

Procedure

Every one of the five groups was given a 28-day course of treatment that included six albino rats. Group-I served as a control and was provided with water to drink and rat chow to eat. [20] A lithiatic group consisting of 1% tween 80 solutions was administered to group II. From day 15 to day 28, group III received the conventional medicine, Cystone, at a dosage of 750 mg/kg. Groups IV and V were each given two test doses of methanolic extract: 300 mg/kg and 500 mg/kg, respectively. [21] For the purpose of creating kidney stones, all groups were given water containing 0.75% ethylene glycol, with the exception of group I. The rats' metabolic processes were monitored in separate cages. The volume of the urine was determined by collecting urine samples on the 28th day. Once a drop of concentrated hydrochloric acid was added, it was held at 4º C. Several biological parameters, such as calcium, creatinine, phosphate, magnesium, and oxalate content, were measured in a urine sample.

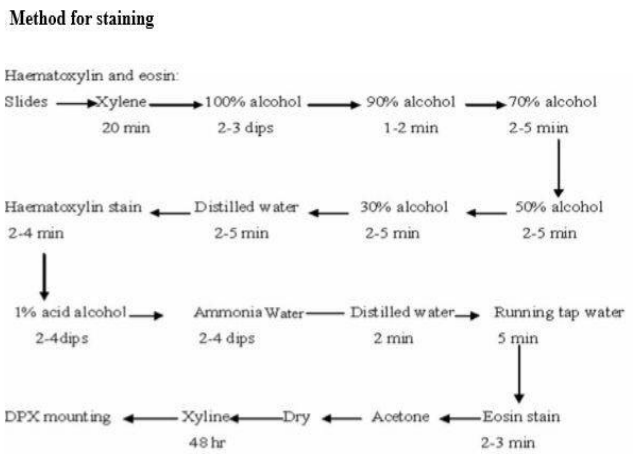
Blood Collection

After the trial was over, blood was taken retro-orbitally under the influence of diethyl ether for anaesthesia on the 29th day. Centrifugation was used to separate the serum (R-4R-V/FA centrifuge, Plastograft industries Pvt. Ltd.) at 10,000 rpm for 10 minutes before creatinine analysis.

Surgical pathology

Following scarification, the kidneys were removed from each group. Their washing process used saline and a fixative solution containing 8-10% phosphate-buffered formalin. The paraffin technique was used to take sections. They were stained with hemotoxylin and eosin. Photographs were taken and histopathological alterations were detected using a light microscope.

Staining technique



- **Assessment of calcium levels**

Principle

Due to lactone synthesis in the phthalein half of the molecule, the dye-O-cresolphthalein complex one forms a strongly coloured complex by binding calcium firmly in an alkaline solution. These performance-enhancing components are part of the reaction mixture. (1) 8-hydroxyquinoline, which binds magnesium and prevents its interference; (2) urea, which decreases the turbidity of a lipemic serum and increases the colour intensity of the calcium-dye complex; and (3) ethanol, which decreases the absorbance of the blank.

Reagents

L1: Agent for Buffering

L2: Colour reagent

S: Standard Reagent (10 mg/dl)

Sample

Urine

Procedure

To prepare the test (T), standard (S), and blank (B) tubes, the following chemicals were pipetted into them in the amounts indicated in the table below:

Additional sequence	Blank (ml)	Standard (ml)	Test (ml)
Buffer Reagent (LI)	0.5	0.5	0.5
Colour Reagent (LI)	0.5	0.5	0.5
Distilled Water	0.02	-	-
Calcium standard (S)	-	0.02	-
Sample	-	-	0.02

Following a thorough mixing, the samples were left to incubate at 25 qC for 5 minutes. Within 60 minutes, the absorbance of the blank was measured at 570 nm using a Shimadzu UV1650PC, and the same was done for the standard and test samples.

Calculation

Calcium in mg/ dl = (Abs T/ Abs S) X10

- **Estimation of Oxalate**

Principle

The process involves titrating with permanganate and then quantitatively precipitating oxalate in the form of its calcium salts.

Procedure

By adding acetic acid or ammonia solution, the pH of a urine sample was brought down from 5.0 to 5.2.

For every 50 ml sample, 2 ml of a calcium chloride solution (5 g/100 ml) was added, and then the solutions were left to remain at room temperature for 16 hours. The precipitated calcium oxalate was isolated by centrifugation, and the liquid that remained after separation was poured off. A solution of weak ammonia was used to hold the precipitates. Titration with 0.01 N KMnO4 was performed after dissolving the precipitates in 1N sulphuric acid.

Calculation

The concentration of anhydrous oxalic acid is 0.45 mg per millilitre of 0.01N KMnO4.

- **Calculating Inorganic Phosphate Concentration**

Principle

When inorganic phosphate-containing samples are treated with phosphorus reagent, phosphomolybdate is produced. The amount of inorganic phosphate in this solution is determined by its intensity. Here is a brief summary of the reaction:

Phosphorus + Ammonia molybdate → Acid Ph Ammonium Phosphomolybdate

Reagents

Preparation of phosphorus reagent

In 1 litre of distilled water, dissolve the following chemicals.

Chemicals	Amount
Ammonium molybdate	1.98 gm
Conc. H_2SO_4	20.5 ml
NaCl	4.5 gm
Twin-20 (Surfactant)	10 gm

Inorganic phosphorus standard (5 mg/dl)

Dissolved in 1 litre of distilled water was 0.2197 gramme of potassium dihydrogen phosphate.

Sample: urine (Diluted 1:20)

To prepare the test (T), standard (S), and blank (B) tubes, the following chemicals were pipetted into them in the amounts indicated in the table below:

Additional sequence	Blank (ml)	Standard (ml)	Test (ml)
Phosphorus reagent	1.0	1.0	1.0
Sample (ml)	-	-	0.02
Standard sample	-	0.02	-
Distilled water	0.02	-	-

For two minutes, all of the samples were well mixed. At 340 nm, using a Shimadzu UV1650PC filter, the absorbance of the test and standard was compared to a blank.

Calculation:

$$\text{Urine Inorganic Phosphorus (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

- **Calmagite technique for magnesium determination**

Principle:

In an acidic environment, magnesium forms a red complex with calmagite. By incorporating targeted chelating agents and detergents, the presence of calcium and protein interference is created. As the magnesium concentration in the sample increases, so does the saturation of the hue.

$$\text{Magnesium} + \text{Calmagite} \xrightarrow[\text{medium}]{\text{Alkaline}} \text{Red colored complex}$$

Reagents

L1: Agent for Buffering

L2: Colour reagent

S: Magnesium solution (2.0 mEq/L)

Samples

Adjust the pH to 2-3 HCl and dilute the urine with distilled water, 1:3.

Procedure

After rinsing with high-quality deionised water, all of the glassware was washed with 0.1 N hydrochloric acid. To prepare the test (T), standard (S), and blank (B) tubes, the following chemicals were pipetted into them in the amounts indicated in the table below:

Additional sequence	Blank (ml)	Standard (ml)	Test (ml)
Buffer Reagent (LI)	0.5	0.5	0.5
Colour Reagent (L2)	0.5	0.5	0.5
Distilled Water	0.01	-	-
magnesium standard	-	0.01	-
Sample	-	-	0.01

After thoroughly mixing all of the samples, they were incubated at 25 qC for 5 minutes. Within 30 minutes, the absorbance of the blank, standard, and test samples were measured at 510 nm using a Shimadzu UV-1650PC.

Calculation:

Magnesium in mEq/L = (Abs T/Abs S) X2

- **Creatinine Assessment:** (Mod. Jaffe's kinetic method):

Principle:

A protein-free solution containing picric acid combines with creatinine to produce an orange-colored complex with alkaline picrate in an acidic media. The quantity of creatinine in the sample is measured calorimetrically at 520 nm, and the intensity of the colour that forms during the given period is directly proportional to that amount.

Creatinine + alkaline picrate → orange colour complex

Reagents

Regent 1: Picric Acid

Reagent 2: Buffer reagent (Sodium Hydroxide, 0.75N)

Reagent 3: Stock Creatinine Standard, 150mg%

Samples

Urine and serum (Before testing, dilute specimens with distilled water 1:50).

Procedure

In clean, dry test tubes designated as Blank (B), Standard (S), and Test (T), the following chemicals were pipetted according to the amounts shown in the table below:

Addition sequence	(S) / (T)30qC/37qC
Picric acid reagent (L1)	0.5 ml
Buffer reagent (L2)	0.5 ml
Bring reagents to the assay temperature and add	
Creatinine standard(S)/sample/dilute urine	0.1 ml

After precisely thirty seconds of mixing, the standard and test were tested for initial absorbance A1. Just sixty seconds after that, we measured a different absorbance A2 for both the control and the test.The absorbance change 'A' was determined at 520 nm using a Shimadzu UV-1650PC spectrophotometer for both the control and experimental groups.

Calculation:

Creatinine in mg/dl = ('AT/ 'AS)* 2

Urine creatinine in g/L = ('AT/'AS)*1

Urine creatinine in g/24 hrs. = urine creatinine in g/L X volume of urine in 24 hrs

- **Crystalluria**

After centrifuging 1 millilitre of fresh urine at 3,000 revolutions per minute for 10 minutes, 950 microlitres of the resulting supernatant were removed and discarded. Into a hemocytometer went ten microlitres of silt. A microscope was used to count the crystals. The following was the scale used to rate the severity of crystalluria: A high power field (100 x) containing 6–20 crystals; "+" containing 1–5 crystals; and "-" indicating no crystals at all.

- **Statistical Analysis**

To analyse the data, one way ANOVA was employed and the results were presented as the mean ± S.E.M. Afterwards, many Dunnett's tests were conducted. It was determined that P< 0.0001 was statistically significant. Software called Graph Pad Prism 6 was used.

1. RESULTS

Table 2: Impact on the body's vital signs

Groups	Group-I (Normal Control)	Group-II (Model Control)	Group-III (standard group, Cystone)	Group-IV (300mg/Kg)	Group-V (500 mg/kg)
Wet kidney Weight(gm)	0.91±0.06	1.12±0.20	0.86±0.10	0.93±0.04	0.98±0.05
Dry kidney Weight(gm)	0.78±0.04	1.34±0.16	0.74±0.07	0.73±0.04	0.74±0.04
% change Body weight	8.03±2.11	-18.14±6.19	10.36±2.34	-6.621±2.51	-4.88±2.71
Urine volume	11.23±0.69	3.76±0.49	9.1±0.66	3.96±0.37	6.5±0.15

- **Ethylene glycol-induced urolithiasis**

In rats with ethylene glycol (EG)-induced urolithiasis, the effect of MEHS on urine calcium levels:

The variation in calcium content in the urine of both the control and experimental groups of rats was shown in Figure 5.49. The normal control group had a calcium concentration of 2.71 ± 0.29 mg/dl, whereas the model group had a significantly higher value of 18. ± 0.22 mg/dl (p < 0.05). "This rise in calcium concentration was effectively averted when Cystone, MEHS 300 mg/kg, and MEHS 500 mg/kg were administered, respectively, with concentrations of 13.56 ± 0.31 mg/dl, 16.38 ± 0.19 mg/dl, and 13.75 ± 0.67 mg/dl, in comparison to the urolithiatic model group. Neither the 300 mg/kg nor the 500 mg/kg doses of MEHS substantially vary in the amount of calcium that is reduced in the urine.

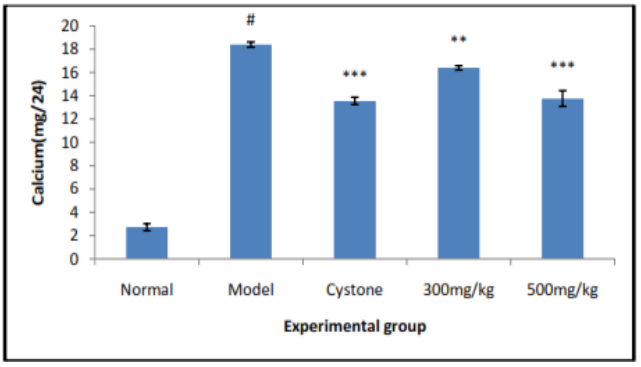


Figure 1: Impact of MEHS on EG-model urinary calcium levels.

Model control = (0.75 % v/v EG, p.o)

Standard control = (0.75 %v/v EG, p.o + 750 mg/kg, Cystone, p.o)

Test–1 = EG (0.75 % v/v, p.o) + MEHS (300 mg/kg, p.o) Test–2 = EG (0.75 % v/v, p.o) + MEHS (500 mg/kg, p.o)

Note: P<0.0001 compared to Normal experimental group,*** P<0.0001, ** P<0.001 *** P<0.0001 compared to Model experimental group, using one-way ANOVA followed by Dunnett's multiple comparisons tests.P< 0.001 was noted as statistically important."

- **Impact of MEHS on oxalate levels in rat urine during urolithiasis produced by ethylene glycol (EG)**

The content of oxalate in the urine of the model animals was considerably higher (12.41 ± 0.39 mg/dl) compared to the normal control animals (6.84 ± 0.15 mg/dl). Treatments with cystone and MEHS 500 mg/kg resulted in a significant ($p < 0.05$) reduction in oxalate concentration (mg). "The oxalate concentrations with Cystone, MEHS 300 mg/kg, and MEHS 500 mg/kg were 4.23 ± 0.09 mg/dl, 7.31 ± 0.37 mg/dl, and 5.91 ± 0.44 mg/dl, respectively. Neither the 300 mg/kg nor the 500 mg/kg doses of MEHS substantially vary in the amount of oxalate that is reduced in the urine.

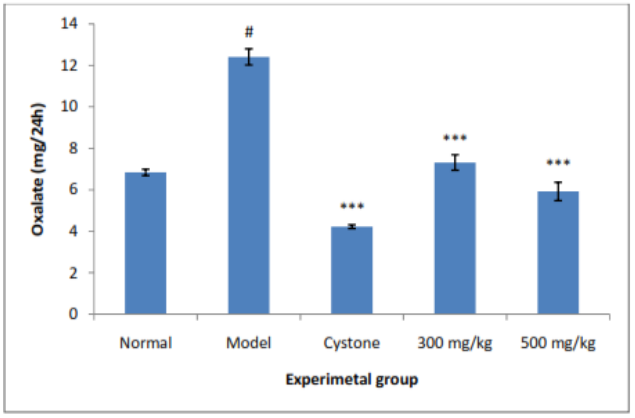


Figure 2: Urine oxalate levels as affected by MEHS in an EG model

Model control = (0.75 % v/v EG, p.o)

Standard control = (0.75 %v/v EG, p.o + 750 mg/kg, cystone, p.o)

Test–1 = EG (0.75 % v/v, p.o) + MEHS (300 mg/kg, p.o)

Test–2 = EG (0.75 % v/v, p.o) + MEHS (500 mg/kg, p.o)

Impact of MEHS on inorganic phosphate levels in rat urine during urolithiasis produced by ethylene glycol (EG)

In comparison to the normal control group, the model group had a significantly higher concentration of urine inorganic phosphate (6.94±0.19 mg/dl) with a p-value of less than 0.05. Both the conventional and test medications were able to halt this growth. A notable alteration was seen with cystone, MEHS 300 mg/kg, and MEHS 500 mg/kg, respectively, with concentrations of 3.41±0.17 mg/dl, 6.19±0.20 mg/dl, and 2.82±0.26 mg/dl. There is a statistically significant difference between the 300 mg/kg and 500 mg/kg reductions of inorganic phosphate in urine.

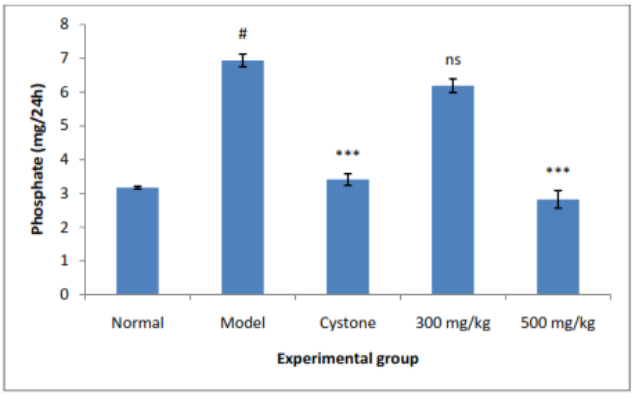


Figure 3: The impact of MEHS on inorganic phosphate levels in the EG model of urine

Model control = (0.75 % v/v EG, p.o)

Standard control = (0.75 %v/v EG, p.o + 750 mg/kg, cystone, p.o)

Test–1 = EG (0.75 % v/v, p.o) + MEHS (300 mg/kg, p.o)

Test–2 = EG (0.75 % v/v, p.o) + MEHS (500 mg/kg, p.o)

- **Impact of MEHS on magnesium excretion in rat urolithiasis caused by ethylene glycol (EG)**

In comparison to the normal control group, the model group exhibited a statistically significant ($p \leq 0.05$) decline in the concentration of magnesium in the urine (mg/dl). In comparison to the control group, all of the treated groups had an elevated blood magnesium content; however, the cystone and MEHS 500 mg/kg (5.89±0.22 mg/dl and 6.24±0.69 mg/dl, respectively) treated groups showed a considerably higher concentration of magnesium. Magnesium increases in urine with 300 mg/kg MEHS and 500 mg/kg MECR are substantially different.

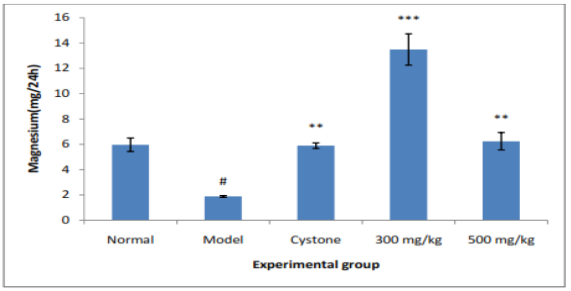


Figure 4: Analysis of magnesium excretion by electrolytes in an EG model

Model control = (0.75 % v/v EG, p.o)

Standard control = (0.75 %v/v EG, p.o + 750 mg/kg, cystone, p.o)

Test–1 = EG (0.75 % v/v, p.o) + MEHS (300 mg/kg, p.o)

Test–2 = EG (0.75 % v/v, p.o) + MEHS (500 mg/kg, p.o)

- **Rats with ethylene glycol (EG)-induced urolithiasis: the impact of MEHS on urine creatinine levels**

0.75% ethylene glycol in drinking water demonstrated statistically significant ($p<0.05$) decrease in urinary creatinine levels as compared to normal control animals as shown in figure. Creatinine levels in urine were found to be significantly ($p<0.05$) higher in cystone, MEHS 300 mg/kg & MEHS 500 mg/kg groups.

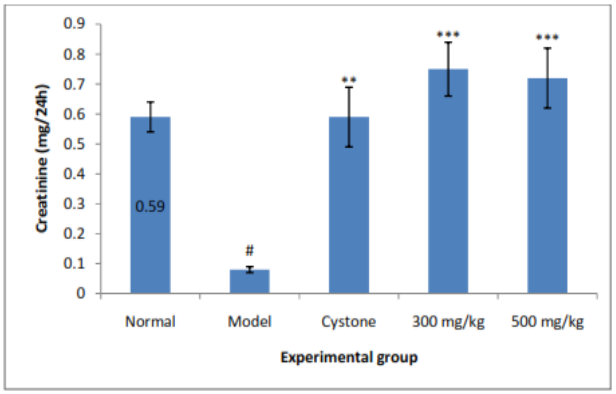


Figure 5: Impact of MEHS on EG model creatinine levels

Model control = (0.75 % v/v EG, p.o)

Standard control = (0.75 %v/v EG, p.o + 750 mg/kg, cystone, p.o)

Test–1 = EG (0.75 % v/v, p.o) + MEHS (300 mg/kg, p.o)

Test–2 = EG (0.75 % v/v, p.o) + MEHS (500 mg/kg, p.o)

- **Impact of MEHS on serum creatinine levels in rats with urolithiasis produced by ethylene glycol (EG)**

In comparison to the control animals in group I, the lithiatic group had substantially higher serum creatinine levels ($p<0.05$). When compared to the model group, pretreatment with Cystone and herbal medicine extract significantly reduced blood creatinine levels ($p<0.05$).

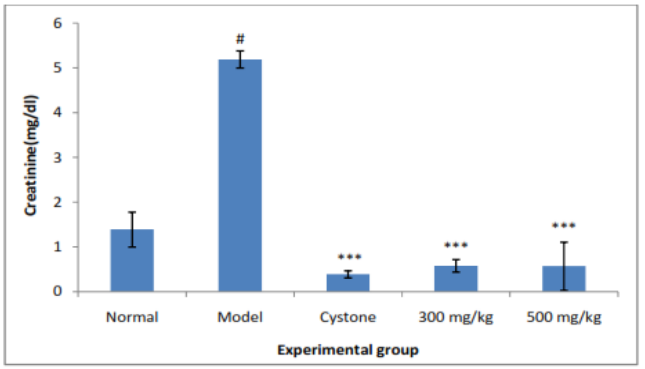


Figure 6: Implications of MEHS on serum creatinine levels in an EG model

Model control = (0.75 % v/v EG, p.o)

Standard control = (0.75 %v/v EG, p.o + 750 mg/kg, cystone, p.o)

Test–1 = EG (0.75 % v/v, p.o) + MEHS (300 mg/kg, p.o)

Test–2 = EG (0.75 % v/v, p.o) + MEHS (500 mg/kg, p.o)

- **Hazards of Crystalluria**

The model group had a larger concentration of crystals than the control group, according to microscopic analysis. Crystalluria as a result of cystone and MEHS 500 mg/kg treatment was reduced.

Table 7: Methylethylene glycol's Impact on EG Model Crystalluria

Parameter	Normal	Model	Standard	MESH 300 mg/kg	MESH 500 mg/kg
No. of crystals per high power field	——	+++	+	++	+

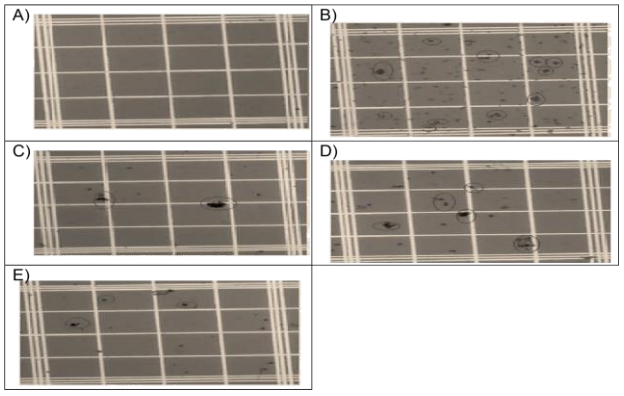


Figure 8: Methylethylene glycol's impact on crystalluria in an EG model.

Rattanine crystalluria. (A) Untreated urine (control group): no crystals found; (B) Crystals in urine (model group; EG): pronounced growth and aggregation; (C) Crystals in urine (EG+STD): same as control group;" (D) Crystals in urine (EG+MEHS 300 mg/kg): fewer aggregated crystals (more than 8

in number); (E) Crystals in urine (EG+MEHS 500 mg/kg): same as control group; (original magnification X100).

- **Analysis of EG model histopathology**

Tissue samples from the control group showed tubules of normal size, according to histopathological examination of the kidney. The accumulation of crystals inside the tubules caused several complications, including inflammation, flattening of renal tubular cells, tubular necrosis, and tubular dilatation. In contrast to the model group, those treated with cystone and MEHS 500 mg/kg exhibited reduced tubule dilatation and crystal deposition.

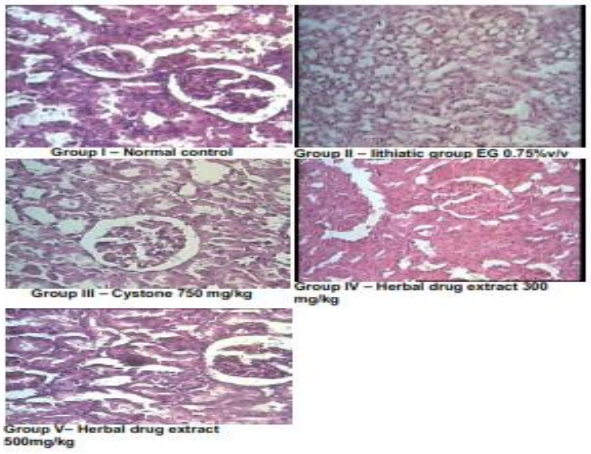


Figure 9: Histopathology of Rat Kidneys and the Impact of MECR

Observations on the kidney histopathology in rats. (A) The control group shows normal renal tubules without inflammation or dilation. (B) The model group (EG) has inflammation and marked tubule dilation due to crystal deposits. (C) The EG+STD group is the same as the control group. (D) The EG+MEHS 300 mg/kg group shows less tubule dilation and interstitial inflammatory infiltrate than the model group. (E) The EG+MEHS 500 mg/kg group is the same as the control group. The data shown above show that, as compared to the control group, animals given 500 mg/kg of MEHS had considerably lower levels of the stone inhibitor magnesium and significantly higher levels of the stone promoters calcium, oxalate, and inorganic phosphate.

A) **Control group:** typical renal tubules and glomeruli, at a 40X magnification

B) **Model group (EG):** Magnification 40X showing enlarged renal tubules caused by stone accumulation.

C) **EG+Cystone:** identical to a control group, at a 40X magnification

D) **EG+MEHS 300mg/kg:** reduced dilation of the renal tubules, as seen at a magnification of 40X (E) EG+MEHS 500 mg/kg: observed similarly to the control group, as observed at a magnification of 40X.

CONCLUSION

Hygrophila Salicifolia methanolic extract substantially inhibited COM crystal growth compared to other subsequent plant extracts. A travelling microscope took the measurement. In another experiment conducted in a controlled environment, researchers used conductometric titration of CaCl2 with Na2C2O4 both with and without different *Hygrophila Salicifolia* extracts. As a result of complexation with the methanolic extract of *Hygrophila Salicifolia*, the free Ca2+ level decreased, and the end point of the conductometric titration curves shifted to the lower side. Results from these in vitro investigations suggest that the methanolic extract of *Hygrophila Salicifolia* reduces the concentration of free calcium ions by complexing with calcium ions in the solution, which in turn affects calcium oxalate crystals. In order to assess the efficacy of a methanolic extract of *Hygrophila Salicifolia* against renal calculi in experimentally produced urolithiasis, in vivo tests were conducted using the available data.

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