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Research Article

**EVALUATION OF *IN-VIVO* ANTIUROLITHIATIC ACTIVITY
OF METHANOLIC LEAF EXTRACTS OF ACALYPHA
INDICA LINN****Radhika Patel^{1*}, Akriti Kushawaha²**¹. Associate Professor, Department of Pharmacology, SHEAT College of Pharmacy, Ghani Ayer, Varanasi, U. P.². Research student, Department of Pharmacology, SHEAT College of Pharmacy, Ghani Ayer, Varanasi, U. P.**Abstract:**

The present study is designing to evaluate the *in vivo* anti-urolithiatic activity of methanolic leaf extracts of *Acalypha indica* L. Urolithiasis is a common and a major cause of morbidity worldwide. After administering 0.75% ethylene glycol (EG) in the drinking water, all mice consistently developed crystalluria, marked by the presence of both calcium oxalate monohydrate (COM) and dihydrate (COD) crystals. COM crystals typically appeared as distinctive biconcave ovals, whereas COD crystals displayed dipyramidal shapes. Notably, untreated nephrolithiatic mice exhibited a greater abundance of large COM crystals in their urine. Urinary microscopy confirmed that EG treatment significantly increased both the number and size of calcium oxalate crystals. Treatment were given from 1st - 21st day administer 0.75% EG in Drinking Water with Standard drug (Cystone 40 mg/kg) and test extract at doses 20 mg/kg and 40 mg/kg respectively. Results of all biochemical test were statistically significant as compare to disease control group. In contrast, mice treated with test extract showed a clear reduction in both the number and size of calcium oxalate crystals. Nevertheless, when compared head-to-head with a group receiving Cystone at 40 mg/kg, the Cystone-treated mice demonstrated a more pronounced decrease in crystalluria.

Keywords: Urolithiasis, *Acalypha indica*, Calcium oxalate, Ethylene glycol, and Cystone.**Corresponding author:****Mrs. Radhika Patel,**

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INTRODUCTION:

Urolithiasis is a widespread and painful disorder of the urinary tract, frequently associated with metabolic syndrome. The symptoms experienced in cases of kidney stones depend on their location within the kidney and the extent of kidney function deterioration. The creation of solid crystalline structures within the kidneys and the steps leading to stone formation vary based on the type of stone and the chemical composition of urine. This process is called calculogenesis. When crystals form within the kidneys, it is known as nephrolithiasis, whereas stones that move from the renal pelvis into the urinary tract—including the ureters, bladder, and urethra—are referred to as urolithiasis. The formation and recurrence of kidney stones remain leading causes of morbidity. Additionally, an increased risk of end-stage renal failure is associated with urolithiasis, affecting approximately 12% of the global population.

**Figure No. 1: Kidney****Worldwide Scenario of Urolithiasis**

The incidence and prevalence of urolithiasis exhibit a clear geographical variation, influenced by a number of factors, including latitude, comorbidities, direct occupational exposure, dietary practices, water quality, inheritance and genetic constitution, and climate and seasonal temperature variation. Overall, the incidence was 5.7% in Iran in 2005, 8.8% in North America between 2007 and 2010, 5% to 10% in Europe in the decade before to 2011, and 7.54% in mainland China between 1990 and 2016⁴.

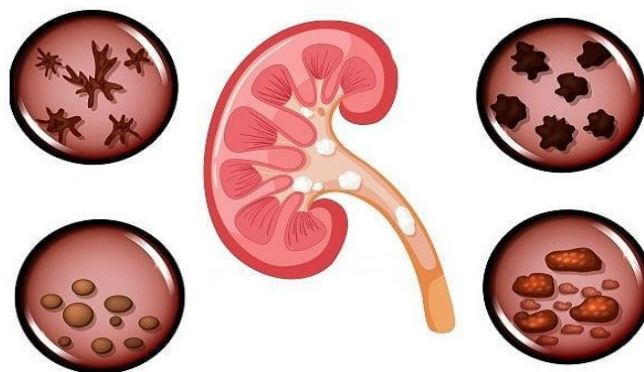
Indian Scenario of Urolithiasis

Many people in India, a poor socioeconomic nation, labor on building sites in hot weather where there is a considerable risk of dehydration in an effort to make ends meet. Additionally, many don't pay enough attention to how much water they drink each day. Since calcium and oxalate-containing foods are common in Indian diets, calcium oxalate stones are more common there than cystine stones, which are rarely reported. Because purine, an amino acid that is a precursor to uric acid, can significantly increase

the acid load in the kidney, a high protein diet increases the risk of uric acid kidney stones.

Type of Kidney Stones

- i. Calcium stone
- ii. Struvite stone
- iii. Uric acid stone
- iv. Cystine stone

**Figure No.2: Types of kidney stone**

A plethora of novel therapeutic strategies, notably urolithiasis therapy, are currently being spoke of into traditional medicine. As per the World Health Organization, the utilization of traditional medicine in conjunction with modern medical remedies can yield multiple benefits for a variety of medical illnesses.(23) These advantages include expanded treatment options, hospice care for the management of modern medical treatment adverse effects, enhanced psychological and emotional wellness and heightened patient satisfaction. A particularly significant herbal remedy that is employed in indigenous medicine for its various benefits such as antiurolithiatic, anti-venomous, wound-healing and anti-malarial properties is *Acalypha indica* Linn., which is a member of the Euphorbiaceae family.

**Figure No.3: Acalypha indica leaves**

The plant is used for various diseases in Ayurveda, Siddha and Unani. It is found that *Acalypha indica* L. shows various pharmacological activities such as Anti-inflammatory activity, antimicrobial activity,

Anti-ulcer Activity, Analgesic Activity, Antiarthritic Activity, HIV-1 reverse transcriptase inhibitory activity, Antiuro lithiatic Activity, Kidney protective effect, Cytotoxic Activity, Anthelmintic Activity, Anticonvulsant activity, Antianxiety activity, Anti-malarial activity, Lipid lowering activity and Anti-venom Activity.

Material & Method

Drugs and chemicals

Ammonia solution, Benzene Solution, Ethylene glycol, Cystone tablet, 3, 5- dinitro benzoic acid and Chloroform.

Collection, Identification and Authentication of plant material

The fresh leaves of *Acalypha indica* linn were collected from the field of Varanasi district in the month of their natural habitat. All leaves were cleaned of extraneous matter and ground into a fine powder. Plant and plant material was identified by comparing morphological features of plant. The herbarium of collected plant material was prepared & the taxonomical identification (authentication) of the plant is done by Botanical Survey of India, Pune, Maharashtra, India.

The collected leaves of *Acalypha indica* L were dried in shade to avoid loss of phytoconstituents. After drying, leaves were coarsely powdered with grinding mill and kept in air tight container for use in the study.

Based upon the extensive literature survey, we decide to use the alcoholic solvent such as methanol for extraction of phytoconstituent from powder of leaves of *Acalypha indica* for further study.

Extraction of plant materials:

Continuous hot extraction using soxhlet apparatus. The procedure is to grind coarsely ground plant material in a paper thimble and place it in a Soxhlet apparatus. The solvent is put into the thimble and collected into the Round Bottom Flask (RBF). Extractions are carried out at elevated temperatures until the bead mark of the siphon tube is attained. The solvent vapors condense and raise the level of the solvent until all coloring material is removed. The solutions obtained are combined and the plant material is extracted using methanol. The dried extracts are weighed and placed in screw-capped vials and labelled for quantification.

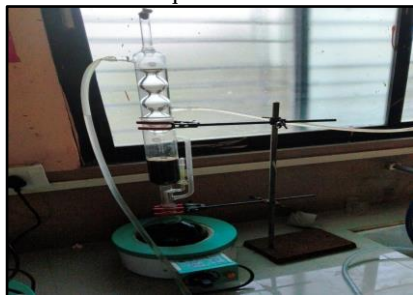


Figure 4: Soxhlet extraction of *Acalypha indica* leaves

Pharmacognostic study

Physicochemical parameters

The examination of organoleptic qualities, such as color, Shape, Odor, Taste and aroma, was conducted on the crude extract and its fractions. Water, ethyl alcohol, chloroform, anhydrous ether, petroleum ether, and methanol were used to test solubility.

Preliminary Phytochemical screening

The *Acalypha indica* leaves extract was screened for the possible presence of secondary metabolites including alkaloids, tannins, flavonoids, terpenoids, and Saponin, phenols using qualitative phytochemical screening procedures are given in K. R. Khandelwal book.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) which utilizes silica gel and alumina as a stationary phase and a mobile phase, is a widely used method for the identification of phytoconstituents. Preparative commercial TLC plates are prepared using standard particle size ranges, which are formed by mixing water, an adsorbent such as silica gel, and an inert binder such as calcium sulfate.

Pharmacological Study

Experimental Animals

The study used 20–30 g Swiss Albino mice that were received from animal house of SHEAT College of pharmacy, Varanasi. The mice were kept in rooms with standard room temperature ($23 \pm 1^\circ\text{C}$) and relative humidity of 67%. They also received unrestricted access to water and standard rodent food pellets. One day before to the experiment and every day for at least one hour before the experiment, they were acclimated to the laboratory conditions. Every experiment was carried out between 09.00 and 17.00 hours. All research was carried out in compliance with the guideline set forth by government of India committee for the purpose of control and supervision on experiments on animal (CCSEA), and prior approvals were obtained from the IAEC committee of SHEAT College of Pharmacy, Varanasi.

Acute oral toxicity of *Acalypha indica* leaves extract

Acute toxicity studies for methanolic extract of leaves was conducted according to OECD guideline 420 using wistar albino rats. The extracts were dissolved in Tween 80 before administration into the animals. Female Adult Wistar albino rats weighing around 180–200 g were used for the testing acute oral toxicity. The animals were divided into 5 groups; each group containing 6 animals. The initial dose was chosen at 2000 mg/kg with the consideration that there were in vivo and in vitro toxicity data. One animal in each group is from the sighting study dosed at 500, 1000, 1500 and 2000 mg/Kg. Animals (e.g. with the rat, food but not water should be withheld) were fasted overnight prior to administration. Group I was considered as control,

whereas group II to V acted as test groups. After administration of the extracts, food was withheld for a further 3 to 4 hr. The animals were then individually observed (with special attention during the first 4 hr) for possible behavioural changes, allergic reactions (skin rash, itching), eyes and mucous membrane, or any alterations unceasingly and mortality for next 24 hr and the observation was continued for 14 days.

Experimental Protocol and Procedure

0.75% Ethylene glycol induced urolithiasis

model was used to assess the antiurolithiatic activity in mice. Mice were randomly divided into 5 main groups (n=6).

Group I consisted of normal mice receiving only the vehicle as a supplement. Group II consisted of mice

with urolithiasis treated with ethylene glycol (0.75% v/v) in drinking water ad libitum for 21 days. Group III received ethylene glycol (0.75% v/v) in drinking water ad libitum for 21 days, followed by treatment with a standard drug (cystone tablet, 40 mg/kg I.P.) from days 11 to 21, which is a remedy for kidney stones. Group IV consisted of mice treated with ethylene glycol (0.75% v/v) in drinking water ad libitum for 21 days, followed by treatment with a low dose of the test drug (20 mg/kg) from days 11 to 21. Group V received ethylene glycol (0.75% v/v) in drinking water ad libitum for 21 days, followed by treatment with a high dose of the test drug (40 mg/kg) from days 11 to 21. Food was prepared and replaced daily because additives can break down and spoil the food. Water and food consumption as well as mice weights were monitored regularly.



Figure No. 5: Intraperitoneal Route Dosing (i.p.)



Figure No. 6: Induction of urolithiasis with 0.75% Ethylene Glycol

Urine collection

Place the mice in a metabolic cage, which is designed to separate urine from feces and usually includes a funnel system that directs urine into a beaker contain ice. Ensure that the metabolic cage has a non-absorbent bedding material to prevent absorption of urine and contamination. Leave the mouse in the cage for 8 hours. Collect the urine at the end of the period. Analyse the urine volume and pH using urine strip and also analyse electronic microscope for stone by using prepared urine slides.

Urine microscopy

Fresh 3 hours morning samples of urine were used for the microscopy. 1 ml of fresh urine sample from each group was collected in centrifuge tube and centrifuged at 2500 rpm for 5 minutes. The supernatant was discarded and the 0.1 ml of sediment was taken on the glassslide, covered with cover slip and observed using Digital Microscope (Labomed LX 300 Binocular microscope).

Blood collection

Insert the needle at a 30-45 degree angle behind the eye into the retro-orbital sinus of mice. Gently aspirate 50-200 μ L of blood. Apply pressure with sterile gauze after withdrawal to prevent bleeding.



Figure No.7: Blood collection with retro orbital puncture

Biochemical estimations

The urine and serum samples were analysed for biochemical parameters like urine pH, Urine output, Blood Urea Nitrogen (BUN), Creatinine by using commercially available kits listed earlier.

Histopathological studies

Euthanize the mouse, dissect the kidneys, and fix them in 10% neutral-buffered formalin for 24 hours at room temperature. Dehydrate the kidneys through a series of alcohol solutions (70%, 80%, 95%, 100% ethanol), clear the tissue in xylene, and then embed in paraffin wax using a tissue embedding station. Cut the paraffin-embedded tissue into 3-5 μ m

sections with a microtome and mount on glass slides. Deparaffinize the tissue sections in xylene, rehydrate in graded ethanol, and stain with Hematoxylin for the nuclei and Eosin for the cytoplasm. Rehydrate, clear, and mount the slides with a coverslip. Finally, examine the stained slides under a microscope to analyze the kidney structure for pathological changes.

Statistical analysis

The results were shown as mean $\pm$ SEM. The inter-group variation was examined using the Student's t-test between two groups and one-way analysis of variance (ANOVA), followed by Dunnett's test for more than two treatments.

RESULTS:**Pharmacognostic Evaluation****Table 1: Pharmacognostic evaluation of *Acalypha indica* leaves**

Sr. No.	Parameters	Results
Physicochemical parameter		
1	Colour	Dark green, light green
2	Shape	long and slender
3	Texture	Leathery and shiny
4	Odour	Not particularly strong or distinctive.
5	Taste	Slightly bitter taste
Ash value		
1	Total Ash	9 %
Moisture content		
1	Moisture content	1.680%
Extractive values		
1	Alcohol soluble extractive value	23 gm w/w
2	Water soluble extractive value	18 gm w/w

The percentage of Moisture content for *Acalypha indica* leaves was found to be 1.680%. The percentage of alcohol soluble extractive and water soluble extractive were found to be 23 gm and 18 gm. The *Acalypha indica* leaves extract are dissolve in Ethyl alcohol, Ethyl acetate, Chloroform, Petroleum ether and Methanol.

Preliminary Phytochemical screening of extract

The Phytochemical analysis of *Acalypha indica* leaves showed the presence the presence of various secondary metabolites, including carbohydrates, alkaloids, flavonoids, saponins and terpenoids. These compounds play important roles in the biological activities and therapeutic potential of plant extracts.

Table 2: Preliminary Phytochemical test of extract

Sr. No.	Phytochemicals	Methanolic leaf extract
1	Carbohydrates	-
2	Proteins	+
3	Alkaloids	+
4	Saponin	+
5	Tannin	-
6	Flavonoids	+
7	Glycosides	+
8	Steroids	+
9	Terpenoids	-

– indicates absence of the constituents, + indicates the presence

Thin layer chromatography

The R_f value of extracts was evaluated using the following formula,

$R_f \text{ value} =$

$$\frac{\text{Distance travelled by the com(cm)}}{\text{Distance travelled by the solvent front(cm)}}$$

The thin layer chromatography studies of the methanolic extract of *Acalypha indica* leaves were performed by using

Mobile phase : n-hexane: Ethyl acetate (7:3)

Stationary phase : silica gel D60 F254 Aluminium sheets

In *Acalypha indica* leaves extract, one spot are UV active with R_F value of 0.50cm were found in the UV cabinet at 254nm.

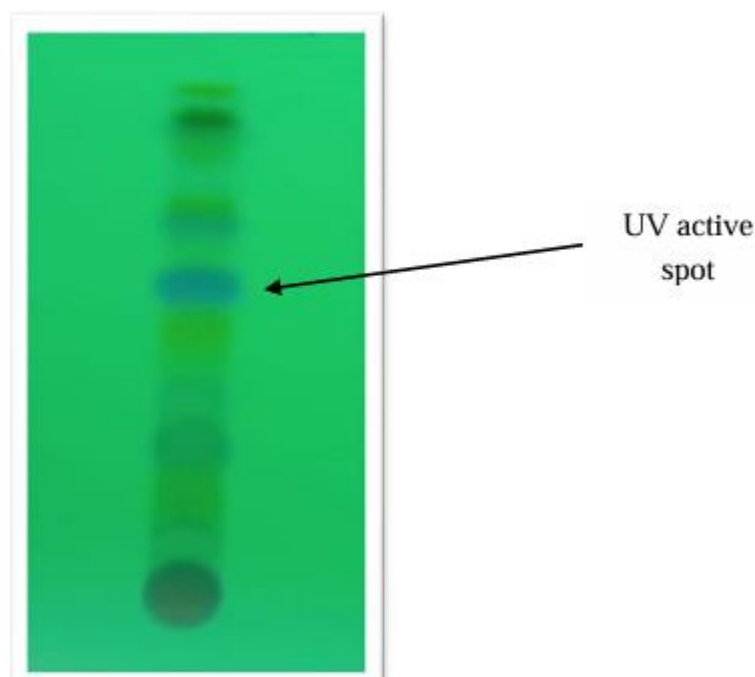


Figure No.8: Thin layer chromatography of isolated fraction from *Acalypha indica* leaves extract in UV cabinet at 254 nm

Acute Oral Toxicity of *Acalypha indica* leaves extract

The initial investigation involved assessing the potential toxicity of methanolic leaf extracts of *Acalypha indica*. A single rat was administered a relatively high dosage of 2000 mg/kg of extract and the results revealed no signs of toxicity. This indicates that the tested extracts did not cause adverse effects at the given concentration within the observed timeframe. Specifically, there were no reported deaths and no notable behavioural abnormalities were observed in any of the groups studied. The absence of mortality and abnormal behaviour is a crucial indicator of the safety of the extracts at the tested dosage. This suggests that the extracts, derived from the leaves and regardless of the solvent used methanol did not induce acute toxic effects under the conditions of the experiment.

Biochemical estimation

Urine analysis

Estimation of Urine pH

As represented in Table 7.7 & Figure 7.7, Urine pH of animals in all groups were performed at 0th day, 7th day, 14th day and, 21th day of the study. Urine pH of animals was significantly ($p < 0.05$) increases in Low, High Dose and Standard treatment groups but the Disease Control Group in that Urine pH of animal was decreased.

Table No. 3: Effect of *Acalypha indica* extract on urine pH levels in 0.75% ethylene glycoside-induced urolithiasis in mice

Days	0 th p ^H	7 th p ^H	14 th p ^H	21 th p ^H
Groups				
Normal	6±1.16	5.9±0.01	5.9±0.03	6.2±0.13
Disease control	6.5±0.13#	4.5±0.13#	5.1±0.13#	5±0.163#
Standard	6.2±0.13	5.3±0.12	6±0.16*	6.3±0.12
Low Dose	6.6±0.13	4.1±0.08	6.1±0.12***	6.5±0.15***
High Dose	6±0.15	5.5±0.13	5.9±0.15***	6.2±0.11***

Each Value represents Mean ± SEM, n = 6, # $p < 0.001$ as compared to vehicle treated groups, *** $p < 0.001$ as compared with Disease control (0.75% EG)

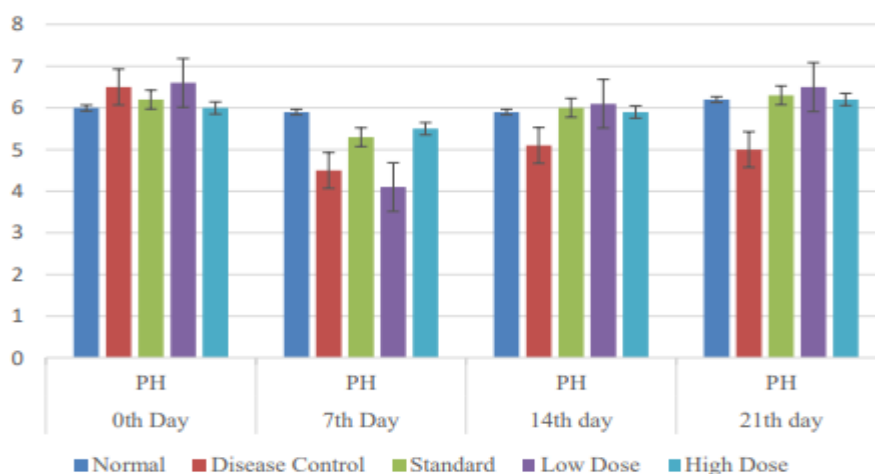


Figure 9: pH of Urine in Normal and Urolithiatic mice

Estimation of Urine Volume

As represented in Table 7.8 & Figure 7.8, Urine volume of animals in all groups were performed at 0th day, 7th day, 14th day and, 21th day of the study. Urine volume of animals was significantly ($p < 0.05$) increases in Low, High Dose and Standard treatment groups but the Disease Control Group in that Urine volume of animal was decreased.

Table No. 4: Effect of *Acalypha indica* extract on urine volume in 0.75% ethylene glycol-induced urolithiasis in mice

Days	0 th	7 th	14 th	21 th
Groups	Urine Volume (ml)	Urine Volume (ml)	Urine Volume (ml)	Urine Volume (ml)
Normal	1.6±0.0	1.2±0.0	1.4±0.0	1.6±0.0
Disease control	1.7±0.0	0.9±0.1#	1±0.1	0.±0.1
Standard	1.8±0.0	0.8±0.01	1.4±0.1*	1.5±0.0
Low Dose	1.9±0.6	1±0.1	1.5±0.1***	1.6±0.1***
High Dose	1.7±0.3	1.1±0.1	1.4±0.0***	1.6±0.2***

Each Value represents Mean $\pm$ SEM, n = 6, # $p < 0.001$ as compared to vehicle treated groups, 1.4±0.0*** *** $p < 0.001$ as compared with Disease control (0.75% EG)

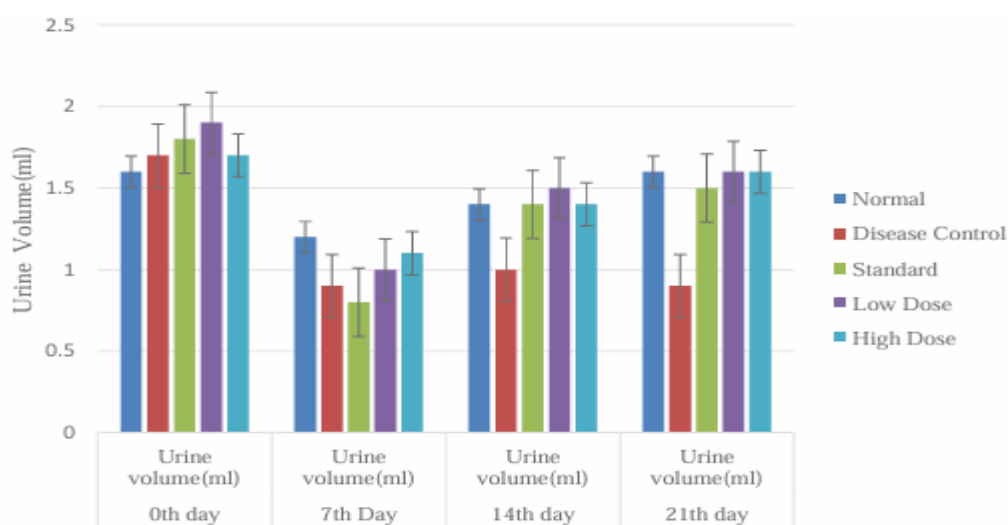


Figure No. 10: Urine volume in Normal and Urolithiatic mice

Estimation of Blood Urea Nitrogen (BUN) in Urine

As compared to Normal group there is a significantly increase in the level of Blood Urea Nitrogen in Group B-Disease control as compared to Group B there is more significant decrease in the level of Blood Urea Nitrogen in group C-Standard, as compared to Group B there is significantly decrease in the level of Blood Urea Nitrogen in Group D-Low dose and Group E-High dose respectively, 20mg/kg i. p. and 40mg/kg i.p. So, we can conclude that $P < 0.001$.

Table No.5: Effect of *Acalypha indica* extract on urine BUN levels in 0.75% ethylene glycol-induced urolithiasis in mice

Groups	Urine BUN Level
Normal	20.23±0.115
Disease control	40.80±0.995#
Standard	10.95±0.190***
Low Dose	6.780±0.479***
High Dose	7.890±0.490***

Each Value represents Mean $\pm$ SEM, n = 6, # p< 0.001 as compared to vehicle treated groups, ***p< 0.001 as compared with Disease control (0.75% EG)

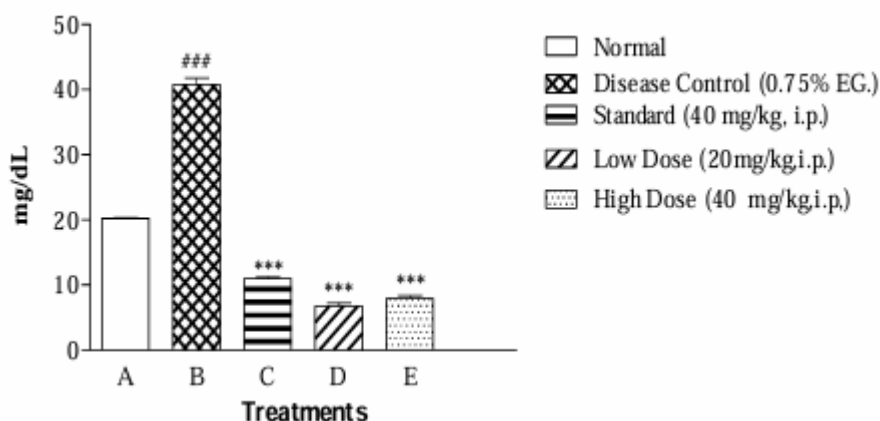


Figure No.11: BUN level of Urine in Normal and Urolithiatic mice

Estimation of Creatinine in Urine

As compared Normal Group there is a significantly increase in the level of creatinine in Group B-Disease control as compared to Group B there is more significant decrease in the level of creatinine in Group C-Standard, as compared to Group B there is significantly decrease in the level of creatinine in Group D-Low dose & Group E-High dose respectively, 20mg/kg i. p. & 40mg/kg i.p. So, we can conclude that $P < 0.001$.

Table No.6: Effect of *Acalypha indica* extract on urine Creatinine levels in 0.75% ethylene glycol-induced urolithiasis in mice.

Groups	Urine Creatinine Level
Normal	2.315±0.485
Disease control	18.29±1.960#
Standard	3.475±0.815***
Low Dose	7.055±0.388***
High Dose	5.295±0.380***

Each Value represents Mean $\pm$ SEM, n = 6, # p< 0.001 as compared to vehicle treated groups, ***p< 0.001 as compared with Disease control (0.75% EG)

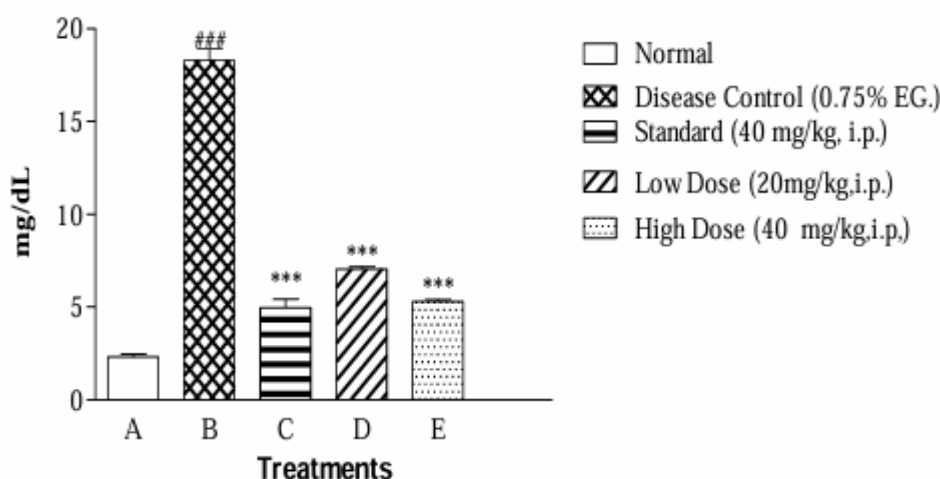


Figure No.12: Creatinine level of Urine in Normal and Urolithiatic mice

Blood plasma Analysis

Estimation of Blood Urea Nitrogen (BUN) in plasma

As compared to Normal Group there is a significantly increase in the level of Plasma BUN in Group B-Disease control as compared to Group B there is more significant decrease in the level of Plasma BUN in Group C-Standard, as compared to Group B there is significantly decrease in the level of Plasma BUN in Group D-Low dose and Group E- High dose respectively, 20mg/kg i. p. and 40mg/kg i.p. So, we can conclude that ($P < 0.001$), Hence therefor this result is significant. ($P < 0.001$) followed by one way ANOVA (Dunnett's Multiple Comparison test)

Table No.7: Effect of *Acalypha indica* extract on Plasma BUN levels in 0.75% ethylene glycol-induced urolithiasis in mice

Groups	Plasma BUN Level
Normal	23.315 $\pm$ 2.120
Disease control	29.605 $\pm$ 3.465#
Standard	15.975 $\pm$ 1.835***
Low Dose	18.615 $\pm$ 1.425***
High Dose	17.790 $\pm$ 0.129***

Each Value represents Mean $\pm$ SEM, n = 6, # $p < 0.001$ as compared to vehicle treated groups, *** $p < 0.001$ as compared with Disease control (0.75% EG)

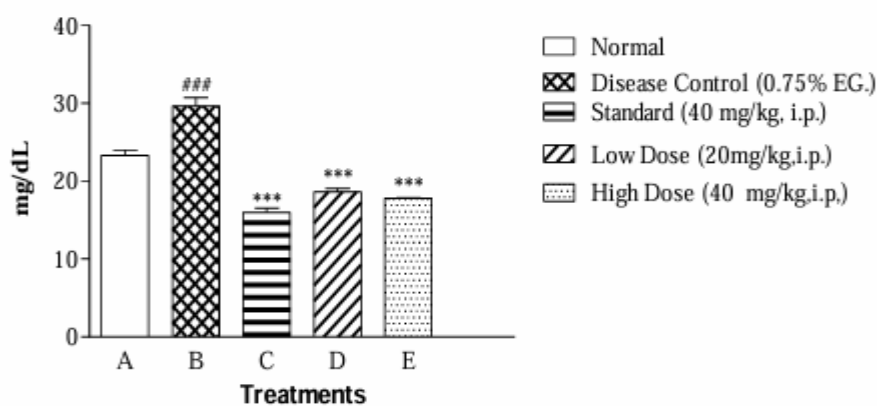


Figure No.13: BUN level of plasma in Normal and Urolithiatic mice

Estimation of Creatinine in Plasma

As compared to Normal Group there is a significantly increase in the level of Plasma creatinine in Group B- Disease control as compared to Group B there is more significant decrease in the level of Plasma creatinine in Group C-Standard, as compared to group B there is significantly decrease in the level of Plasma creatinine in Group D-Low dose and Group E- High dose respectively, 20mg/kg i. p. and 40mg/kg i.p. So, we can conclude that ($P < 0.001$), Hence therefor this result is significant. ($P < 0.001$) followed by one way ANOVA (Dunnett's Multiple Comparison test)

Table No.8: Effect of *Acalypha indica* extract on Plasma Creatinine levels in 0.75% ethylene glycol-induced urolithiasis in mice

Groups	Plasma Creatinine Level
Normal	11.815 $\pm$ 0.040
Disease control	24.105 $\pm$ 0.514#
Standard	13.975 $\pm$ 0.063***
Low Dose	13.850 $\pm$ 0.225***
High Dose	14.630 $\pm$ 0.098***

Each Value represents Mean $\pm$ SEM, n = 6, # $p < 0.001$ as compared to vehicle treated groups, *** $p < 0.001$ as compared with Disease control (0.75% EG)

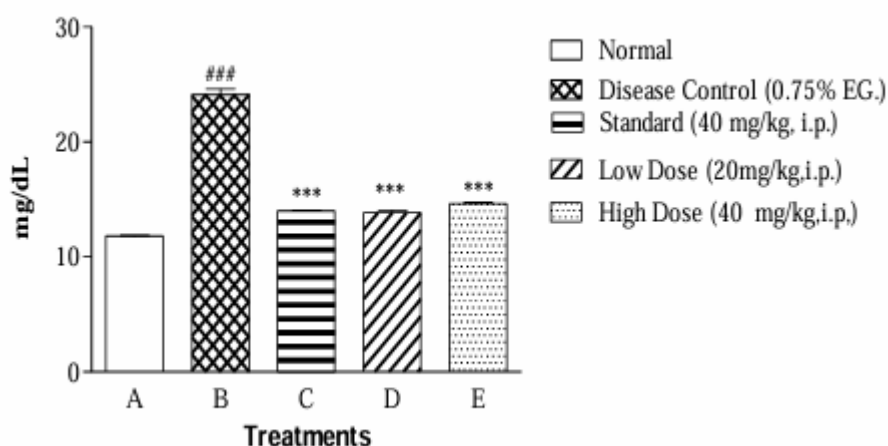
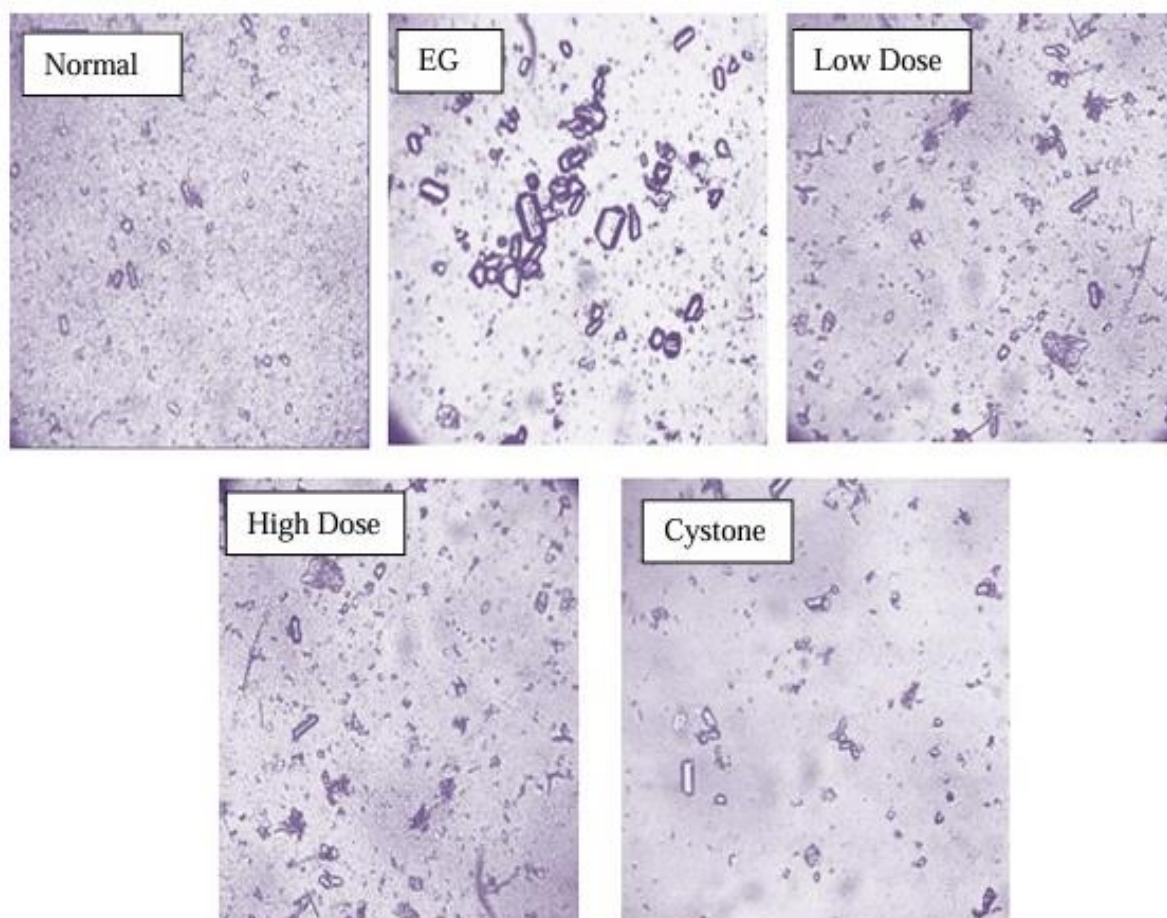


Figure No. 14: Creatinine level of plasma in Normal and Urolithiatic mice

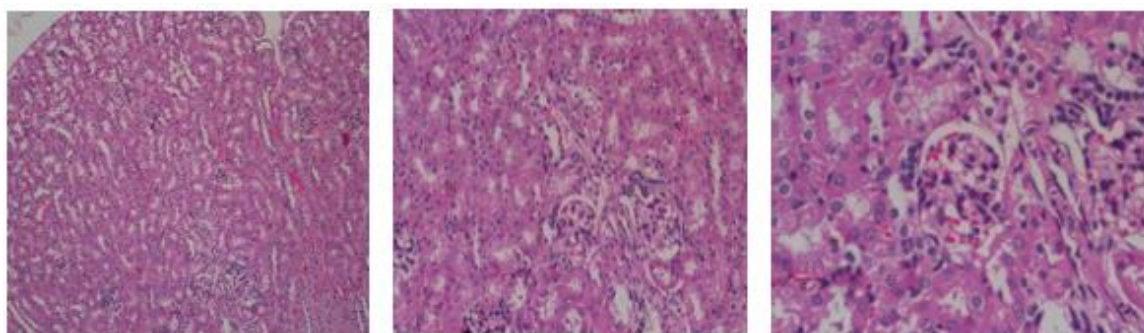
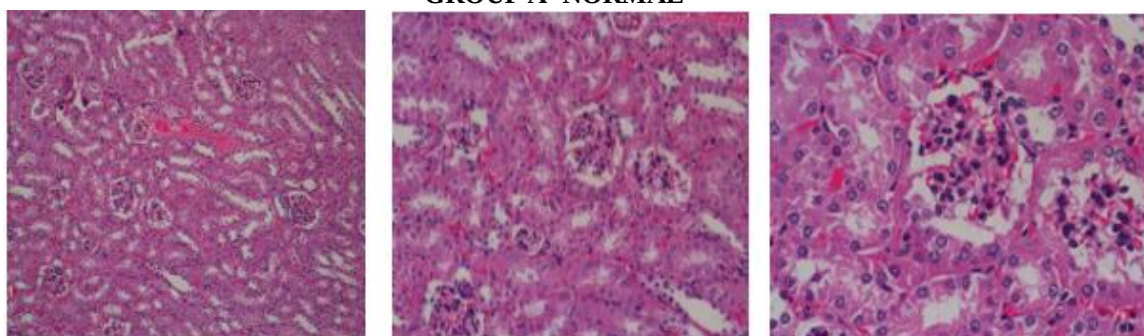
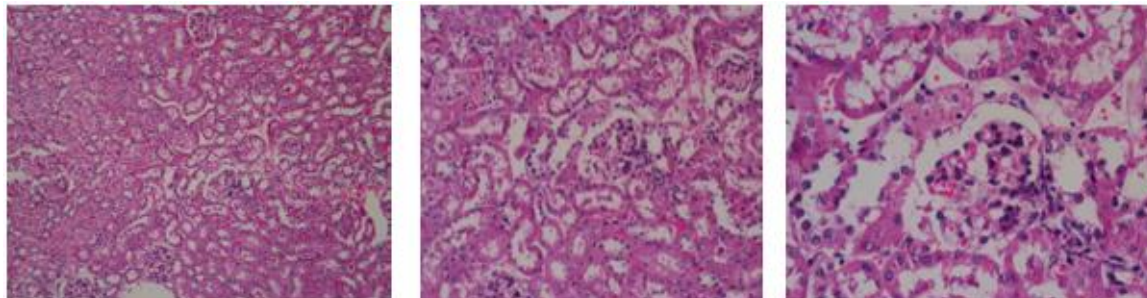
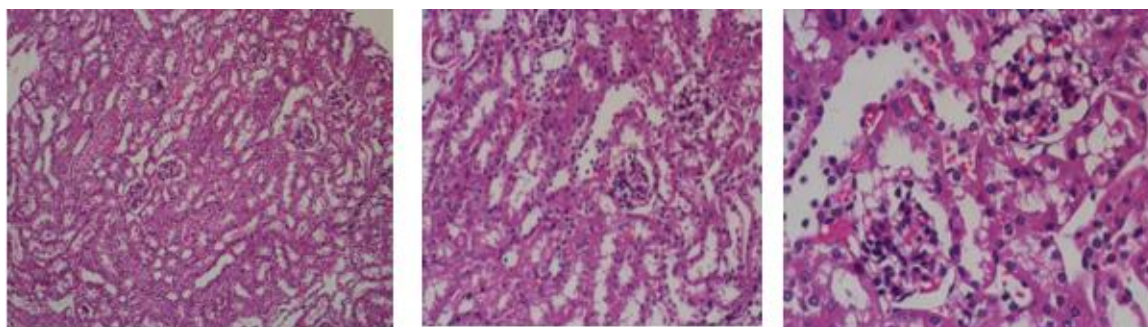
Urine Microscopy:

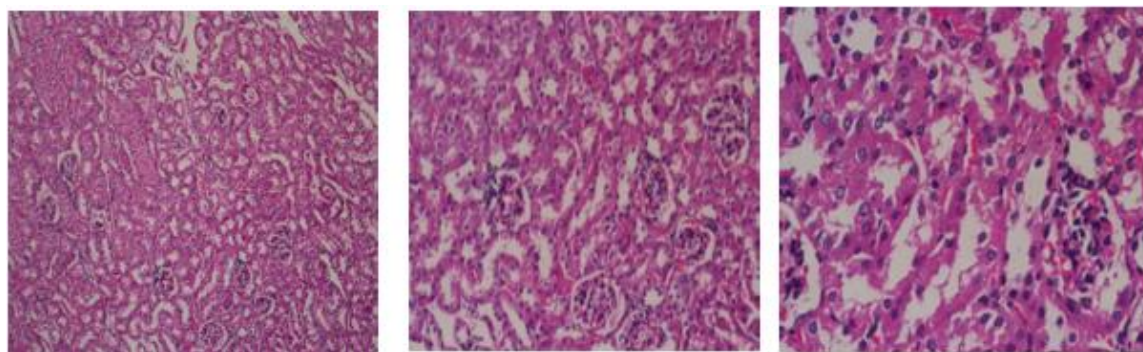
Mice urine microscopic analysis revealed presence of more number of calcium oxalate crystals (calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) crystals) (Fig. 6.8 b) in the only ethylene glycol treated group, also the crystals observed were comparatively larger in size. Whereas, in case of all the Urolithiatic mice treated with *Acalypha indica* extract, Cystone crystalluria composed mostly of COD particles and only with few COM particles (Fig. 6. 8c, d and e). Interestingly, the number and size of both the type of crystals was smaller as compared to the mice in the only ethylene glycol treated group.

Figure No.15: Urine microscopy

**Histopathological Examination:**

The Histopathological examinations of kidneys excised from the mice of different groups in ethylene glycol induced urolithiasis model revealed following alteration: 1) The Normal group had normal histology of kidney- outer cortex and inner medulla- showing normal glomeruli and tubules with no deposition of crystals. 2) The ethylene glycol treated group showed the greatest amount of CaOx deposition, and this was present in all parts of the kidney. Intratubular and interstitial crystals were observed in the cortex region. There was marked dilation of tubules, tubular damage and infiltration of inflammatory cells into the interstitial space. 3) The Cystone treated group shows the normal cortex and medulla. 4) The 0.75% ethylene glycol + low dose of test extract treated group had less number of crystals as compared to Toxicant group along with dilated vessels and small areas of hemorrhage. 5) The 0.75% ethylene glycol + High dose of test extract treated group had normal histology of kidney, few inflammatory cells in interstitium with small area of hemorrhage.

**GROUP A- NORMAL****GROUP B- DISEASE CONTROL****GROUP C- STANDARD****GROUP D- LOW DOSE**



GROUP E- HIGH DOSE

Figure 16: Histopathological microscopy of mice kidney of different groups in antiurolithiatic activity (H&E, 100x, 200x & 400x)

DISCUSSION:

The purpose of the current investigation was to determine if the antiurolithiatic action of bioactive molecule that was extracted from the leaves of *Acalypha indica* could effectively prevent renal stones in mice.

The most prevalent component of kidney stones, calcium oxalate (CaOx), is one of the urinary salts that supersaturate urine, leading to urinary stone disease. (128) Up to 80% of stones in the urinary tract are calculi, which are caused by common urine components like CaOx. Numerous *in vivo* models have been created to determine the effects of different therapeutic drugs on the onset and progression of the disease as well as to comprehend the mechanisms underlying the creation of urinary stones. (129) Because male mice urinary systems are similar to those of humans and because previous research has demonstrated that female mice had far less stone deposition, male mice were chosen for this investigation in order to develop urolithiasis. (130) Mice are the most commonly utilized animals in urolithiasis research because they resemble the pathophysiology of stone production in people and exhibit CaOx accumulation in the kidneys.

In the current investigation, the excretion of calcium and oxalate has gradually risen in animals with calculi. Changes in urine oxalate levels are comparatively more significant than those in calcium since it is widely acknowledged that hyperoxaluria is a significantly more significant risk factor in the etiology of renal stones than hypercalciuria. One of the factors that promotes the nucleation, precipitation, and subsequent crystal development of calcium oxalate or apatite (calcium phosphate) from urine is elevated urinary calcium.

Treatment with 0.75%EG produces metabolic acidosis in male mice. This acidification of urine along with decreased urine volume favors the formation of urinary stones. Extract of *acalypha indica* increases the pH of urine and this may, at least partially, hinder the process of crystal formation.

After administering 0.75% ethylene glycol (EG) in the drinking water, all mice consistently developed crystalluria, marked by the presence of both calcium oxalate monohydrate (COM) and dihydrate (COD) crystals. COM crystals typically appeared as distinctive biconcave ovals, whereas COD crystals displayed dipyramidal shapes. Notably, untreated nephrolithiatic mice exhibited a greater abundance of large COM crystals in their urine. Urinary microscopy confirmed that EG treatment significantly increased both the number and size of calcium oxalate crystals.

In contrast, mice treated with test extract showed a clear reduction in both the number and size of calcium oxalate crystals. Nevertheless, when compared head-to-head with a group receiving Cystone at 40 mg/kg, the Cystone-treated mice demonstrated a more pronounced decrease in crystalluria.

CONCLUSION:

Acalypha indica demonstrates remarkable efficacy in ameliorating urolithiasis and protecting renal function in ethylene glycol (EG)-induced models. In mice treated with EG, it significantly diminishes both the number and size of urinary crystals, a key indicator of its antiurolithiatic potential. This reduction is paralleled by an improvement in serum biomarkers: creatinine and urea levels decline, corroborating its nephroprotective effects as further confirmed by histopathological examinations. These studies reveal marked preservation of renal tissue

architecture compared to untreated, EG-exposed controls. Moreover, extract of *Acalypha indica* exhibits diuretic properties, contributing to its multifaceted role in urolithiasis management. By promoting urinary output, it aids in flushing out calcium and other lithogenic substances, reducing stone formation and facilitating their elimination. Notably, EG induced calcium excretion is curtailed in emodin-treated subjects, further underscoring its dual diuretic and antiurolithiatic activities. In head-to-head assessments with Cystone—a popular herbal formulation used in urolithiasis. Taken together, the cumulative evidence suggests that methanolic extract of *Acalypha indica* combats EG-induced urolithiasis through a comprehensive therapeutic profile: it is antiurolithiatic, diuretic, nephroprotective, and antioxidant. These synergistic actions not only prevent stone formation and enhance excretion but also safeguard against renal injury and oxidative damage. Given these promising outcomes, further exploration via rigorous preclinical and clinical trials is warranted to fully evaluate its safety, mechanism of action, and clinical efficacy, particularly in comparison to conventional and herbal treatment options for urolithiasis.

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