

## Effect of Leaves of *Dendrophthoe Elastica* on Kidney Stone and Nephron Protective Activity by Rat

Ankita Mishra\*, Shreya Singh, Radhika Patel, Rajeev Shukla

*Saraswati Higher Education and Technical College of Pharmacy, Varanasi, UP, India.*

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### ABSTRACT

WHO reported that nearly 75% global population, most in the developing world depends on the botanical medicines for their basic health care needs with around 800 plants being used in indigenous systems of medicines. Human body, for its normal functioning deals with hundreds of thousands of independent deals which involves interactions of a large no of proteins and cells working with environmental factors. Urolithiasis (urinary calculi or stone), one of the most common urinary tract disease with worldwide prevalence and incidence refers to the calcification that form in the urinary system, primarily in the kidney referred to as nephrolithiasis or ureter as ureterolithiasis and may also form in or migrate into the lower urinary system either in the bladder or urethra. Urolithiasis is a worldwide problem

A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome because there is potent therapeutic medicine like NSAID's, aminoglycoside antibiotics, chemotherapeutic agents have been added to the therapeutic arsenal in recent years.

**Keywords:** Urolithiasis, Nephrotoxicity, urethra, nephritic, renal failure,

### I. INTRODUCTION KIDNEY

The kidneys are reddish, kidney-bean-shaped located just above the waist between the peritoneum and the posterior wall of the abdomen. The kidneys are located between the levels of the last thoracic and third lumbar vertebrae.

### Anatomy of the kidneys

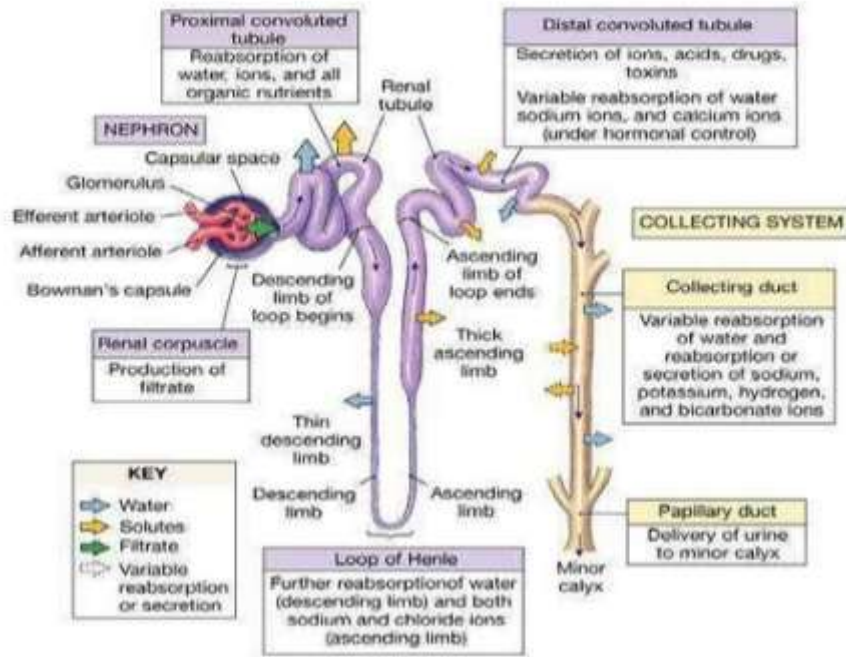
An adult kidney is 10-12cm long, 5-7 cm wide and 3 cm thick. The concave medial border of each kidney faces the vertebral column. Three layer of tissue surround each kidney. Deep layer of renal capsule is a smooth, transparent sheet of dense irregular connective tissue. The middle layer is adipose capsule is a mass of fatty tissue surrounding the renal capsule. Superficial layer is renal fascia is another layer of dense irregular connective tissue.

### Functions of the kidneys

#### • Formation of urine.

The production of urine is vital to the health of the body. Most of us have probably never thought of urine as valuable, but we could not survive if we did not produce it and eliminate it. To produce urine, nephrons and collecting ducts perform three basic processes.

- Glomerular filtration
- Tubular reabsorption
- Tubular secretion



### CLASSIFICATION OF STONES

Table 1: Common types of stones

Name of stone	Constituents	Incidence
Calcium oxalate	Calcium, oxalate	34%
Calcium phosphate	Calcium, phosphate	33%
Uric acid	Uric acid	8%
Struvite	Ammonium, magnesium, phosphate	15%
Cystine	Cystine	3%
Medication induced stones	Depends on the medication (eg; Indinavir, ephedrine, Guaifensin, silica etc)	1%

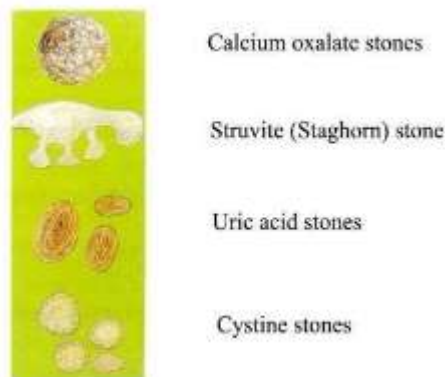


Figure 4: Different types of Stones

- **Calcium oxalate stones**
- Most common type and represent about 80%
- Contains calcium oxalate alone or in combination with calcium phosphate in the form of apatite or brushite
- They appear as 'envelopes' microscopically. They may also form 'dumbbells'
- Hydrated forms of the compound occur naturally as three mineral species: whewellite (monohydrate, known from some coal beds), weddellite (dihydrate) and a very rare trihydrate called caoxite.<sup>27</sup>
- **Struvite stones (Ammonium magnesium phosphate,  $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ )**
- Constitute about 10-15% of urinary calculi.
- Also known as infection stones
- They form most often in the presence of infection by urea-splitting bacteria.
- **Uric acid stones**
- About 5–10% of all stones are formed from uric acid.
- They appear as pleomorphic crystals, usually diamond-shaped. They may also look like squares or rods which are polarizable.<sup>28</sup>
- **Cystine stones**
- Constitutes about 3% of the urinary calculi.
- Mainly caused due to cystinuria, an inherited (genetic) disorder of the transport of an amino acid (a building block of protein) called cystine that results in an excess of cystine in the urine (cystinuria) and the formation of cystine stones.
- Although cystine is not the only overly excreted amino acid in cystinuria, it is the least soluble of all naturally occurring amino acids.
- **Silicate stones or drug induced stones**
- Very difficult to observed.

#### ETIOLOGY OF STONE FORMATION

- Stone formation is usually multi-factorial with more than one element increasing a patient's risk for stone formation and is strongly related to dietary lifestyle habits or practices.
- A higher rate of renal stone disease is in males Comparison than females. Men are 4 times more affected than women. The presence or absence of certain trace elements in water has been implicated in the formation of urinary calculi. For example, zinc is an inhibitor of calcium crystallization.

#### Major causes of calcium stone formation

- **Hypercalciuria:-** Urinary calcium excretion  $>200$  mg/dl
- **Absorptive hypercalciuria:**  $\uparrow$ GI calcium absorption renal.

- **Hypercalciuria:** impaired renal Calcium absorption
- **Resorptive hypercalciuria:** Primary hyperparathyroidism.
- **Hyperoxaluria:-** Urinary oxalate excretion 40mg/dl
- **Primary hyperoxaluria:** genetic Oxalate overproduction dietary
- **Hyperoxaluria:** excessive dietary intake.
- **Enteric hyperoxaluria:**  $\uparrow$ GI oxalate absorption
- **Hypercitraturia:-** Urinary citrate excretion 320 mg/dl.
- **Distal renal tubular acidosis:** impaired renal tubular acid Excretion.
- **Hyperuricosuria:-** Urinary acid excretion  $>600$  mg/dl.

#### PATHOPHYSIOLOGY OF UROLITHIASIS

A number of crystalloids of different types (oxalate, uric acid, calcium, cystine etc) are present in the urine which is kept in the solution by means of colloids like mucin and  $\text{H}_2\text{SO}_4$  by the process of absorption.

Urolithiasis/Nephrolithiasis results in increased oxalate (hyperoxaluria), calcium, phosphate, uric acid and cysteine in the urine. Hyperoxaluria being a major risk in urolithiasis leads to the activation of renin angiotensin which in turn increases the angiotensin II levels. Angiotensin II causes the activation of NADPH oxidase and as a result reactive oxygen species are generated which causes lipid peroxidation in the renal cell membrane.

#### SYMPTOMS OF KIDNEY STONE

- Severe loin pain that comes and goes in the back or side between the pelvis and lower rib.
- Nausea and vomiting
- Difficulty in passing urine
- Sweating, fever and shivers.

#### TREATMENT OF KIDNEY STONES

Many allopathic agents like Thiazide diuretics (e.g. Hydrochlorothiazide), Alkali (e.g. Potassium citrate), Allopurinol, Sodium cellulose phosphate (SCP), Penicillamine (Cuprimine), Analgesic (Diclophenac sodium), Bisphosphonates, Potassium phosphate, Oxalobacter Formigenes and other probiotics are used in treating the stones formed. The ayurvedic medicines used in the treatment are Cystone, Calcuri, Chandraprabhabati, Trinapanchamool, Rencare Capsule, Patherina tablet, Ber Patthar Bhasma, Chander Prabhavati.

### Surgical Method

Currently there are four methods for stone removal

- Extracorporeal shockwave lithotripsy.
- Percutaneous Nephrolithotomy(PCNL)
- Ureteroscopic stone removal
- Open (incisional) Surgery

### NEPHROTOXICITY

There are various forms of toxicity. It is one of the most common kidney problems and occurs when body is exposed to a drug or toxin.

#### Nephrotoxic agents

- Heavy metals : Mercury, arsenic, lead, bismuth
- Antineoplastic agents : Alkylating agents, Cisplatin, Cyclophosphamide, Antimetabolites, Antitumor antibiotics
- Antimicrobial agents : Tetracycline, Acyclovir, Rifampicin

- Amino glycosides : Gentamicin, Amikacin, Kanamycin, Streptomycin (Tubular cell toxicity)
- Miscellaneous : Non-steroidal anti-inflammatory Ibuprofen, Indomethacin

### AIM & OBJECTIVE

#### AIM

To evaluate the kidney stone and Nephroprotective activity of the methanolic extract of leaves of *Dendrophthoe elastica*

#### OBJECTIVES

- Identification and authentication of *Dendrophthoe elastica*.
- Collection, shade drying and powdering of leaves.
- Preparation of the methanol (80%) extract by Soxhlet extraction.

To evaluate the nephroprotective activity Cisplatin induced nephrotoxicity Statistical evaluation-ANOVA and scientific documentation.



Fig 1. *Dendrophthoe elastica*

### PLANT PROFILE

#### Synonyms

*Dendrophthoe elastica* (Desr.) Danser  
*Helicanthus elastica* (Desr.) Danser  
*Loranthus elasticus* Desr.  
*Loranthus euphorbiae* Wight

#### IBP Taxonomy Hierarchy

Kingdom : Plantae Phylum  
 Class : Tracheophyta  
 Order : Magnoliopsida  
 Family : Santalales  
 Genus : *Dendrophthoe*  
 Species : *elastica*

#### Uses

Traditional use: The leaves of *Dendrophthoe elastica* were used to check abortion.

Pharmacologically reported activities: -

- Antimicrobial activity
- Antioxidant activity

Antihyperglycemic activity

Hepatoprotective activity Diuretic and natriuretic activity

#### Chemical Constituents

The whole plant of *Dendrophthoe elastica* is found to contain sterols, terpenoids, flavones, tannins and glycosides. Alkaloids, Quinone's and Coumarins were absent.

The presence of compounds viz, 1-octadecene, neophytadiene, hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester, stigmasterol,  $\gamma$ -stigmasterol,  $\beta$ -stigmasterol-3-ol, pentacosane and -sitost-4-en-3-one by GC-MS analysis were also reported. The column chromatography of the ethyl acetate extract also showed the presence of friedelin, epifriedelinol,  $\beta$  amyryl,  $\beta$  sitosterol, ethyl.

## II. MATERIALS AND METHOD

### PLANT MATERIALS

#### Collection and identification of the plant materials

The plant *Dendrophthoe elastica* (Loranthaceae) growing on *Mangifera indica* (Anacardiaceae) were collected during the month of September – October from South zone.

#### Preparation of methanolic extract of *Dendrophthoe elastica*

The collected plant materials were washed with water to remove the dirt and other materials and then dried under shade. After confirming the dryness of the plant material, they were grounded with a mechanical grinder to get a coarse powdered plant material and then extracted with methanol using Soxhlet extraction apparatus. The methanol (80%) extract was concentrated using rota vapour under vacuum pressure.

#### Preliminary phytochemical screening

##### • Detection of Carbohydrates

###### • Molisch's test

Test extract was dissolved in 1ml of distilled water and added 2 drops of alcoholic  $\alpha$ -naphthol solution in a test tube and added 2ml of Conc. Sulphuric acid carefully along the sides of the test tube. The formation of violet ring at the junction indicates the presence of carbohydrates.

###### • Benedict's Test

Mixed equal volumes of the Benedict's reagent and the test extract and heated on a water bath. The formation of orange red precipitate indicates the presence of reducing sugars.

###### • Fehling's Test

The extract was hydrolysed with HCl, Neutralized with alkali and heated with Fehling's A and B solutions. The formation of red precipitate indicates the presence of reducing sugars

##### • Detection of proteins

###### • Biuret test

Treated a small amount of the extract with 4% (w/v) NaOH and 1% (w/v)  $\text{CuSO}_4$ . The formation of violet colour indicate the presence of protein.

###### 2. Ninhydrin test

0.5ml of the extract was treated with 3 drops of Ninhydrin reagent and boiled for few minutes. Formation of blue colour indicate the presence of protein.

#### C. Detection of alkaloids.

##### 1. Dragendroff's test

The extract was treated with Dragendroff's reagent (solution of potassium bismuth iodide). The formation of red precipitate indicates the presence of alkaloids.

##### 2. Mayer's Test

The extract was treated with Mayer's reagent (Potassium mercuric iodide) and the formation of yellow precipitate indicate the presence of alkaloids.

##### 3. Wagner's Test

The extract was treated with Wagner's reagent (iodine in potassium iodide) and the formation of brown or reddish brown precipitate indicates the presence of alkaloid.

##### • Hager's Test

The extract was treated with Hager's reagent (saturated picric acid solution). The formation of yellow coloured precipitate indicate the presence of alkaloids.

#### D. Detection of glycosides

##### 1. Keller kiliani test

2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to 0.5 ml of extract diluted with 5 ml water.

##### 2. Bromine water test

The extract was treated with bromine water. The formation of yellow colour indicates the presence of glycosides.

##### 3. Legal's test

The extract was treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red blood colour indicates the presence of cardiac-glycosides

#### E. Detection of Flavanoids

##### 1. Alkaline reagent test

The extract was treated with few drops of NaOH solution. The formation of intense yellow colour that becomes colourless on addition of few drop of dilute acid indicates the presence of flavonoids.

##### • Lead acetate test

The extract was treated with few drops of lead acetate solution. The formation of yellow colour indicates the presence of flavonoids.

##### • Shinoda test

To an alcoholic solution of the extract, few pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. The appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

##### • Detection of tannins



#### 1. Ferric chloride test

0.5ml of extract was boiled in 10ml of water and filtered. To the filtrate added few drops of 0.1% ferric chloride solution. The formation of brownish green or bluish black colour indicates the presence of phenolic compounds.

#### 2. Gelatin test

To the extract added 1% gelatin solution containing sodium chloride. Observed for the formation of white precipitate which indicates the presence of tannins.

Lead acetate solution was added to the extract. The formation of yellow colour indicates the presence of tannins.

#### • Detection of saponins

##### 1. Foam test

0.5ml of the extract was shaken with 5ml of water and then checked for the foam formation that persist for ten minutes which indicates the presence of saponins.

### B. Experimental Animals and Exposure conditions

Healthy Wistar rats of either sex weighing about 180-250g procured from the Government veterinary animal house, Mannuthy, approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were used for the study.

The animals were kept in a clean environment with 12 hours light and 12 hours dark cycles. The temperature was maintained at  $25\pm 3^{\circ}\text{C}$  and the relative humidity was maintained at  $45\pm 5\%$ . Animals were fed ad libitum with normal laboratory chow standard pellet diet and drinking water in polypropylene bottles with a stainless-steel sipper tube throughout the study. The animals were selected randomly. All the animals were acclimatized for 7 days prior to the commencement of the experiment.

#### Instruments

- Digital colorimeter (SYSTRONICS)
- High speed refrigerated centrifuge (KEMI)
- Micro centrifuge (SPINWIN)
- Digital balance (SHIMADZU AY220)
- UV-Spectrometer (SHIMADZU 1700)
- Vertical laminar air flow (KEMI)
- BOD incubator (ROTEK) Hot air oven (KEMI)
- Autoclave (KEMI)

#### Drugs And Chemicals

- Ethylene glycol (Sigma)
- Cystone (Himalaya) Cisplatin
- Tween 80 Ethyl ether

#### Selection of Dose

Dose selected for the present was based on the acute toxicity studies conducted on the methanolic fractions of *Dendrophthoe elastica*.<sup>15</sup> They reported that neither death nor any observable neuro behavioural effects were observed in the limit test. Due to the lack of any observable toxicity at the 2000mg/kg dose, LD<sub>50</sub> was not determined. So two doses were chosen to carry out the present study. The two doses were chosen in such a way that, the first dose was approximately 1/10<sup>th</sup> of the maximum dose administered during acute toxicities (i.e., 1/10<sup>th</sup> of 2000mg/kg body weight-200mg/kg body weight) and a high dose which was twice that of 1/10<sup>th</sup> dose i.e., 400mg/kg body weight.

### EXPERIMENTAL DESIGN

#### In- vivo methods-Anti urolithiatic activity

For the assessment of antiurolithiatic activity, two dose levels 200mg/kg and 400mg/kg were chosen. Male Wistar rats were elected from the entire group of animals and grouped in different cages. The dried crude methanolic extracts of whole plant of *Dendrophthoe elastica* were suspended in 5% tween 80 for oral administration. Antiurolithiatic activity was performed using Ethylene Glycol Induced Urolithiasis rat models having curative and preventive treatment schedules.

#### Ethylene Glycol Induced Urolithiasis Model<sup>46,47,48</sup>

The experimental animals were divided into eight groups (n=6) to study the curative and preventive effect of the methanolic extract of *Dendrophthoe elastica* on ethylene glycol induced urolithiasis in rats.

**Group I (Normal control)** - Animals were fed with regular rat food and drinking water ad libitum from day 1 to 28. 0.75% ethylene glycol with 1% w/v ammonium chloride in drinking water ad libitum for a period of 3 days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for next 25 days was fed to group II to group VIII for induction of renal calculi.

**Group II (Toxic control)** - Animals received 0.75% ethylene glycol in drinking water till 28<sup>th</sup> day.

**Preventive treatment**

**Group III (standard)**- Animals received daily single oral dose of standard antiurolithiatic drug Cystone (750mg/kg) first day till 28<sup>th</sup> day

**Group IV** - Animals received a single oral dose of the plant extract (200mg/kg body weight p.o) from first day till 28<sup>th</sup> day.

**Group V** - Animals received a single oral dose of the plant extract (400mg/kg body weight p.o) from first day till 28<sup>th</sup> day.

**Curative treatment**

**Group VI (Standard)** - Animals received daily single oral dose of standard antiurolithiatic drug Cystone (750mg/kg) from 15<sup>th</sup> day till 28<sup>th</sup> day.

**Group VII** - Animals received a single oral dose of the plant extract (200mg/kg body weight p.o) from 15<sup>th</sup> day till 28<sup>th</sup> day.

**Group VIII** - Animals received a single oral dose of the plant extract (400mg/kg body weight p.o) from 15<sup>th</sup> day till 28<sup>th</sup> day.

On day 28 animals of all the groups were kept in metabolic cages and urine samples were collected for 24 hours and analyzed for calcium, magnesium and total standard methods. The animals are anaesthetized using ethyl ether and blood samples were collected by cardiac puncturing and serum creatinine, blood urea nitrogen and uric acid were analyzed. The urinary output volumes of all groups were also noted. The animals were sacrificed under ethyl ether anaesthesia and the kidneys are isolated and carried out the Histopathological studies.

**In vivo methods- Nephroprotective activity Cisplatin induced nephrotoxicity mode**

The experimental animals were divided into five groups (n=6) to study the curative effect of methanolic extract of *Dendrophthoe elastica* on cisplatin induced renal toxicity in rats.

**Group I (Normal control)** – Animals received daily oral dose of the vehicle (CMC 0.6% w/v) from day 1 to day 15(10ml/kg body weight, p.o).

**Group II (Toxic control)** – Animals were administered with single i.p. dose of cisplatin 5mg/kg body weight on day 1.

**Group III (Standard group)**-Animals were administered with single i.p dose of cisplatin 5mg/kg body weight on day 1 and Cystone 500mg/kg body weight orally from day 2 till 14.

**Group IV** – Animals received a single i.p dose of cisplatin 5mg/kg body weight on day 1, followed by daily a single oral dose of the plant extract (200mg/kg body weight, p.o) from day 2 to 14.

**Group V** – Animals received a single i.p dose of cisplatin 5mg/kg body weight on day 1, followed by daily a single oral dose of the plant extract (400mg/kg body weight, p.o) from day 2 to day 14. On day 15, animals of all the groups were anesthetized using ethyl ether and the blood samples were collected by cardiac puncture and serum creatinine, total protein and blood urea nitrogen were analysed. The animals are sacrificed at the end of the study and isolated the kidneys and carried out the Histopathological studies on the samples.

**BIOCHEMICAL ESTIMATION**

**G.1.MAGNESIUM**

**Principle**

Calcium is excluded from the reaction by complexing with ethylene glycol tetra acetic acid.

**Procedure**

Pre warm at room temperature the required amount of reagent before use.

	Sample	Standard	Blank
	0.01ml	0.01ml	-
<b>Reagent</b>	1.0ml	1.0ml	1.0ml

**Incubation**

Incubate the assay mixture for 5 minutes at 37°C.After the incubation measure the absorbance of assay mixture against blank at 546nm.Final colour is stable for 30 minutes if not exposed to direct light.

**Principle**

Calcium forms a purple coloured complex with cresol phthalein complex one in alkaline medium. This complex absorbs light at 575(570-580) nm.

**Procedure**

Pre warm at room temperature (25-30°C) the required amount of working solution. Perform the

assay as given below.

	Sample	standard	Blank
	0.02ml	0.02ml	-
<b>Working solution</b>	1.0ml	1.0ml	1.0ml

### CREATININE

#### Principle

Creatinine in alkaline medium reacts with picrate to produce orange colour. This colour absorbs light at

492nm.(490-510nm). The rate of increase in absorbance is directly proportional to the concentration of creatinine in specimen. Creatinine + Picrate → Orange colour

#### Procedure

<b>Standard/sample</b>	0.05ml
<b>Working solution</b>	1.0ml

Mix and aspirate. After the initial delay of 30 seconds record the absorbance of the test at an interval of 60 seconds at 492nm. Determine the mean change in absorbance and calculate the test results.

This colour complex absorbs light at 546nm.(530-570nm). The intensity of the colour is directly proportional to the protein concentration in specimen.

Protein → Blue colour complex

#### Procedure

Prewarm at room temperature (25-30° C) the required amount of working solution before use. Perform the assay as given below

### TOTAL PROTEIN

#### Principle

	Sample	Standard	Blank
	0.01ml	0.01ml	-
<b>Working Solution</b>	1.0ml	1.0ml	1.0ml

### URIC ACID

#### Principle

Uricase converts uric acid into allantoin and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidatively couples with phenolic chromogens to form a red coloured compound, which has maximum absorbance at 510nm.(500-530nm). The concentration of the red

coloured compound is proportional to the amount of uric acid in specimen.

H<sub>2</sub>O<sub>2</sub> + Phenolic chromogen → Red colour compound

#### Procedure

Prewarm at room temperature (25-30°C) the required amount of reagent before use. Perform the assay as given below

	Sample	Standard	Blank
	0.025ml	0.025ml	-
<b>Reagent</b>	1.0ml	1.0ml	1.0ml



**Incubation**

Incubate the assay mixture for 5 minutes at 37° C or 10 minutes at room temperature. After completion of the incubation measure the absorbance of assay mixture against blank at 510nm. Final colour is stable for 30 minutes if not exposed to direct light.

Crude methanolic extract of the whole plant of *Dendrophthoe elastica* was prepared by soxhlet extraction with methanol (hydro alcoholic mixture (20:80)).

**III. RESULTS**

**Preparation of Crude Extract**

**Preliminary Phytochemical Screening of Extract**

The results of preliminary phytochemical screening of MDE are given below in the table. The MDE extract revealed the presence of carbohydrates, sterols, terpenoids, flavones, phenols, tannins and glycosides.

Table 3: Results of Phytochemical Screening

Sl.No	Test	Inference
<b>1</b>	<b>Carbohydrates</b>	
a.	Mollisch's test	+
b.	Benedict's test	+
c.	Fehling's test	+
<b>2</b>	<b>Proteins and aminoacids</b>	
a.	Biuret test	-
b.	Ninhydrin test	-
<b>3</b>	<b>Alkaloids</b>	
a.	Mayer's test	-
b.	Wagner's test	-
c.	Hager's test	-
d.	Dragendorff's test	-
<b>4</b>	<b>Glycosides</b>	
a.	Keller kiliani test	+
b.	Legal's test	+
c.	Bromine water test	+
<b>5</b>	<b>Flavanoids</b>	
a.	Alkaline reagent test	+
b.	Lead acetate test	+
c.	Schinoda test	+
<b>7</b>	<b>Tannins</b>	
a.	Ferric chloride test	+
b.	Gelatin test	+
c.	Lead acetate test	+

8 a.	Saponins Foam test	-
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**Key:** + indicates the presence of constituents  
 - indicates the absence of constituents

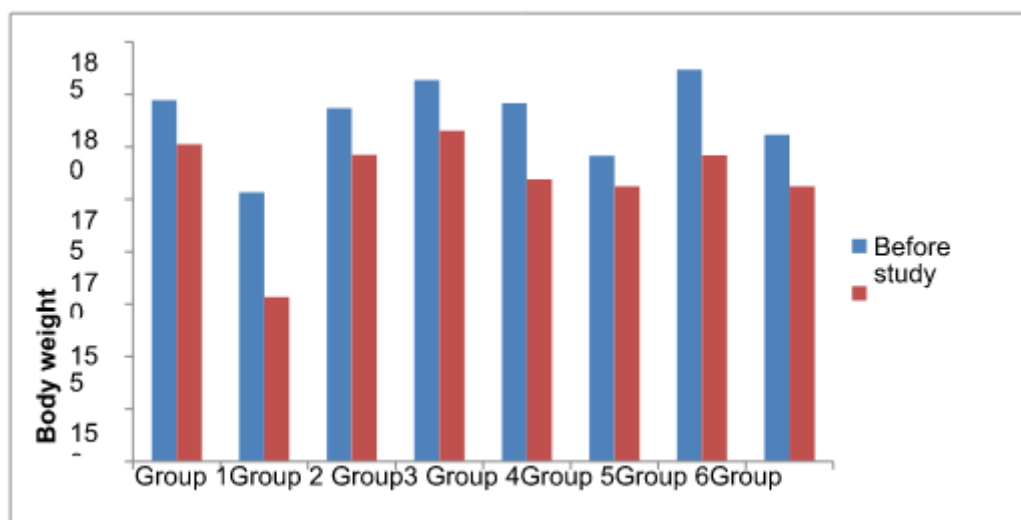
**ANTIUROLITHIATIC ACTIVITY**

**Determination of body weight**

Table 4 : Mean ± SEM of body weight determination

GROUPS		BODY WEIGHT Mean ± SEM (g)	
		Before study	After study
Group I	Normal control	178.432±4.3214	174.21±6.2312
Group II	Toxic control	171.65±4.3541	161.66±4.2456
Group III	Preventive standard	177.665±5.2156	173.232±6.3215
Group IV	Preventive 200mg/kg	180.367±5.2453	175.553±1475
Group V	Preventive 400mg/kg	178.167±3.4566	170.893±4.8742
Group VI	Curative standard	173.165±5.2457	170.235±5.4758
Group VII	Curative 200mg/kg	181.357±4.5641	173.23±6.1452
Group VIII	Curative 400mg/kg	176.14±3.847	170.235±3.4574

**Effect of MDE on body weights of rats**



Graph 1: Body weights of rats before and after study period

Group1: Normal control; Group 2: Toxic control;  
 Group 3: Preventive standard; Group 4: Preventive  
 200mg/kg; Group 5: Preventive MDE 400mg/kg;  
 Group 6:  
 Curative standard; Group 7: Curative MDE  
 200mg/kg; Group8: Curative MDE 400mg/kg

Rats were kept in individual metabolic cages for 24 hours for urine collection on 28<sup>th</sup> day of the study. The normal control rats did not show any significant variation in the urinary output level throughout the experimental period. The urine output was found to increase significantly (**P< 0.001**) by ethylene glycol treated group. Results are shown in table5 and graph 2

**Urine output determination**

Table 5: Statistical analysis of urine output

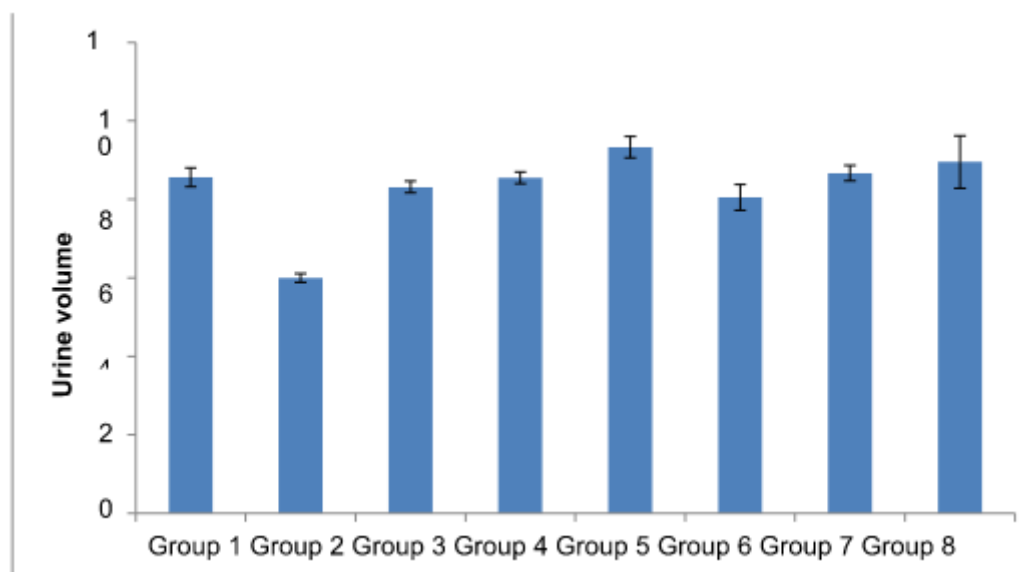
GROUPS		URINE OUTPUT (ml/24 hr)
Group I	Normal control	8.57±0.2402
Group II	Toxic control	6.01±0.1126***
Group III	Preventive standard	8.32±0.1493***
Group IV	Preventive MDE 200mg/kg	8.56±0.1478***
Group V	Preventive MDE 400mg/kg	9.34±0.2729***
Group VI	Curative standard	8.06±0.3272***
Group VII	Curative MDE 200 mg/kg	8.6±0.1968***
Group VIII	Curative MDE 400mg/kg	8.88±0.6684***

All the values are expressed as Mean ± SEM (n=6)

P values \*\*\* for P<0.001; \*\* for P<0.01; \* for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II.

**Urine Volume**



Graph 2: Effect of MDE on urine volume

**SERUM PARAMETERS**

**Table 6 : Serum parameters of rats used in antiurolithiatic study**

Groups	Treatment	Serum Creatinine mg/dl	Serum Blood Nitrogen mg/dl	Urea mg/dl	Uric Acid mg/dl
Group I	Normal control	0.89± 0.142	37.52±1.052		1.87±0.159
Group II	Toxic control	2.51±0.257** *	52.54±1.482 ***		3.98±0.228***
Group III	Preventive treatment	Standard	0.94±0.300** *	42.92±1.464 ***	2.53±0.356**
Group IV		200mg/kg	1.27±0.205**	48.76±1.619	3.49±0.1956
Group V		400mg/kg	0.97±0.190***	43.57±1.154 ***	2.62±0.210**
Group VI	Curative treatment	Standard	1.08±0.247**	44.88±1.854 **	2.77±0.238**

Group VII		200mg/kg	1.42±0.195*	48.88±1.468	3.77±0.322
Group VIII		400mg/kg	1.17±0.356**	45.30±0.846 **	2.95±0.229*

All the values are

expressed as Mean ± SEM (n=6)

P values \*\*\* for P<0.001; \*\* for P<0.01; \* for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II

### URINE PARAMETERS

Table 7: Urine parameters of rats used in antiurolithiatic study

Groups	Treatment	Calcium mg/dl	Magnesium mg/dl	Total Protein g/dl	
Group I	Normal control	4.92±0.4135	2.93±0.2133	2.12±0.1459	
Group II	Toxic control	9.54±0.483***	0.87±0.302***	5.60±0.184***	
Group III	Preventive treatment	Standard	5.60±0.345***	2.21±0.115**	2.60±0.233***
Group IV		200mg/k g	8.93±0.720	1.45±0.114	4.21±0.245**
Group V		400mg/k g	5.98±0.475***	2.16±0.659**	2.88±0.227***
Group VI	Curative treatment	Standard	5.83±0.517***	2.19±0.303**	2.87±0.287***
Group VII		200mg/k g	8.10±0.614	1.51±0.367	4.39±0.323**
Group VIII		400mg/k g	6.81±0.821**	2.10±0.057**	3.15±0.173***

All the values are expressed as Mean ± SEM (n=6)

P values \*\*\* for P<0.001; \*\* for P<0.01; \* for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II.



**NEPHROPROTECTIVE ACTIVITY**

Table 8 : Effect of MDE on % change in body weight

Groups	Treatment	% Change in body weight
Group I	Normal Control	-2.18±0.3791
Group II	Toxic control	18.71±2.9696***
Group III	Standard drug	-3.45±0.6511***
Group IV	MDE 200	-8.21±2.0000**
Group V	MDE 400	-4.00±0.6168***

All the values are expressed as Mean ± SEM (n=6)

P values \*\*\* for P<0.001; \*\* for P<0.01; \* for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II

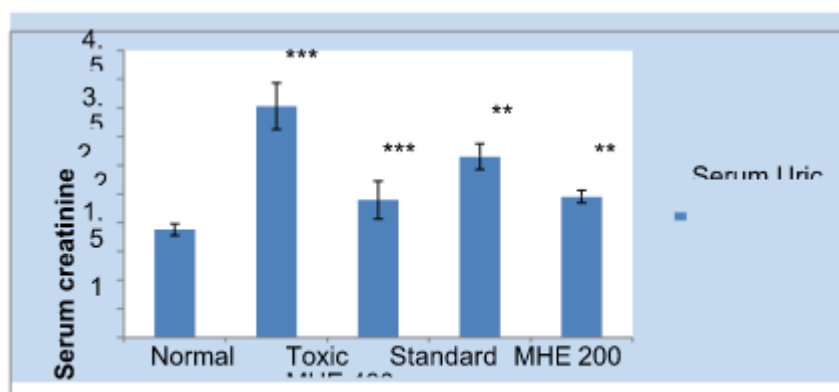
**Table 9 . Effect of methanolic extract of Dendrophthoe elastica on Serum urea, Serum creatinine, Serum Total protein and Serum Uric acid in cisplatin induced renal toxicity**

Group s	Treatme nt	Serum urea mg/dl	Serum creatinine mg/dl	Total protein gm/dl	Uric acid mg/dl
Group I	Normal control	29.86±1.060	0.60±0.103	3.09±0.239	1.87±0.160
Group II	Toxic control	59.25±3.376***	3.10±0.402** *	5.60±0.171***	4.03±0.287***
Group III	Standard	35.87±3.611***	1.03±0.328** *	3.45±0.328***	2.39±0.323**
Group IV	MHE 200	49.07±2.675*	2.13±0.223**	4.41±0.381	3.15±0.242
Group V	MHE 400	40.04±4.203**	1.53±0.111**	3.93±0.233**	2.45±0.371**

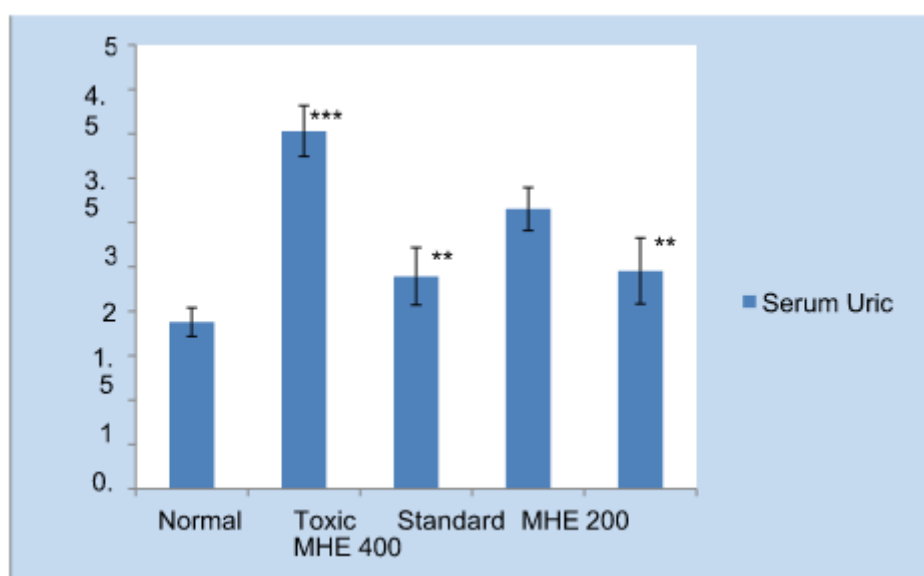
All the values are expressed as Mean ± SEM (n=6)

P values \*\*\* for P<0.001; \*\* for P<0.01; \* for P<0.05

Values of Group II were compared with Group I and those of Group III to V with Group II.



**Graph 10: Effect of methanolic extract of *Dendrophthoe* creatinine in cisplatin induced renal toxicity**



**Graph 12: Effect of methanolic extract of *Dendrophthoe elastica* on serum uric acid in cisplatin induced renal toxicity**

#### IV. DISCUSSION

##### Antirolithiatic activity

A number of animal models using rats have been used to induce calcium oxalate urolithiasis. The most reliable and hence commonly employed method is to provide ethylene glycol and ammonium chloride in drinking water to rats. Therefore, in the present study the anti urolithiatic activity of methanolic extract of *Dendrophthoe elastica* is evaluated using the ethylene glycol induced urolithiasis in rat models.

The biochemical mechanism of ethylene glycol and ammonium chloride induced urolithiasis are related to an increase in the urinary concentration of oxalate. Ethylene glycol is readily absorbed along the intestine and is metabolized in the liver to oxalate leading to hyperoxaluria. Due to

the poor solubility of oxalate it precipitates in the urine as calcium oxalate. High oxalate levels and calcium oxalate crystals especially in the nephron damage epithelial cells leading to heterogeneous nucleation followed by causing aggregation of crystals. Furthermore ammonium chloride has been reported to accelerate lithiasis.

Male Wistar rats were selected to induce urolithiasis because the urinary system of male Wistar rats has more resemblance to that of humans. In addition earlier studies have reported that the amount of stone deposition in female rats was significantly less compared to male rats due because of enhancing capacity of testosterone and inhibiting capacity of oestrogen in stone formation.

As reported in the previous antirolithiatic studies<sup>48,49</sup> decrease in urine volume was observed

in ethylene glycol induced urolithiatic rats. The methanolic extract of DE treatment also increased urine output but less than the stone induced group. The urine output of the test group was high when compared with the normal group. This may be due to the diuretic activity of *Dendrophthoe elastica*. Consistent with previous reports,<sup>46,47</sup> increased lipid peroxidation has been reported in the kidneys of the rats treated with ethylene glycol.

Magnesium is one of the urinary inhibitors of crystallization. Low levels of magnesium are encountered in stone-forming rats as well as in patients with renal stones. Magnesium is reported to form a complex with oxalate and reduce the supersaturation of calcium oxalate by reducing the saturation of calcium oxalate and as a consequence it reduced the growth and nucleation rate of calcium oxalate crystals.<sup>125,126</sup> In the present study, the MDE restored the magnesium excretion near to the normal and thus reduced the growth of calcium oxalate crystals.

#### Nephroprotective Activity

Nephrotoxicity is an undesired side effect of chemotherapy in general. Most chemotherapy drugs targets pathways that are essential to dividing cells. Several studies have now documented the importance of reactive oxygen metabolites (ROM) in cisplatin and gentamicin induced renal damage. Nephrotoxicity of the drugs is usually associated with their accumulation in renal cortex, dependent upon their affinity to kidneys and on kinetics of drug trapping process.

Creatinine is a spontaneously generated cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys through glomerular filtration and proximal tubular secretion.

In the present study, serum urea, BUN, and total protein level were significantly decreased by MDE at both doses showed its nephroprotective action on cisplatin-induced nephrotoxicity. The present investigation showed SOD activity increased by the administration of MDE (400 mg/kg) implicates its antioxidant and nephroprotective activity.

Cystone a polyherbal formulation inhibit the lipid peroxidation by cisplatin in renal corticles at a dose between 500 and 1000mg/kg. Cystone treated animals regained the normal blood urea nitrogen, creatinine and uric acid levels. The main mechanism behind the protective effect against cisplatin is through its lipid peroxidation inhibition.

#### V. CONCLUSION

- The present study was designed to evaluate the antiurolithiatic and nephroprotective activity of methanolic extract of *Dendrophthoe elastica*.
- Phytochemical analysis of the extract reported positive result for carbohydrates, flavonoids, phenolics, glycosides, tannins, steroids and triterpenoids.
- In the antiurolithiatic study, ethylene glycol was given orally to induce urolithiasis in two different doses for preventive and curative treatment regimen.
- The effect of the plant extract was assessed in terms of serum markers like blood urea nitrogen, uric acid and creatinine and urine markers like calcium, magnesium and total protein. Histopathological studies were also done to support the results.
- Extract treated groups of animals showed significant improvement in serum and urine markers and the kidney damage due to urolithiasis produced by ethylene glycol.
- Based on the results obtained, it can be concluded that methanolic extract of *Dendrophthoe elastica* possess antiurolithiatic activity against ethylene glycol induced urolithiasis.
- In the nephroprotective study, Cisplatin was given to induce kidney toxicity.
- Extract was orally administered to experimental animals in two different doses.
- The effect of the extract was assessed in terms of serum markers like urea, creatinine, uric acid and total protein. Histopathological studies were also done to support the results.
- Extract treated groups of animals showed significant improvement in serum markers and the kidney damage caused by cisplatin.
- Based on the results obtained, it can be concluded that methanolic extract of *Dendrophthoe elastica* possess nephroprotective activity against cisplatin induced nephrotoxicity.

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