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Antilithiatic Activity of Ethanolic Extract of *Hamelia patens* (Leaves) on Ethylene Glycol Induced Lithiasis in Rats

K. Yitzhak*, Sumasri Guntupalli, Venugopalaiah P, B. Venkata Ramana, B. Kumar, M. Gobinath

Ratnam Institute of Pharmacy, Pidathapolur, Muthukur (M), Nellore, Andhra Pradesh, India-524346

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Abstract

Indian traditional medicine is based on various systems including ayurveda, siddha and unani. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. These traditional systems of Indian medicine have their uniqueness no doubt but there is a common thread running through these systems in their fundamental principle and practices with the emerging interest in the world to adopt and study the traditional and to exploit their potentials based on different health care system. The ethanolic extract of leaves of *Hamelia patens*. (EEHP) Jacq was evaluated for its antilithiatic activity in rats. Lithiasis was induced by oral administration of ethylene glycolated water (0.75%) in Adult male albino Wistar rats for 28 days. The ionic levels of urine was altered by ethylene glycol (EG), which elevated the urinary concentration of crucial ions, Viz. calcium, phosphate, uric acid and oxalate there by contributing to renal stone formation. The EEHP, however significantly ($P<0.05$) reduced the elevated levels of these ions in urine. Also, it elevated concentration of urinary magnesium, which is considered as one of the inhibitor of crystallization. It also increased urinary volume thereby reducing the tendency for crystallization. The histopathological studies confirmed the induction as degenerated glomeruli, necrotic tubule and inflammatory cells was observed in section of kidney from animals treated with ethylene glycol. This was reduced; however after treatment with EEHP. These observations enable to conclude that EEHP is effective against ethylene glycol induced Lithiasis.

Keywords: Ethanolic extract, *Hamelia patens*, antilithiatic activity.

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***Corresponding author**
K. Yitzhak
Ratnam Institute of Pharmacy,
Pidathapolur, Muthukur (M), Nellore,
Andhra Pradesh, India-524346
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1. Introduction

Indian traditional medicine is based on various systems including ayurveda, siddha and unani. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. These traditional systems of Indian medicine have their uniqueness no doubt but there is a common thread running through these systems in their fundamental principle and practices with the emerging interest in the world to adopt and study the traditional and to exploit their potentials based on different health care system. Herbal drugs constitute a major part in all the traditional systems of medicine. Herbal medicine is a triumph of popular therapeutic diversity. Indian medicinal plants used in different traditional system of medicine are being study in different universities and institutes. The WHO estimates that about 80 per cent of world population relies on TM for primary health care. Although modern medicine is widely spread, TM still exists in all countries. It is interesting to note that 25 per cent of modern medicines are derived from plants that were used traditionally. For example, the Chinese herbal remedy *Artemisia annua*, used in China for almost 2000 years, has been found to be effective against resistant malaria, and has created a breakthrough in preventing almost a million deaths annually, most of them of children, from severe malaria.

Urolithiasis is the condition where urinary stones are formed or located anywhere in the urinary system. Urinary stones are typically classified by their location or by their chemical composition (calcium-containing, struvite, uric acid, or other compounds). In humans, calcium oxalate is a major constituent of most urinary stones. About 80% of those with kidney stones are men. Men most commonly experience their first episode between 20-30 years of age, while for women the age at first presentation is somewhat later.

Present treatment for lithiasis is not effective, so treatment through Indian traditional medicine is gaining popularity. Indian traditional medicine is based on various systems including ayurveda, siddha and unani. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. These traditional systems of Indian medicine have their uniqueness no doubt but there is a common thread running through these systems in their fundamental principle and practices with the emerging interest in the world to adopt and study the traditional and to exploit their potentials based on different health care system. Herbal drugs constitute a major part in al the traditional systems of medicine. Herbal medicine is a triumph of popular therapeutic diversity.

Indian medicinal plants used in different traditional system of medicine are being study in different universities and institutes. The WHO estimates that about 80 per cent of world population relies on TM for primary health care. Although modern medicine is widely spread, TM still exists in al countries. It is interesting to note that 25 per cent of modern medicines are derived from plants that were used traditionally. For example, the Chinese herbal remedy Artemisia annuals, used in China for almost 200 years, has been found to be effective against resistant malaria, and has created a breakthrough in preventing almost a million deaths annually, most of them of children, from severe malaria. Rats are the suitable species for study of calcium oxalate deposition in kidneys, as that process mimics the etiology of kidney stone formation in humans.

Plant Profile

Botanical name : *Hamelia patens*.Jacq
Synonyms : *Hamelia erecta*. Jacq
Family : Rubiaceae

Botanical description [2]

Class : Magnoliopsida – Dicotyledons
Subclass : Asteridae
Order : Rubiales
Family : Rubiaceae
Genus : Hamelia
Species : patens

Vernacular names [9]

Bengali **:** *Muna*

Geogaraphical distribution [10]

A genus of woody shrubs indigenous to tropical and subtropical America. One species ,*Hamelia patens Jacq* introduced in to India and is widely grown as ornamental plant in gardens.It is large,evergreen shrub, with dense attractive foilage of greenish bronze leaves,flowers orange-red,tubular ,brone in profusion during hot and rainy seasons ;berries blood-red,edible. The plant is easily propagated by cuttings or by seeds. It stands pruning well and can be trimmed to any shape and makes a good ornamental hedge

Figure 5: Leaves of *Hamelia patens* Jacq

2. Materials and methods

Preparation of plant extract [20]

The leaves of *Hamelia patens* were air-dried and powdered leaves, was passed through sieve no 40 to get coarse powder. About 360 g of powder was then subjected to successive extraction using 95%v/v ethanol in soxhelt apparatus at a temperature of (60- 70) for 72 hours. The Solvent elimination was done using rotary evaporator under reduced pressure. The yield was about 5% w/w and it was stored at $4^{o}C$ in freeze dryer.

Preliminary phytochemical analysis [21, 22, 23]

The Ethanolic extract of *Hamelia patens.* Jacq was subjected to preliminary phytochemical screening.

1. Test for Alkaloids

The extract was treated with diluted HCl and filtered. The filtrate was treated with various alkaloidal agents.

Mayer's Test: Sample were treated with Mayer's reagent, appearance of cream color indicates presence of alkaloids.

**Dragendroff`s Test**: Sample were treated with Dragendroff`s reagent, appearance of reddish brown precipitate indicates presence of alkaloids.

Hager's Test: Sample was treated with Hanger's reagent; appearance of yellow color indicates presence of alkaloids.

Wager's Test: Sample was treated with wager's reagent; appearance of brown precipitate indicates presence of alkaloids.

2. Test for Carbohydrates

The extracts were treated with 3 ml of alpha naphthol in alcohol and Conc. Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of two liquids indicates presence of carbohydrates.

**Fehling`s Test:** To the sample Fehling`s solution A and B was added and heated for two minutes. Appearance of reddish brown color indicates presence of reducing sugars.

Benedict's test: To the sample benedict's solution was added and heated; appearance of reddish orange precipitate indicates presence of reducing sugars.

**Barfoed`s Test:** The sample were treated with Barfoed`s reagent and heated, appearance of reddish orange precipitate indicates presence of reducing sugars.

3. Test for Proteins

Biuret`s Test: To the extracts copper sulphate solution followed by sodium hydroxide solution, a violet color precipitates indicates presence of proteins.

Million's Test: To the extracts million's reagent was added, appearance of pink color indicates presence of proteins.

4. Test for Steroids

Libermann Burchard`s Test: The extracts were treated with Conc.Sulphuric acid and glacial acetic acid followed by acetic anhydride, a violet ring appears at the junction of the liquids and appearance of green color in the aqueous layer indicates presence of steroids.

5. Test for Sterols

The extracts were treated with 5%KOH solution; appearance of pink color indicates the presence of sterols.

6. Test for Phenols

The extracts were treated with neutral ferric chloride solution, appearance of violet color indicates presence of phenols. The extracts were treated with 10%sodium chloride solution, appearance of cream color indicates presence of phenols.

7. Test for Tannins

The extract were treated with 10%lead acetate solution appearance of white precipitate indicates presence of tannins. The extracts were treated with aqueous bromine water; appearance of white precipitate indicates presence of tannins.

8. Test for Flavanoids

5ml of the extracts solution was hydrolyzed with 10%sulphuric acid and cooled. it was then extracted with diethyl ether and divided in to 3 portions in three separate test tubes .1ml of diluted sodium carbonate, 1ml of 0.1 n sodium hydroxide and 1 ml of diluted ammonia solutions were added to the first second and third test tube respectively. Development of yellow color in each test tube indicates presence of flavanoids.

Shindoa`s test

The extracts were dissolved in alcohol, to which a piece of magnesium followed by drop wise addition of Conc. HCL and heated. Appearance of magenta color indicates presence of flavanoids.

9. Test for Gums and Mucilage

The extracts were treated with 25 ml absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties

10. Test for Glycosides

A pinch of the extract were dissolved in glacial acetic acid and few drops of ferric chloride solution was added followed by the addition of Conc.Sulphuric acid, formation of red ring at the junction of the two liquids indicates presence of glycosides.

11. Test for Saponins

Foam test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes presence of saponins.

12. Test for Terpenes

The extracts were treated with tin and thionyl chloride, appearance of pink color indicates presence of terpenes.

Methodology

Animal

Albino rats (wistar strain), weighing 150-200g, were procured from the Central Animal House Facility, Ratnam Institute of Pharmacy, Nellore. The animals were kept in polypropylene cages (6 in each cages) under standard laboratory condition (12 hr light and 12 hr dark:day:night cycle) and had free access to commercial pellet diet with water *ad libitum.* The animal house temperature was maintained at 25 ± 2^0C with relative humidity at (50 ±15%). The study was approved by the institutional animal ethical committee, CPCSEA (IAEC 99/2009). Ethical norms were strictly followed during all experiments.

Experimental design

Grouping of animals

Table 2

Groups	No. of animals	Drug	Dose/route of administration
I (Control)	6	Vehicle	(*P.O*)
II (Lithiatic control)	6	Ethylene glycol	0.75% (*P.O*)
III (Standard)	6	Ethylene glycol +Cystone	0.75% +750mg /kg (*P.O*)
IV (Test-I)	6	Ethylene glycol+ EEHP	0.75%+150mg/kg (*P.O*)
V (Test-II)	6	Ehylene glycol +EEHP	0.75% +300mg/kg (*P.O)*

Induction of kidney stones by ethylene glycol in rats

Lithiasis was induced by administering by gastric intubation of 0.75% ethylene glycolayed water to the animals except control animals up to 28 days. The control animals received vehicle.

Estimation of total urinary volume [19]

All animals were kept in an individual metabolic cage and urine samples of 24Hrs are collected on 28th day. Animals had free access to drinking water during the urine collection period.

Lists of kits and manufacturers

Table 3

S.No	Name of the kit	Manufacturer
1	Calcium	(AGAPPE)
2	Phosphate	(AGAPPE)
3	Creatinine	(MERCK)
4	BUN	(MERK)
5	Magnesium	(ACCUREX)

Estimation of ionic contents in urine

All animals are kept in a individual metabolic cages and urine samples of 24Hrs were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated HCl is added urine

before being stored at 4^0c. And the urine is analyzed for magnesium, calcium, phosphate, oxalates and Creatinine content by using kits.

Estimation of phosphates [24, 25]

Principle: Determination of inorganic phosphorus according to the following reaction.

Ammonium molibdate + sulphuric acid ⟶ phosphor molybdic complex

Reagent composition

Inorganic phosphorus reagent

Sulphuric acid : 210mmol/L

Ammonium molibdate : 650mmol/L

Inorganic phosphorus standard

Phosphorus standard concentration 5mg/dl

General system parameters

Table 4

Mode of reaction	End point
Slope of reaction	Increasing
Wave length	340nm
Temperature	37°C
Standard	5mg/dL
Linearity	15mg/dL
Blank	Reagent
Reaction time	1 min
Sample volume	20µL
Reagent volume	1000µl
Cuvette	1cm light path

Laboratory procedure

Table 5

	Blank	Standard	Sample
Reagent	1000µl	1000µl	1000µL
Standard	-	20µL	_
Sample	-	-	20µL

Calculations

$$\text{Phorphous conc (mg/dL)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 5$$

Estimation of calcium [26, 27, 28]

Principle

Colorimetric measurement with ortho-cresopthalein complex. The 8-hydroxy-quinoline prevents Mg from interference up to 4mmol/L

Reagent composition

Calcium dye reagent

Diethylamine : 360mmol/L

Calcium base reagent

O-Cresolphtalein : 0.15mmol/L

8-hydroxy quinoline : 17.2mmol/L

Calcium standard

Calcium standard concentration : 10mg/dL

Laboratory procedure

Table 6

	Blank	Standard	Sample
Working reagent	1000µL	1000µL	1000µL
Standard	-	10µL	_
Sample	-	-	10µL

General system parameters

Table 7

Mode of reaction	End point
Slope of reaction	Increasing
Wave length	578nm(565-580nm)
Temperature	Room temperature
Standard concentration	10mg/dL
Linearity	15mg/dL
Blank	Reagent
Incubation time	5min
Sample volume	10µL
Reagent volume	1000µL
Cuvette	1cm light path

Mix and incubate for 5minutes at room temperature. Read the absorbance of the standard and sample against reagent blank.

Calculations

$$\text{Calcium concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 10$$

Estimation of creatinine [29, 30, 31]

Photometric determination of Creatinine based on Jaffekinetic method

Principle

Creatinine forms a yellow orange compound in alkaline solution with picric acid. As result a rapid reaction between Creatinine and picric acid, the secondary reaction don't cause interferences.

Reagents

Reagent 1 Buffer solution
Reagent 2 Picric acid
Reagent 3 Standard solution

Reagent preparation: Pre - warm the reagents as well as sample (serum or plasma and urine samples).

Mono reagent preparation

Mix reagent I and II in the ratio of 1:1 the mixing ratio should be observed exactly. Leave the mono reagent for at least 30min, at room temperature before using. The reaction solution is 5hrs at 15 – 25° C and 4 weeks at 2 – 8°C.

General system parameters

Table 8

Wave length	Hg 492nm, (490 - 510)
Light path	1cm
Temperature	20 – 25° C

Assay procedure

Table 9

Reagents	Blank	Standard	Test
Mono reagent	1000µL	1000µL	1000µL
Standard	-	100µL	-
Test	-	-	100µL
Mix and read the absorbance AI at 60sec and AII after 120sec			

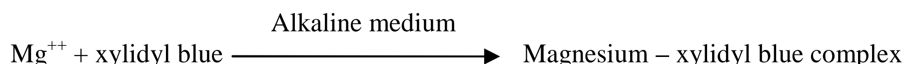
Calculation

$$\text{Creatinine (mg/dL)} = \frac{\text{A sample}}{\text{A Standard}} \times \text{con. Std. (mg/dL)} \times 50$$

Estimation of magnesium [32, 33, 34]

Principle

Magnesium ions reactive withxylidyl blue in an alakaline medium to form purple colored complex.the intensity of the purple color is directly proportional to the concentration of magnesium in specimen.



Mg^{++} + xylidyl blue —(Alkaline medium)→ Magnesium – xylidyl blue complex

Components and concentration of working solution

Table 10

Components	Concentration
Tris buffer(pH 11.0)	200mmol/L
EGTA	60µmol/L
Xylidyl Blue	110 µmol/L
Stabilizers/Surfactants	

Procedure

Table 11

Reaction type	End point
Reaction time	5min.at 37ºC
Wave length	546nm(520-570nm)
Zero setting with	Reagent blank
Blank absorbance limit	1.100Abs
Sample volume	0.01ml(10µl)
Reagent volume	1.0 ml
Standard concentration	2mg/dl
Linearity	5mg/dl

Manual assay procedure

Prewarm at room temperature the required amount of reagent before use.

1.0 ml procedure

2.0

Table 12

	Serum/Urine	Standard	Blank
	0.01ml	0.1 ml	----
Reagent	1.0ml	1.0ml	1.0ml

Incubate the assay mixture for 5min. at 37 ºC. after the incubation measure the absorbance of assay mixture against the blank at 546nm. Final color is stable for 30min if not exposed to direct light.

Calculation

$$\text{Magnesium in mg \%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 2$$

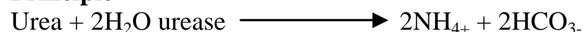
Serum analysis [19]

After the experimental period, blood was collected from the retro orbital under anesthetic conditions and serum separated by centrifugation at 10000rpm for 10 min and analyzed for calcium, Creatinine, BUN, uric acid by using kits.

Estimation of blood urea nitrogen [35, 36, 37]

Photometric estimation of BUN in plasma on Urease GLDH method as described in manufacturer's instruction manual (Merck Specialties Pvt Ltd)

Principle



Urea + $2H_2O$ urease → $2NH_{4+}$ + $2HCO_{3-}$

Monoreagent preparation

Mix reagent I and II in the ratio of 4:1.the mixing ratio should be observed exactly. Leave the Monoreagent for at least 30min, at room temperature before using. The reaction solution is 5 hrs at 15 – 25°C and 4 weeks at 2 – 8°C.

Assay

Wavelength: 340 – 365nm
Light path: 1cm
Temperature: 15 – 25°C

Table 13

Reagents	Blank	Standard	Test
Mono reagent	1000µL	1000µL	1000µL
Standard	-	10µL	-
Test	-	-	10µL
Mix incubate for approximately for 60sec, at15 – 25°C and at AI and AII after 1min			

Dilution limit
When values exceed 300mg/dL, the sample should be diluted with NaCl solution (9gm/l) and the result multiplied by 3.

Estimation of creatinine [29, 30, 31]

Photometric determination of Creatinine based on Jaffekinetic method

Principle
Creatinine forms a yellow orange compound in alkaline solution with picric acid. As result a rapid reaction between Creatinine and picric acid, the secondary reaction doesn't cause interferences.

Reagents
Reagent 1 Buffer solution
Reagent 2 Picric acid
Reagent 3 Standard solution

Reagent preparation
Pre - warm the reagents as well as sample (serum or plasma samples).

Mono reagent preparation
Mix reagent I and II in the ratio of 1:1 the mixing ratio should be observed exactly. Leave the mono reagent for at least 30min, at room temperature before using. The reaction solution is 5hrs at 15 – 25° C and 4 weeks at 2 – 8°C.

General system parameters

Table 14

Wave length	Hg 492nm, (490 - 510)
Light path	1cm
Temperature	20 – 25° C

Assay procedure

Table 15

Reagents	Blank	Standard	Test
Mono reagent	1000µL	1000µL	1000µL
Standard	-	100µL	-
Test	-	-	100µL
Mix and read the absorbance AI at 60sec and AII after 120sec			

Calculation

$$\text{Creatinine (mg/dL)} = \frac{\text{A sample}}{\text{A Standard}} \times \text{con. Std. (mg/dL)} \times 50$$

Estimation of uric acid [39, 40, 41, 42]

Principle
Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

uric acid + H_2O + O_2 uricas allantoin + H_2O_2 + CO_2

In presence of peroxidase, hydrogen peroxide oxidatively couples with 4 – aminoantipyrine and DHBS to produce red quinoneimine dye. The intensity of the red colour formed is directly proportional to the concentration of uric acid in the specimen and is measured photometrically

H_2O_2+ 4 - aminoantipyrine + DHBS peroxidase red quinoneimine dye + H_2O

DHBS = 3, 5 – dichloro – 2 hydroxy benzene sulfonic acid.

Components & Concentration of Working Solution

Component	**Concentration**
Hepes buffer; pH 7.8	50 mmol/l
Peroxidase	2500 lU/l
Uricase	400 lU/l
Ascorbate Oxidase	300 lU/l

4 –Amino antipyrine 0.1 mmol/l
DHBS 1 mmol/l
Stabilizers, excipients & surface active agents

Specimen Collection & Preservation

Collect sample using standard sampling tube. Serum, heparinized plasma or EDTA - plasma can be used. Uric Acid in serum / plasma is stable for 5 days at 2°– 8°C and 6 months at -20°C. Centrifuge samples containing precipitate before performing assay.

Calculations

Fully automated systems automatically calculate the uric acid concentration of each sample.
Results in mmol/l = Results in mg/dl x 0.059
Results in mg/dl = Results in mmol/l x 16.81Results in µmol/l = Results in mg/dl x 59.5

Kidney homogenate analysis for (calcium, phosphate) 'The abdomen is cut open to remove both kidneys from each animal. Isolated kidneys are cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys are dried at 80^0c in hot air oven. A sample of 100mg of the dried kidney was boiled in 10ml of 1N hydrochloric acid for 30min and homogenized. The homogenate is centrifuged at 2000rpm. 10min and the supernatant is separated and the calcium, phosphate, oxalate contents in kidney homogenate are determined by using kit.

Estimation of calcium (26, 27, 28)

Principle

Colorimetric measurement with ortho-cresopthalein complex. The 8-hydroxy-quinoline prevents Mg from interference up to 4mmol/L

Reagent composition

Calcium dye reagent

Diethylamine : 360mmol/L
Calcium base reagent
O-Cresolphtalein: 0.15mmol/L
8-hydroxy quinoline: 17.2mmol/L

Calcium standard

Calcium standard concentration: 10mg/dL

General system parameters

Table 16

Mode of reaction	End point
Slope of reaction	Increasing
Wave length	578nm(565-580nm)
Temperature	Room temperature
Standard concentration	10mg/dL
Linearity	15mg/dL
Blank	Reagent
Incubation time	5min
Sample volume	10µL
Reagent volume	1000µL
Cuvette	1cm light path

Laboratory procedure

Table 17

	Blank	**Standard**	**Sample**
Working reagent	1000µL	1000µL	1000µL
Standard	_	10µL	_
Sample	_	_	10µL

Mix and incubate for 5minutes at room temperature. Read the absorbance of the standard and sample against reagent blank.

Calculations

$$\text{Calcium concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 10$$

Estimation of phosphates [24, 25]

Principle

Determination of inorganic phosphorus according to the following reaction.

Ammonium molibdate + sulphuric acid ⟶ phosphor molybdic complex

Reagent composition
Inorganic phosphorus reagent

Sulphuric acid 210mmol/L
Ammonium molibdate 650mmol/L

Inorganic phosphorus standard

Phosphorus standard concentration 5mg/dl

General system parameters

Table 18

Mode of reaction	End point
Slope of reaction	Increasing
Wave length	340nm
Temperature	37^oC
Standard	5mg/dL
Linearity	15mg/Dl
Blank	Reagent
Reaction time	1 min
Sample volume	20µL
Reagent volume	1000µl
Cuvette	1cm light path

Laboratory procedure

Table 19

	Blank	**Standard**	**Sample**
Reagent	1000µl	1000µl	1000µL
Standard	-	20µL	-
Sample	-	-	20 µL

Calculations:

$$\text{phorphous conc (mg/dL)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times 5$$

Histopathological studies

Kidney samples were weighed and fixed rapidly with 10% neutralized formalin (p^H 7.4) section of the kidney fixed in paraffin was prepared and stained with hematoxylin & eosin and observed for pathological changes.

3. Results and Discussion

Results

Preliminary Phytochemical Investigation

The results of Phytochemical analysis of Ethanolic extract of *Hamelia patens Jacq* were tabulated.

Table 20: Preliminary phytochemical test for EEHP

SI.No.	Phytochemical Tests	Results
1	Test for Alkaloids	+Ve
2	Test for Carbohydrates	+Ve
3	Test for Proteins	+Ve
4	Test for Steroids	-Ve
5	Test for Sterols	-Ve
6	Test for Phenols	-Ve
7	Test for Flavonoids	+Ve
8	Test for Gums and mucilage	-Ve
9	Test for Glycosides	-Ve
10	Test for Saponins	-Ve
11	Test for Terpenes	-Ve

+Ve: indicates the presence of compounds
-Ve: indicates the absence of compounds

Table 21: Total urinary volume

Groups	Urine Output (ml/day/rat) Mean ± SEM
(I) Control	32±1.73**
(II) Lithiatic Control	14.66±0.05
(III) Standard	30±1.50***
(IV) Test-I	25±0.035***
(V)Test-II	27±2.14**

The data were analyzed by Newman-Keuls multiple range test ($p < 0.05$) and the values were expressed as mean ± SEM for six animals in each group
Newman-Keuls multiple range test ($p < 0.05$) was used
*** Values were significantly different from lithiatic control (G-II), $p<0.001$.
** Values were significantly different from lithiatic control (G-II), $p<0.01$.

Table 22: Change in The urinary excretion of stone forming constituent in control and EEHP treated animals

Groups	Dose (mg/kg)	Urine parameters(mg/dL)	
		Calcium	Phosphate
I (Control)	Vehicle	1.20±0.07***	3.50±0.03***
II (Lithiatic control)	Ethylene glycol (0.75%)	4.20 ±0.09	6.90±0.05
III (Standard)	750	1.50±0.05**	3.50±0.08
IV Test I (EEHP)	150	2.03 ± 0.05***	4.05±0.09***
V Test II (EEHP)	300	1.70 ±0.06***	3.89±0.05***

The data were analyzed by Newman-Keuls multiple range test ($p < 0.05$) and the values were expressed as mean ± SEM for six animals in each group
Animals in each group. Newman-Keuls multiple range test ($p < 0.05$) was used
*** Values were significantly different from lithiatic control (G-II), $p<0.001$.
** Values were significantly different from lithiatic control (G-II), $p<0.01$.

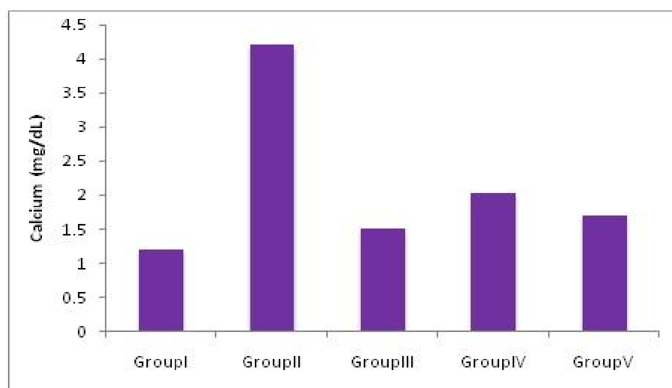


Figure 4: Effect of EEHP on Calcium excretion

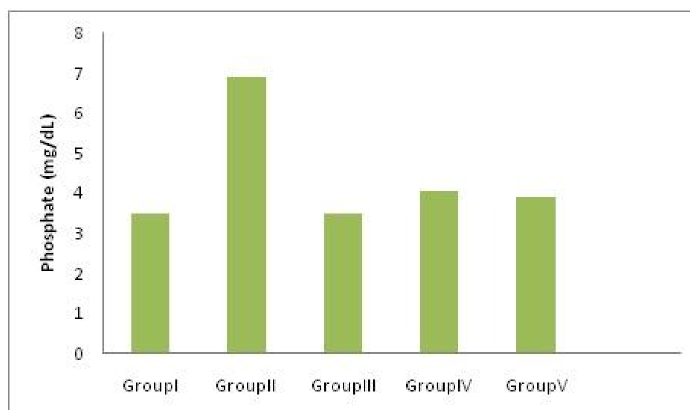


Figure 5: Effect of EEHP on Phosphate excretion

Table 23: Change in the Urinary excretion of stone forming constituent in control and treated animals

Groups	Dose (mg/kg)	Urine parameters(mg/dL)	
		Magnesium	Creatinine
I (Control)	Vehicle	1.20±0.03***	6.20±0.10*
II (Lithiatic control)	Ethylene glycol (0.75%)	0.520±0.07	6.62±0.19
III Standard	750	1.08± 0.07***	6.30±0.22***
IV Test I (EEHP)	150	0.90±0.05***	4.50±0.20
V Test II (EEHP)	300	1.02±0.19***	3.72±0.05***

The data were analyzed by Newman-Keuls multiple range tests ($p < 0.05$) and the values were expressed as mean ± SEM for six animals in each group
*** Values are significantly different from lithiatic control (G-II), p<0.001.
** Values are significantly different from lithiatic control (G-II), p<0.01.
* Values are significantly different from lithiatic control (G-II), p<0.05.

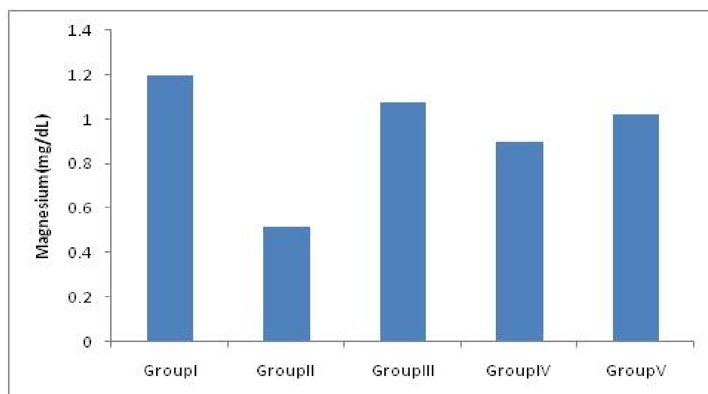


Figure 6: Effect of EEHP on Magnesium excretion

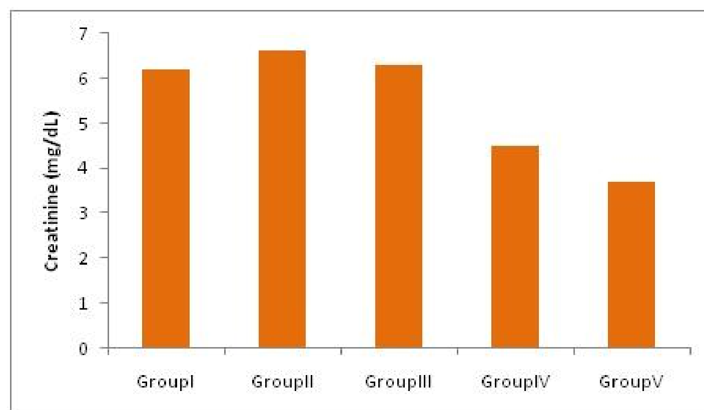


Figure 7: Effct of EEHP on Creatinine excretion

Table 24: Changes in serum parameters in control and EEHP treated animals

Groups	Dose(mg/kg)	Serum parameters(mg/dl)		
		BUN	Creatinine	Uric acid
I Control	Vehicle	3.50±0.014***	0.72±0.01***	1.40 ± 0.07***
II Lithiatic control	Ethylene glycol (0.75%)	45.69±0.46	0.90±0.03	3.35±0.01
III Standard	750	38.02±0.46***	0.81±0.02**	1.51±0.04*
IV Test I (EEHP)	150	43.56±0.01***	0.86±0.01***	2.01±0.06***
V Test II (EEHP)	300	41.76±0.01***	0.83±0.01***	1.85±0.04***

The data were analyzed by Newman-Keuls multiple range test ($p < 0.05$) and the values were expressed as mean ± SEM for six animals in each group
*** Values were significantly different from lithiatic control (G-II), p<0.001.
** Values were significantly different from lithiatic control (G-II), p<0.01.
* Values were significantly different from lithiatic control (G-II), p<0.05.

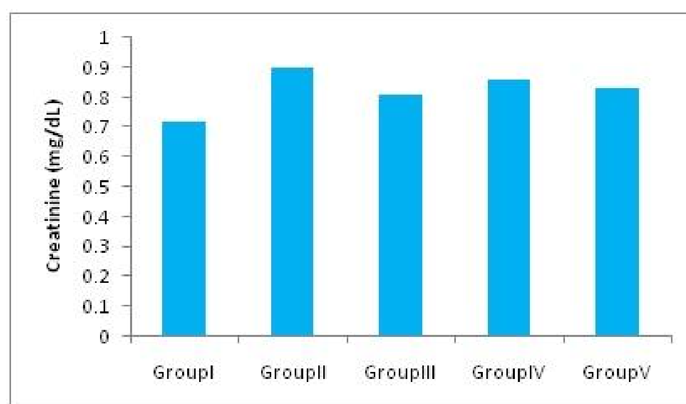


Figure 8: Effect of EEHP on Creatinine level in Serum

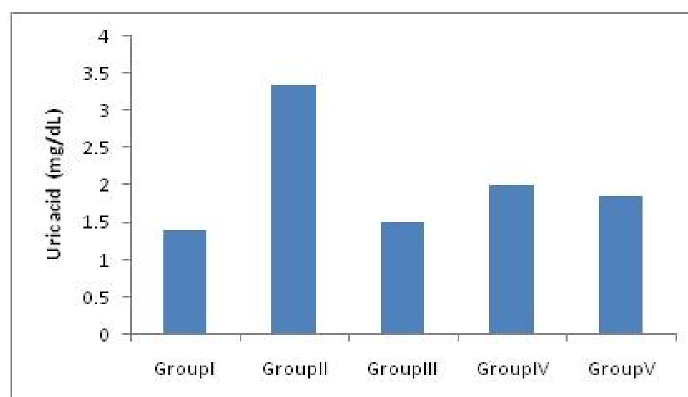


Figure 9: Effect of EEHP on Uric acid level in serum

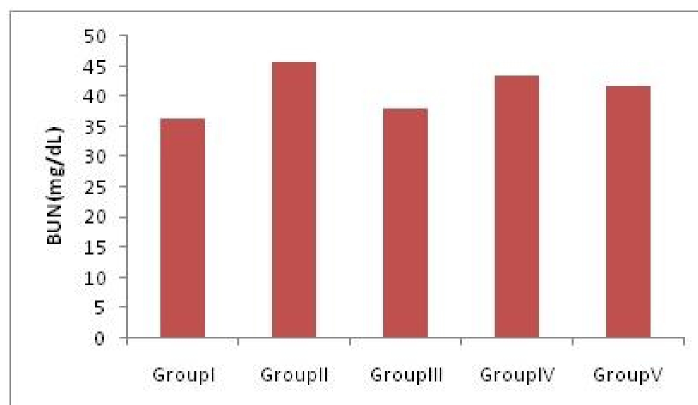


Figure 10: Effect of EEHP on Blood urea nitrogen in serum

Table 25: Changes in kidney retention of stone formining constiuents in control and EEHP treated animals

Groups	Dose (mg/kg)	Kidney parameters(mg/g)	
		Calcium	Phosphate
I Control	Vehicle	3.10 ±0.04***	2.20±0.03***
II Lithiatic control	Ethylene glycol (0.75%)	4.60 ±0.15	3.70±0.09
III Standard	750	3.41 ±0.06*	2.50±0.06**
IV Test I (EEHP)	150	4.40 ± 0.04***	3.05±0.08***
V Test II (EEHP)	300	3.72 ±0.07***	2.70±0.06***

The data were analyzed by Newman-Keuls multiple range test ($p < 0.05$) and the values were expressed as mean ± SEM for six animals in each group

*** Values are significantly different from lithiatic control (G-II), p<0.001.

** Values are significantly different from lithiatic control (G-II), p<0.01

* Values are significantly different from lithiatic control (G-I), p<0.05.

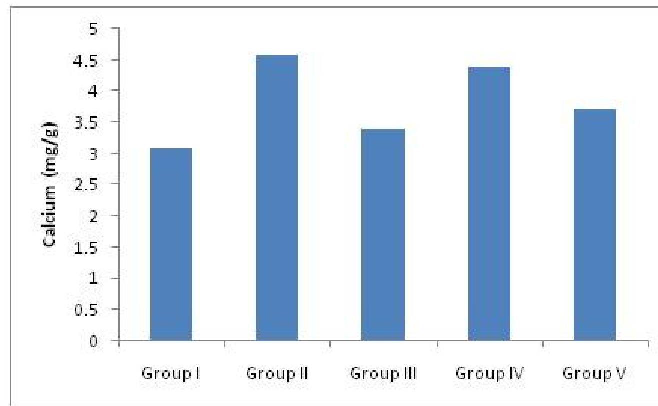


Figure 11: Effect of Calcium level in Kidney Homogenate

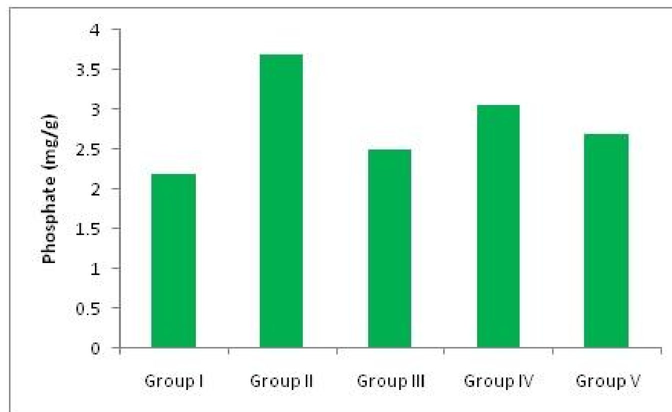
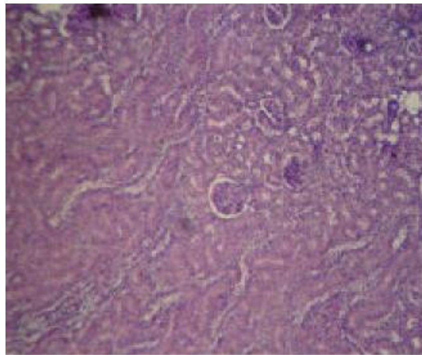
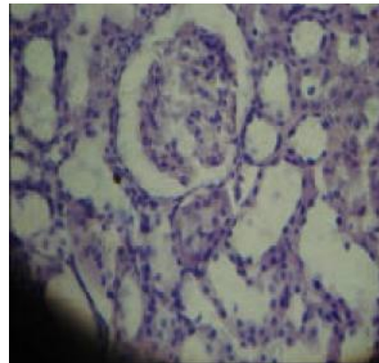


Figure 12: Effect of EEHP on Phosphate levels in kidney Homogenate

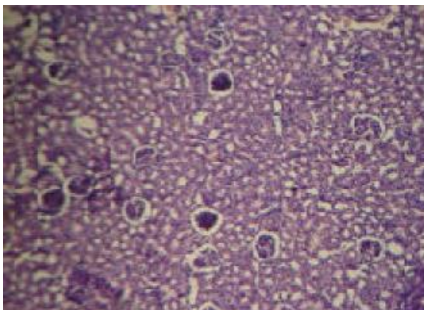
Histo pathological study



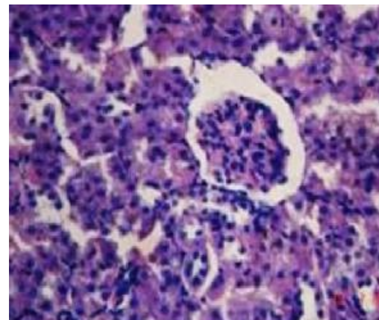
Group I: Normal tubles of epithelial lining



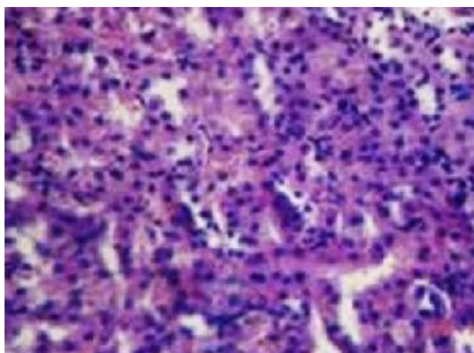
Group II: Degenerated epithelial lining & dilated tubles



Group III: Tubles of epithelial lining similar to Group I



Group IV: Tubles of epithelial lining Similar Group I



Group V: Tubles of epithelial lining similar to group I

Figure 13

Discussion

In the present study male rats were selected to induce Lithiasis because the urinary system of male rats resembles that of humans and earlier studies showed that the amount of stone deposition in female rats was significantly less. Different chemicals used to induce Lithiasis in experimental animals include ethylene glycol (EG) glycolic acid and ammonium oxalate[45]. Kidney being the principal target for ethylene glycol toxicity and its administration to the experimental administration more than 4 weeks resulted in substantial excretion of oxalate and deposition of micro crystals in kidney[46] therefore, in this study EG was preferred to induce Lithiasis. Alteration in urine volume and composition of urine, blood were observed after induction of Lithiasis. As the volume of urine excreted by Group IV and Group V animals were more when compared to that of volume excreted the Group II animals, this reinforces the plants having diuretic[47, 48] activities such an effect may be advantage in Lithiatic condition. As increased urine output is recommended to reduce the possibility of stone formation.

It was observed that the amount of calcium excreted following administration of ethylene glycol in Group II animals increases. Previous study report states that more than 80 % that the renal stones made up of with calcium oxalate and calcium phosphate. Increased urinary calcium is factor favoring the nucleation and precipitation of calcium oxalate or apatite. But on administration of EEHP to the animals, the amount of calcium in the urine were reduced in Group IV and V. Moreover the amount of phosphate excreted in urine was increased in urine Group II animals an increased urinary phosphate excretion seems to provide an environment appropriate for stone formation by forming Calcium phosphate crystals[49]. Following EG administration, after treatment with EEHP there is a decrease in phosphate level of the animals levels in Group IV and V.

In urolithiasis, the glomerular filtration rate (GFR) decreases, due to the obstruction to the out flow of urine by stones in urinary system. Due to the waste products particularly nitrogenous substances such as urea, Creatinine and uric acid get accumulated in the blood[50] in Lithiatic control rats marked renal tissue damage was seen by the elevated serum levels of Creatinine, and uric acid and BUN. However the extract treated animals has a process of dissolving the performed stones and prevention of new stone formation in urinary system[51]. In Group II the excretion of Creatinine level is high where as in group III, IV and V the levels were decreased. In serum of Group II animals where the BUN Creatinine and uric acid levels are increased where as in Group III, IV and V are decreased. In Group II the magnesium level in the urine was decrease which is the common future in the stone formers. Where as in Group IV and V were increased thus reducing the intensity of the crystallization.

4. Conclusion

The Ethanolic extract of *Hamelia patens* significantly reduced the elevated level of calcium Oxalate, and Phosphate ions. The urinary concentration of Magnesium was increased which is considered as one of the inhibitor of crystallization. The Histo pathological finding also shows sign of improvement after with EEHP. All these observations provided the basis for the conclusion those *Hamelia patens*. Jacq leaves extract inhibit the stone formation. Further research need to be carried out in isolation of active constituents report for the same.

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