WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 1063-1081. Research Article ISSN 2277– 7105

ANTIUROLITHIATIC ACTIVITY OF *VITIS VINIFERA* ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

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Article Received on
01 Oct. 2018,
Revised on 22 Oct. 2018,
Accepted on 12 Nov. 2018
DOI: 10.20959/wjpr201819-13759

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ABSTRACT

Introduction: Urolithiasis is a common problem afflicted for centuries with high recurrence and is predominant in males with no satisfactory method available for complete cure. *Vitis vinifera* is a plant commonly used as a traditional herbal medicine and posses the wide range of pharmacological applications. **Objective:** The present study is to investigate the anti-urolithiatic activity of *Vitis vinifera* against ethylene-glycol induced urolithiasis in rats. **Methods:** Urolithiasis was induced by oral administration of 0.75%v/v ethylene glycol and 2%w/v ammonium chloride in drinking water for 21days. 20 Male Albino rats were randomly divided into 5 groups. Group-1 received normal drinking water. Group:2-5 received ethylene glycol and ammonium chloride for 21days. Group 3&4 received leaf extract of *Vitis vinifera* at oral dose of 200mg/kg & 400mg/kg respectively from 1st day to 21st day. Group 5 received standard drug cystone(750mg/kg p.o). After completion of 21days respective treatments, the levels of various urolithiatic parameters in the urine, serum and kidney homogenate were used as criteria for assessing antiurolithiatic effect of *Vitis vinifera*. **Results:** The urolithiatic control group showed significant decrease in urine volume. The uric acid, calcium and creatinine levels were increased. Rats treated with *vitis vinifera* leaf extract significantly decreased the urinary excretion of calcium, serum creatinine and uric acid levels. Histopathological studies confirmed the crystal deposition in sections of kidney from animals treated with ethylene glycol and this was reduced in treatment with leaf extract. **Conclusion:** These results indicate that *Vitis vinifera* leaves are effective against ethylene glycol induced urolithiasis.

KEYWORDS: Urolithiasis; ethylene glycol; ammonium chloride; *Vitis vinifera*; cystone; calcium oxalate.

1. INTRODUCTION

Urolithiasis is derived from the Greek word "Ouron" which means urine and "lithos" which means stone. Urolithiasis refers to the growth of solid, hard and non metallic minerals in the urinary tract. It is the formation of urinary stones or calculi which are located or formed at any level of the urinary system. It is estimated that renal stone disease is experienced by the 12% of the world population with a recurrence rate of 47-60% in female and 70-80% in male. Urolithiasis is more common in men than in women.

The formation of calculi in the kidney is termed as nephrolithiasis. The formation of stones in the ureters is termed as ureterolithiasis and the formation of bladder stones is cystolithiasis. Urolithiasis comprises of nephrolithiasis, ureterolithiasis and cystolithiasis.[1]

The standard drugs used in the prevention and treatment of urolithiasis are not so effective in all the patients and most of them show adverse effects that compromise their long term use. Hence the investigation for antilithiatic drugs to be effective without adverse effects from natural sources has gained a great potential.[2]

Formation of renal calculi is a complex process. It occurs due to the imbalance between promoters and inhibitors in the kidneys. The factors affecting stone formation are urine pH, urine output, concentration of specific constituents present in the urine and damage or infection in the urinary system.

The development of renal calculi is determined by two key processes i.e crystal formation and retention of the crystals. The changes in the composition of ions, osmolarity and intratubular fluid volume may frequently change the solubility of urinary calcium salts, calcium phosphate, calcium oxalate and crystals may form in the tubular fluid as a result of supersaturation.[2]

2. MATERIALS AND METHODS

2.1. Plant material and preparation of extract

2.1.1. *Vitis vinifera*

Vitis vinifera is generally termed as grape or vine. It is a type of woody perennial vine belongs to the family vitaceae.[4]

2.1.2. Collection of plant

The leaves of *vitis vinifera* plant were collected from the village kammarkanpet, mandal: choppadandi, District:karimnagar and authenticated by Botanical survey of India, Deccan regional centre, Attapur, Hyderabad.(Authentication no:BSI/DRC/2017-18/TECH./697).

2.1.3. Plant drying

The leaves of Vitis vinifera were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no. #30. Dried samples was extracted with ethanol using soxhlet process. The extracts was concentrated under reduced pressure by using rota vapour.[5]

2.1.4. Method of Extraction of Plant material

Hot Continuous Extraction (Soxhlet)

The crude drug was finely ground and the powder was placed in thimble made up of filter paper and inserted in to the wide central tube of extractor. Ethanol is placed in the round bottom flask and brought to its boiling point up to 78^{o}C. Its vapour passed through the larger right hand tube in to the upper part of extractor and then to the condenser where they condensed and dropped back on to the drug. During this period, the soluble constituents are extracted when the level of the extract reaches the top of the siphon tube, the entire volume of extract siphons over in to the flask. The process was continued until the drug is completely extracted then the extract processed for evaporation. After the evaporation the semi-solid jelly is formed.[6]

Figure 1: Extraction of vitis vinifera leaves by soxhlet.

2.1.5. Phytochemical screening

Table 1: Phytochemical screening of vitis vinifera leaf extract.[7]

Test	Observation	Result
Test for Flavanoids: To small quantity of residue, lead acetate solution was added.	Yellow colour precipitate was observed.	Presence of flavanoids
Test for Polyphenols: To 2-3ml of aqueous extract 5%$FeCl_3$ solution was added.	Deep blue colour was observed.	Presence of polyphenols
Test for Tannins: To 2-3ml of aqueous extract 5%$FeCl_3$ solution was added.	Deep blue colour was observed.	Presence of tannins
Test for Alkaloids: Dragendroff's test: To the aqueous extract dilute Hcl was added and filtered. To the filtrate few drops of Dragendroff's reagent was added.	Orange brown precipitate was observed.	Presence of alkaloids
Test for Glycosides: Legal's test: To aqueous extract 1ml pyridine and 1ml sodium nitropruside was added.	Pink to red colour is not observed.	Absence of glycosides
Test for Carbohydrates; Fehling's test: To 2-3ml of aqueous extract, few drops of alpha-naphthol solution in alcohol was added and shaked. From sides of test tube conc.H_2SO_4 was added.	Violet ring was formed at the junction of two liquids.	Presence of carbohydrates.

Figure 2: Phytochemical screening.

2.1.6. Thin Layer Chromatography

Thin layer chromatography was carried out on all the fractions using TLC pre coated plates by using one way ascending techniques. The plates were cut with scissors and marked with pencil about 1cm from the bottom of the plate. Each sample was faintly dissolved in distilled water and capillary tubes were used to uniformly apply the dissolved samples on the plates and allowed to dry. The plates were developed in a chromatographic tank using the different solvent systems.[7]

TLC profile

1. TLC for flavanoids

n-butanol: acetic acid: water = 4:1:5

Detection: UV light

2. TLC for alkaloids

Toluene: ethylacetate: diethylamine= 7:2:1

Detection: Dragondroff's reagent

3. TLC for phenols

Chloroform: ethylacetate: formic acid= 5:4:1

Spray: $FeCl_3$ (2% in ethanol)

Detection: UV at 254nm

The plates were dried and visualized under normal day light, UV(254nm and 366nm) and by spraying with 10%H_2SO_4 followed by heating at 105^{O}C for 5-10 minutes in an oven.

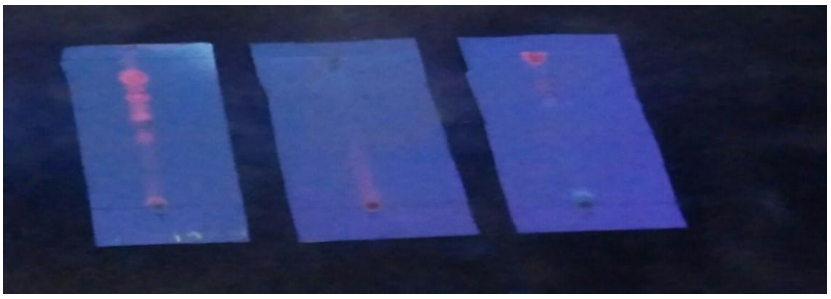


Figure 3: TLC plates.

2.2. Chemicals and apparatus

Ethylene glycol and formaldehyde were purchased from Finar fine chemicals, Hyderabad, India. Other chemicals and reagents used were procured from central drug house, New Delhi. Apparatus such as the Metabolic cage, Electronic balance(Electronic compact scale virgo), UVSpectrophotometer(Shimadzu), Microcentrifuge(ElektrotechinkLtd) and Incubator(Teknik AN10) were used in this study. Various kits for biochemical estimation of urine and serum were purchased from M/S Excel diagnostics Pvt Ltd, Hyderabad.

2.3. Animals

Male wistar albino rats weighing 150-200g were used for this study. They were kept in polypropylene cages. 4Rats per each cage were placed. Controlled environment such as temperature and light was maintained. Standard diet was provided for the animals. The study protocol was approved by Institutional Animal Ethics Committee(IAEC) (Approval

No:vcp/cology/001/11/2017) which was constituted in accordance with the rules and guidelines of the committee for the Purpose of Control and Supervision of Experimental on Animals, India.

2.4. Method for induction of urolithiasis in rats

2.4.1. Ethylene glycol and ammonium chloride

Ethylene glycol(0.75%v/v) and ammonium chloride(2%w/v) induced urolithiasis in rats. Urolithiasis inducing treatment received ethylene glycol in drinking water for 21days and further ammonium chloride was co-administered with ethylene glycol for first 3days in order to augment lithiasis effect of ethylene glycol.[8]

Ethylene glycol is widely used as a solvent.

MOA: Ethylene glycol is rapidly absorbed and metabolised in liver via alcohol dehydrogenase and aldehyde dehydrogenase to glycolic acid. This is oxidised to glyoxylic acid which is further oxidised to oxalic acid or oxalate by glycolate oxidase or lactate dehydrogenase thus promoting hyperoxaluria. Hyperoxaluria is the major risk factor for urolithiasis.[9]

Dose:0.75%v/v in drinking water for 21days.

The administration of ethylene glycol is a common method for the induction of urolithiasis in experimental rats. Calcium oxalate urolithiasis in rats was induced by ethylene glycol alone or in combination with ammonium chloride. Ethylene glycol is a metabolic precursor of oxalate. Ethylene glycol administration to rats result in hyperoxaluria, calcium oxalate crystalluria and crystal deposition in the kidney.

Ingestion of ammonium chloride, induces metabolic acidosis. So, it has been used in combination with ethylene glycol to promote the deposition of calcium oxalate crystals in rat kidneys. Ammonium chloride ingestion resulted in higher urinary acidification and a corresponding decrease in urinary citrate excretion.[10]

2.5. Acute toxicity test

Acute oral toxicity study for the test extract of the plant was carried out using OECD guidelines 425. The test procedure minimises the number of animals required to estimate the oral acute toxicity. The test allows the observation of signs of toxicity.

The healthy young adult albino wistar rats were used for this study. Animals should be fasted prior to dosing. The fasted body weight of each animal is determined and the dose is calculated according to the body weight.

Limit Test at 2000mg/kg

To one animal the extract at a dose of 2000mg/kg body weight was administered orally. The first tested animal was survived. Since, four other animals were dosed(orally) sequentially, so that a total of five animals were tested. Individually animals are observed at least once during the first 30min after dosing, periodically during the first 24hrs and daily thereafter, for a total of 14days. No animals were died. So the LD_{50} is greater than 2000mg/kg.[5]

2.6. Experimental design

Male wistar albino rats were randomly divided into 5groups containing four rats in each.

Group 1: Served as normal control group received normal drinking water.

Group 2: Served as urolithiatic control group received 0.75%v/v ethylene glycol and 2%w/v ammonium chloride in drinking water for 21days.

Group 3: Served as preventive low dose group received vitis vinifera leaf extract (200mg/kg; p.o) for 21days.

Group 4: Served as preventive high dose group received vitis vinifera leaf extract (400mg/kg;p.o) for 21days.

Group 5: Served as standard drug control received cystone (750mg/kg;p.o) for 21days.[8]

2.7. Collection and analysis of urine

After 21days of an experimental period, all animals were kept in individual metabolic cages which were hydrated with 5ml of distilled water orally and urine samples of 24hr were collected from normal, standard and preventive treated groups. A drop of concentrated hydrochloric acid was added to the collected urine and stored at $4^{o}C$. After urine collection, total urinary excretion of calcium and uric acid were measured by various biochemical kits.[11]

2.8. Collection of blood samples

At the end of the experiment, blood samples were collected from all animals through retro orbital puncture under mild anaesthetic conditions. Serum was separated by centrifugation at

3000 rpm for 10 minutes and analysed for creatinine and uric acid. Creatinine kit and uric acid kit were used to estimate the levels.[11]

2.9. Collection of organs

Rats were killed by cervical decapitation. To remove both the kidneys from each animal the abdomen was cut and opened. Isolated kidneys were then cleaned off extraneous tissue and rinsed in ice-cold physiological saline. One kidney from each rat was separated and fixed in 10% formalin.

2.10. Histopathological examination

The abdomen of the rat was cut, opened and kidney was removed from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10%formalin solution. The isolated kidneys was embedded in paraffin using conventional methods and cut into 5µm thick sections and stained using hematoxylin eosin dye and finally mounted in diphenyl xylene. Then the sections were observed under microscope for histopathological changes in kidney and their photomicrographs were taken.[12]

2.11. Statistical analysis

All the data was expressed in mean ± standard deviation(SD) (n=4). The data obtained by the various parameters are statistically evaluated by ANOVA (analysis of variance) followed by Dunnett's multiple comparision test using graph pad prism software. P value less than 0.05 was considered as statistically significant.[8]

3. RESULTS

In this study, urolithiasis in rats was induced by administration of ethylene glycol(0.75%v/v) and ammonium chloride(2%w/v) in drinking water for 21days. To assess the antiurolithiatic activity of vitis vinifera the parameters such as urine volume, calcium, creatinine and uric acid levels in serum were estimated. Tissue homogenate was also analysed for calcium, creatinine and uric acid levels.

3.1. Urine volume

Urine volume was estimated in all the groups of rats.

Table-2: Effect of vitis vinifera on urine volume(ml).

Groups	Urine volume
Normal control	4.99±0.04
Urolithiatic control	3.00±0.09 #
Standard drug treatment	4±0.16*
Plant low dose treatment(200mg/kg)	4.10±0.36*
Plant high dose treatment(400mg/kg)	4.25±0.27*

All values are expressed as mean ±SD (n=4). #P<0.01 as compared to normal control. *p<0.01 as compared to urolithiatic control.

In urolithiatic control rats urine volume was decreased significantly when compared to normal control rats. It indicates the obstruction of crystals in the urinary tract. On preventive (low & high doses) treatments significant increase in urine volume were observed.

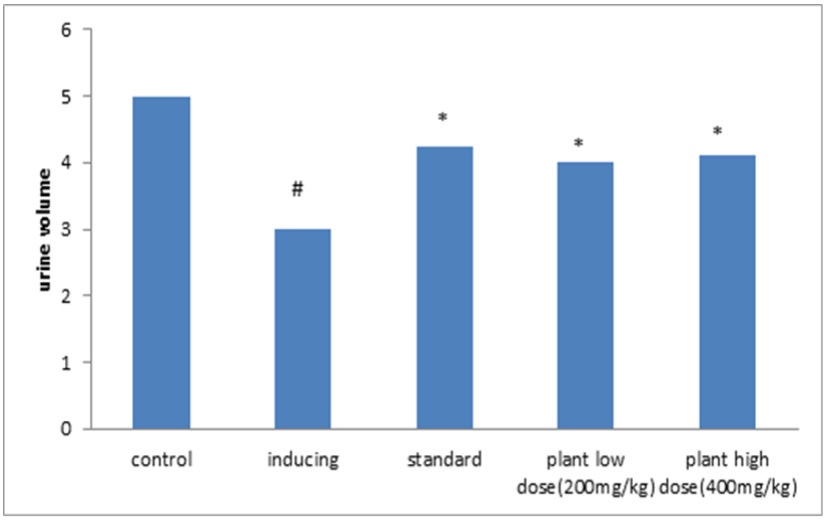


Figure-4: Graphical representation of effect of vitis vinifera on urine volume.

#P<0.01 when compared to normal control. *P<0.01 when compared to urolithiatic control.

3.2. Serum calcium

Serum calcium levels were estimated in all the group of rats at 0th day, 14th day and 21st day and the results were shown in the table.

Table 3: Effect of vitis vinifera on serum calcium.

Groups/days	Normal control	Urolithiatic control	Standard drug control	Plant low dose(200mg/kg)	Plant high dose(400mg/kg)
0th day	10.64±1.52	10.67±0.57	10.59±0.47	10.47±0.53	10.50±0.55
14th day	10.85±1.28	11.47±0.67#	10.91±0.63*	11.26±0.51*	11.03±1.01*
21st day	10.65±0.45	12.07±0.88#	10.86±1.24*	11.10±1.28*	10.96±0.97*

All the values are expressed as mean ± SD. #P<0.01 as compared to normal control. *p<0.01 as compared to urolithiatic control.

The serum calcium levels were significantly elevated in group of rats treated with ethylene glycol and ammonium chloride than normal rats. similar to cystone, treatment with vitis vinifera leaf extract, serum calcium levels were significantly reduced. In treatment groups of 14th and 21st day significant decrease in serum calcium levels were observed.

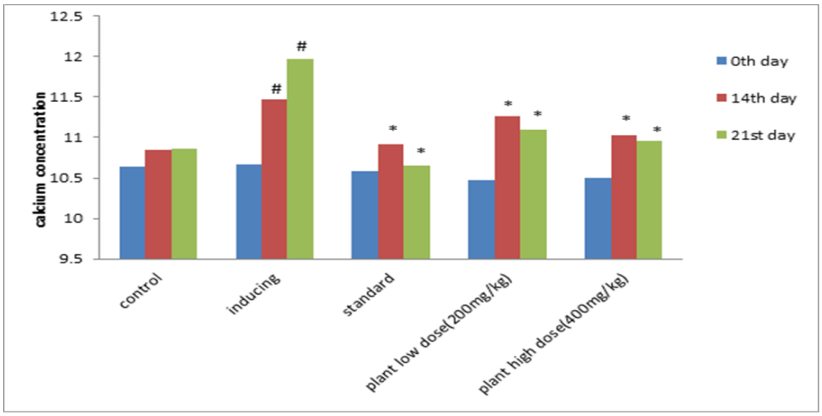


Figure 5: Graphical representation of serum calcium levels in rats.

#p<0.01 when compared to normal control.*p<0.01 when compared to urolithiatic control.

3.3. Serum creatinine

Serum creatinine levels were estimated in all groups of rats at 0th day, 14th day and 21st day and the results were shown in the table.

Table 4: Effect of vitis vinifera on serum creatinine.

Groups/days	Normal control	Urolithiatic control	Standard drug control	Plant low dose(200mg/kg)	Plant high dose(400mg/kg)
0th day	1.66±0.21	1.74±0.29	1.59±0.42	1.75±0.30	1.68±0.22
14thday	1.61±0.41	1.83±0.20#	1.63±0.39*	1.79±0.30*	1.74±0.25*
21st day	1.68±0.22	1.91±0.14#	1.66±0.38*	1.85±0.28*	1.79±0.29*

All values are expressed as mean ±SD (n=4). #P<0.01 as compared to normal control. *p<0.01 as compared to urolithiatic control.

The administration of ethylene glycol and ammonium chloride to rats resulted in elevation of serum creatinine levels significantly than the 0th day. In treatment groups of 14th day and 21st day significant decrease in serum creatinine levels were observed. The significant dose dependent reduction of serum creatinine levels were also observed between low dose and high dose treatments.

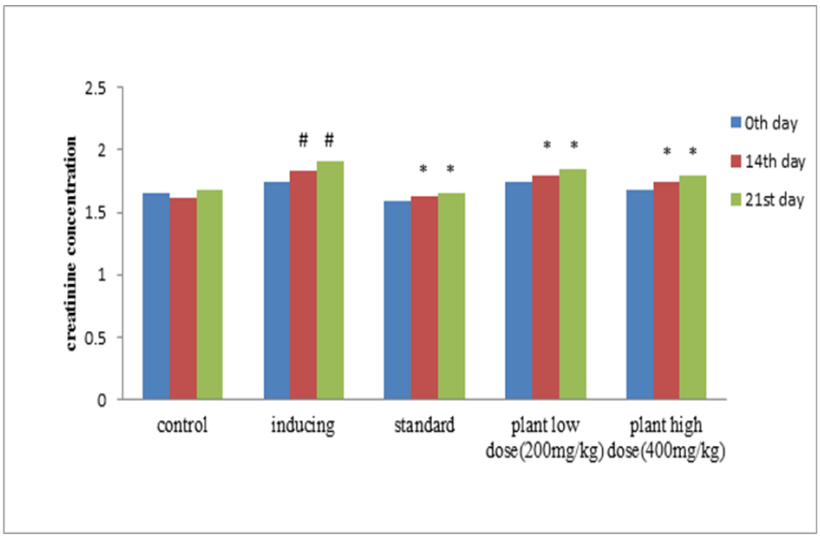


Figure 6: Graphical representation of serum creatinine levels in rats.

#p<0.01 when compared to normal control. *p<0.01 when compared to urolithiatic control.

3.4. Serum uric acid

Table 5: Effect of vitis vinifera on serum uric acid levels.

Groups/days	Normal control	Urolithiatic control	Standard drug treatment	Plant low dose(200mg/kg)	Plant high dose(400mg/kg)
0th day	4.95±0.15	4.94±0.31	4.84±0.60	4.73±0.12	4.98±0.40
14thday	4.94±0.22	5.55±0.56#	4.98±0.16*	5.41±0.40*	5.24±0.32*
21st day	4.92±0.20	5.85±0.80#	4.95±0.52*	5.31±0.22*	5.19±0.54*

All values are expressed as mean ±SD (n=4). #P<0.01 as compared to normal control. *p<0.01 as compared to urolithiatic control.

Uric acid levels were evaluated as a renal biomarker. The serum uric acid levels were significantly elevated in groups of rats induced with ethylene glycol and ammonium chloride than normal rats. In treatment groups on 14th and 21st day significant decrease in serum uric acid levels were observed. The significant dose dependent reduction of serum uric acid levels were also observed. The significant dose dependent reduction of serum uric acid levels were also observed between low dose and high dose treatments.

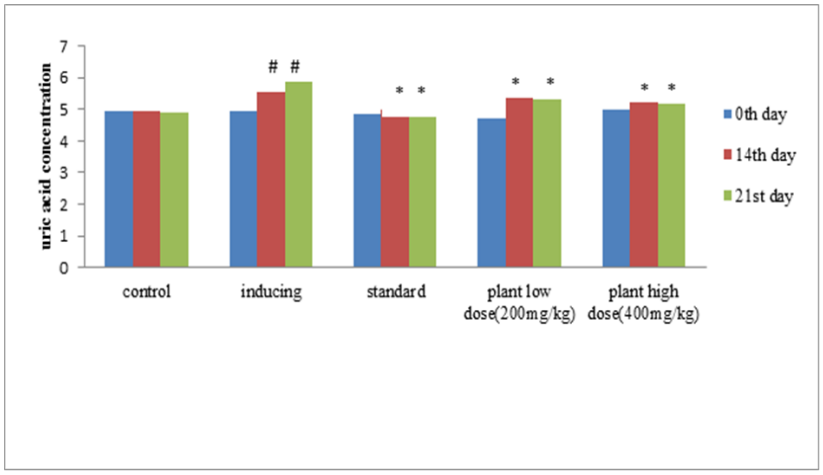


Figure 7: Graphical representation of serum uric acid in rats.

#p<0.01 when compared to normal control. *p<0.01 when compared to urolithiatic control.

3.5. Analysis of tissue homogenate

The tissue (kidney) homogenate was analysed for deposition of calcium, creatinine and uricacid levels and the results were as follows.

3.5.1. Deposition of calcium in kidney homogenate

Calcium deposition levels were estimated in all the groups of rats at last day. Increased levels indicate more deposition of crystals within the renal tubules and renal damage state.

Table-6: Calcium values in kidney homogenate.

Groups	Calcium concentration(mg/dl) in kidney
Normal control group	10.65±0.88
Urolithiatic control	12.65±1.36#
Standard drug treatment	10.71±0.91*
Plant low dose(200mg/kg)	11.14±1.13*
Plant high dose(400mg/kg)	11.00±0.86*

All values are expressed as mean ±SD (n=4). #P<0.01 as compared to normal control. *p<0.01 as compared to urolithiatic control.

Retention or deposition of calcium in kidneys was significantly increased in urolithiatic control group when compared to normal control rats. Accumulation of high amount of calcium in kidneys indicates more deposition of calcium oxalate or calcium phosphate crystals within the renal tubules and renal tissue damage. On treatment with vitis vinifera leaf extract significantly reduced the retention or deposition of calcium levels in kidneys. Significant dose dependent reduction of calcium deposition was also observed between high and low dose treatment.

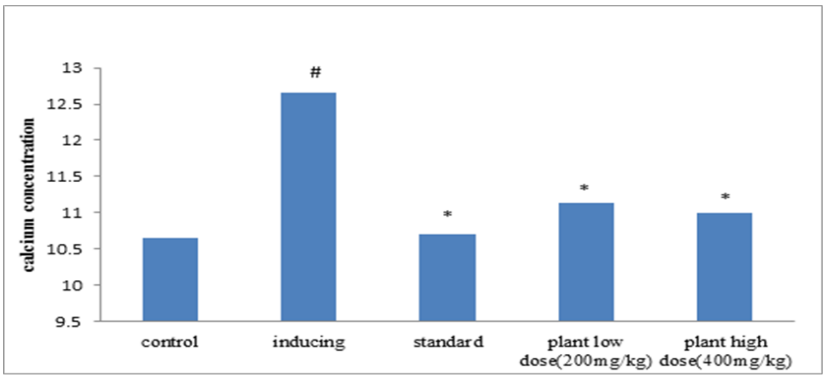


Figure-8: graphical representation of calcium deposition levels in kidney homogenate.

#p<0.01 when compared to normal control. *p<0.01 when compared to urolithiatic control.

3.5.2. Deposition of creatinine in kidneys

Creatinine deposition levels were estimated in all the groups of rats at last day. Increased levels indicate more deposition of creatinine within the renal tubules and renal damage.

Table 7: Creatinine values in kidney homogenate.

Groups	Creatinine concentration(mg/dl)
Normal control	1.33±0.76
Urolithiatic control	2.28±0.23#
Standard drug treatment	1.34±0.42*
Plant low dose(200mg/kg)	1.59±0.43*
Plant high dose(400mg/kg)	1.41±0.36*

All values are expressed as mean ±SD (n=4). #P<0.01 as compared to normal control. *p<0.01 as compared to urolithiatic control.

In urolithiatic control the retention or deposition of creatinine in kidneys was significantly increased when compared to normal control rats. Accumulation of high amount of creatinine in kidneys indicates renal tissue damage. On treatment with leaf extract of vitis vinifera significant reduction of deposition of creatinine levels in kidney was observed.

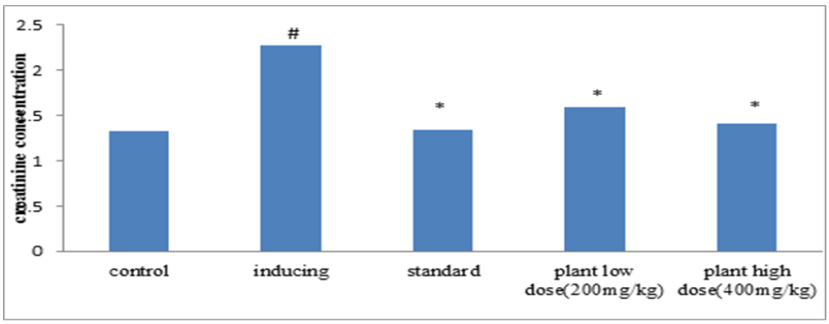


Figure 9: Graphical representation of creatinine deposition levels in kidney homogenate.

#p<0.01 when compared to normal control. *p<0.01 when compared to urolithiatic control.

3.5.3. Deposition of uric acid in kidney homogenate

Uric acid deposition levels were estimated in all the groups of rats at last day. Increased levels indicate more deposition of uric acid within the renal tubule and renal damage.

Table-8: Uric acid levels in kidney homogenate.

Groups	Uric acid concentration(mg/dl) in kidneys
Normal control	3.67±0.44
Urolithiatic control	5.45±0.44#
Standard drug treatment	3.70±0.38*
Plant low dose(200mg/kg)	3.92±0.06*
Plant high dose(400mg/kg)	3.80±0.36*

All values are expressed as mean ±SD (n=4). #P<0.01 as compared to normal control. *p<0.01 as compared to urolithiatic control.

In urolithiatic control the retention or deposition of uric acid in kidneys was significantly increased when compared to normal control rats. accumulation of high amount of uric acid in kidneys indicates renal tissue damage. On treatment with leaf extract of Vitis vinifera significant reduction of deposition of uricacid levels in kidney was observed.

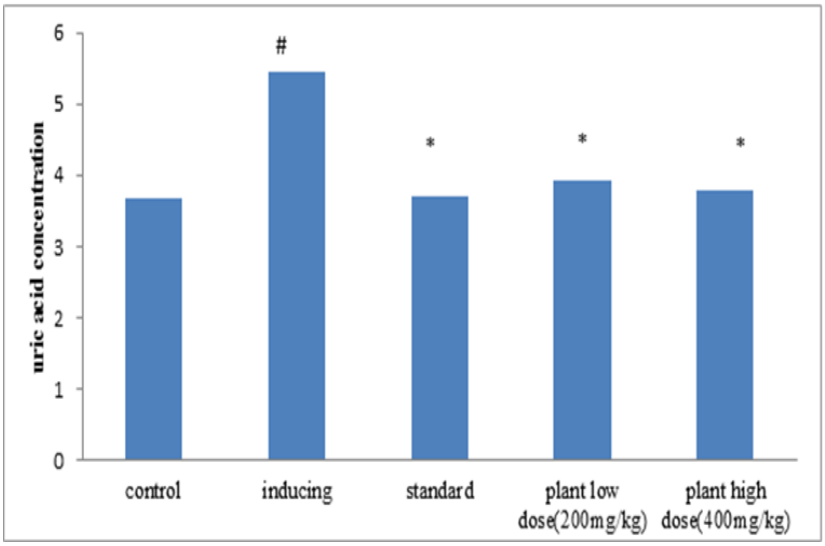


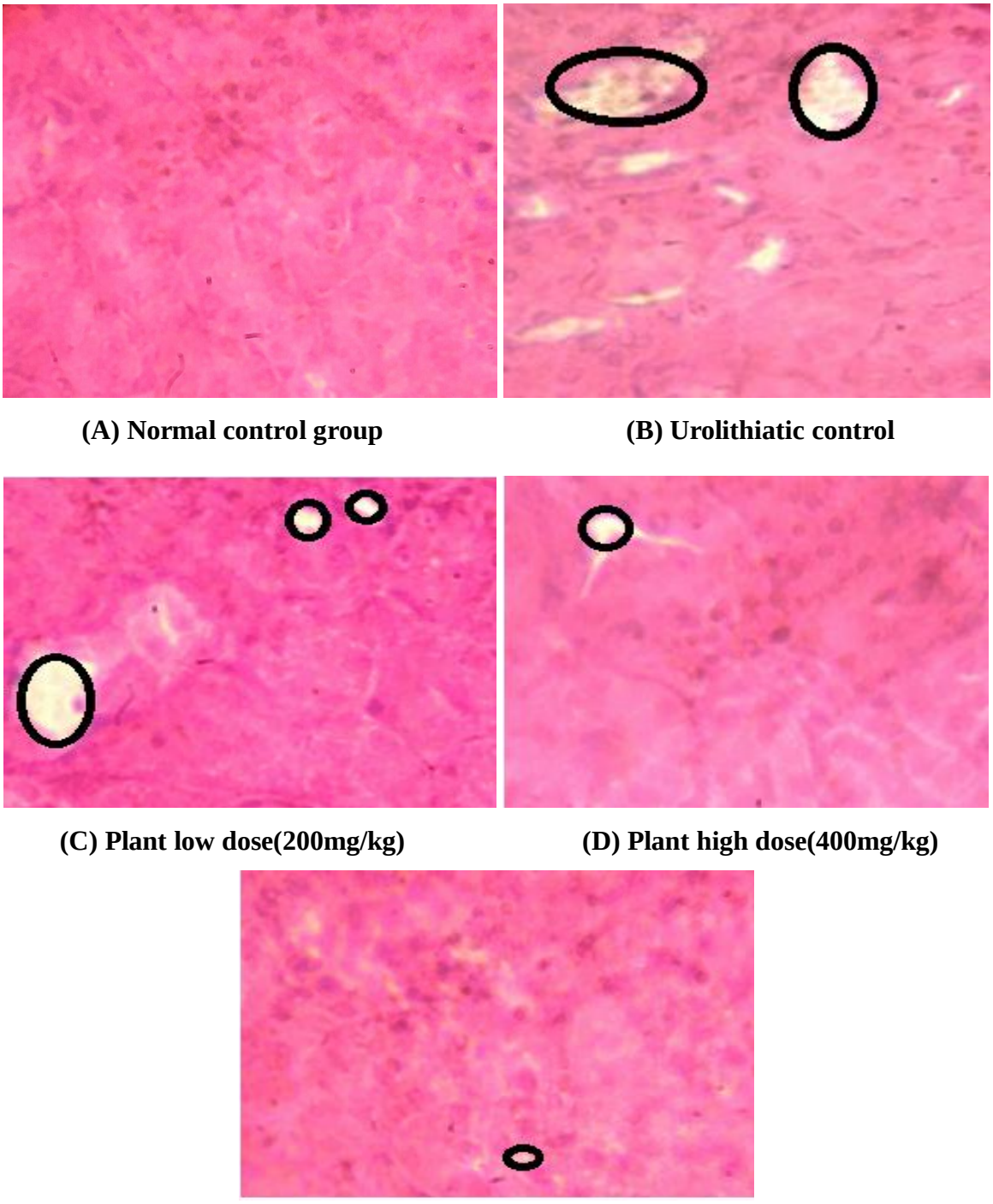
Figure-10: Graphical representation of uric acid deposition levels in kidney homogenate.

#p<0.01 when compared to normal control. *p<0.01 when compared to urolithiatic control.

3.6. Histopathological analysis of kidney

The antiurolithiatic effect was further confirmed by kidney histopathological studies.

The kidney tissues of treated animals from different groups were evaluated for histopathological examination and the results were shown in following figures.



(A) Normal control group **(B) Urolithiatic control**

(C) Plant low dose(200mg/kg) **(D) Plant high dose(400mg/kg)**

(E) Standard group

Figure-11: Light micrograph of kidney tissue from rats treated with (A) normal drinking water, (B)0.75%v/v ethylene glycol and 2%w/v ammomium chloride, (C) vitis vinifera leaf extract(200mg/kg), (D) vitis vinifera leaf extract(400mg/kg), (E) standard drug cystone(750mg/kg).

GROUP 1: (Normal control)

The analysis of histopathological examination of rat kidney treated with normal drinking water showed no damage to the tissue and lesions were not observed.

GROUP 2: (Urolithiatic control)

Rats treated with 0.75%v/v ethylene glycol and 2%w/v ammonium chloride showed renal epithelial cells with more tubular dilation and damage shown by large spaces in the tissue with crystal deposition.

GROUP 3 (plant low dose)

In kidney sections of rats treated with leaf extract of Vitis vinifera(200mg/kg), less crystal depositions were seen compared to untreated animals and the necrosis as well as the tubule dilation are very limited.

GROUP-4: (plant high dose)

Rats treated with leaf extract of Vitis vinifera(400mg/kg) showed less dilation of tubule, less damage to the tissue and crystal deposition is very limited compared to low dose treated rats.

GROUP-5 (Standard)

In kidney sections of the rats treated with cystone, no crystal depositions were seen and the renal epithelial tissue had less tubular dilation and the tissue damage is very limited.

4. DISCUSSION

The present study was aimed to evaluate the anti-urolithiatic activity of Vitis vinifera against ethylene glycol (0.75%v/v) and ammonium chloride (2%w/v) induced urolithiasis in rats by measuring the various urolithiatic biomarkers, renal biomarkers and by carrying the histopathological examination in rats. In this study male rats were selected to induce urolithiasis as Mohammad Reza Naghil et al., 2014 stated that testosterone appears to enhance stone formation by inhibiting osteopontin (inhibitor of CaOX crystal growth) in the kidneys and by increasing urinary oxalate excretion.

Previous studies by Atul Makasana et al., 2014 indicated that renal calculi was induced by treating male wistar albino rats with ethylene glycol(0.75%v/v) in drinking water for 21days. Further ammonium chloride (2%w/v) was co-administered with ethylene glycol for first 3days in order to induce lithiasis effect of ethylene glycol. Therefore, in the present study rats

were treated with ethylene glycol(0.75%v/v) and ammonium chloride(2%w/v) for 21days to induce urolithiasis.

Ethylene glycol is a metabolic precursor of oxalate. Administration of ethylene glycol to rats resulted in hyper-oxaluria. Ingestion of ammonium chloride induces metabolic acidosis. So, it has been used in combination with ethylene glycol to promote the deposition of calcium oxalate crystals in rat kidneys.

This model was used to evaluate the preventive effect of vitis vinifera against urolithiasis. The oral administration of ethylene glycol and ammonium chloride causes significant elevation in urine calcium, serum creatinine, uric acid and reduction in the urine volume when compared with normal control group. Microscopic examination of kidney sections derived from ethylene glycol induced urolithic rats showed polymorphic irregular crystal deposits inside the tubules which causes renal damage.

In this study, treatment with leaf extract of Vitis vinifera for 21days as a preventive regimen causes significant increase in the urinary volume and in high dose treatment groups, there was a significant decrease in the serum calcium, creatinine and uric acid levels. The significant reduction in deposition (tissue homogenate) of calcium, creatinine and uric acid levels in the kidneys when compared to urolithiatic group. It concludes that Vitis vinifera can prevent the formation of urinary stones.

Due to the obstruction to the flow of urine caused by renal calculi in urinary system, the glomerular filtration rate decreases in urolithiasis. As a result, the substances like urea, creatinine and uric acid accumulates in blood. In rats induced with renal calculi, the serum levels of creatinine and uric acid are increased which indicates the renal damage.

Tissue injury and inflammation in these animals is due to exposure to crystals, leading to the generation of reactive oxygen species, development of oxidative stress, lipid peroxidation and depletion of antioxidant enzymes. Renal epithelial injury further promotes crystal retention, as epithelial injury exposes a variety of crystal adhesion molecules on epithelial surfaces and promotes stone formation. Probably antioxidant constituents of herbal formulation restore the renal antioxidant enzyme and prevent renal cell injury.

5. CONCLUSION

The current study was able to show the antiurolithiatic effect of leaf extract of vitis vinifera in ethylene glycol induced renal calculi model. The vitis vinifera leaf extract showed remarkable protection by strongly suppressing various urolithiatic promoters in serum, urine and kidney tissue. It has a significant diuretic action that can further help to flush those promoters in urine and prevention of new stone formation(recurrence). The mechanism underlying this effect is possibly mediated through antioxidant property and lowering the concentration of urinary stone forming constituents by diuretic activity. Vitis vinifera possess potential medicinal value and beneficial in the prevention of renal calculi.

Further studies need to be undertaken to explain the exact mechanism of this action of Vitis vinifera and can be used as a drug of choice in kidney stones.

ACKNOWLEDGEMENTS

I am thankful to Head of the department of Pharmacology and Principal of Vaageswari College of Pharmacy, Karimnagar, Telangana, India for providing me the facilities to carry out my research work.

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