Available online on 15.06.2024 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access

Research Article

Evaluation of Anti-Urolithiatic Activity of Ethanolic Extract of *Salacia Reticulata* against Ethylene Glycol Induced Urolithiasis in Wistar Albino Rats

Rajesh Asija[1]*, Amandeep Swami[2], Kanha Ram Sharma[3]

[*1] Principal & Professor, Maharshi Arvind Institute of Pharmacy, Jaipur, Rajasthan India
[2] Associate Professor, Maharshi Arvind Institute of Pharmacy, Jaipur, Rajasthan India
[3] PG Scholar , Maharshi Arvind Institute of Pharmacy , Jaipur, Rajasthan India

A B S T R A C T

Urolithiasis is a common disease estimated to affect approximately 12% of the population, with recurrence rates of 70-81% in men and 47-60% in women. It is one of the three most common diseases found in humans. The effects of oral administration of Salacia Reticulata ethanol extract were studied in Wistar rats. Ethylene glycol feeding not only induced hyperoxaluria but also increased renal excretion of serum calcium, creatinine, urea, uric acid, magnesium, and phosphorus. Supplementation with ethanol extract of Salacia Reticulata significantly reduced urinary oxalate, serum uric acid creatinine and increased levels of serum calcium, creatinine, urea, uric acid, magnesium and phosphorus. Increased deposition of stone-forming components in the kidneys of stone-induced rats was also significantly reduced by the ethanol extract of Salacia Reticulata. The results indicate that the ethanolic extract of Salacia Reticulata has anti-urolithiatic activity.

KEYWORDS *Salacia Reticulata*, Hyperoxaluria; Urolithiasis; Ethylene glycol.

A R T I C L E I N F O: Received 20 Dec 2023.; Review Complete 15 March 2024; Accepted 20 April 2024 ; Available online 15 June. 2024

Cite this article as:
Asija R, Swami A, Sharma KR, Evaluation of anti-urolithiatic activity of ethanolic extract of *Salacia Reticulata* against ethylene glycol induced urolithiasis in wistar albino rats, Asian Journal of Pharmaceutical Research and Development. 2024; 12(3):58-65.

DOI: http://dx.doi.org/10.22270/ajprd.v12i3.1394

*Address for Correspondence:
Rajesh Asija, Principal & Professor, Maharshi Arvind Institute of Pharmacy, Jaipur, Rajasthan India

INTRODUCTION:-

S. reticulata WIGHT (Sinhala: Kothala himbatu) is a large woody climbing shrub belongs to family Hippocrateaceae. The green- ish grey color bark of the plant is smooth, with white inside. The average dimension of a leaf is 3 – 6 inches long and 1 – 2 inches broad They are opposite and elliptic- oblong, base acute, apex abruptly acuminate. The plant has traditional uses which include Diabetes, Dysentery, Flu, Headache, Fever, Conjunctivitis, Menstrual Disorder, Anticancer, Blenorrghia, Jaundice, Vaginitis and Dyspepsia. Urolithiasis, one of the most painful ailments of the urinary tract disorder, has beset humans from centuries. Calcium oxalate (CaOx) is the primary constituent of the majority of stones formed in the urinary system of patients with urolithiasis. The medical managementof urolithiasis mainly involves techniques like extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy however; the prevention of recurrence of stone formation is not assured.

Kidney stones may have a combination of medications. Most types of stones contain a mixture of calcium and oxalates or phosphates. These substances are part of normal human nutrition and form important parts of the body such as bones and muscles. Kidney stones consist of crystals and proteins that grow until they break and enter the urinary tract. Stones contain calcium oxalate, calcium phosphate, or both and account for approximately 80% of all stones. It contains about 15% magnesium ammonium phosphate (struvite; these are often associated with infections) and small amounts of pure cystine or uric acid are present.

Among the different types of kidney stones, calcium oxalate is the most common. The formation of these stones involves many physicochemical events, from crystal nucleation and aggregation to storage in urine. Cystinuria and hyperoxaluria are two rare causes of metabolic diseases that often lead to kidney stones. In cystinuria, large amounts of the insoluble amino acid cystine are excreted in the urine, causing cystine stones to form. People with hyperoxaluria produce too much oxalates (a type of salt) in their bodies. When the solubility of oxalate in the urine is exceeded, the crystals precipitate and form stones. Hypercalciuria is hereditary and can lead to stone formation in more than half of patients. Calcium is absorbed from food and lost in urine. Excess calcium in the urine can cause calcium oxalate or calcium phosphate crystals to form in the kidneys or elsewhere in the urine.

Therefore, *Salacia Reticulata* became our study target and we prepared the drug with a standardized protocol under regulation and used it for further investigation.

MATERIALS AND METHODS

The evaluation of anti- urolithiactic activity was done on wistar albino rats. This research would need different material and equipment's explained as follows:-

Plant collection

Plant material - Plant material: The plant of *Salacia Reticulata* plants were collected from the certified ayurvedic wholesaler from jaipur. The plant was identified and authenticated by Dr Bhima Ram choudhary , Department of Forest , Sikar

Preparation of *Salacia Reticulata* extract

The collected fresh plant material was dried in shade (2 days) and then dried in a hot air oven at 25ºC for three days and they were made in to coarse powder with the use of mixer. The powder of *salacia Reticulata* obtained were weighed separately and transferred to a round bottomed flask and then to continuous heat extraction with soxhlet apparatus using 95% ethanol for 24 hours. Then the extract of ethanol was concentrated. Extract obtained was dried by placing it on a big petri plate on electric water bath (70ºC) and then kept in an oven at 30ºC for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators.

Equipment

Autoanalyzer (Robonik), Refrigerator centrifuge (MPW-350R), UV-Spectro-phot ometer (UV-1601, Shimadzu Corporation, Kyoto, Japan), Mini Lyotrap (LTE Scientific Ltd.), Research centrifuge (Remi industries, Mumbai) and homogenizer (Remi Motors, Mumbai). Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India).

Phytochemical screening of extract

Phytochemical screening of plant extract shows the presence of carbohydrate, protein, flavonoids, alkaloids and saponins.

Drugs and Chemicals

- Cystone (Himalaya Pharmaceutical, Bangalore)
- Ethylene glycol (SRL Mumbai)
- Tween 80 (Merck Pvt Ltd, B, Mumbai)
- Anaesthetic ether (SD Fine chem Ltd., Mumbai)
- Chloroform (SD Fine chem Ltd .Mumbai)
- Formaline (SD Fine chem Ltd., Mumbai) and all chemicals and reagents were ofanalyticalgrade

Diagnostic kits

Diagnostic kits used for estimation of Creatinine, Urea, Uric acid, Calcium, Phosphorus, Calcium oxalate were procured from **Robonik Diagnostic Ltd India.**

Preparation of Acute toxicity study

Procedure: Acute toxicity studies were performed according to OECD-423guidelines category IV substance (acute toxic class method). Swiss albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fastedfor 4 hrs with free accessto water only. The plant extracts of *salacia Reticulata* were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed in only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose.

Pharmacological screening for antiurolithic activity

Animals: Wistar albino male (180–220 g) was obtained from the central animal house of Sigma Institute of Clinical Research and administration Pvt Ltd Hyderabad. The animals were housed at room temperature (22-28 ºC) for 12 hr dark and 12 hr light cycle and given standard laboratory feed and water *ad-libitum*. The study was approved and conducted as per the norms of the Accuprac research Laboratory .

Experimental Design:-Ethylene glycol induced urolithiasis model

Thirty healthy adult Wistar albino strain rats of either sex weighing 180-220g were randomly divided into five groups. Each group consisted of 6 animals. The treatment period was considered for 10 days.

Group 1: Normal rats were fed with standard rat chow diet and tap water ad libitum for 10 days.

Group 2: EG and ammonium chloride intoxicated rats were given normal lab diet + drinking water containing 0.75% [v/v] ethylene glycol (EG) for 10 days to induce urolithiasis.

Group 3: Standard group were fed with normal diet and drinking water containing 0.75% [v/v]EG and Cystone (5 ml/kg) for 10 days.

Group 4: the test groups treated with ethanolic extract of *Salacia Reticulata* 200 mg/kg with normal lab diet and drinking water containing 0.75% [v/v] EG.

Group 5: the test groups treated with ethanolic extract *Salacia Reticulata* 400 mg/kg of body weight were fed with normal diet and drinking water containing 0.75% [v/v].

Assessment of Anti-urolithic Activity

Collection and Analysis of Urine: The urine samples of the test animals in different groups were collected in their respective end day of the experiment (1%) EG model on 10th day in (0.75%) EG model. The collected urine sample volume and PH were measured followed by centrifugation at 3000 rpm for 10 minutes. After centrifugation the urine samples were examined under light microscope (LAICA, DME Germany 400X) to ensure the presence of oxalate microcrystal followed by biochemical analysis (urine oxalate, calcium and uric acid, creatinine, urea, magnesium and phosphorus).

Serum Analysis: The blood samples were collected from the animals under anaesthesia (ether) before sacrificing. The collected blood samples were then centrifuged to obtain serum for the analysis of serum creatinine and serum calcium, creatinine, urea, uric acid, magnesium and phosphorus

Kidney homogenate analysis: A portion of kidney was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of protein, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA).

Histopathology: Normal rat's kidney showed normal cellular structure. The histopathology of kidney samples of rats treated with EG (0.75) **control** group showed loss of normal architecture with presence of white chalky coloured calcium oxalate crystals in several tubules and glomeruli in sections. The histopathology of kidney rats treated with **standard** drug cystone 5ml/kg and EG for 10 days showed normal architecture of the kidney. The histopathology of kidney samples of rats treated with **ESR 200mg/kg** and EG for 10 days showed mild colloidal cast inside tubules and **ESR 400mg/kg** showed cloudy changes and congestion of these glomeruli.

Statistical analysis: Results were indicated in terms of mean ± SEM. Statistical significance of data were assessed by analysis of variance (One way-ANOVA), followed by comparison between different groups using 'Dunnett's multiple comparison test. The significance was considered at the level of $P<0.05$.

Biochemical parameters: The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kit using Auto analyzer.

The results of preliminary phytochemical studies of the plant extract are presented in **Table follows:**

Phytoconstituents	**Presence or Absence**
Carbohydrates	+
Glycosides	+
Fixed oils and fats	+
Gums & mucilage	-
Potein & amino acids	-
Saponins	++
Tannins	+
Phytosterols	+
Flavonoids	+++
Alkaloids	+++

Presence: +, Absence: -

RESULTS

Effect of ethanolic extract of Salacia Reticulata on urine biochemical parameters against EG induced urolithiasis.

Urinary Creatinine

Administration of EG (0.75%) for 10 days caused significant increased ($p<0.001$) in urine creatinine concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p<0.01$) in urine creatinine concentration when compared to EG alone treated group. Pretreatment with ESR 200 and 400mg/kg causes significant reduction (not significant and $p<0.001$) in urine creatinine concentration when compared to EG alone treated group.

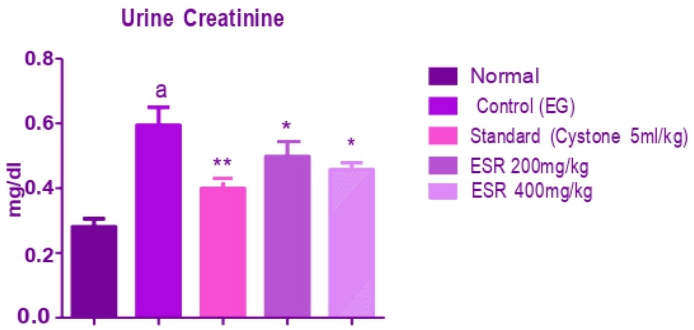


Figure1: Effect of ethanolic extract of *Salacia Reticulata* on urine creatinine parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, **p<0.01 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Urea

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine urea concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine urea concentration when compared to EG alone treated group. Pretreatment with *ESR* 200 and 400mg/kg causes significant reduction (p<0.01 and p<0.001) in urine urea concentration when compared to EG alone treated group.

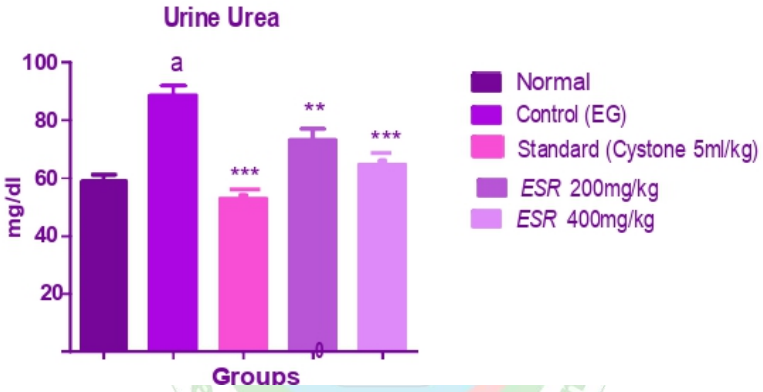


Figure 2: Effect of ethanolic extract of *Salacia Reticulata* on urine urea parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001, as when comparedto normal.*

Urinary Calcium

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine calcium concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine calcium concentration when compared to EG alone treated group. Pretreatment with *ESR* 200 and 400mg/kg causes significant reduction (p<0.05 and p<0.001) in urine calcium concentration when compared to EG alone treated group.

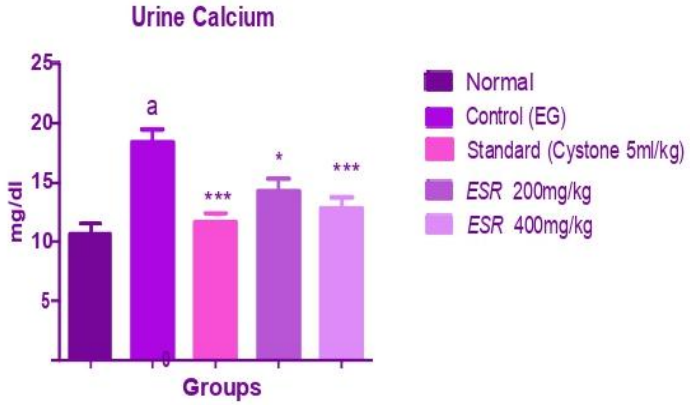


Figure 3: Effect of ethanolic extract of Salacia Reticulata on urine calcium parameters against EG induced urolithiasis..

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Oxalate

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine oxalate concentration compared to normal one. Standard cystone5ml/kg causes significant reduction (p<0.001) in urine oxalate concentration when compared to EG alone treated group. Pretreatment with *ESR* 200 and 400mg/kg causes significant reduction (p<0.001 and p<0.001) in urine oxalate concentration when compared to EG alone treated group.

.

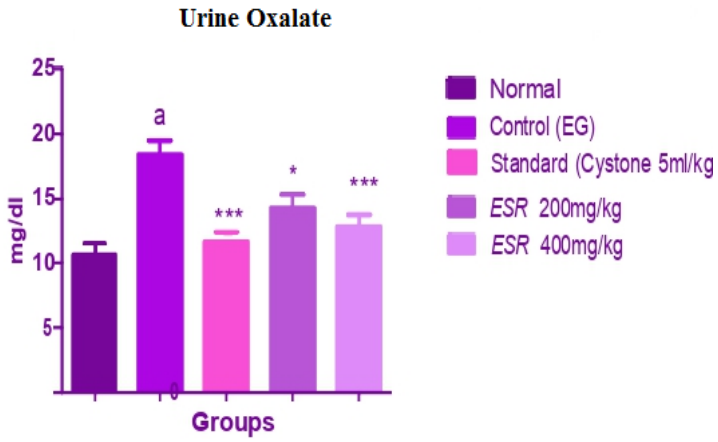


Figure 4: Effect of ethanolic extract of *Salacia Reticulata* on urine oxalate parameters against EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Phosphorus

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine phosphorus concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine phosphorus concentration when compared to EG alone treated.

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine phosphorus concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction (p<0.001) in urine phosphorus concentration when compared to EG alone treated group. Pretreatment with *ESR* 200 and 400mg/kg causes significant reduction (p<0.05 and p<0.001) in urine phosphorus concentration when compared to EG alone treated group.

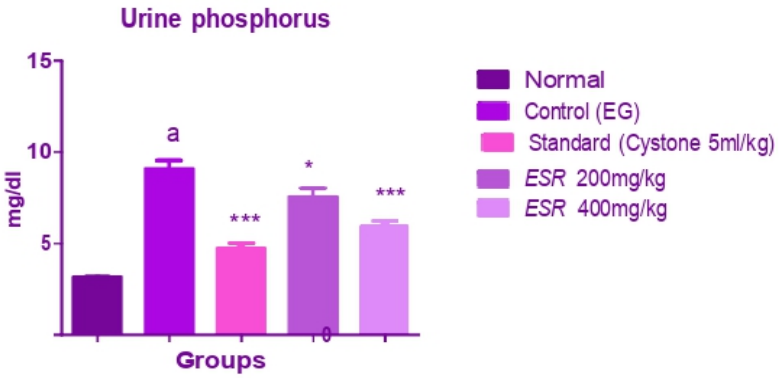


Figure 5: Effect of ethanolic extract of *Salacia Reticulata* on urine phosphorus parametersagainst EG induced urolithiasis.

*All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, ***p<0.001 as compared to control and [a]p<0.001, as when compared to normal.*

Urinary Magnesium

Administration of EG (0.75%) for 10 days caused significant increased (p<0.001) in urine magnesium concentration compared to normal one. Standard cystone 5ml/kg causes significantreduction (p<0.001) in urine magnesium concentration when compared to EG alone treated group. Pretreatment with *ESR* 200 and 400mg/kg causes significant reduction (p<0.05 and p<0.001) in urine magnesium concentration when compared to EG alone treated group.

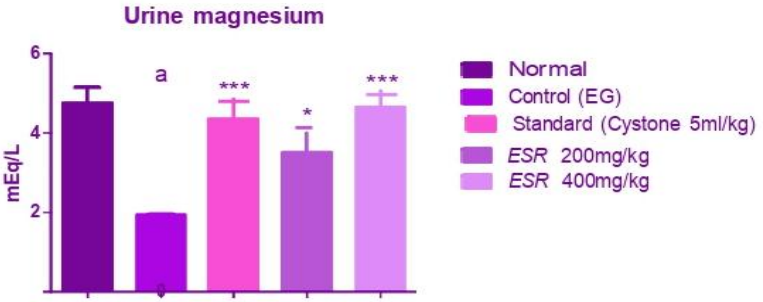


Figure 6: Effect of ethanolic extract of Salacia Reticulata on urine magnesium parameters against EG induced urolithiasis.

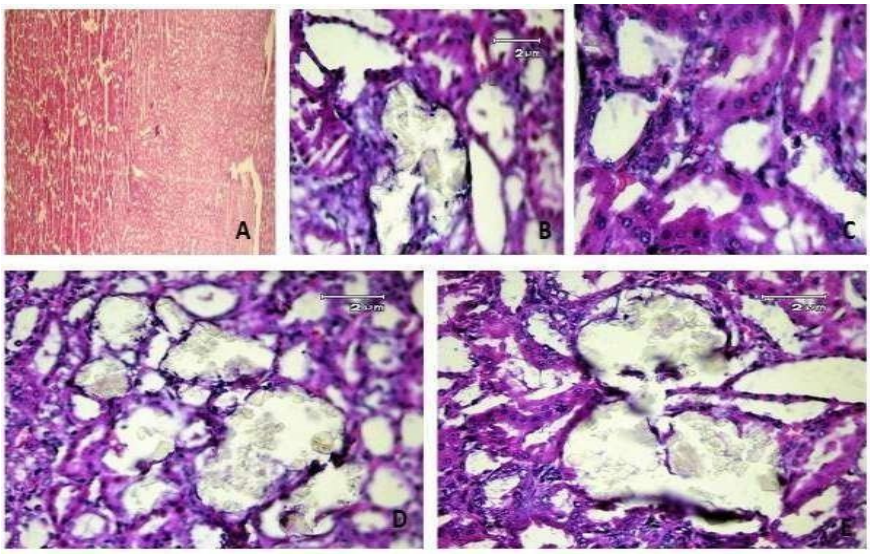
All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, ***p<0.001 as compared to control and ap<0.001, as when compared to normal.

Table 2: Effect of ethanolic extract of *Salacia Reticulata* on urine biochemical parameters against EG induced urolithiasis.

Treatmentgroup	**Urine Biochemical Parameters**						
Normal	**Creatinine mg/dl**	**Urea mg/dl**	**Uric Acid mg/dl**	**Calcium mg/dl**	**Oxalate mg/dl**	**Phosphorus mg/dl**	**Magnesium mg/dl**
Control (EG0.75%)	0.28±0.02	58.94±2.35	2.31±0.16	10.65±0.83	6.81±0.41	3.195±0.01	4.77±0.37
Standard Cystone(5ml/kg)	0.59±0.05a	88.82±3.19a	6.01±0.28a	18.49±1.03a	15.65±1.22a	9.093±0.46a	1.94±0.01a
ESR 200mg/kg	0.40±0.01**	53.05±2.10***	2.99±0.36***	11.62±0.72***	7.03±0.46***	4.758±0.27***	4.36±0.48***
ESR 400mg/kg	0.49±0.04ns	73.48±3.53**	3.56±0.25***	14.37±0.98*	7.60±0.57***	7.58±0.45*	3.51±0.48*

HISTOPATHOLOGY OF KIDNEY

Normal rat kidneys had normal cellular structure. Histopathology of rat kidney samples treated with control EG (0.75) showed loss of normal architecture with the presence of crystalline structures in the dilated collecting ducts. In the same section, white, chalky calcium oxalate crystals were found in several tubules and glomeruli when observed under a polarizing microscope. Interstitial hyperemia and inflammation of the pelvic cup system were also observed in these groups. Kidney histopathology of rats treated with the standard drug Cystone at a dose of 5 ml/kg and EG for 10 days showed normal kidney architecture. Histopathology of kidney samples from rats treated with 200 mg/kg doses of ESR and EG for 10 days showed light colloidal casts within the tubules, while 400 mg/kg doses of ESR showed cloudy changes and hyperemia in the glomeruli. Appeared. However, the kidney structure was almost normal.



A: Normal group, B: EG group , C: Standard (Cystone 5ml/kg) group ,

D: group *ESR* 200mg/kg , E :group *ESR* 400mg/kg

DISCUSSION

Urinary stone disease, which is both painful and costly, is a prevalent medical condition. While extracorporeal shock wave lithotripsy has facilitated the removal of stones and reduced the associated morbidity, recurrence is unfortunately common. Several experimental and clinical studies have demonstrated the efficacy of certain plants used in the Indian traditional system of medicine for managing renal stone disease. Therefore, it is advisable to evaluate these plants for Antiurolithiatic activity, which may also help reduce the recurrence rate of stones.

Rats are commonly used to study the pathogenesis of human CaOx kidney stones, as oxalate metabolism is similar in rats and humans. Ethylene glycol (EG) has been found to be a reliable inducer of oxalate lithiasis in rats, as it is converted to endogenous oxalic acid by the liver enzyme glycolate oxidase and acetum induces urinary acidification, which is supposed to disrupt the enzyme sorting mechanism in the tubular cells of the kidney, thereby favoring the adhesion and retention of CaOx particles within the renal tubules. In this study, EG in drinking water was used to induce hyperoxaluria in rats. The urinary super saturation level in relation to stone-forming constituents, mainly urinary oxalate, is a critical factor in renal calculi formation, as it complexes with calcium to form insoluble CaOx crystals.. Enhanced deposition and urinary excretion of calcium and oxalate in the preventive and curative control group animals indicate that administration of EG induced hyperoxaluria. An increase in the kidney weight and enhanced urinary creatinine excretion in the control group animals also substantiated these results.

On administration of *ESR* , the dose-dependent reduction in calcium and oxalate deposition inthe kidneys and their urinary excretion in control groups implies the potential of *ESR* in preventing the formation and dissolving the preformed CaOx stones.

On treatment with the extract and standard cystone, the significant reduction in the elevated urinary creatinine, urea, uric acid, calcium, phosphorus, oxalate and magnesium in the treated groups reflects the improvement in hyperoxaluria induced renal impairment. Dissolution of calculi can be achieved by alteration in urinary pH. If the pH is 5.0 or below, the stones likelyto form are of uric acid type, if 5.0-6.5, calcium oxalate type and if above 7 indicates crystals of magnesium ammonium phosphate. In the present study, a decrease in the normal urine pH of 7.0-7.5 to 5.5-6.0 in the control groups, indicates hyperoxaluria induced CaOx stone formation. In the treated groups, *ESR* and cystone 5ml/kg administration restored the pH to 6.5-7.5, supporting the decrease in the deposition and excretion of calcium and oxalate.

Mucoproteins exhibit a significant affinity for the CaOx surface, which promotes the growth of crystals and cements them in place. Flavonoids, on the other hand, disintegrate mucoproteins, thereby preventing calcium and oxalate deposition and excretion. Preliminary phytochemical screening of ESR revealed the presence of flavonoids, suggesting that they may have reduced calcium and oxalate deposition by pre-coating CaOx crystals and disintegrating mucoproteins in the ESR-treated groups. The stone-forming effects of EG are also attributed to its hyperoxaluria- induced oxidative damage. Oxalate has been reported to induce LPO and cause renal tissue damage due to its high polyunsaturated fatty acid content, making the kidney susceptible to ROS attack. Excessive generation of ROS and/or a reduction in cellular antioxidant levels can result in the development of OS, with MDA being one of the most common byproducts of ROS-induced OS. Increased levels of MDA, diminished levels of GSH, and catalase in the control groups indicate that EG administration promoted extensive generation of ROS, which may have consumed GSH and catalase excessively, impairing antioxidant protection. Unquenched ROS may have provoked cellular damage and enhanced OS, which might have further favored the accumulation and retention of oxalate and subsequent deposition of CaOx. Studies have shown that treatment with antioxidants prevents CaOx deposition in the kidney and reduces Ox excretion. Daily consumption of tea has been shown to reduce the risk of kidney stone formation in women by 8%. Low concentration of renal cellular glutathione also promotes LPO and subsequent retention of calcium and oxalate in the kidneys.

Health benefits of tea are due to its antioxidant properties of flavonoids which act by quenching ROS and also by chelating metal ions like iron and copper. In the present study, lowered levels of MDA and enhanced levels of antioxidant enzymes, GSH and catalase in the kidneys of the *ESR* treated animals indicate attenuation of hyperoxaluria induced LPO and oxidative damage. Flavonoids may have minimized ROS by free radical scavenging and prevented further generation, by metal chelating property. Thus, the flavonoid principles of *Salacia Reticulata* might have been responsible for the inhibition of CaOx crystal aggregation and stone formation. The results support the use of *Salacia Reticulata* plant as an effective alternative in treating CaOx urolithiasis. Disintegration of the mucoproteins and pre-coating of CaOx crystals by antioxidant effect of flavonoid principles may be responsible for the possible antiurolithiatic activity of *Salacia Reticulata*. Further studies are necessary to find out the chemical components responsible for the antiurolithiatic activity of *Salacia Reticulata*

CONCLUSION

To summarize, the data suggests that administering ESR plant to rats with ethylene glycol/Ammonium chloride led to the prevention of urinary stone formation and the induction of lithiasis. This finding supports the traditional beliefs about the antiurolithiatic activity of the plant. The specific mechanism underlying this effect is not yet understood, but it seems to involve diuresis and the reduction of stone-forming compounds in the urine. Additionally, the plant may provide protection against oxalate-induced lipid peroxidation, which could contribute to the recovery of renal damage. In light of these findings, it is possible to conclude that *Salacia Reticulata* possesses antiurolithiatic properties.

SUMMARY

In this study, the powdered form of Salacia Reticulata was subjected to extraction with 90% ethanol. Part of the extracted substance was reserved for preliminary phytochemical analysis, while the rest was used for pharmacological screening. The findings of the phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, saponins, and flavonoids. The pharmacological screening included an evaluation of the antiurolithiatic activity of the extract in a male Wistar albino rat model using 0.75% ethylene glycol-induced urolithiasis. The results showed that the kidney stone formation induced in rats by chronic administration of 0.75% ethylene glycol was significantly inhibited by oral administration of the extract.

Administration of the extract and Cystone 5ml/kg caused a significant increase in urine output and urine pH compared to the control group. Pretreatment with standard Cystone 5ml/kg and ESR 200 and 400mg/kg caused a significant reduction in serum creatinine, urea, uric acid, calcium, oxalate, phosphorus, and magnesium concentrations in the treated groups compared to the control group. Additionally, significant reductions in urinary creatinine, urea, uric acid, calcium, oxalate, phosphorus, and magnesium concentrations were observed in the treated groups compared to the control group. After treatment with standard Cystone 5ml/kg and ESR 200 and 400mg/kg, significant reductions in kidney MDA levels were observed in the treated groups compared to their respective control groups.

Catalase and GSH levels in the kidney were significantly decreased in the control group after administration of 0.75% ethylene glycol for 10 days compared to the normal group. However, after treatment with standard Cystone and ESR 200 and 400mg/kg, significant increases in renal catalase and GSH levels were observed in the treated groups.

REFERENCES

1. Viel TA, Domingos CD, Monteiro APDS, Lima-Landman MTR, Lapa AJ, Souccar C. Evaluation of the antiurolytic properties of costus extracts in rats. Journal of Ethnopharmacology, 1999; 66:193-8.
2. Kaur T, Bijarnia RK, Singla SK, Tandon C. In vivo activity of anticalcific proteins in the mouse model of Trachyspermum ammiurolithiasis. Journal of Ethnopharmacology, 2009; 126:459–62.
3. Hossein Hosseinzodeh, Ali-Roza khooi, Zahra khashayavmanesh, Vahideh motamed-shariaty. Urinary tract anticalculus activity of Pinus Eldarica Medw. Rat fruit extract. Journal of Urology, 2010; 7(4): 232-37.
4. Butterweck V. Khan SR. Herbal treatment of urolithiasis: alternative or complementary? Phytomedicine, 2009; 75: 1095-1103.
5. Kaur T, Bijarnia RK, Singla SK, Tandon C. Trachispermum ammiIn vivo activity of anticalcific proteins in the rat urolithiasis model. Journal of Ethnopharmacology, 2009; 126:459– 62.
6. Grases F, Costa Bauza A, Ramis M, Montesinos V, Conte A. Simple classification of kidney stones is closely related to their micromorphology and etiology. Acta Clin Chem 2002; 322:29-36.
7. Bihl G, Meyers A. Recurrent nephrolithiasis - advances in pathogenesis and treatment. Lancet, 2001; 358:651-56.
8. Smith LH. Calcium kidney stones. Nephrology, 1978; 13:383-89.
9. Borsatti A. Calcium oxalate nephrolithiasis: Impaired oxalate transport. Renal International, 1991; 39: 1283-98.
10. Branch Florida. Uric acid and calcium oxalate kidney stones. Renal International, 1983; 24: 392-403.
11. Seyedzadeh A, Momtaz HE, Moradi MR, Moradi A. Pediatric cystine stones in Western Iran. Urrol Magazine, 2006; 3(3): 134-38.
12. Fleisch H. Inhibitors and accelerators of rock formation. Kidney International, 1978; 13:361- 71.
13. Wilska. Kidney stones with bad symptoms at first. Manual Journal of Physiology, 2000; 23(3):196-01.
14. Portis AJ, Sundaram CP. Diagnosis and initial treatment of kidney stones. My Family Doctor, 2001; 63(7): 1329-38.
15. Del Vecchio Football Club, General Manager Preminger. Treatment of stone disease. Current Opinion in Urology, 2003; 13(3): 229-33.
16. Siener R, Ebert D, Nicolay C, Hesse A. Dietary intervention for hyperoxaluria in old calcium oxalate stones. Renal International, 2003; 63: 1037-43.
17. Prasad KVSRG, Bharthi K, Srinivasan KK. A review of Ammania baccifera Linn. Anti- urolytic activity in albino rats. Ind J Expt Biol, 1994; 32(5): 311-13.
18. Keller T, Janssen B, Hesse A. The effects of blackcurrant, cranberry and plum juice are beneficial on the risk associated with kidney stone formation. European Journal of Clinical Nutrition, 2002; 56:1020-23.
19. Bhaskar R, Varalakshmi P, Amsaveni R. Changes in tissue enzymes produced by Coleus in experimental urolithiasis. Indian Medicine, 1992; 29:254-58.
20. Harg TM, Rodgers A, Charlton K. Effect of cranberry juice on urinary action on calcium oxalate kidney stone formation. Br J Urrol, 2003; 92:765-68.
21. Christiana AJ, Lakshmi MP, Nagarajan M, Kurian S. Regulatory effects of Cyclea pertata lam.Ethylene glycol treatment causes stone formation in rats. Exp Clin Pharmacol 2002; 24:77-79.