

Evaluation of Anti Urolithic and Antioxidant Activities of Glochidion Velutinum Leaves

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ABSTRACT

Urolithiasis is a common urinary disorder characterized by the formation of renal calculi, predominantly calcium oxalate crystals. Hyperoxaluria, altered urinary mineral composition and oxidative stress play important roles in the development and progression of kidney stones. Glochidion velutinum is a medicinal plant reported to possess antioxidant and other pharmacological activities.

The present study was undertaken to evaluate the antiurolithiatic and antioxidant activities of aqueous and ethanolic leaf extracts of Glochidion velutinum.

The leaves of Glochidion velutinum were collected, shade-dried, powdered and extracted separately using water and ethanol. Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, carbohydrates and proteins/free amino acids. Antiurolithiatic activity was evaluated using an ethylene glycol-induced urolithiasis model in male Wistar rats. The extracts were administered orally at a dose of 200 mg/kg, while Cystone was used as the standard drug. Urinary and serum biochemical parameters, renal histopathology and renal antioxidant parameters including catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) were evaluated. In-vitro antioxidant activity was assessed by DPPH, hydrogen peroxide scavenging, nitric oxide scavenging, reducing power and anti-haemolytic methods.

Keywords: Glochidion velutinum, Urolithiasis, Antiurolithiatic activity, Calcium oxalate, Hyperoxaluria, Antioxidant activity, DPPH, Ethylene glycol, Renal protection.

INTRODUCTION

Kidney Stones

The incidence of kidney stones has been increasing in western societies in the last five decades, in association with economic development. Most calculi in the urinal system arise from a common component of urine, e.g. calciumoxalate (CaOx), representing upto 80% of analyzed stones. Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of Extracorporeal Shock Wave Lithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones.

However, in addition to the traumatic effects of shock waves, persistent residual stone fragments and the possibility of infection, suggest that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence (Begun, F.P. 1991). Urolithiasis is still a mysterious disease even after extensive research in urology. Sophisticated instruments, investigation etc., have failed to trace out the exact mechanism of urolithiasis, but they are manifesting this condition. The treatment in modern medicine is not

only expensive but also not easily affordable to the needy poor. Actually there is no satisfactory drug in modern medicine which can dissolve the stone and the physician remains to be depending on alternative systems of medicine for better relief.

The ionic composition of urine i.e. decreasing the calcium ion concentration or increasing the magnesium and citrate excretion. These remedies also express diuretic effect or lithotriptic activity. Drug with multiple mechanisms of protective action may be one way forward in minimizing tissue injury in human. Herbal medicines have several phytoconstituents and exert their beneficial effects in urolithiasis by multiple mechanisms.

1. Helps in spontaneous passage of calculi by increasing urine volume, PH and anti-calcifying activity.
2. Balance the inhibitors and promoter of the crystalisation in urine and effects the crystal nucleation, aggregation and growth (crystallisation inhibition activity)
3. Relieves the binding mucine of calculi (lithotriptic activity)
4. Improve renal functions.
5. Regulate oxalate metabolism.
6. Regulate the crystalloid colloid imbalance and improve renal function, thus prevents recurrence of urinal calculi
7. Improve renal tissue antioxidant status anscell membrane integrity and prevent reoccurrence (Antioxidant activity)
8. Exerts noteworthy anti-infective action in aligned with the chief causative organisms (antimicrobial activity).
9. Reveals marked improvement in symptoms of urinal calculi like pain, burning micturation and haematuria (Analgesic and anti-inflammatory activity)
10. In Herbal treatment of kidney stones, drugs used to dissolve the stone or aid their passing to guard aligned with further retention. Diuretic action is also needed to increasing the amount of fluid going through the kidneys and flush out the deposits (Arafat, OM, 2008). Lithotripsy means breaking and disintegrating or dissolution of the preformed stones. Some of the drugs increasing the urine volume decreasing the saturation of the salts and prevents the precipitation of the crystals at physiological pH. Some of the herbal drugs disaggregate mucoproteins, which actually binds the crystal to the renal cells (Atmani.F.2003). Stones occur when urinal chemistry consequences in saturation (SS) and exceeds the limit of metastability for that salt in solution. Increased urine volume decreases the saturation of the salts and prevents the precipitation of the crystal at physiological pH. All herbal medicines used for the treatment of the urolithiasis also have diuretic action and some are known to alkalize the urine.

Inhibitors are defined as molecules that increase the Super Saturation (SS) required to initiate nucleation, decrease crystal growth rate and aggregation, and inhibit secondary nucleation. In contrast promoters reduce the formation product of the supersaturated solution. Some of the common promoters are oxalate, calcium, cystine, uric acid and inhibitors are citrate and magnesium. An imbalance between urinal-promoting and inhibiting factors has been suggested as more important in urinal stone formation than a disturbance of any single substance (adhirai. M 1997). An assortment of physiological inhibitors of urolithiasis found in urine including inorganic (e.g., magnesium) and organic (e.g., Citrate, Urinal prothrombin fragment, Glycosaminoglycans (GAGs) and other macromolecule) substances are known to inhibit stone formation. Organic inhibitory compounds adsorb to the surface of the crystal, thereby inhibiting crystal nucleation, growth and aggregation. Interference with crystal growth and aggregation therefore seems a possible therapeutic strategy for the prevention of recurrent stone disease. The medicinal plants contain chemical

compounds like Glycosaminoglycans (GAGs) which themselves possess an inhibitor effect in the crystallization of calcium oxalate. Macromolecule of higher molecular weight of plant extract excerpts their action similar to natural urinal inhibitors and inhibits crystal.

In urine there are a number of crystalloids of different types (oxalate, uric acid, calcium, cystine) which are kept in solution by the presence of colloids (mucin and sulphuric acid) in the urine by the process of absorption. When there is imbalance in the crystalloid-colloid ratio, i.e., increase in crystalloid and fall in colloid level leading to formation of renal stones or when the colloid lose the solvent action or adhesive property, urinal stones are formed. In this condition the Glomerular Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinal system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood. Herbal therapy improves the renal function by increasing the excretion of urea and creatinine. Most of the phytotherapeutic agent exerts their antiurolithiatic effect through this mechanism given.

for precipitation and deposition of CaOx crystals. Herbal extract interfere with Hyperoxaluria is a most noteworthy risk factor in the pathogenesis of renal stone. It has been perceived and documented that oxalate play an important role in stone formation and has about 15-fold greater effect than urinal calcium. Increased oxalate concentration is responsible the metabolism of oxalate in male rats fed sodium glycolate. Glycolate feeding consequenced in hyperoxaluria as well as increased activities of oxalate synthesizing enzymes of the liver i.e., glycolate oxidase (GAO), glycolate dehydrogenase (GAD) and lactate dehydrogenase (LDH), and lessened kidney LDH activity. Increased excretion of phosphorus has been perceived and documented in stone formers.

Increase durinal phosphorus excretion along with oxalate estress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which induces calcium oxalate deposition. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of GAGs. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Supersaturation of these urinal colloids consequences in precipitation as crystal initiation particle which when trapped acts as a nidus leading to subsequent crystal growth and Cystone (polyherbal formulation) maintain crystalloid-colloid balance by decreasing excretion of urinal calcium, oxalate, uric acid, phosphorus and protein in urolithiasis.

In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of aqueous and alcoholic extract of Glochidion velutinum using ethylene glycol induced hyperoxaluria model in rats and some relevant symptoms like anti-analgesic, anti-inflammatory, antioxidant and antimicrobial activity studies also validated.

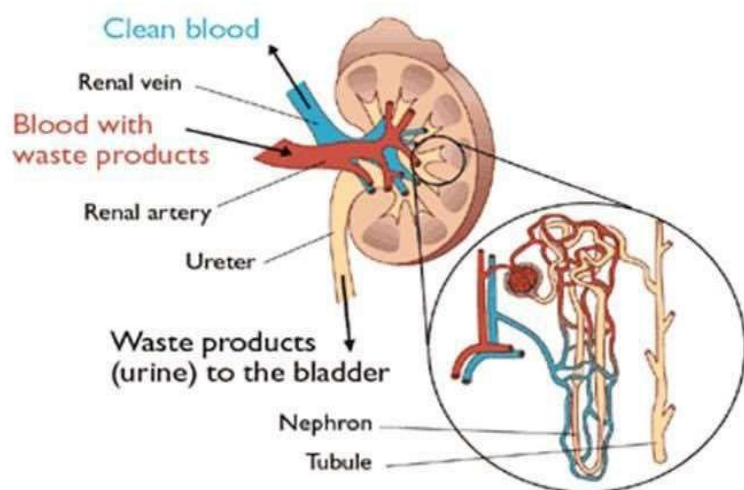
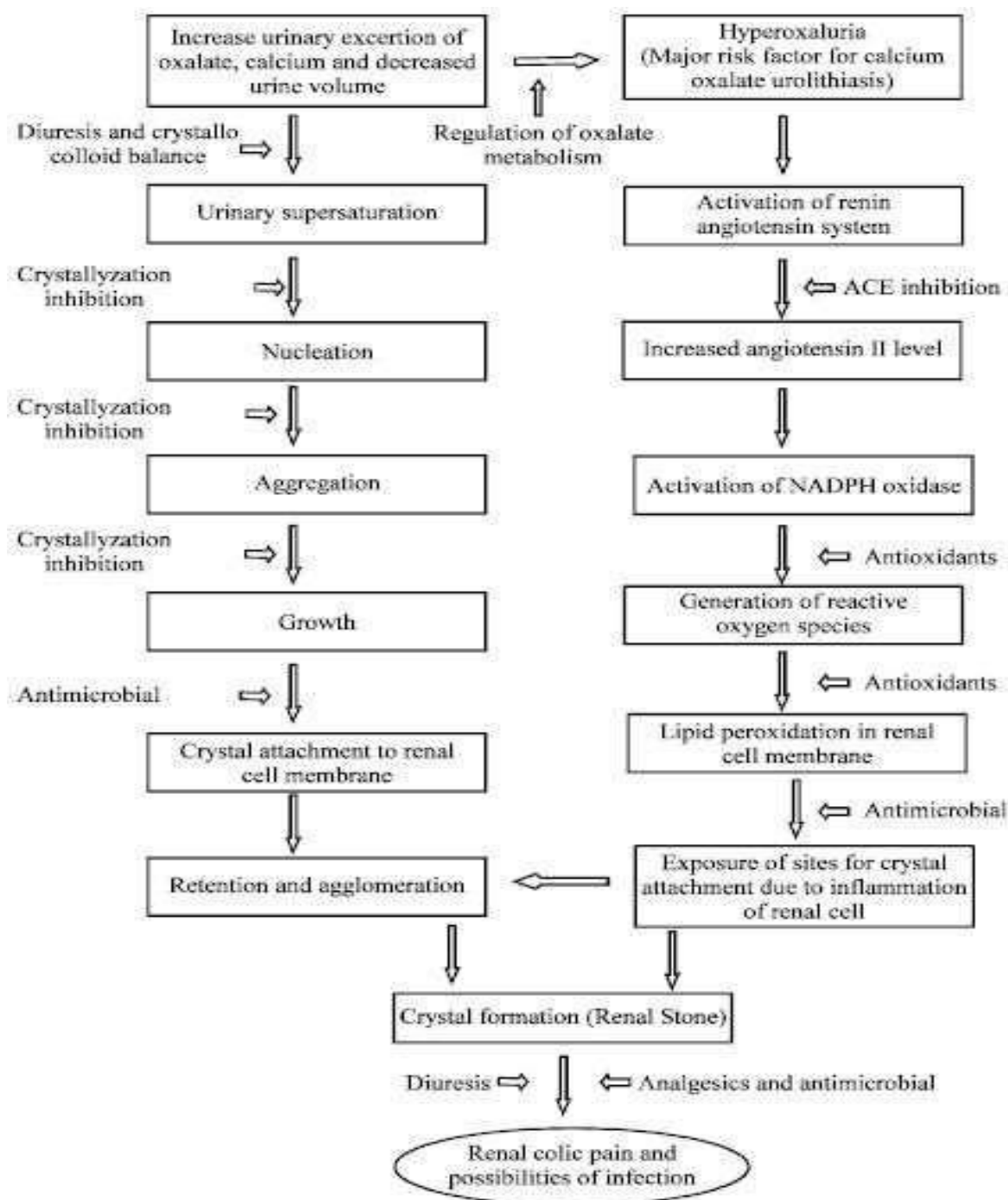


Fig.No:1: Functions of Kidney

Fig.No:2: Mechanism of Kidney Stone Formation



Classification of renal stones

Kidney stones may contain an assortment of combination of chemicals. The four most common types of kidney stones contain (Mukharjee.T. et al 1984)

- Calcium
- Struvite
- Uric acid
- Cystine

Aetiology of stone formation

An etiology of the urinary calculi is by no means clear, but the following possible factors may be

considered. Stones can be classified into those caused by infection, or non-infectious causes, genetic defects or adverse drug effects (drug stones)

- Non-infection stones
- Calcium oxalate
- Calcium phosphate (including brushite and carbonate apatite)
- Uric acid
- Infection stones
- Magnesium ammonium phosphate
- Carbonate apatite
- Ammonium urate
- Genetic causes
- Cystine
- Xanthine

2, 8-dihydroxyadenine

- Drug stones
- Dehydration
- pH of the urine
- Concentration of urinary salts
- Vitamin a deficiency
- Parathyroid hormone
- Prolonged immunity
- Nephrocalcinosis

Stone composition

Metabolic aspects are important in stone formation, and metabolic evaluation is required to rule out any disorders. Analysis in relation to metabolic disorders is the basis for further diagnostic and management decisions. Stones are often formed from a mixture of substances. Most renal calculi contain calcium, usually in the form of calcium oxalate (CaC_2O_4) and often mixed with calcium phosphate (CaPO_4). In most instances no specific cause can be identified, although most patients have idiopathic hypercalcuria without hypercalcaemia. Brushite is a unique form of calcium phosphate stones that tends to recur quickly if patients are not treated aggressively with stone prevention measures and are resistant to treatment with shock wave lithotripsy. Interestingly hyperuricosuria is also associated with increased calcium containing stone formation, and is thought to be related to the uric acid crystals on which calcium oxalate and calcium phosphate can precipitate. Rarely the underlying cause is primary oxaluria a liver enzyme deficiency leading to massive medullary nephrocalcinosis and renal failure. Small asymptomatic stones in the kidney can be safely ignored, and if patients maintain good states of hydration, the risk of recurrent symptoms can be dramatically abridged. In all settings a search for a possible underlying cause of hyperoxaluria/hypercalcuria should be sought and if present corrected when possible. Struvite stones Struvite (magnesium ammonium phosphate) stones are usually seen in the setting of infection with urease producing bacteria (e.g. *Proteus*, *Klebsiella*, *Pseudomonas* and *Enterobacter*), consequencing in hydrolysis of urea into ammonium and increase in the urinary pH. They can grow very large and form a cast of the renal pelvis and calices consequencing in so -called staghorn calculi. The struvite accounts for approximately 70% of these calculi, and is usually mixed with calcium phosphate thus rendering them opaque. Uric acid and cystine are also found as minor components. Struvite stones are usually large (staghorn calculi) and consequence from infection. These stones need to be treated surgically and the entire stone removed, including small fragments, as otherwise these residual fragments act as a reservoir for infection and recurrent stone formation. Uric acid Hyperuricosuria is not always associated with hyperuricoemia, and is seen in a variety of settings, although in most instances uric acid stones occur in patients with no identifiable underlying aetiology. Uric acid crystals form and remain insoluble at acidic urinary pH below 5.

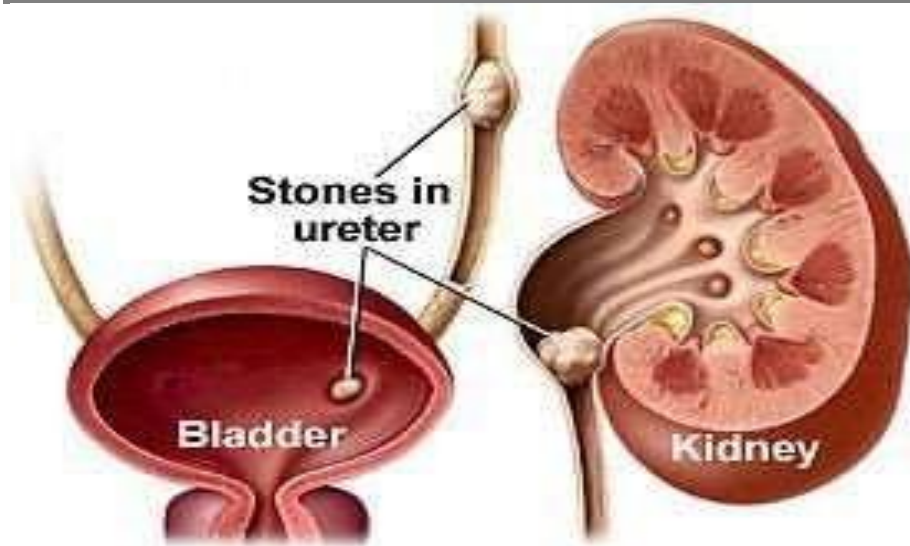


Fig. No: 3: Location of Kidney Stone

Cystine stones: Cystine stones are also formed in acidic urine, and are seen in patients with congenital cystinuria. Cystine stones may be difficult to treat and are difficult to shatter with ESWL. Hydration and alkalinisation are usually first line therapy

Drug-Induced Nephrolithiasis Ephedrine Calculi:- Ephedrine and its metabolites (norephedrine, pseudoephedrine, and norpseudoephedrine) are sympathomimetic agents that have been used for the treatment of enuresis, myasthenia gravis, narcolepsy, and rhinorrhea (Powell, Hsu, Turk, &Hruska, 1998). In addition to numerous side effects, ephedrine and its derivatives have been associated with the production of urinary stones (Blau, 1998). The diagnosis of these calculi is similar to that of other radiolucent calculi. Twenty-four hour urine metabolic analyses can aid in identifying ephedrine or its respective metabolites.

Guaifenesin Calculi:- Guaifenesin is a widely used expectorant that has been recently associated with nephrolithiasis. Guaifenesin calculi are radiolucent and present in patients who ingest this medication in excess. Twenty-four hour urine metabolic analysis can aid in the identification of guaifenesin or b-2-methoxyphenoxy-lactic acid.

Indinavir Calculi:- Indinavirsulfate (Crixivan) is currently one of the most frequently used protease inhibitors used aligned with human immunodeficiency virus, the virus that causes AIDS. The incidence of calculi in patients taking indinavir ranges from 3% to 20% (Schwartz, Schenkman, Armenakas, &Stoller, 1999). Indinavir calculi are radiolucent when they are pure, and are radiopaque when they contain calcium.

Xanthine Calculi:- These stones occur due to a rare hereditary condition with xanthine oxidase deficiency. The deficiency in this enzyme consequences in lessened levels of serum and urinary uric acid. Acidic urine causes crystal precipitation, consequencing in stone formation (Bernier, 2005). These stones are also seen in patients treated with iatrogenic inhibition of xanthine oxidase with xanthine oxidase inhibitors for hyperuricosuria such as allopurinol.

Causes of kidney stones

- Age
- Gender
- Diet
- Family history
- Urinal infections and blockage of the urinary tract
- Kidney diseases, such as cystic kidney disease
- Medicinal condition like gout
- Excess vitamin d intake
- Metabolic disorders, such as hyperparathyroidism.
- Certain medications such as diuretics, calcium based antacids and Inherited

disease such as cystinuria, hyperoxaluria, hypercalciuria or hyperuricosuria. Kidney stone s diagnosis In order to diagnose a patient with kidney stones, doctors will typically:

- Gather a complete medical history
- Ask about the patient's occupation
- Ask about the patient's food habits
- Order laboratory tests, which include urine and blood tests

Laboratory tests:

- X-rays
- Ultrasound (sonogram)
- CT (computed tomography) scan
- Intravenous pyelogram IVP)

Treatments for kidney stone

- Most of the kidney stones pass through the urinary system with plenty of water.
- Extracorporeal shock wave lithotripsy
- Ureteroscopy

Larger stones may be treated with:

- Extra corporeal Shock Wave lithotripsy(ESWL)
- Percutaneous nephrostomynt
- Tunnel surgery

Medical Management:

Effective kidney stone prevention is dependent on the stone type and identification of risk factors for stone formation. An individualized treatment plan incorporating dietary changes, supplements, and medications can be developed to help prevent the formation of new stones. Certain conservative recommendations should be made for all patients regardless of the underlying etiology of their stone disease. Patients should be instructed to increase their fluid intake in order to maintain a urine output of at least 2,000 ml/day. Patients should also limit their dietary oxalate and sodium intake, thereby decreasing the urinary excretion of oxalate and calcium. A restriction of animal proteins is encouraged for patients with "purine gluttony" and hyperuricosuria (SastryJ.L.N.2004).

Chemical Composition of Stones

There are several types of renal stones that differ in composition and pathogenesis. The most common type of kidney stone is composed of calcium oxalate and is caused by metabolic disorders that are often treatable. Calcium stones Most stones contain calcium combined with oxalate and phosphate or occasionally uric acid. All calcium stones are radio -opaque, and calcium oxalate and calcium phosphate stones are black, grey, or white and small dense and sharply circumscribed on radiographs Hypercalciuria Hypercalciuria or hypercalcinuria is the condition of elevated calcium in the urine. Chronic hypercalcinuria may lead to impairment of renal function nephrocalcinosis, and renal insufficiency. Hypocitrauria It's also associated with renal lithogenesis. Citrate acts in the tubular lumen combining with calcium to form a non -dissociable but soluble complex. Hypocitraturia could consequence from a cause of intracellular acidosis such as nephritic failure potassium deficiency, distal renal tubular acidosis, chronic diarrhea state, and drugs such as acetazolamide. Some studies suggest people who take supplemental calcium have a higher risk of developing kidney stones, and these findings have been used as the basis for setting the recommended daily intake for calcium in adults. In the Womens Health Initiative, postmenopausal women who consumed 1000 mg of auxiliary calcium and 400 units of vitamin D per day in seven years had a 17% higher risk of developing kidney stones than subjects taking a placebo. Uric acid stone Uric acid stones are smooth, round, yellow orange and nearly radio graphically transparent unless mixed with calcium crystals or struvite. Diets high in purines, especially those containing meats and fish, consequence in hyperuricosuria, and in combination with low urine volume and low urinary pH can exacerbate uric acid stone formation. Struvite or phosphate stones Struvite is a crystalline substance

composed of magnesium ammonium phosphate. Radiographs depict Struvite stones as large, gnarled, and laminated. They are associated with substantial morbidity infection. Signs of Struvite stones include urinary pH greater than 7, stag horn calculi, and urease that grow bacteria on culture. Cystine stone Formation of cystine stone is the only clinical expression of cystinuria an autosomal recessive intestinal and renal tubular disorders of four amino acids. Cystine, Arginine, Lysine, Ornithine. People who are homozygous for Cystinuria excrete more than 600mg per day of insoluble cystine. The stones are greenish –yellow flecked with shiny crystals and are moderately radio –opaque rounded appearance. Protease –related stone This the newest type of stone described. The increasing incidences of HIV- positive patients have led to widespread use of the protease inhibitor Indinavir sulphate. Although the drug is generally well tolerated, it can be associated with urolithiasis 4 -12% of patients. It thus may coexist or from a nidus for indinavir stones vice versa.

Prevention of stone

The first step in preventing kidney stones is to understand what is causing the stones to form. The health care provider may ask the person to try to catch the kidney stone as it passes, so it can be sent to a lab for analysis. Stones that are retrieved surgically can also be sent to a lab for analysis. Kidney stones may be prevented through changes in eating, diet, and nutrition and medications. Diet and Nutrition People can help prevent kidney stones by making changes in their fluid intake. Depending on the type of kidney stone a person has, changes in the amounts of sodium, animal protein, calcium, and oxalate consumed can also help. Drinking enough fluids each day is the best way to help prevent most types of kidney stones. Health care providers recommend that a person drink 2 to 3 liters of fluid a day. People with cystine stones may need to drink even more. Though water is best, other fluids may also help prevent kidney stones, such as citrus drinks.

The following recommendations based on the specific type of kidney stones, Calcium Oxalate Stones

- Reduction of sodium intake
- Reduction of animal protein, such as meat, eggs, and fish
- Getting enough calcium from food or taking calcium supplements with food
- Avoiding foods high in oxalate, such as spinach, rhubarb, nuts, and wheat bran

Calcium Phosphate Stones

- Reduction of sodium intake
- Reduction of animal protein
- Getting enough calcium from food or taking calcium supplements with food

Uric Acid Stones

- Limiting animal protein

Medications

RESULT AND DISCUSSION

The Phytochemical screening results are as follows

Table 3. Phytochemical screening

S.no	Chemical Test	GV	
		Aqueous	Alcohol
(1)	Alkaloids		
A	Mayer's test	+	+
B	Dragendroff's test	+	+

C	Wagner'stest	+	+
D	Hager'stest	+	+
(2)	Carbohydrates		
A	Molisch'stest	+	+
B	Fehling'stest	+	+
C	Barfoed'stest	+	+
(3)	ProteinsandFree Amino acids		
A	Ninhydrintest	—	-
B	Biuretttest	+	+
C	XanthoProteintest	+	+
(4)	TanninsandPhenolic Compounds		
A	Ferricchloride test	+	+
B	Leadacetatetest	+	+
C	Gelatintest	+	+
(5)	Phytosterols		
A	LibermannBurchard test	-	+
B	Salkowskitest	-	+
(6)	Flavanoids		
A	Shinodatest	+	+
(7)	Saponins	+	+
(8)	Glycosides	-	-
9	Terpenoids	-	-

The phytochemical screening result shows Alkaloids, Carbohydrates, Proteins and aminoacids, Tannins and phenolic compounds, Phytosterols, Flavanoids, Saponins, etc

Antiurolithiatic Activity

In the present study, the urine amount augmented in the treated group's animals than that of the control and it abridged in the untreated lithiatic animals when comparing to the standard and urinary concentration of the an assortment of ions investigated varied drastically, subsequent ethylene glycol treatment in the lithiatic control. The oxalate, calcium, uric acid, creatinine and phosphate excretion were notably increased on day 14th& 28th respectively for GROUP -II following ethylene glycol treatment. Management with Ethanolic and aqueous extracts of Glochidion velutinum abridged the excretions notably on 14th day of treatment and supplementary abridged on 28th day, like standard. In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were augmented notably in GROUP -II (Lithiatic control) following ethylene glycol treatment. Treatment with Ethanolic and aqueous extracts of Glochidion velutinum abridged all above mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP -II (Lithiatic control) following ethylene glycol action. After management with Ethanolic and aqueous extracts of Glochidion velutinum the magnesium level was restored near to regular and standard levels. Low urinary magnesium content is a general attribute in stone formers. An alike condition was observed in the (GROUP-II) rats. Management with Ethanolic and aqueous extracts of Glochidion velutinum elevated the urinary magnesium level, and consequently, abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has

been distinguished in hyperoxaluric rats and stone formers. In this study before estimating the ionic concentration of the urine on 14th and 28th day, the total quantity of urine excreted by the 9 groups were estimated. The treated groups depicted augmented the amount of urine volume. The consequences were expressed in table no1. In the 14th and 28th day after treatment the Urine parameters such as calcium, uric acid, creatinine, oxalate, phosphate and magnesium levels were estimated and documented in the table no. 2 and table no3. Then the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were estimated on 28th day of the treatment and that was documented in the table no.4. The kidney was prepared for the estimation of glutathione (GSH) Super oxide dismutase (SOD) catalase (CAT) and malondialdehyde (MDA) that enzyme level was documented in the table no.2.

Table2. Effect of Glochidion velutinum on urinary output in urolithiasis induced rats.

S. No	Days	Group-I	Group-II	Group-III	Group-IV
1	0	7.25±0.52	7.30±0.60	7.35±0.66	7.90±0.70
2	14	7.89±0.60	5.35±1.36	6.45±1.50	6.20±1.20
3	28	7.56±0.76	4.95±1.60	6.95±1.86	7.75±1.30

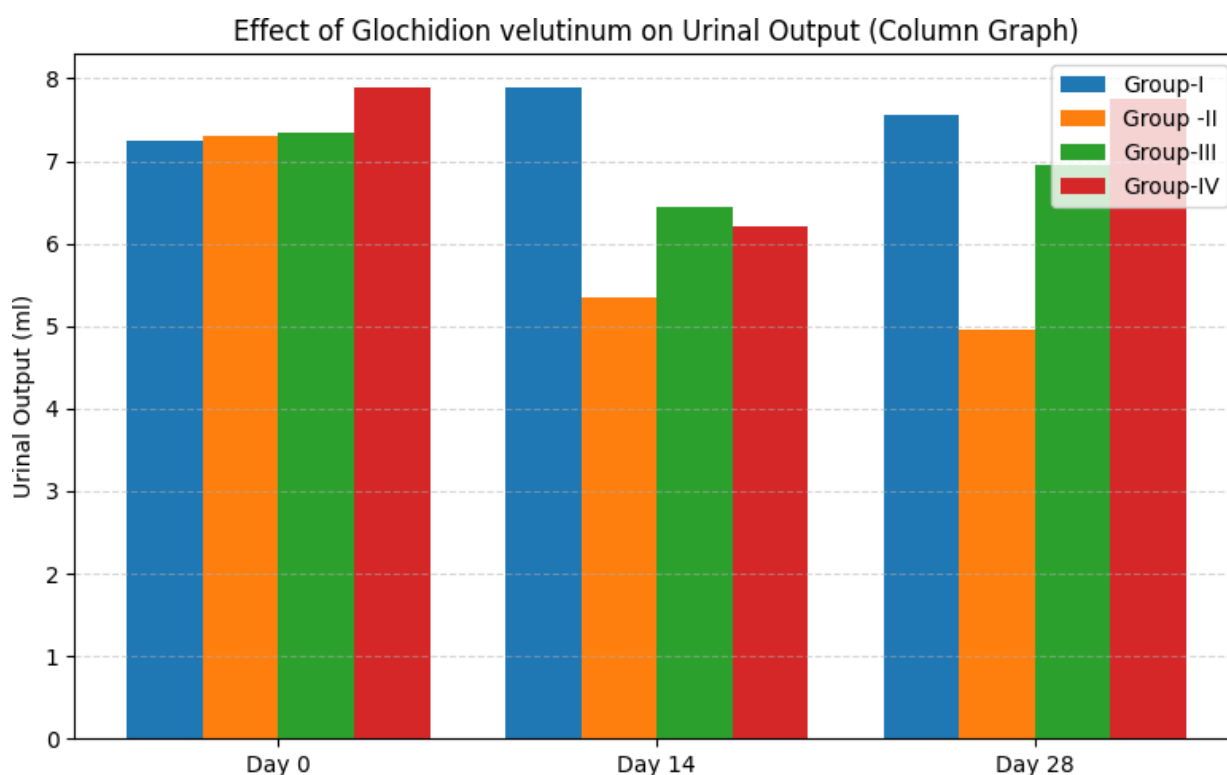


Table 3. Effect of Glochidion velutinum on urinary Biochemical parameters on the 14th day

Groups	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	70.90±3.76	4.42±0.58	6.15±0.70	3.30±0.62	0.90±0.08	16.30±1.50	33.60±2.26
Group-II	158.40 ±7.30	1.35 ±0.11	22.15 ±1.60	13.60±1.32	1.86±0.24	48.20 ±4.45	78.66±4.26
Group-III	93.12 ±5.78	2.95 ±0.38	12.65 ±0.95	8.40 ±0.86	1.22 ±0.16	24.38 ±2.68	44.82 ±3.32
Group-IV	88.56 ±5.26	3.26±0.45	10.66±0.76	6.05±0.76	1.08 ±0.10	18.10 ±2.50	37.55±3.06

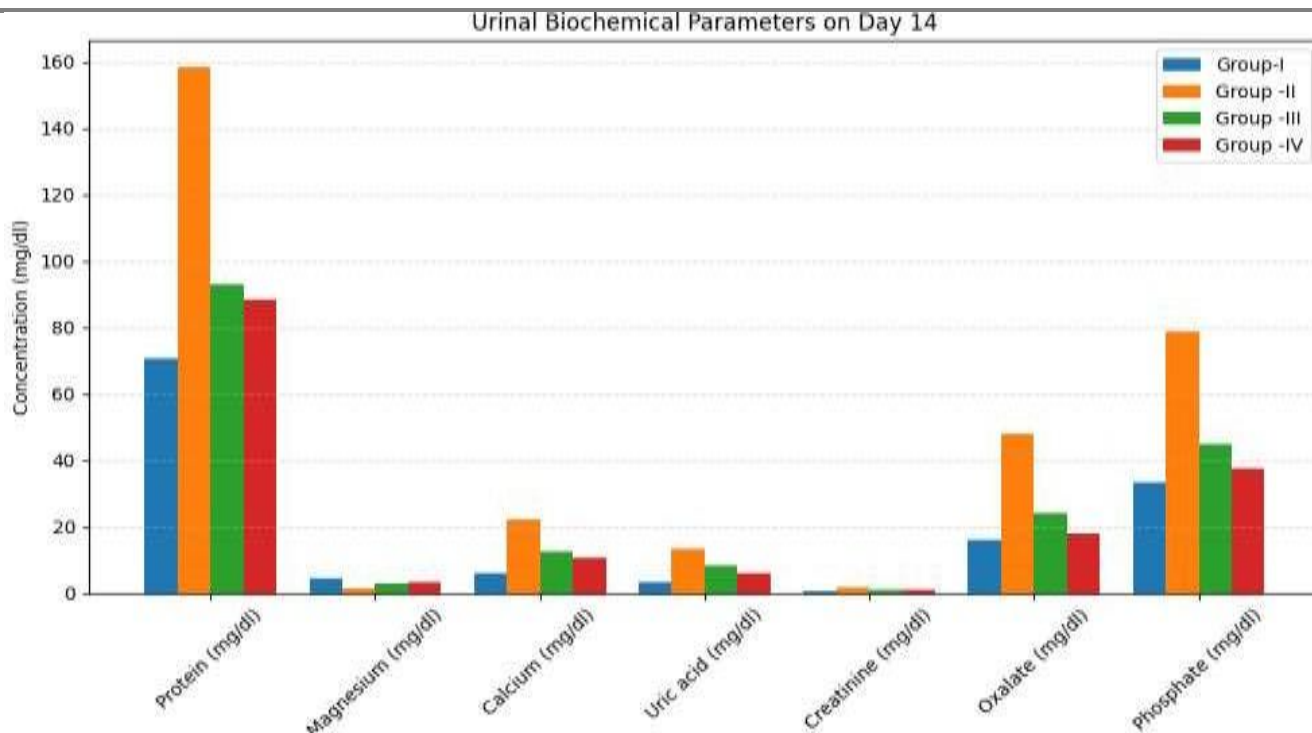


Table 4. Effect of Glochidion velutinum on urinary Biochemical parameters on the 28th day

Groups	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	65.96±2.86	4.20±0.52	5.63±0.54	3.22±0.65	0.80±0.08	15.80±1.83	32.90±2.20
Group-II	152.22 ±6.30	0.98 ±0.14	20.15±1.98	12.5 ±1.62	1.56 ±0.14	32.65 ±3.42	73.60 ±4.26
Group-III	86.20 ±4.20	2.50 ±0.36	9.45 ±1.10	5.5 ±0.94	0.92 ±0.09	23.22 ±2.76	43.55 ±3.73
Group-IV	82.66 ±3.55	2.88 ±0.40	8.90 ±0.92	4.20 ±0.85	0.86 ±0.11	19.30 ±2.32	37.80 ±3.15

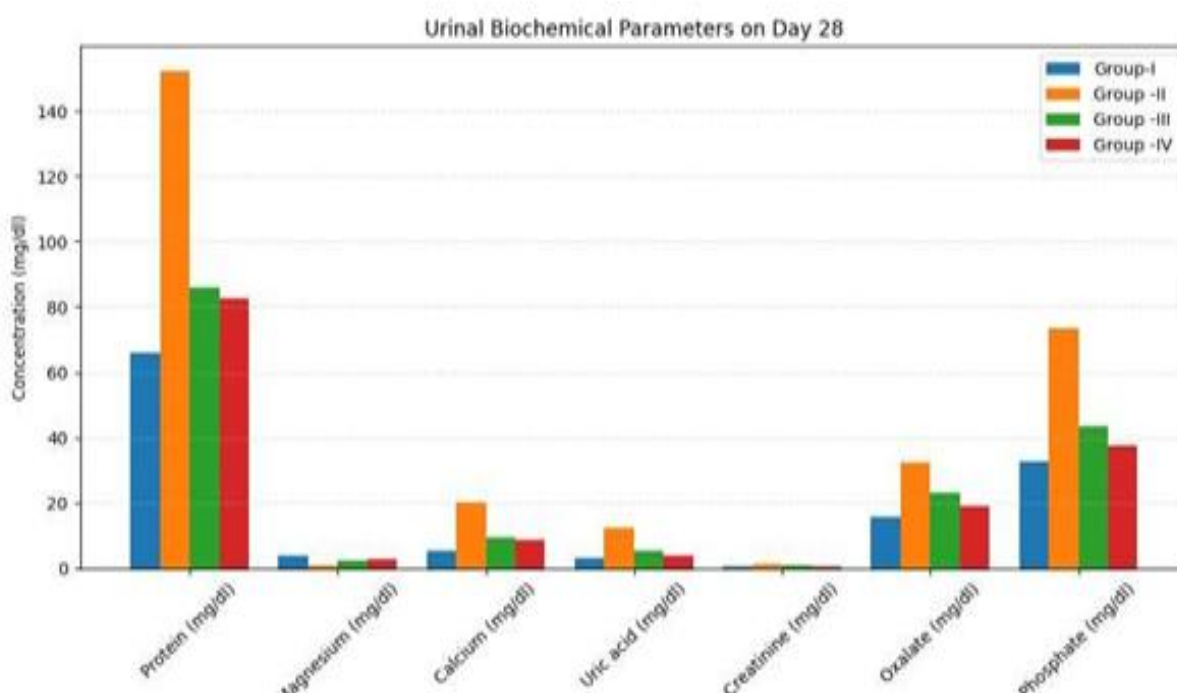


Table 7. Effect of Glochidion velutinum on serum Biochemical parameters on 28day

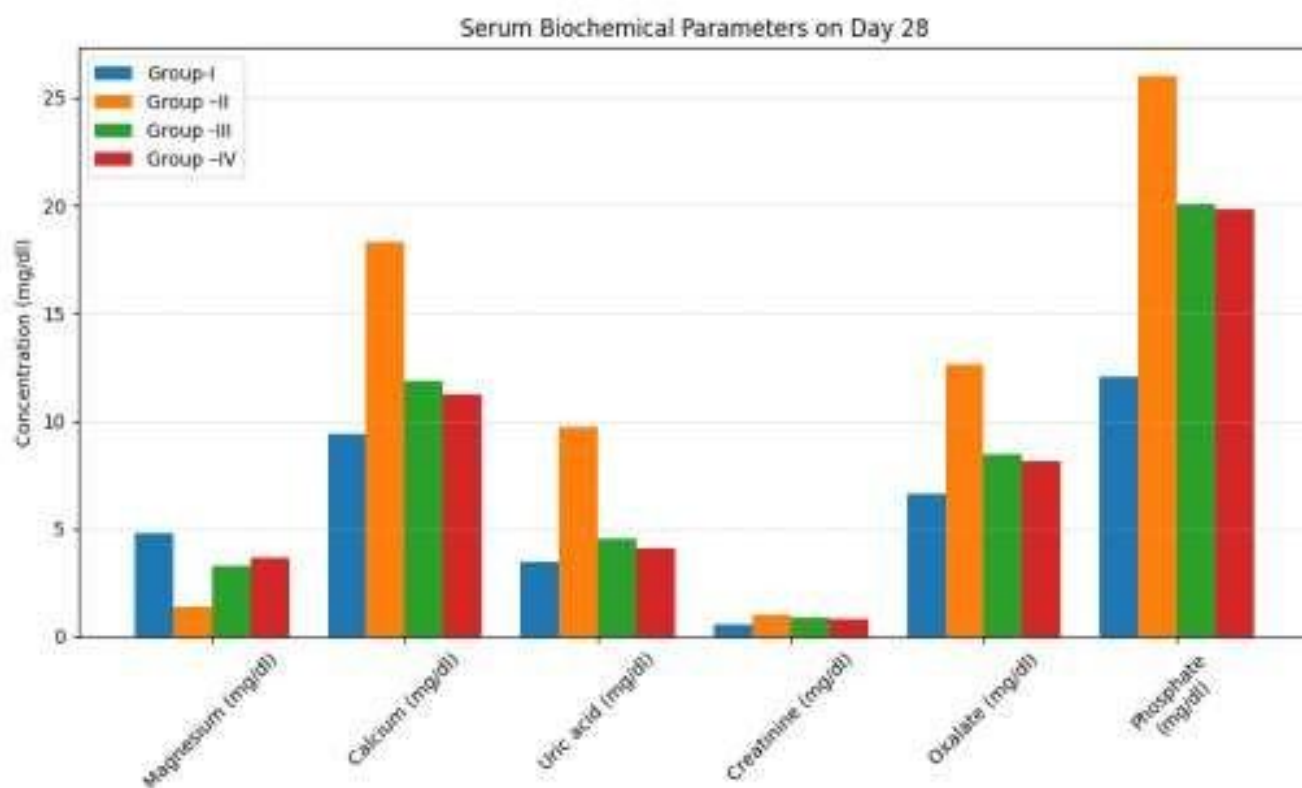
Groups	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
Group-I	4.80±0.86	9.40±1.32	3.45±0.40	0.56±0.03	6.6±0.57	12.06±1.43
Group-II	1.38±0.25	18.30±2.34	9.7±1.10	1.01±0.13	12.60±1.61	26.01±3.25
Group-III	3.28±0.46	11.85±1.88	4.55±0.55	0.91±0.09	8.45±0.88	20.10±2.65
Group-IV	3.67±0.52	11.22±1.60	4.10±0.46	0.80±0.07	8.12±0.78	19.85±2.05

GP1- Normal; GROUP-II- Lithiatic Control; GP3- EEGV (200mg/kg); GP4- AEGV(200mg/kg);

GP- Cystone herbal tablets (100mg/kg)

- Values are expressed in ml/24 h urine sample mean ± SEM
- Values were originate out by by means of ONE WAY ANOVA Followed by Newman keul's multiple range tests.

- ****(a)** Values were notably different from normal control (GP1) at P< 0.01
- ****(b)** Values were notably different from Lithiatic control (GROUP-II) at P<0.01



Histopathological study:

The abdomen was cut open to take away both kidneys from each animal. Secluded Kidneys were made tidy away from extraneous tissue and rinsed in ice-cold physiological saline. The right kidney was prepared and stained for histopathological examination. The slides were examined under light microscope to study microscopic design of the kidney and calcium oxalate deposits. The photo from the histopathological

examination gave a clear consequence that depicts the reduction in the stone development and the inflammations also abridged when comparing with the standard drug and the untreated group depicted large quantity of microcrystal deposition and stern dilation of most tubules and mass tubulointerstitial inflammatory infiltration with lesion area. It has been noted in the figure 6,7,8,9

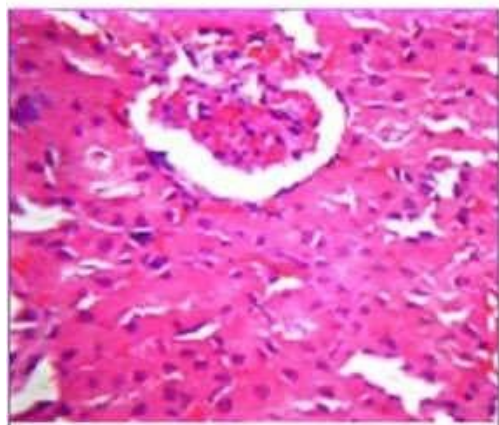


Fig6. Section of kidney glomeruloi-I

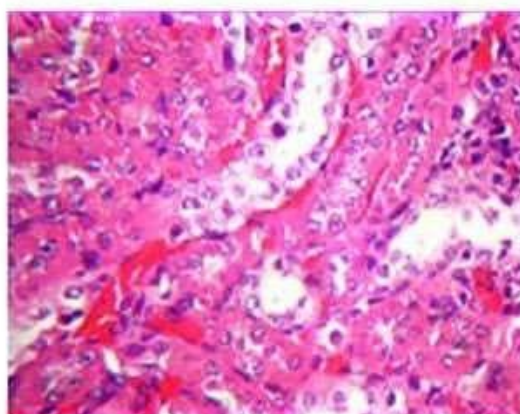


Fig7.Section of kidney glomeruloi-II

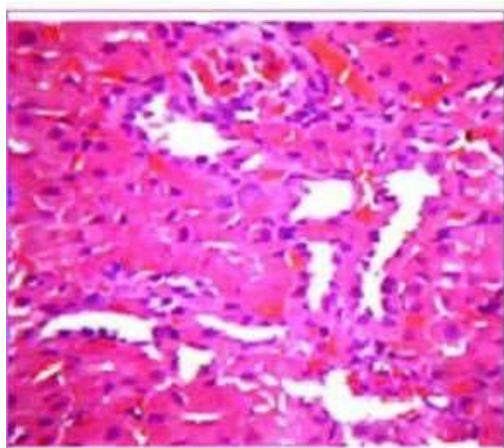


Fig8.Sectionof kidney glomeruloi-IIIb

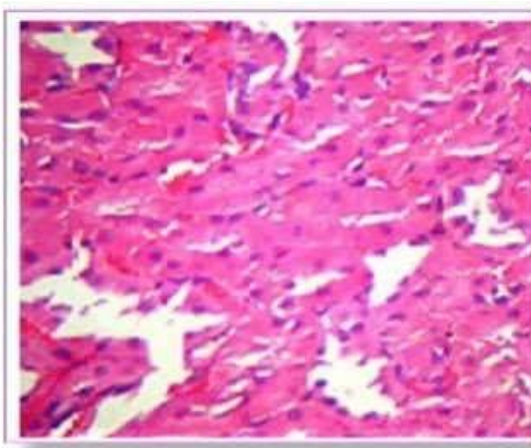


Fig9.Section of kidney glomeruloi-IV

In Vivo Antioxidant Activity

For in vivo antioxidant action, ethylene glycol treatment significantly augmented MDA levels ($P < 0.01$) and decreased GSH ($P < 0.01$), SOD ($P < 0.01$), and CAT ($P < 0.01$) levels in control animals. Aqueous and ethanolic extracts of *Glochidion velutinum* at a dose of **200 mg/kg** produced a significant ($P < 0.001$) reduction in MDA levels and increased GSH and antioxidant enzymes such as SOD and CAT compared with the standard group treated with Cystone (Table 8).

Table 8. Effect of Aqueous and Ethanolic Extracts of *Glochidion velutinum* on Antioxidant Enzymes in Renal Tissue

Treatment	Catalase (A/protein)	SOD (B/mg protein)	GSH (n moles/mg protein)	MDA (n moles/mg protein)
Normal Control	3.20 ± 0.18	9.20 ± 0.18	3.72 ± 0.16	3.88 ± 0.28
Ethylene Glycol	0.98 ± 0.02	3.68 ± 0.07	0.52 ± 0.06	10.05 ± 0.46
Cystone	2.80 ± 0.16	7.05 ± 0.11	2.68 ± 0.14	4.20 ± 0.30
AEGV 200 mg/kg	2.32 ± 0.09	6.55 ± 0.10	2.12 ± 0.10	5.68 ± 0.35
EEGV 200 mg/kg	2.28 ± 0.08	6.60 ± 0.12	2.20 ± 0.11	6 0.45

- Values are expressed as Mean $\pm$ SEM
- Values were found out by using ONE WAY ANOVA followed by Newman–Keuls multiple range tests.
- Values were notably different from normal control (GP-I) at $P < 0.01$.
- Values were notably different from lithiatic control (Group-II) at $P < 0.01$.

In-Vitro Antioxidant Activity

All these three plants are having antioxidant action. It was evaluated by five techniques.

In the Hydrogen Peroxide Scavenging Action Method, the IC_{50} values are calculated and compared with the standard Ascorbic Acid. It depicts that 140 μ g of Glochidion velutinum is corresponding to that of **12.5 μ g of Ascorbic Acid**. The Anti-haemolytic Activity Method, IC_{50} values are calculated and compared with the standard Quercetin. The consequence depicts 211.29 μ g of Glochidion velutinum is corresponding to 57.06 μ g of Quercetin. Flavonoids are phenolic components, abundant in several plants, which inhibit lipid peroxidation and lipoxygenases in vitro and in the presence of Fe^{3+} . In the Reducing Power Action Method, the IC_{50} values are calculated and compared with the standard Epicatechin. The outcome depicts that 114 μ g of Glochidion velutinum is equivalent to 12.5 μ g of Epicatechin action. NO is an imperative component intermediary generated by endothelial structures, macrophages, neurons, and other cells in the regulation of an assortment of physiological processes. Excess concentration of NO is implicated in the cytotoxic effects observed in an assortment of disorders like AIDS, cancer, Alzheimer's disease, and arthritis. Oxygen reacts with excess NO to produce nitrite and peroxynitrite anions, which act as free radicals. [23] From the consequences of the Nitric Oxide Scavenging Method, it was proved that the aqueous leaf extract of AC had effective antioxidant action. These extracts fight with oxygen to act in reaction with NO and accordingly inhibit the generation of nitrite and peroxynitrite anions. In the NO Scavenging Action Method, the IC_{50} values are calculated and compared with the standard Quercetin IC_{50} value. It depicts that 294.16 μ g of Glochidion velutinum is corresponding to 25 μ g of Quercetin. Free radical scavenging action of the aqueous leaf extract of AC is concentration-dependent. As the concentration of the test compounds increases, the radical scavenging activity also increases. In the **DPPH Scavenging Action Method**, ...method the IC_{50} values are calculated and it lends a good consequence of 384 μ g of Glochidion velutinum is equivalent to 24 μ g of Ascorbic acid.

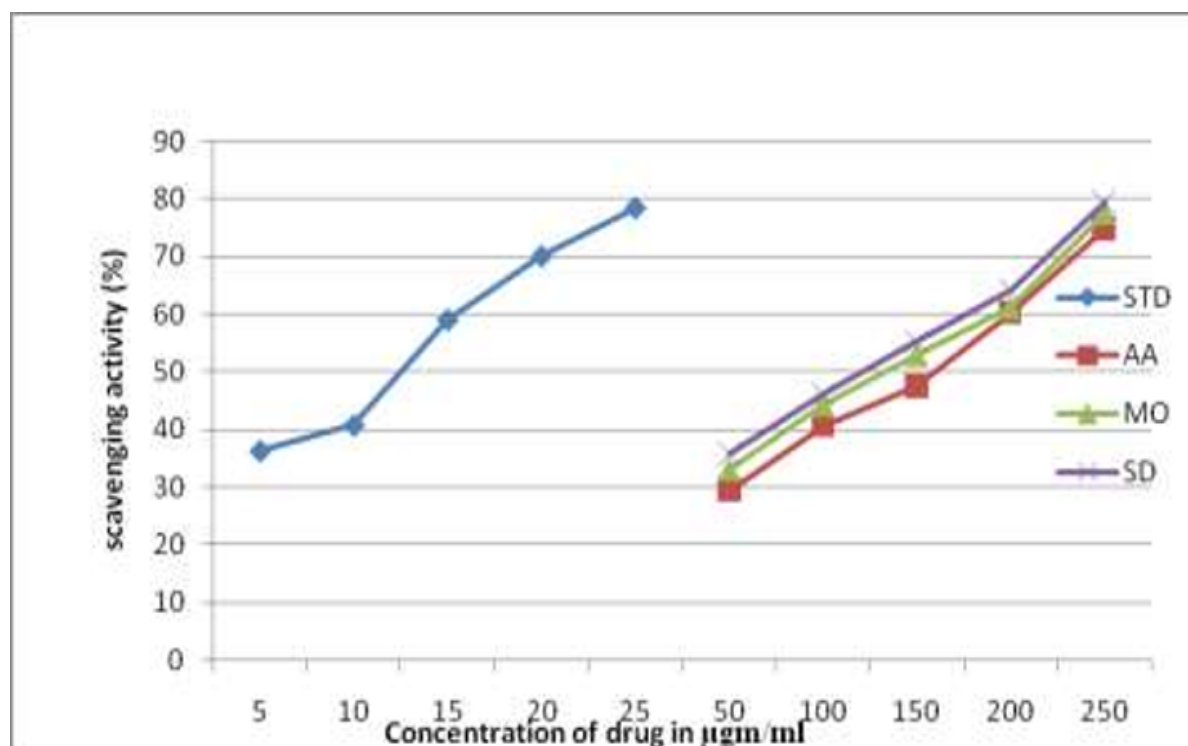


Fig10. Hydrogen Peroxide Method

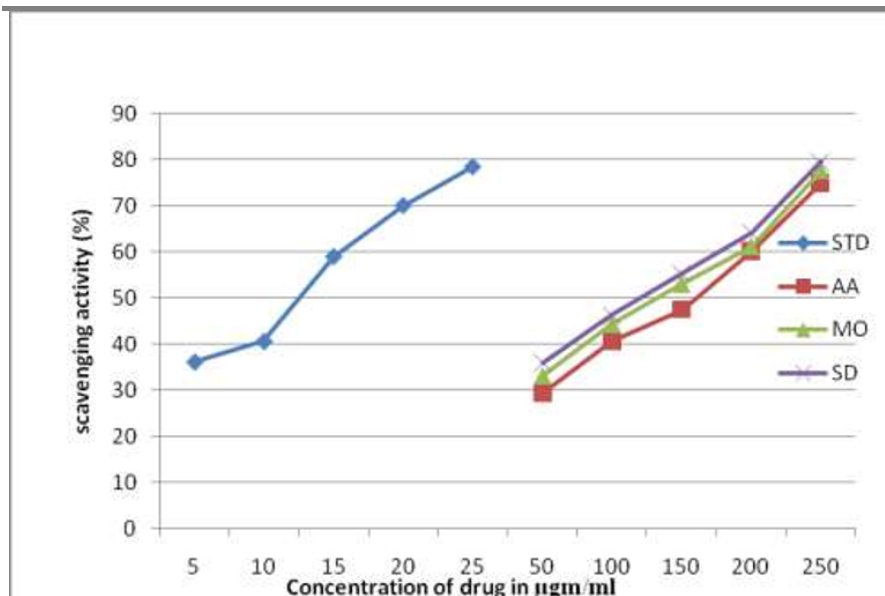


Fig11.Scavenging of hydrogen peroxide

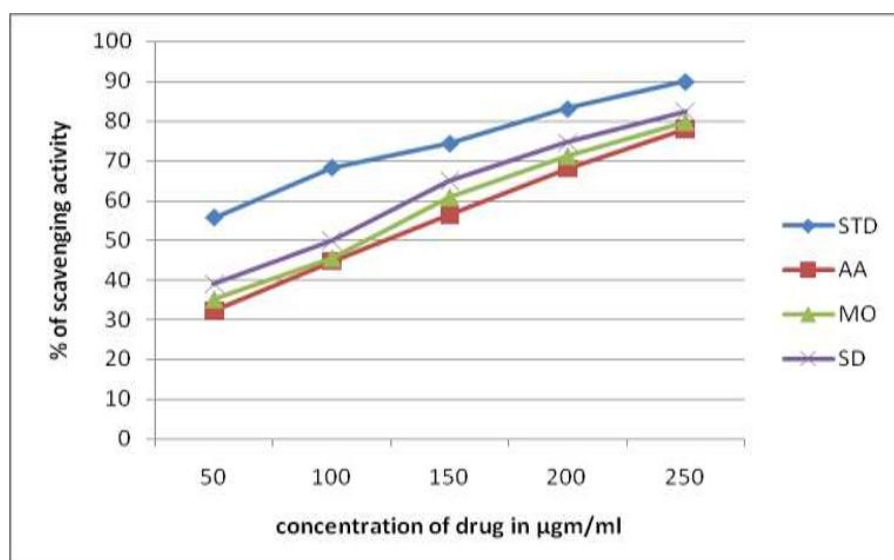


Fig12.Reducing power determination

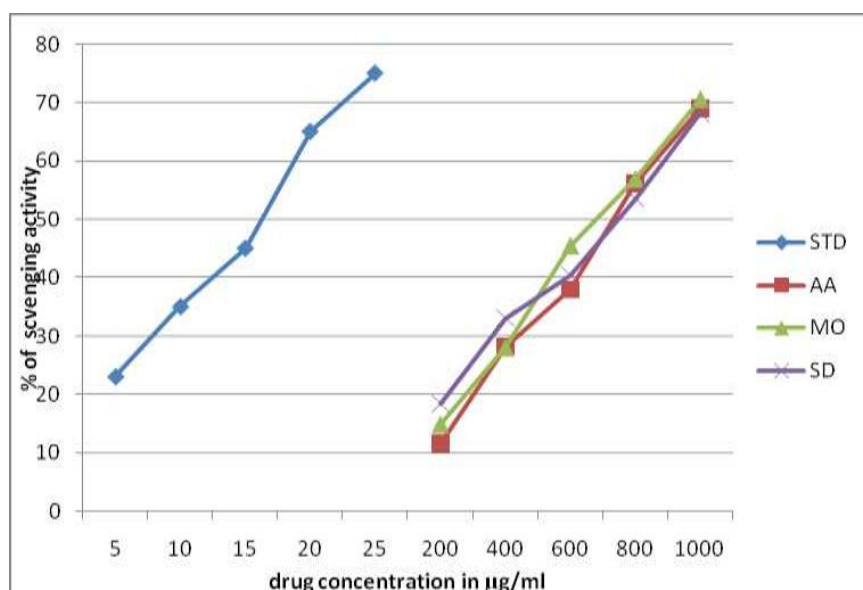


Fig13.Nitric oxide scavenging activity

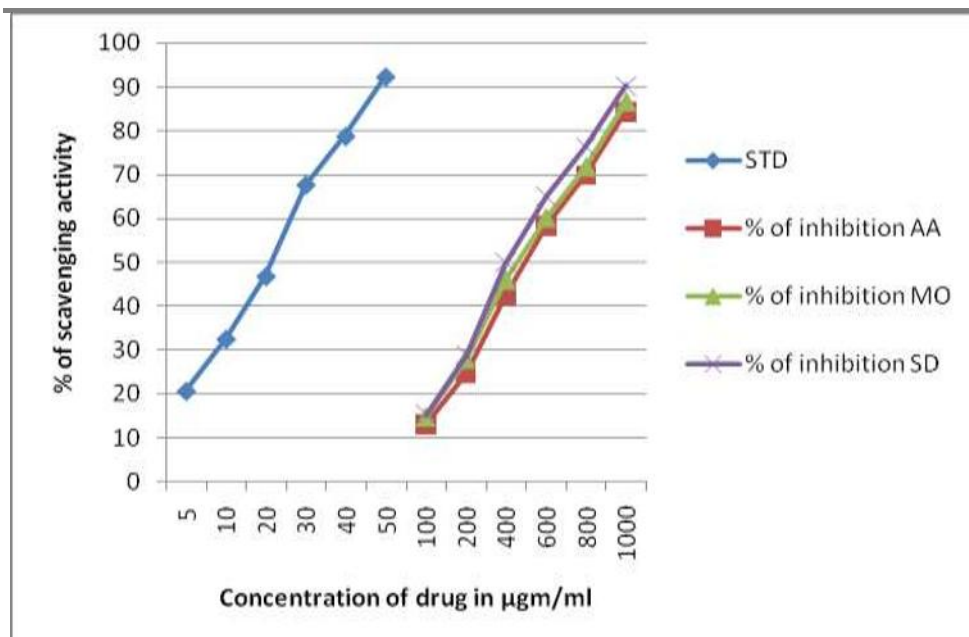


Fig14.DPPH radical scavenging activity

Studies also revealed the augmented lipid peroxidation and lessened levels of antioxidant impending in kidneys of mice provided with ethylene glycol. The chief stone oxalate-forming constituent has been perceived and documented to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes. Phenolic compounds present in *Glochidion velutinum* extracts may prevent the lipid peroxidation-induced renal damage caused by calcium oxalate crystal setting up in the renal tubules. Consequently, *Glochidion velutinum* extracts can prevent calcium oxalate crystal attachment as well as stone formation. *Glochidion velutinum* extracts treatment produced noteworthy decrease in MDA and increased GSH, SOD, and CAT. These consequences indicate the protective effects of *Glochidion velutinum* extracts aligned with the oxidative damage induced through ethylene glycol. These traits have been attributed to the triterpenes. Lupeol and polyphenolic compounds like quercetin may be present in *Glochidion velutinum* extracts. Thus, the consequences reveal that the *Glochidion velutinum* extracts hold a robust antioxidant and anti-urolithiatic action similar to pomegranate juice. In the present study, long-term delivery of 1% (v/v) ethylene glycol solution to the Wistar rats resulted in hyperoxaluria. Urinal concentration of the assortment of ions investigated varied drastically following ethylene glycol treatment in the lithiatic groups. Uric acid, oxalate, ... Calcium, creatinine, and phosphate excretion were notably increased on the 14th and 28th days, respectively, for GROUP-II following ethylene glycol treatment. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* abridged the excretions notably on the 14th day of treatment and more abridged on the 28th day, like standard. In GP-I normal rats, the magnesium emission was assessed as 4.20 ± 0.52 mg/dl/24hr and 4.42 ± 0.58 mg/dl/24 hr on the 14th and 28th days, respectively. Contrary to this, in GROUP-II lithiatic control rats, the magnesium level in urine gradually lessened following ethylene glycol treatment on the 14th and 28th days. Subsequent delivery of the extract improved the magnesium emission notably on the 14th and 28th days. In the prophylactic study, the serum parameters such as calcium, uric acid, creatinine, oxalate, and phosphate levels were increased notably in GROUP-II (lithiatic control) following ethylene glycol treatment. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg reduced all the above-mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP-II (lithiatic control) following ethylene glycol treatment. Subsequent to management with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg, the magnesium level was restored near to normal and standard levels. In stone-induced models, the subsequent modifications were observed: damaged epithelial cells at the inner layer of the tubules, dilation of the tubules, and presence of crystals. Scores were assigned according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol depicted a large quantity of microcrystal deposition, severe dilation of tubules, and tubulointerstitial inflammatory infiltration, with lesion area > 40% (score 3). On the other hand, kidney sections of animals subjected to extract treatment depicted obvious dilation of many tubules and

tubulointerstitial inflammatory infiltration, with abrasion area $< 40\%$ (score 2). As customary medicines are usually taken by the oral route, the same route of administration was used for evaluation of the antilithiatic effect of the ethanolic and aqueous extracts of three drugs. In the present study, male rats were chosen to induce urolithiasis because the urinary system of male rats resembles that of humans, and earlier studies have depicted that the amount of stone deposition in female rats was notably less. Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence from previous studies indicated that in response to a 14-day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate. The biochemical mechanisms for this process are related to an augment in the urinary concentration of ...Oxalate. Stone formation in ethylene glycol-fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Similar consequences have been obtained when rats were treated with ethylene glycol and ammonium oxalate. Therefore, this model was used to evaluate the antilithiatic effect of ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg in urolithiasis. In the present study, oxalate and calcium excretion progressively increased in calculi-induced animals (GROUP-II), since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria, and the changes in urinary oxalate levels are relatively much more important than those of calcium. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth. However, ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg lowered the levels of oxalate as well as calcium excretion. An increase in urinary phosphate is observed in calculi-induced rats (GROUP-II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which, through epitaxial processes, induce calcium oxalate deposition. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg restored phosphate levels, thus reducing the risk of stone formation. The increases in urinary uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility, and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid-binding proteins are capable of binding to calcium oxalate and modulating its crystallization also suggest its primary role in stone formation. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg lowered the excretion of uric acid and reduced the risk of stone formation. Supersaturation, a step in the pathogenesis of nephrolithiasis, occurs when substances that make up the stone are found at high concentrations in urine, when urine volume decreases, and when urinary concentrations of chemicals that inhibit stone formation decrease. Inhibitors of ... Crystallization include citrate, magnesium, phosphate, nephrocalcin, etc. Low urinary magnesium content is a common feature in stone formers. A similar condition was observed in the GROUP-II rats. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg elevated the urinary magnesium level and thus abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers. A high urinary colloidal concentration favours crystal growth. Such a condition was observed in ethylene glycol-treated rats in this study. Administration of the ethanolic and aqueous extracts of *Glochidion velutinum* abridged the urinary protein excretion in the treated group of rats and hence minimized the conditions favorable for crystal growth. In urolithiasis, the Glomerular Filtration Rate (GFR) decreases due to obstruction to the outflow of urine by stones in the urinary system. Due to this, waste products, particularly nitrogenous substances such as creatinine and uric acid, get accumulated. Also, increased lipid peroxidation and lessened levels of antioxidant potential have been perceived and documented in the kidneys of rats supplemented with a calculi-producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane. In calculi-induced rats (GROUP-II), marked renal damage was seen, as indicated by the elevated serum levels of creatinine and uric acid. However, prophylactic treatment with ethanolic and aqueous extracts causes diuresis and hastens the process of dissolving the preformed stones and preventing new stone formation in the urinary system. An increase in calcium and oxalate levels in the renal tissue of EG-treated rats was observed. Prophylactic treatment with ethanolic and aqueous extracts of *Glochidion velutinum* suppressed this increase in intracellular calcium. Several studies have perceived and documented that flavonoids, polyphenols, and triterpenes have anti-

inflammatory and antioxidant effects. It can be expected that antilithiatic activity might be mediated through antioxidant activity and free radical scavenging mechanisms. Microscopic examination of kidney sections derived from ethylene glycol-induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules, which caused dilation of the proximal tubules along with interstitial inflammation that might be attributed to ... Treatment with these extracts abridged the oxalate excretion notably. The urinary calcium excretion was 5.63 ± 0.54 mg/dl/24hr and 6.15 ± 0.70 mg/dl/24hr on the 14th and 28th days, respectively, for GP-I. It increased notably to 20.25 ± 1.98 mg/dl/24hr and 22.15 ± 1.60 mg/dl/24hr ($P < 0.01$) on the 14th and 28th days in GROUP-II following ethylene glycol treatment. The calcium excretion was notably abridged following treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg. The calcium excretion was reduced notably ($P < 0.01$) on the 14th day of treatment. Likewise, on the 28th day, calcium excretion was notably abridged in rats and was nearer to that of the standard drug-treated rats. Likewise, phosphate excretion values of 73.60 ± 4.26 mg/dl/24hr and 78.66 ± 4.26 mg/dl/24hr, and creatinine excretion values of 1.56 ± 0.14 mg/dl/24hr and 1.86 ± 0.24 mg/dl/24hr gradually increased in GROUP-II on the 14th and 28th days. However, in the treated animals, these elevated values were brought down. Likewise, urinary protein and uric acid concentrations increased following ethylene glycol treatment in GROUP-II, and they reached maximum excretion on the 14th and 28th days, respectively. On treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg (GP-III to GP-VIII), the protein and uric acid excretion was restored to near-normal limits in GP-III to GP-VIII for protein when compared with the standard drug-treated animals ($P < 0.001$). In GP-I normal rats, the magnesium excretion was estimated as 4.20 ± 0.52 mg/dl/24 hr and 4.42 ± 0.58 mg/dl/24 hr on the 14th and 28th days, respectively. Contrary to this, in GROUP-II lithiatic control rats, the magnesium level in urine gradually lessened to 0.98 ± 0.14 mg/dl/24hr and 1.35 ± 0.11 mg/dl/24hr following ethylene glycol treatment on the 14th and 28th days, respectively. Subsequent administration of the extract enhanced the magnesium excretion notably compared to the standard drug ($P < 0.01$) on the 14th and 28th days, respectively.

Effect of Glochidion velutinum on Serum Parameters on Day 28

In the prophylactic study, the serum parameters such as calcium, uric acid, creatinine, oxalate, and phosphate levels were increased notably in GROUP-II (lithiatic control) following ethylene glycol treatment. Treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of ... Treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg reduced all the above-mentioned parameters notably. On the contrary, the magnesium levels ... The magnesium levels were lessened notably in GROUP-II (lithiatic control) following ethylene glycol treatment. After treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg, the magnesium level was restored near to normal levels.

Effect of Glochidion velutinum on Histopathological Studies on Day 28

In stone-induced models, the following changes were noted:

1. Damaged epithelial cells at the inner layer of the tubules.
2. Dilatation of the tubules.
3. Presence of crystals in the tubules.

Scores were given according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol depicted a large quantity of microcrystal deposition and severe dilation of most tubules and mass tubulointerstitial inflammatory infiltration, with lesion area $> 40\%$ (score 3). However, kidney sections of animals treated with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg depicted obvious dilation of many tubules and tubulointerstitial inflammatory infiltration, with lesion area $< 40\%$ (score 2). In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to Wistar rats resulted in hyperoxaluria. Urinary concentration of the assortment of ions investigated varied drastically following ethylene glycol treatment. The oxalate excretion was increased on the 14th and 28th days, respectively, for GP-I. It increased notably on the 14th and 28th days in GROUP-II

following ethylene glycol treatment. Treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg and Cystone herbal tablets at a dose of 100 mg/kg (GP-III to GP-IX) abridged the oxalate excretion notably on the 14th day of treatment. Likewise, on the 28th day, treatment ... The urinary calcium excretion was 5.63 ± 0.54 mg/dl/24hr and 6.15 ± 0.70 mg/dl/24hr on the 14th and 28th days, respectively, for GP-I. It increased notably on the 14th and 28th days in GROUP-II following ethylene glycol treatment. The calcium excretion was notably abridged following treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg and Cystone herbal ... Cystone herbal tablet at a dose of 100 mg/kg reduced the calcium excretion notably in rats, respectively. Likewise, phosphate and creatinine excretion values gradually increased in GROUP-II on the 14th and 28th days. However, in the grouped treated animals, these elevated values were brought down on the 14th and 28th days. Regarding creatinine, these elevated values were also brought down. Likewise, urinary protein and uric acid concentrations increased following ethylene glycol treatment in GROUP-II and reached high levels on the 14th and 28th days. On treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg and Cystone herbal tablet at a dose of 100 mg/kg, the protein and uric acid excretion was restored to near-normal limits on the 28th day. In the prophylactic study, the serum parameters such as calcium, uric acid, creatinine, oxalate, and phosphate levels were increased notably in GROUP-II (lithiatic control) following ethylene glycol treatment. Treatment with ethanolic and aqueous extracts of Glochidion velutinum reduced all the above-mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP-II (lithiatic control) following ethylene glycol treatment. After treatment with ethanolic and aqueous extracts of Glochidion velutinum at a dose of 200 mg/kg and Cystone herbal tablet at a dose of 100 mg/kg, the magnesium level was restored near to normal levels.

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herbal tablet at a dose of 100 mg/kg restored the phosphate level, thus reducing the risk of stone formation. The increases in urinary uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility, and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid-binding proteins are capable of binding to calcium oxalate and modulating its crystallization also suggest its primary role in stone formation. Treatment with extracts of *Glochidion velutinum* at a dose of 200 mg/kg and Cystone herbal tablet at a dose of 100 mg/kg lowered the excretion of uric acid and reduced the risk of stone formation. Low urinary magnesium content is a common feature in stone formers. A similar condition was observed in ...the (GROUP-II) rats. Treatment with extracts of *Glochidion velutinum* at a dose of 200 mg/kg and Cystone herbal tablet at a dose of 100 mg/kg elevated the urinary magnesium level and thus abridged the propensity to crystallize, thereby creating an ambience unfavourable for precipitation. Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers. A high urinary colloidal concentration favours crystal growth. Such a condition was observed with ethylene glycol-treated rats in this study. Administration of the extracts of *Glochidion velutinum* at a dose of 200 mg/kg and Cystone herbal tablet at a dose of 100 mg/kg abridged the urinary protein excretion in the treated group of rats and hence minimized the conditions favourable for crystal growth. In urolithiasis, the Glomerular Filtration Rate (GFR) decreases due to obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid, get accumulated. Also, increased lipid peroxidation and lessened levels of antioxidant potential have been perceived and documented in the kidneys of rats supplemented with a calculi-producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane. Microscopic examination of kidney sections derived from ethylene glycol-induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules, which caused dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with extracts of *Glochidion velutinum* and Cystone herbal tablet lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damage to the tubules and calyces. In stone-induced models, the following changes were noted: damaged epithelial cells at the inner layer of the tubules, dilatation of the tubules, and presence of crystals in the tubules. Scores were given according to the severity of changes in the tubules. In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to Wistar rats resulted in hyperoxaluria. Urinary concentration of the assortment of ions investigated varied drastically following ethylene glycol treatment in the lithiatic control group. The oxalate, calcium, uric acid, creatinine, and phosphate excretion were notably increased on the 14th and 28th days, respectively, in GROUP-II following ethylene glycol treatment. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* abridged the excretions notably on the 14th day of treatment and more abridged on the 28th day, like standard. In the prophylactic study, the serum parameters such as calcium, uric acid, creatinine, oxalate, and phosphate levels were increased notably in GROUP-II (Lithiatic control) following ethylene glycol treatment. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg reduced all the above-mentioned parameters notably. On the contrary, the magnesium levels were lessened notably in GROUP-II (Lithiatic control) following ethylene glycol treatment. After treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg, the magnesium level was restored near to normal and standard levels. In the present study, oxalate and calcium excretion progressively increased in calculi-induced animals (GROUP-II), since it is accepted that hyperoxaluria is a far more risk factor in the pathogenesis of renal stones than hypercalciuria, and the changes in urinary oxalate levels are relatively much more important than those of calcium. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth. However, ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg lowered the levels of oxalate as well as calcium excretion. An increase in urinary phosphate is observed in calculi-induced rats (GROUP-II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induce calcium oxalate deposition. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg restored phosphate levels, thus reducing the risk of stone formation. The increases in urinary uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been

perceived and documented in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility, and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid-binding proteins are capable of binding to calcium oxalate and modulating its crystallization also suggest its primary role in stone formation. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* lowered the excretion of uric acid and thus abridged the conditions favourable for stone formation. Risk of stone formation. Supersaturation, a step in the pathogenesis of nephrolithiasis, occurs when substances that make up the stone are found in high concentration in urine, when urine volume decreases, and when urinary concentration of chemicals that inhibit stone formation decreases. Inhibitors of crystallization include citrate, magnesium, phosphate, nephrocalcium, etc. Low urinary magnesium content is a common feature in stone formers, and a similar condition was observed in the GROUP-II rats. Treatment with ethanolic and aqueous extracts of *Glochidion velutinum* at a dose of 200 mg/kg elevated the urinary magnesium level and thus abridged the propensity to crystallize, thereby creating an ambience unfavorable for precipitation. Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers. A high urinary colloidal concentration favours crystal growth. Such a condition was observed with ethylene glycol-treated rats in this study. Administration of the ethanolic and aqueous extracts of *Glochidion velutinum* abridged the urinary protein excretion in the treated group of rats, and hence minimized the conditions favourable for crystal growth. In urolithiasis, the Glomerular Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system (Table No. 11). Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid, get accumulated. Also, increased lipid peroxidation and lessened levels of antioxidant potential have been perceived and documented in the kidneys of rats supplemented with a calculus-producing diet (CPD). Elevated oxalate has been perceived and documented to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane. In calculi-induced rats (GROUP-II), marked renal damage was seen, as indicated by the elevated serum levels of creatinine and uric acid. However, prophylactic treatment with ethanolic and aqueous extracts caused diuresis and has tensed the process of dissolving the preformed stones and prevention of new stone formation in the urinary system. An increase in calcium and oxalate levels in the renal tissue of EG-treated rats was observed. Prophylactic treatment with ethanolic and aqueous extracts of *Glochidion velutinum* suppressed this increase in intracellular calcium. Microscopic examination of kidney sections derived from ethylene glycol-induced urolithiasis rats depicted polymorphic irregular crystal deposits inside the tubules, which cause...Dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with ethanolic and aqueous extracts of *Glochidion velutinum* lessened the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damage to the tubules and calyces. This study also revealed increased lipid peroxidation and lessened levels of antioxidant potential in the kidneys of rats supplemented with ethylene glycol. Oxalate, the chief stone-forming constituent, has been perceived and documented to induce lipid peroxidation and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes. Phenolic compounds present in the extracts may prevent lipid peroxidation-induced renal damage caused by calcium oxalate crystal deposition in the kidney. Hence, these extracts can prevent calcium oxalate crystal attachment as well as stone formation. The extract treatment produced a noteworthy decrease in MDA and increased GSH, SOD, and CAT. These consequences indicate the protective effects of *Glochidion velutinum* extracts against the oxidative changes induced by ethylene glycol. Thus, the consequences revealed that the three extracts possess potent antiurolithiatic and antioxidant activities.

In-Vivo Antioxidant Activity

Ethylene glycol treatment increased MDA ($P < 0.01$) and lessened GSH ($P < 0.01$), SOD ($P < 0.01$), and CAT ($P = 0.01$) levels in control animals. Aqueous and ethanolic extracts of *Glochidion velutinum* produced a noteworthy reduction ($P < 0.001$) in MDA and increased GSH and antioxidant enzymes such as SOD and CAT compared to the standard group treated with Cystone.

In-Vitro Antioxidant Activity

The antioxidant activity of the ethanolic extract was estimated by five methods: DPPH radical scavenging activity, reducing power determination, scavenging of hydrogen peroxide, assay of nitric oxide scavenging

activity, and comparison with different standards such as ascorbic acid, quercetin, and epicatechin. The three plant extracts depicted good antioxidant activity. The IC_{50} values for all methods were calculated and documented. Flavonoids are a heterogeneous group of phenolic compounds present in the plant world. The functions of flavonoids in plants include pigmentation, protection against UV light and microorganisms, defence against grazing animals, and regulatory functions for enzymes and signal substances involved in nitrogen-fixing bacteria. Flavonoids are abundant in the human diet. They are principally found in fruits, vegetables, and popular drinks such as red wine, tea, coffee, and beer. Many flavonoids, purified from medicinal plants and herbs used in customary medicine, are endowed with biological effects. Flavonoids may directly scavenge some radical species by acting as chain-breaking antioxidants, or they may recycle other chain-breaking antioxidants such as tocopherols by donating a hydrogen atom to the tocopherol radical. Transition metals such as ferric and copper ions are important pro-oxidants, and some flavonoids can chelate divalent metal ions, hence preventing free radical formation. Glochidion velutinum possesses antioxidant activity. It was evaluated by five methods. In the hydrogen peroxide scavenging activity method, the IC_{50} values were calculated and compared with the standard ascorbic acid. It was observed that 140 μ g of Glochidion velutinum was equivalent to 12.5 μ g of ascorbic acid. Flavonoids are phenolic compounds present in several plants, which inhibit lipid peroxidation and lipoxygenases in vitro and in the presence of free metal ions (Fe^{3+}). By the reducing power activity method, the IC_{50} values were calculated and compared with the standard epicatechin. The results indicated that 114 μ g of Glochidion velutinum was equivalent to 12.5 μ g of epicatechin activity. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, and neurons and is involved in the regulation of an assortment of physiological processes. Excess concentrations of nitric oxide are implicated in the cytotoxic effects observed in an assortment of disorders such as AIDS, cancer, Alzheimer's disease, and arthritis. Oxygen reacts with excess NO to generate nitrite and peroxynitrite anions, which act as free radicals. From the results of the nitric oxide scavenging method, it was observed that the aqueous leaf extract of Glochidion velutinum possesses effective antioxidant activity. These extracts compete with oxygen to react with NO and thus inhibit the generation of nitrite and peroxynitrite anions. In the nitric oxide scavenging activity method, the IC_{50} values were calculated and compared with the standard quercetin IC_{50} value. It was observed that 294.16 μ g of Glochidion velutinum was equivalent to 25 μ g of quercetin. DPPH is a relatively stable nitrogen-centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, as a consequence of which the electrons become paired off, forming the corresponding hydrazine. The solution therefore loses colour stoichiometrically depending on the number of electrons taken up. Substances capable of donating electrons or hydrogen atoms are able... DPPH (purple) is converted into its non-radical form, 1,1-diphenyl-2-picrylhydrazine (yellow), through a reaction that can be followed spectrophotometrically. The free radical scavenging activity of the methanolic leaf extract of Glochidion velutinum is concentration-dependent. As the concentration of the test compounds increases, the radical scavenging activity also increases. In the DPPH scavenging activity method, the IC_{50} values were calculated, and the results showed that 384 μ g of Glochidion velutinum was equivalent to 24 μ g of ascorbic acid.

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