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PHAMACOGNOSTICAL ANALYSIS AND INVIVO ANTILITHIATIC ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *CANAVALIA GLADIATE* AGAINST ETHYLENE GLYCOL INDUCED LITHIASIS IN WISTAR RATS

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ABSTRACT

The objective of the study is to investigate pharmacognostical analysis & antiurolithiatic activity of Hydroalcoholic extract of *Cannavalia Gladiata*. Pharmacognostic study include determination of physiochemical, fluorescence analysis & phytochemical screening of *cannavalia gladiate*. For antilithiatic study, 30 male wistar rats were segregated into 5 groups. All groups receive 1% Ethylene glycol for 28 days except normal control. G1(Normal control) receive normal diet and water, G2 (Toxic control) receive only ethylene glycol, G3(Standard) treated with Cystone at 100mg/kg whereas G4 & G5 treated with 200 & 400mg/kg of Hydroalcoholic extract of *Cannavalia gladiata*. After 28 days, various parameter like calcium, phosphate, magnesium, oxalate, protein, uric acid and creatinine concentration were estimated using Serum & Urine analysis by standard methods. Histopathological studies were carried out using Kidney section. Treatment with Hydroalcoholic extract of *Canavalia gladiate* at 200 and 400 mg/kg significantly reduced the serum & urinary levels of protein, uric acid, calcium, phosphate, oxalate & creatinine in dose dependent manner along with significant increase in magnesium. The histopathological examinations revealed that deposition of calcium oxalate crystals in renal tubules and glomerular congestion were reduced after HAECG treatment. This study demonstrate that Hydroalcoholic extract of Cannavalia gladiate reduces and inhibits the growth of urinary stones showed potent antiurolithiatic activity.

INTRODUCTION

Urolithiasis is a condition which involves the process of stone formation in kidney, bladder and urethra that results in renal colic, urine retention and pain in the abdomen and flank. The pathogenesis of urolithiasis involves the imbalance between promoters and inhibitors of crystallization in kidneys, where hyperoxaluria and hypercalciuria are the major risk factors. Epidemiological studies revealed that urolithiasis is more common in men than in women and is more prevalent between the ages of 20-40 in both sexes.[1] It is also reported that, overall probability of forming stones differs in various parts of the world 1-5% in Asia, 5-9% in Europe, 13% in North America, 20% in Saudi Arabia.[2] The cause of urolithiasis is still unknown but probably positive family history, overweight, obesity, or increased BMI. Some other causes include low urine volume <1500 mL/day, high dietary animal protein intake, increased urine excretion of calcium oxalate, uric acid and cystine. In modern medical field, various remedies like Lithotripsy, Kidney Dialysis and Surgical operations are followed, but the reoccurrence is common, need a regular follow up. Hence, the search for effective antilithiatic drugs without side effects from natural sources has assumed a greater importance. The plant *cannavalia gladiate* commonly used as a traditional herb claimed to possess various medicinal properties which has not been scientifically validated for lithiatic treatment. It's also important to standardize the plant material to be used as a medicine. So the present study was intended to evaluate the pharmacognostical analysis & antiurolithiatic activity of hydroalcoholic extract *of Cannavalia gladiate*.

MATERIALS AND METHODS

Plant collection

Canavalia gladiate were collected from Alagar hills and Plant material was identified and authenticated by Dr.D. Stephan, Dept of Botany, American college in Madurai.

Physiochemical analysis

Pharmacognostical values such as the percentage of total ash value, acid insoluble &water soluble ash value were performed according to the WHO guidelines on quality control methods for medicinal plant materials.[3]

Fluorescence Analysis[4-6]

The plant was washed thoroughly with tap water, shade dried and homogenized to fine powder. Fluorescence study of different plants powder was performed as per WHO guideline.[7]A small quantity of the plants powder was placed on a grease free clean microscopic slide and

1-2 drops of freshly prepared reagent solution were added, mixed by gentle tilting of the slide and waited for a few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365nm) ultra violet radiations. The colors observed by application of different reagents in different radiations were recorded.

Preparation of plant Extract

The whole plants were cleaned and chopped into small pieces. It is dried under shade and pulverized. Extraction is carried out by Soxhlet apparatus by using various solvents likes Petroleum ether, ethyl acetate for 40 hours and reflux successively with ethanol and water for 72 hours. The extracts were concentrated on rotary evaporator at 40 °C under reduced pressure to yield the crude extract.

Preliminary phytochemical screening

Preliminary qualitative phytochemical screening of the extracts for the detection of various active ingredients was carried out by using standard conventional procedures.[8] They identified by characteristic color changes and precipitation reactions using standard procedures.

Selection & acclimatization of animals

Albino rats of Wistar strain, of either sex, aged around 2 to 3 months weighing 150-200 g were housed under standard conditions of temperature (25 ± 2°C), relative humidity of 45-55%, and maintained on 12–hour light & dark cycle in animal house. The animals provided with standard pellet diet had free access to water The ethical committee Proposal number is IAEC/TNMGRMU/KMCP/98/2019-20, study approved by Institutional Animal Ethics Committee (IAEC) at K.M. College of Pharmacy, Uthangudi, Madurai.

Experimental design

The acclimatized animals were divided into five groups of six each designated as Group I, II, III, IV, and V. Lithiasis induced by1% ethylene glycol orally with drinking water for 28 days to all groups of animals except normal control.[9] G1 served as normal controls.G2 received 1% ethylene glycol in drinking water for 28 days served as lithiatic control.G3 treated with 'cystone'at a dose of100mg/kg for 28 days.[10] whereas G4 & G5 treated with 200 mg/kg & 400mg/kg of Hydro Alcoholic extracts of *Canavalia gladiate* orally, for 28 days.[11]

24 hour urine samples were collected from rats on 28th days in metabolic cages and the volume noted. Urinary parameters like calcium, phosphate, magnesium, oxalate, protein, uric acid and creatinine concentration were estimated using standard methods.[12-14] All rats were anaesthetized with diethyl ether; blood collected from retro orbital plexus of the animal, centrifuged for 10 minutes at 3000 r.p.m. Serum separated and parameters like calcium, phosphate, magnesium, oxalate, protein, uric acid and creatinine concentration were estimated using standard methods.[15, 16]

Histopathological study

On 28th day, animals sacrificed and their kidneys isolated subjected to histopathological studies. Kidneys were removed & washed in ice cold, 0.15 M KCl. The kidneys were fixed in formaldehyde (10%) stained with Hematoxylin & Eosin. The crystal deposit was visually examined under light microscope.[17]

Statistical Analysis

All the values are expressed as mean ± SEM. The data were statistically analyzed by One-way ANOVA followed Newman keul´s multiple range test. P values < 0.01 were considered significant.

RESULTS

The physicochemical characteristics of the plant *Canavalia gladiate* include ash content measured and shown in **Table 1**.Fluorescence characteristics of plant viewed under visible, short, and long light results shown in **Table 2**. Phytochemical analysis with different chemical reagents detects the presence of various phytoconstituents like alkaloids, flavonoids, phenolics and tannins, phytosterols and triterpenoids, carbohydrates as shown in **Table 3.**

Administration of Ethylene glycol significantly (p < 0.001) increased the protein, uric acid, calcium, phosphorus, oxalate and creatinine levels in urine & serum whereas magnesium levels were reduced when compared to normal control group [**Table 4 & 5**]. Treatment with Hydroalcoholic extract of *Canavalia gladiate* at 200 and 400 mg/kg significantly reduced the Serum creatinine & urinary levels of protein, uric acid, calcium, phosphate, oxalate in dose dependent manner along with significant increase in magnesium levels (p < 0.001) compared to lithiatic control group. ($p<0.001$).Standard treatment with cystone also shows significant changes in urinary calcium, oxalate and magnesium. ($p < 0.01$). Histological section in lithiatic control showed severe deposition of calcium oxalate crystals in lumen of tubules along with marked glomerulus congestion and tubular inflammation whereas treatment with hydroalcoholic extract of *Canavalia gladiate* group showed reduced glomerulus congestion and tubular regeneration.

Table No. 1: MORPHOLOGICAL ANALYSIS.

S.NO	Particulars	Limits	Results	Reference
01	Macroscopic	-	Color-Green	A.P.I (Part1 Vol-1)
02	Total Ash (% by mass)	-	11.795	A.P.I (Part1 Vol-1)
03	Acid insoluble ash (%by mass)	-	2.787	A.P.I (Part1 Vol-1)
04	Water soluble ash (%by mass)	-	11.225	A.P.I (Part1 Vol-1)

Table No. 2: Fluoresence Analysis.

S.NO	Particulars	Treatment	Day Light	Short UV light	Long UV light
1	Fluorescence	Powder as such	Green	_	_
		Powder+1N NaOH(aqueous)	Yellowish green	Light green	Light green
		Powder+1N NaOH (alcoholic)	Turbid yellowish green	Turbid dark green	Turbid dark green
		Powder+1N HCl	Light yellow	Light green	Light green
		Powder+50% H_2SO_4	Light green	Light green	Light green
		Powder+ Conc.HNO_3	Light orange	Light green	Light green

Table No. 3 Phytochemical Analysis.

S.NO	TEST	REPORT
1	**Test for carbohydrates** Molisch's Test	+
2	**Test for Glycosides** Keller –killiani test	_
3	**Test for Saponins** Foam test	_
4	**Test for Alkaloids** a. Mayer's test b. Dragendrodroff's test	+ +
5	**Test for Flavonoids** Alkaline reagent test	+
6	**Test for Phenolics and Tannins** a. Ferric chloride test b. Test for Tannins	+ +
7	**Test for Phytosterols and Triterpenoids** a. Leiberman-Bucharat test b. Salkowaski test	+ +
8	**Test for fixed oils and fats** Oily spot test	_

Table No: 4 Effect of Haecg on Urine Parameter.

PARAMETERS	TREATMENTS				
	G1	G2	G3	G4	G 5
Protein(mg/dl)	64.60±2.30	155.90±4.35**(a)	79.4±2.45**(b)	91.95±2.80**(b)	86.70±2.65**(b)
Magnesium (mg/dl)	4.15±0.36	1.24±0.10**(a)	3.30±0.22**(b)	2.68±0.18**(b)	2.45±0.15**(b)
Calcium (mg/dl)	6.15±0.45	19.64±1.05**(a)	8.20±0.58**(b)	9.88±0.69**(b)	9.65±0.62**(b)
Uric Acid (mg/dl)	3.01±0.14	13.68±0.95**(a)	7.26±0.54**(b)	8.03±0.63**(b)	7.80±0.57**(b)
Creatinine (mg/dl)	0.56±0.07	1.83±0.15**(a)	0.79±0.09**(b)	1.13±0.12**(b)	1.07±0.10**(b)
Oxalate (mg/dl)	14.65±1.25	46.67±3.75**(a)	21.10±1.65**(b)	24.95±1.90**(b)	23.85±1.81**(b)
Phosphate (mg/dl)	31.95±2.86	75.24±3.90**(a)	39.05±3.12**(b)	42.05±3.34**(b)	41.50±3.22**(b)

G1-Normal;**G2**-Toxic; **G3**-Std; **G4**-Trt low dose;**G5**-Trt high dose.

- **a-values are significantly different from normal control at p<0.001.

- **b-values are significantly different from toxic control at p<0.001.

Table No. 5: Effect of HAECG on SERUM PARAMETER.

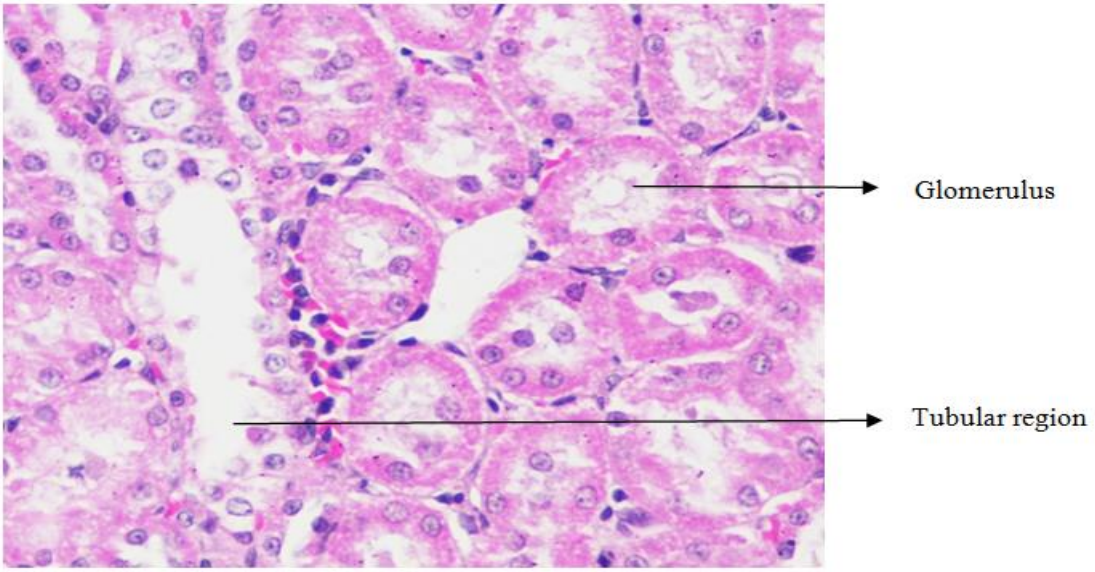
PARAMETER	TREATMENTS				
	G1	G2	G3	G4	G5
Magnesium (mg/dl)	4.26±0.19	1.34±0.13**(a)	3.46±0.12**(b)	3.49±0.10**(b)	3.51±0.13**(b)
Calcium (mg/dl)	9.24±0.19	19.01±0.36**(a)	9.75±0.22**(b)	11.27±0.21**(b)	10.59±0.28**(b)
Uric Acid (mg/dl)	3.67±0.12	10.23±0.26**(a)	4.44±0.15**(b)	4.39±0.12**(b)	4.43±0.14**(b)
Creatinine (mg/dl)	0.51±0.03	1.35±0.09**(a)	0.64±0.04**(b)	0.82±0.02**(b)	0.75±0.02**(b)
Oxalate (mg/dl)	6.28±0.26	13.06±0.30**(a)	7.55±0.11**(b)	8.45±0.15**(b)	8.28±0.08**(b)
Phosphate (mg/dl)	12.64±0.16	26.61±0.87**(a)	16.51±0.31**(b)	19.61±0.23**(b)	19.05±0.36**(b)

G1-Normal, **G2**-Toxic, **G3**-Std, **G4**-Trt low dose, **G5**-Trt high dose.

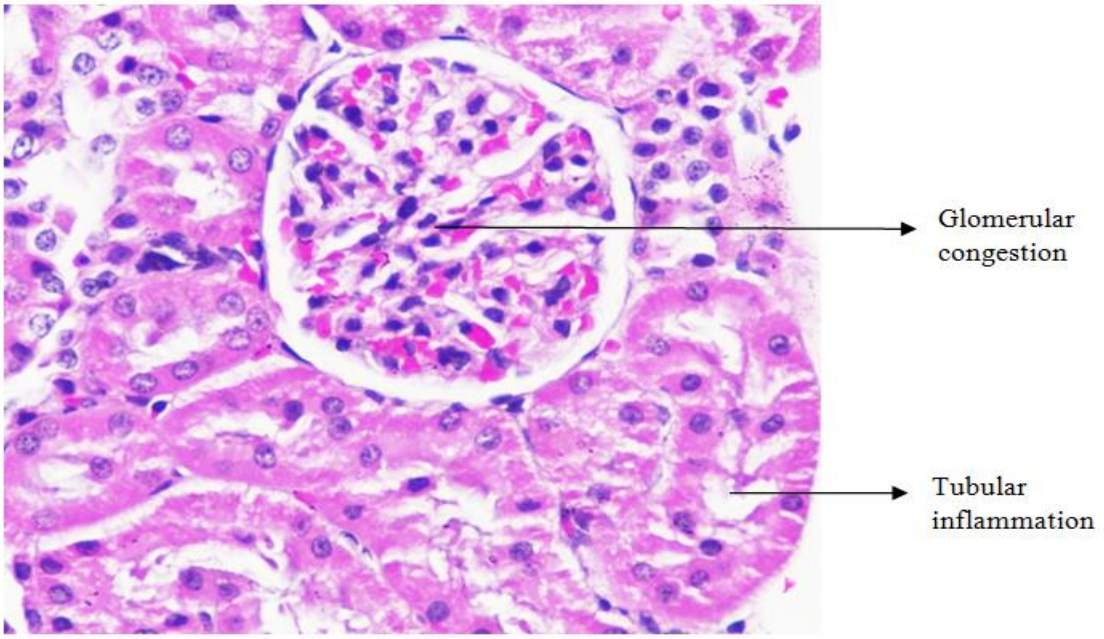
- **a-values are significantly different from normal control at p<0.001.
- **b-values are significantly different from toxic control at p<0.001.

HISTOPATHOLOGICAL REPORT

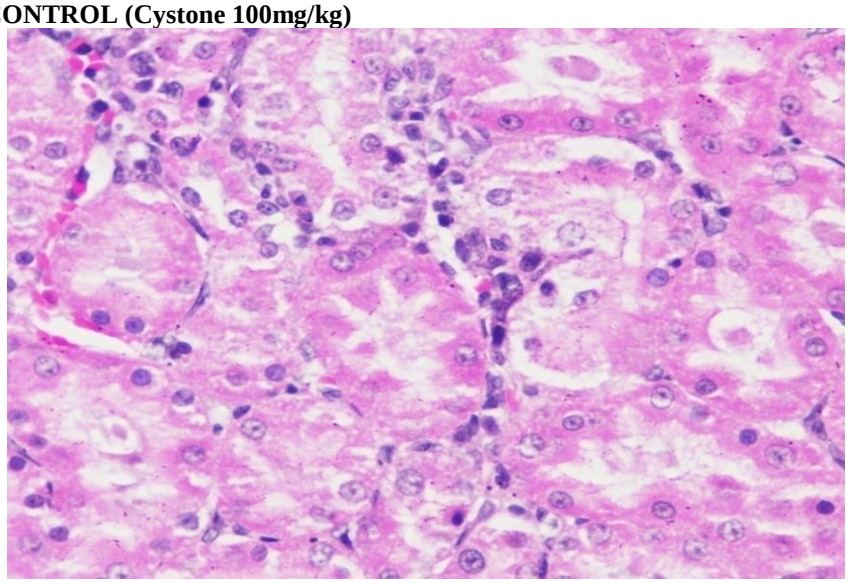
G1- NORMAL CONTROL



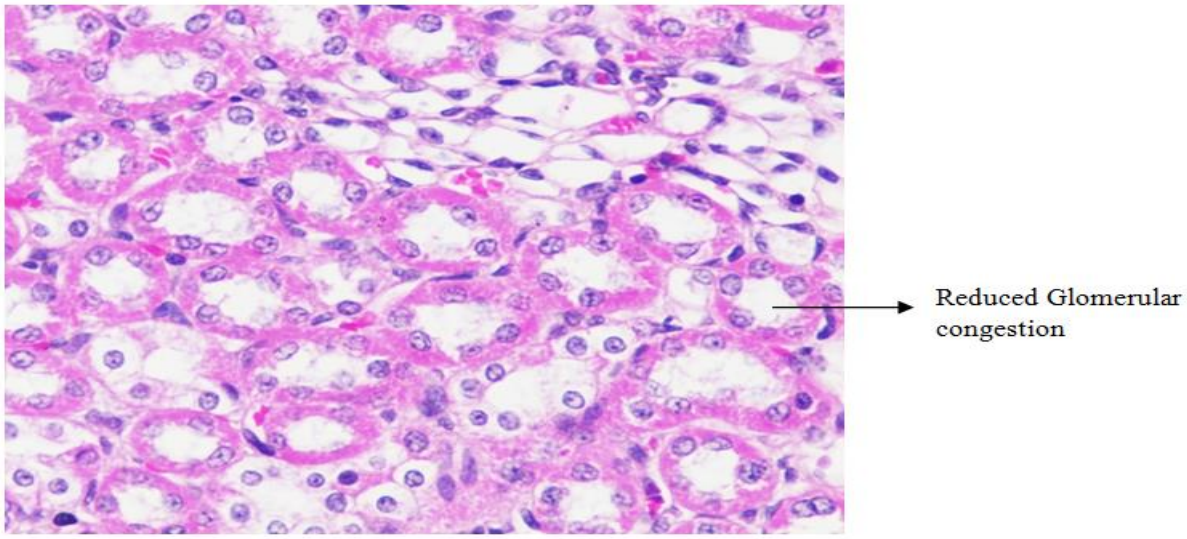
G2-TOXIC CONTROL



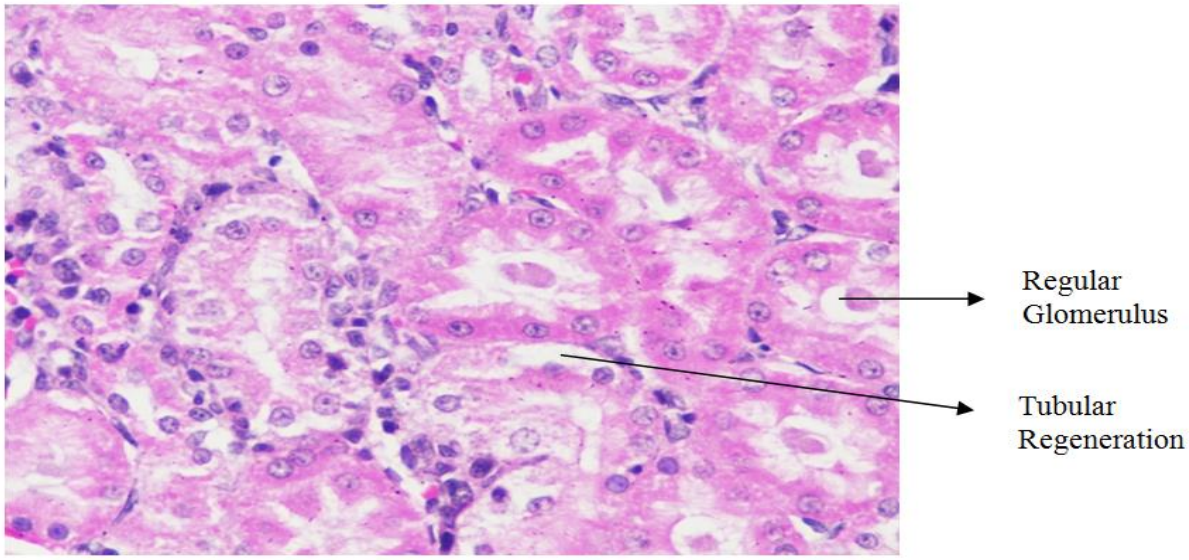
G3-STANDARD CONTROL (Cystone 100mg/kg)



G4- LOW DOSE (HAECG 100mg/kg)



G5-HIGH DOSE (HAECG 200mg/ml)



DISCUSSION

The physico-chemical and phytochemical studies of *Canavalia gladiate* serve as an important tool to identity and determine the quality and purity of the plant material. The ash values of the plant were obtained. Fluorescence analysis of plant powder were also determined. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in many natural products, which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation. The presence of phytoconstituents like flavonoids, alkaloids, triterpenoids & phytosterol helps in authentication of the plant *Canavalia Gladiate* These information could be a diagnostic tool for standardization of plant towards monograph development on the plant.

Administration of 1% ethylene glycol get rapidly absorbed and metabolized in liver via alcohol dehydrogenase or aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxalic acid which is further oxidized to oxalic acid/oxalate by glycolate oxidase or lactate dehydrogenase promoting hyperoxaluria.[18] Oxalate is attracted to cations like Ca2+ to form insoluble calcium oxalate. Among urinary stones, majority of stones are calcium oxalate (CaOx).[19] High calcium oxalate (CaOx) deposition reduced urinary output, damage epithelial showed reduction in glomerular filtration rate, which further decreases excretion of Na+, Cl- and Ca+2, and promotes stone formation.[20, 21]

The increased urinary uric acid, phosphorus, calcium and oxalate levels were observed in lithiatic rats. The urine oxalate levels are relatively more important than those of calcium since it is accepted that hyperoxaluria is a higher risk factor in the formation of renal calculi than hypercalciuria.[22] The decrease in creatinine clearance and urinary magnesium levels indicate their accumulation in blood which further increases the risk of urolithiasis. Treatment with cystone and HAECG elevated the creatinine clearance reduced the tendency to crystallize. The magnesium level returns to normal level after the drug treatment. Magnesium forms complex with oxalate and prevent super saturation of calcium oxalate. The serum creatinine, urea and uric acid levels were elevated in lithiatic group indicating marked renal damages. The decreased levels of these nitrogenous substances due to extracts or cystone treatment suggest the prevention of renal damage by increasing GFR and urinary output. It also restored calcium, phosphorus, oxalate to near normal values in treatment group reducing the risk of lithiasis. This effect was more pronounced in treatment with high dose of *Canavaliagladiate* extract, possibly due to presence of phenolic, phytosterols, triterpenoids& flavonoids components.

Microscopic study of kidney sections in lithiatic control rats showed CaOx crystals in tubular and interstitial spaces with glomerular congestion and tubular necrosis. The large crystals of CaOx might cause the obstruction of renal tubular flow leading to glomerular congestions and tubular degeneration. HAECG prevented the renal damages by inhibiting the accumulation and retention of CaOx crystals in renal tubules. These effects of extracts suggest their stone dissolving property. Treatment with HAECG prevented the deposition of CaOx crystals in different parts of the renal tubules possibly by hastening the dissolution of preformed stone and preventing the formation of new crystals due to the presence of phytochemicals such as flavonoids, phytosterol and triterpenoids. It is reported that the phytosterol & triterpenoids content of plants contributes to diuretic and stone dissolving ability.Therefore,it's confirmed that Hydro alcoholic extract of *Canavalia gladiate* possess significant antilithiatic activity.

CONCLUSION

In Conclusion, this study strongly implies that Hydro alcoholic extract of *Cannavalia gladiate* showed preventive effect on ethylene glycol induced formation of renal calculi might be through dissolution of preformed stones and prevention of formation of CaOx crystals. Thus this study validates the ethnomedicinal use of *Cannavalia Gladiate* in treatment of urinary stones.

Ethics statement

All animal experiments were approved by the Institutional Animal ethical committe (IAEC) Reg no: 661/PO/RE/S/02/CPCSEA .As per the standard practice the rats are segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They are fed on healthy diet maintained under hygienic environment in our animal house.

Declaration of competing interest

The authors declare there is no competing financial interests or personal relationships related to the work.

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