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Research Article

Preventive effect of *Euphorbia thymifolia* Linn against ethylene glycol-induced urolithiasis in male wistar albino rats

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ABSTRACT

The effect of the Ethanolic extract of *Euphorbia thymifolia* Linn (*EEET*) against ethylene glycol induced urolithiasis in albino rats is summarized in this study. Lithiasis was induced in rats by administrating 0.75% ethylene glycol in drinking water for 28 days and was manifested by high Urinary calcium, phosphate, oxalate, protein, uric acid, creatinine and low urinary magnesium Content. The *EEET* was administered in 250 & 500 mg/kg body weight orally for 28 days along with 0.75% ethylene glycol. Results revealed that Urinary calcium, phosphate, oxalate, protein, uric acid, creatinine was reduced and urinary magnesium level was elevated in diseased group. It also increased the urine volume, thereby reducing the tendency for crystallization. The histopathological studies confirmed the induction of lithiasis as microcrystal deposition was observed in section of kidney from animals treated with ethylene glycol. This was reduced, however, after treatment with the extract. These observations enable us to conclude that *EEET* is effective against ethylene-glycol induced urolithiasis in albino rats.

Keywords: *Euphorbia thymifolia*; Hyperoxaluria; Urolithiasis; Ethylene glycol.

INTRODUCTION

Urinary calculi are the third prevalent disorder in the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system (Hadjzadeh, M *et al.*, 2007). Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly costly and with these procedures recurrence is quite common (Prasad, K. *et al.*, 2007). The recurrence rate without reventiv treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years (Doddametiturke, R.B, *et al.*, 200). Many remedies have been employed through the ages to treat urolithiasis. In most cases, the management of urolithiasis involves both surgical and medical approaches, i.e., percutaneous nephrolithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL) and antibiotics (Rivers K, *et al.*, 2000). However; these treatments are relatively costly, painful and require expert hands with availability of appropriate equipment's. This has stimulated research on traditional remedies showing anti-urolithiatic activity. These plant products are reported to be effective in decreasing the recur-

rence rate of renal calculi with no side effects (Prasad, K. *et al.*, 2007).

Euphorbia thymifolia Linn (Family: Euphorbiaceae) recommended for various ailments like The plant is bitter, astringent, demulcent, laxative, diuretic, vermifuge, vulnerary, alexipharmic, expectorant, bronchodilator, stimulant and depurative. It is useful in flatulence, dysentery, bleeding piles, gonorrhoea, dysmenorrhoea, amenorrhoea, helminthiasis, ringworm, wounds, chronic cough, asthma, bronchitis, cardiac debility, graying of hairs, dandruff, diseases of teeth, skin diseases and leprosy. The latex is said to be useful in acne vulgaris. Pharmacological activities are Antimicrobial, antiviral, antispasmodic, bronchodilator, antifungal, anti-inflammatory, hypoglycemic, antibacterial (Agarwal, R. & Baslas, R.K. 1981- Anonymous, 1996).

MATERIALS AND METHODS

Plant material and preparation of extract

The dried plants of *Euphorbia thymifolia* Linn were collected locally and received from Nellore, Andhra Pradesh. Dr. C V S Bhaskars M.sc, Ph.D Govt. Venkataraja's College, authenticated plant. The plants were coarsely powdered and packed into Soxhlet column and extracted with 95 % v/v ethanol at 75–79°C for 72 h. The yield of the extract 7.8% w/w was stored in a refrigerator at 40°C, until use for the biological testing

Chemicals and reagents

Cystone (Himalaya drug company, Bangalore), ethylene glycol (EG) being a chief substitute for alcohol, EG is commonly used as anti-freeze in cooling systems of

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automobiles, aircrafts and has Wide industrial application. Many accidental deaths due to its poisoning have been reported (Aларcon-Aguilara, E.J. *et al.*, 1998). Toxicity from EG is produced from the metabolites such as Glyceraldehydes and oxalate, producing wide spread tissue injury in the kidney. Patients die of acute renal failure due to EG toxicity (Dr K.M.Nadkarni, Indian Materia Medica, V1:185–186). All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers.

Animals

Male Wistar albino rats weighing between 150–200 g each were used for this experiment. They were procured from Sri Venkateswara Enterprises, Bangalore, India. They were housed in polypropylene cages and maintained at $27 \pm 20^{\circ}\text{C}$ relative humidity $65 \pm 10\%$ under 12 h light/dark cycles. The study protocol was approved from the Institutional Animal Ethics Committee constituted in accordance with the rules and guidelines of the CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals), India.

Antiuro lithiatic activity of EEET

Ethylene glycol induced hyperoxaluria model (Mohansundari M, *et al.*, 2005-Pousand C A and Custer R P, 1946) was used to induce urolithiasis. Thirty animals were randomly divided into five groups containing six animals in each. Group I served as a vehicle treated control and maintained on regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to groups II-V for induction of renal calculi until the 28th day. As well as ethylene glycol, groups 2-5 also received the following treatments Groups III received standard antiuro lithiatic drug, cystone (750 mg/kg body weight). Group IV received EEET (250 mg/kg body weight) Group V received EEET (500 mg/kg body weight). All extracts were given once daily by oral route.

Collection of urine and serum analysis

All animals were kept in individual metabolic cages. Urines samples for a 24 h period were collected on 28th day of the protocol. Urine was analyzed for calcium, (Fiske, C.H, 1925) oxalates (Karadi, R.V, *et al.*, 2006) and total proteins (Fried E A, *et al.*, 1962). On the 28th day all animals were sacrificed, blood samples were taken and analyzed for sodium, calcium, Creatinine and phosphorus.

STATISTICAL ANALYSIS

The results were expressed as mean \pm standard error of the mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey test Comparison test ($p < 0.05$ was considered significant).

RESULTS

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Oxalate, calcium and total protein excretion were significantly increased in calculi-induced animals.

Group I: Kidney Section of GP-1 (Normal Control) Rats Section Kidney control Section Show structure of kidney with glomeruli and tubules which appear normal

Group II: Kidney section of GP-2 (Lithiatic control)

Group III: Kidney section of GP- 3 (Cystone Tablet Treatment) Section Show structure of kidney with glomeruli and tubules which appear normal. Tubules show crystals in the lumen indicating stone formation.

Group IV: Kidney section of GP- 4 (*Euphorbia thymifolia* extract treatment Section Show structure of kidney with glomeruli and tubules which appear normal. Small No evidence of crystal deposition seen.

Group V: Kidney section of GP- 5 (*Euphorbia thymifolia* extract treatment) Section Show structure of kidney with glomeruli and tubules which appear normal. No evidence of crystal deposition seen

DISCUSSION

Kidney stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in most societies in the last five decades, especially in association with economic development. In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of nephrolithiasis.

Recently, there is increasing evidence that many healthy natural food and medicinal herbal and supplements have the potential to become valuable complementary therapy in the treatment of various renal disorders and in the protection against iatrogenic nephrotoxicity. Proteinuria reflects proximal tubular dysfunction. Super saturation of urinary colloids results in precipitation crystal initiation particle which when trapped acts as a source leading to subsequent crystal growth (Bashir, S, *et al.*, 2009). Protein excretion was increased in hyperoxaluric rats (Durkin, E.T, *et al.*, 2010). Super saturation of urinary colloids results in precipitation as a crystal initiation particle, which when trapped, act as a nidus and leading to subsequent crystal growth (Selvam R, *et al.*, 2001). Protein urea reflects proximal tubular dysfunction (Vidya. L and Varalakshmi.P, 2000). Renal injury whether a result of renal calculi decreases nephron population. The effect of this reduction in renal mass is to place an increased workload on surviving nephron. Through a series of micro-circulatory adaptations, glomerular blood flow and capillary hydrostatic pressure is increased, and the filtration rate in each glomerulus is augmented. Glomeruli under

Table 1: Effect of oral administration of the Euphorbia thymifolia Linn extract on urinary volume excretion

Group	Urine volume (ml/100g/24 hrs)
Normal control (Saline)	3.4±0.16
Calculi induced (0.75%EG)	3.0±0.21
Standard (Cystone750mg/kg)	5.4±0.12
T ₁ (EEET250 mg/kg)	4.2±0.32
T ₂ (EEET 500 mg/kg)	5.6±0.29

The urinary volume is excretion was deviated in diseased group (3.0±0.21) and the same was improved in the test groups (T₁; 4.2±0.32 ,T₂ ; 5.6±0.29).

Table 2: Estimation of Urinary Electrolytes of Normal and Urolithiatic Rats

S.No	Group & Drug Treatment	Estimation of Urinary Electrolytes			
		Oxalate(mg/dl)	Magnesium(mg/dl)	Calcium(mg/dl)	Phosphate(mg/dl)
1	Normal control (Sa-line)	0.32±0.02	5.62±0.36	2.816±0.170	3.54±0.04
2	Calculi induced (0.75%EG)	2.10±0.07###	1.23±0.11###	8.140±0.33###	7.19±0.06###
3	Standard (Cystone750mg/kg)	1.00±0.04***	4.29±0.72***	3.816±0.240***	3.71±0.09***
4	T ₁ (EEET250 mg/kg)	0.615±0.05***	3.60±0.20**	2.865±0.128***	4.14±0.10**
5	T ₂ (EEET 500 mg/kg)	0.340±0.03 ***	4.06±0.15***	2.882±0.185***	4.13±0.09***

All values are expressed as mean ±S.E.M for six rats in each group; # -Diseased compared with normal group; *-Standard, T₁,T₂ –Compared with diseased group; ### - Indicates –P > 0.001; ***- Indicates –P > 0.001; ** - Indicates –P > 0.001.*- Indicates –P > 0.001; Group II Compared with group I, group III, IV, V compared with group II , One-way ANOVA followed by Tukey test.

Table 3: Estimation of Kidney Homogenate Electrolytes of Normal and Urolithiatic Rats.

S. No	Group & Drug Treatment	Estimation of Kidney Homogenate Parameters		
		Oxalate(mg/dl)	Calcium(mg/dl)	Phosphate(mg/dl)
1	Normal (Saline)	0.181±0.02	4.683±0.38	3.4±0.03
2	Positivecontrol (0.75%EG)	1.550±0.06###	5.216±0.14#	3.54±0.10###
3	Standard (Cystone750mg/)	0.400±0.04***	3.623±0.15***	2.42±0.07***
4	T ₁ (EEET 250 mg/kg)	0.223±0.03***	2.578±0.23***	2.07±0.10***
5	T ₂ (EEET 500 mg/kg)	0.443±0.04***	3.023±0.12***	2.87±0.06***

All values are expressed as mean ±S.E.M for six rats in each group; # -Diseased compared with normal group *-Standard, T₁,T₂ –Compared with diseased group; ### - Indicates –P > 0.001; ***- Indicates –P > 0.001; ** - Indicates –P > 0.001.*- Indicates –P > 0.001; Group II Compared with group I, group III, IV, V compared with group II , One-way ANOVA followed by Tukey test.

Table 4: Estimation of Serum Parameters of Normal and Urolithiatic Rats

S.No	Group & Drug Treatment	Estimation of Serum Parameters		
		BUN(mg/dl)	Creatinine(mg/l)	Uric acid(mg/dl)
1	Normal (Saline)	16.58±0.30	0.621±0.01	5.70±0.12
2	Positive control (0.75% EG)	25.076±0.24###	0.755±0.01###	7.766±0.25#
3	Standard(Cystone750mg/kg)	21.287±0.39***	0.971±0.006***	5.032±0.07***
4	T ₁ (EEET 250 mg/kg)	33.331±0.73***	1.136±0.01***	6.433±0.14***
5	T ₂ (EEET 500 mg/kg)	28.327±0.60***	1.048±0.01***	7.223±0.06 ^{ns}

All values are expressed as mean ±S.E.M for six rats in each group; # -Diseased compared with normal group; *-Standard, T₁,T₂ –Compared with diseased group; ### - Indicates –P > 0.001;***- Indicates –P > 0.001; ** - Indicates –P > 0.001.*- Indicates –P > 0.001; Group II Compared with group I, group III, IV, V compared with group II , One-way ANOVA followed by Tukey test.

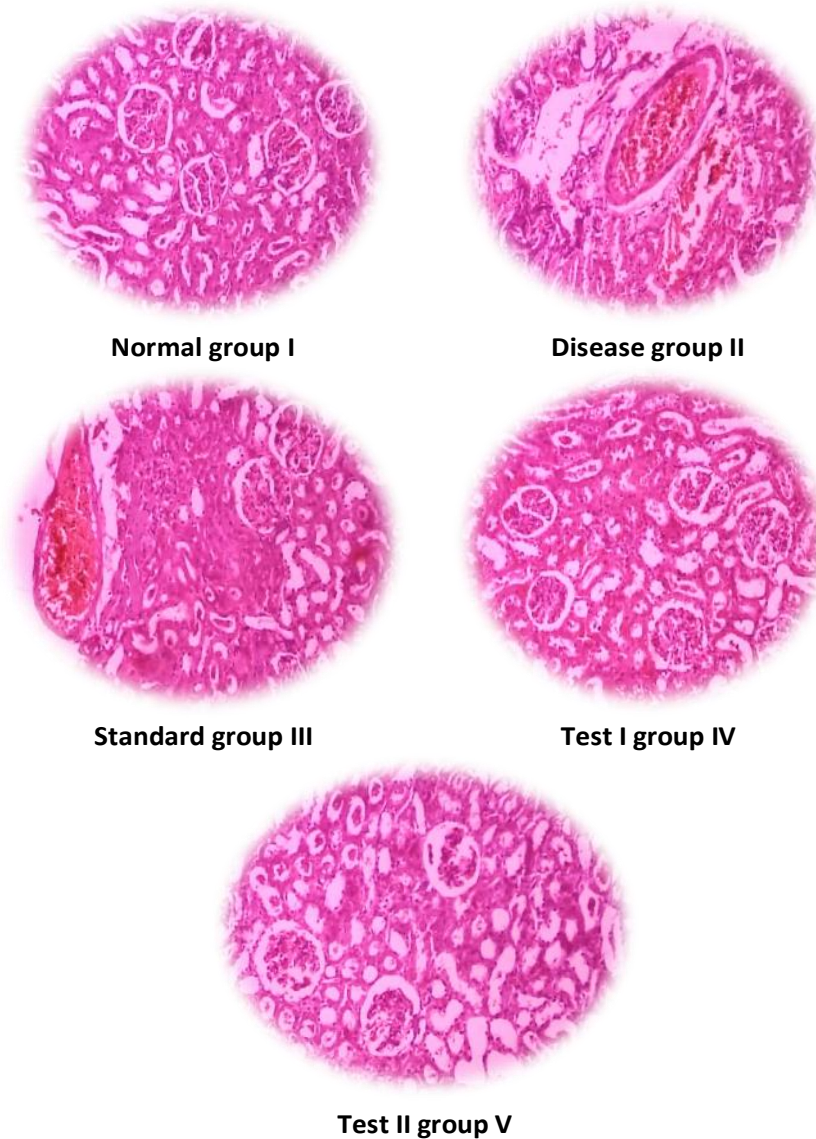


Figure 1: Kidney section of Group-I (Normal Control), Group-II (Lithiatic control), Group-III (Cystone Tablet Treatment), Group-IV (*Euphorbia thymifolia*), Group-V (*Euphorbia thymifolia* extract treatment)

going this hyper filtration process are subjected to hemodynamic stress; which damages capillary integrity and predisposes to leakage of protein into the urine (Soundararajan, *et al.*, 2006).

Sodium and chloride ions excretion from the body is a function of arterial blood pressure (Clive D.M, *et al.*, 1991). Sodium depletion stimulates rennin release and subsequent production of Angiotensin II, a potent vasoconstrictor (Guyton, A.C. and Hall, 2006). Increased blood sodium levels inhibit rennin release from the juxtaglomerular cells and consequent withdrawal of angiotensin II (Jackson, B.A. and T.A. Kotchen, 1984). When modulation of the rennin angiotensin system is pharmacologically prevented, changes in salt intake markedly affect long term levels of arterial blood pressure (Hall J.E, *et al.*, 1999). There is therefore a need to strike a balance in the levels of blood sodium and chloride to avoid either of the extreme of hypotension or hypertension (Kang, S, *et al.*, 2002). Reported that the hypernatremia is rare but does occur when there is

loss of body fluids containing less sodium than blood along with water intake restriction or if there is excessive sodium intake with limited liquid intake (Vogt B, *et al.*, 2009). Reported that the hypernatremia almost always indicates water depletion. The present increase of serum sodium level is suspected to be due to the inability of the kidneys to excrete adequate sodium from the tubular fluid. LPO is a degenerative pathway of membrane components mediated through free radicals produced in the cell (Veena C.K, *et al.*, 200). Membrane injury facilitated the fixation of calcium oxalate crystals and subsequent growth into kidney stones (Selvam R, *et al.*, 2002). Oxalate the major stone forming constituent has been reported to induce free radical generation, which results in peroxidative injury to renal epithelial cells (Tamil selvan. S *et al.*, 2005). Oxalate-induced peroxidative injury is one of the major mechanisms in promoting crystal attachment to renal epithelial cells (Rashed, T, *at al.*, 2004).

CONCLUSION

In conclusion, the presented data indicate that administration of the *Euphorbia thymifolia* extracts to rats with ethylene glycol induced Lithiasis reduced and prevented the growth of urinary stones. The mechanism underlying this effect is still unknown but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. The protective effect against oxalate-induced lipid peroxidation may be contributory to the recovery of renal damage.

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