

Effect of Methanolic Extract of Tribulus Terrestris in Ethylene Glycol Induced Crystalluric Rats

Inmozhi.R¹, Santha.K¹, Balu Mahendran.K¹, Baskaran.K¹, Sethupathy.S¹

1. Department Of Biochemistry, Rajah Muthiah Medical College And Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India.

Abstract

Aim: The aim of the present study is to assess the effect of Tribulus terrestris in ethylene glycol induced crystalluric rats.

Materials and methods: Thirty two wistar male rats aged 16-20 weeks weighing 160-200 gm were randomly divided into four groups. Group I- Controls, Group II – Methanolic extract of Tribulus terrestris, Group III- Ethylene glycol -10% aqueous solution, Group IV- Ethylene glycol + Methanolic extract of Tribulus terrestris with standard chow diet and water ad libitum. After feeding for 15 days blood and urine samples were collected. The blood and urine samples were used for the analysis of calcium, magnesium, phosphorus, creatinine, uric acid and alkaline phosphatase. Serum samples were analyzed for urea, aspartate transaminase, and alanine transaminase. And also urinary samples were analyzed for citrate, oxalate, lactate dehydrogenase, blood haemolysate used for erythrocyte antioxidant status, TBARS levels, tissue homogenate used for hepatic and renal marker enzymes.

Results: Ethylene glycol + Methanolic extract of Tribulus terrestris treated rats were showed significantly decreased calcium, oxalate, hepatic glycolic acid oxidase, hepatic lactate dehydrogenase, hepatic & renal TBARS levels than compared to ethylene glycol treated rats.

Conclusion: On the basis of the results obtained in the present study, we conclude that administration of Tribulus terrestris extract improve the antioxidant status and reduce crystalluria.

Keywords: Tribulus terrestris, Ethylene glycol, crystalluria, Hyperoxaluria

I. Introduction

Urolithiasis is a common condition with a prevalence of 3%–5% and a lifetime risk of 11% in men and 5.6% in women [1,2]. The incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common components of urine, calcium oxalate, representing up to 80% of analyzed stones. This may cause obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system [3-5]. Hyperoxaluria is one of the major risk factors for calcium oxalate kidney stone formation in humans. Oxalate is normally excreted by the kidneys, and 60–80% of renal calculi are composed of calcium oxalate [6-8]. Oxalate present in many foods is poorly absorbed from the intestine, with only 5–15% of dietary oxalate appearing in the urine; the remaining 85% of the oxalate is produced endogenously [9]. Several previous studies showed that oxalate-induced peroxidative injury is involved in the nucleation, aggregation and development of calcium oxalate stone disease [10-12]. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal remedies has risen in the developed countries in the last decades. So, in the present study we aimed to find out methanolic extract of *Tribulus terrestris* can inhibit calculogenesis in ethylene glycol induced crystalluric rats.

II. Materials And Methods

Wistar male rats aged 16-20 weeks weighing 160-200 gm were obtained from Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, Annamalai University. All animals were housed in a temperature controlled room ($27 \pm 2^{\circ}\text{C}$) with a 12: 12 hr light and dark cycle. The animals were provided with standard chow diet and water ad libitum. The experiment were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC), Annamalai University.

Experimental groups

The animals were divided into four groups consisting of eight rats and were maintained for a period of 15 days.

Group I- Controls

Group II – Methanolic extract of *Tribulus terrestris* (2ml//rat)

Group III- Ethylene glycol -10% aqueous solution (2ml/kg body weight)

Group IV- Ethylene glycol (2ml/kg body weight) + Methanolic extract of *Tribulus terrestris* (2ml/rat)

Administration of Ethylene glycol:

Ethylene glycol was given at the dose of 2ml per kg body weight using Ryle tube.

Preparation of Tribulus terrestris Methanolic extract and administration:

The whole plant was washed and air –dried and powdered. *Tribulus terrestris* powder extracted in to methanol (1:5 ratio) soxalation for 48 hours. The extract was air dried and the residue obtained was dissolved in 100ml of water (50%) and 2ml/rat was given using Ryle tube.

Sample collection

Rats were housed in metabolic cages and 24 hours urine was obtained using thymol crystal as preservative. After feeding for 15 days blood and urine samples were collected. The blood samples were used for the analysis of serum calcium, phosphate, magnesium, urea, creatinine, uric acid, aspartate transaminase, alanine transaminase and alkaline phosphatase. Haemolysate used erythrocyte antioxidant status (superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione). Urinary samples were analyzed for calcium, oxalate, citrate, magnesium, alkaline phosphatase, lactate dehydrogenase enzymes, creatinine, phosphorus and uric acid.

Preparation of Haemolysate, analysis of antioxidant enzymes and TBARS

After removal of plasma and buffy coat, the packed red blood cells obtained were washed twice with cold physiological saline. The lysate was prepared and freezing and thawing three times in dry ice made up to 20g/litre Hb, with distilled water and frozen 20⁰ C until the determination of erythrocyte antioxidant enzymes(Super oxide dismutase, Catalase, Glutathione peroxidase , Glutathione) and TBARS .

Preparation of Tissue Homogenate, analysis of hepatic and renal marker enzymes

A portion of the organ was blotted, weighed and homogenized in appropriate ice cold buffer using all glass homogenizer with teflan pestle. Hepatic oxalate synthesizing enzymes (Glycolic oxidase, Lactate dehydrogenase), renal and hepatic TBARS levels were analyzed.

Analytical procedures

TBARS in erythrocyte were estimated by the method of Donnan, 1950 [13]. Glycolic acid oxidase was estimated by Lui and Roels, 1970 [14]. Superoxide dismutase was assayed by the method of Kakkare^{etal.}, 1984[15], Catalase activity was assayed by Sinha 1972[16].Glutathione peroxidase was assayed by the method of Rotruck ^{et al.}, 1973[17], after specified period of enzyme action reduced glutathione was measured by Beutler and Kelly, 1963[18], oxalate was estimated by Hodgkinson and Williams 1972 [19] . Calcium, citrate, magnesium, alkaline phosphatase, lactate dehydrogenase, AST, ALT, ALP enzymes, creatinine, phosphorus and uric acid were assayed by routine standardized methods.

Statistical analysis: Statistical analysis was performed with SPSS (version 17.0). Results are expressed as mean ± SD using student ‘t’ test. Probability level less of 0.05 was considered statistically significant.

III. Results

Table 1: Body weight and urinary biochemical parameters in control and experimental groups

Parameters	Group -I (n=8)	Group -II(n=8)	Group -III(n=8)	Group -IV(n=8)
Body weight changes (gm)	5.29±1.10	5.78±1.49	-23.5±3.89 ^{a*}	-15.5±4.18 ^{b#}
Urinary calcium (mg/24h urine)	0.79±0.14	0.72±0.12	1.30±0.13 ^{a*}	0.84±0.14 ^{b*}
Urinary oxalate (mg/24h urine)	0.29±0.12	0.32±0.06	0.68±0.08 ^{a*}	0.38±0.05 ^{b*}
Urinary Citrate (mg/24h urine)	3.67±0.86	3.89±0.55	1.78±0.352 ^{a*}	2.61±0.062 ^{b#}
Urinary Magnesium (mg/24h urine)	2.39±0.16	2.53±0.26	1.65±0.24 ^{a*}	2.11±0.31 ^{b#}
Urinary Alkaline phosphatase (Units/mg creatinine)	0.14±0.04	0.16±0.03	0.34±0.07 ^{a*}	0.19±0.05 ^{b*}
Urinary Lactate dehydrogenase (Units/mg creatinine)	0.34±0.08	0.31±0.06	0.96±0.13 ^{a*}	0.40±0.08 ^{b*}
Urinary Creatinine (mg/24 h urine)	1.96±0.30	1.79±0.27	1.33±0.35 ^{a*}	2.08±0.32 ^{b*}
Urinary Phosphorus (mg/24 h urine)	8.26±0.13	8.31±0.11	10.74±0.74 ^{a*}	8.75±0.62 ^{b*}
Urinary uric acid (mg/24 h urine)	0.67±0.13	0.74±0.09	0.72±0.22	0.69±0.19

Data are expressed as mean±SD, * p value <0.001, # p value <0.05 was considered statistically significant.
 a= Group I compared with Group II and Group III
 b= Group III compared with Group IV

Table 2: Serum biochemical parameters in control and experimental groups

Parameters	Group -I (n=8)	Group -II(n=8)	Group -III(n=8)	Group -IV(n=8)
Serum calcium (mg/dl)	9.29±0.26	9.10±0.31	8.83±0.24	9.24±0.30
Serum Phosphorous (mg/dl)	5.08±0.32	5.06±0.22	3.67±0.36 ^{a#}	4.88±0.38 ^{b#}
Serum Magnesium (mg/dl)	2.44±0.11	2.28±0.25	3.69±0.13 ^{a*}	3.06±0.16 ^{b*}
Serum Urea (mg/dl)	27.50±3.20	28.25±3.58	30.75±2.60 ^{a*}	32.53±1.94 ^{b*}
Serum Creatinine (mg/dl)	0.83±0.32	0.86±0.08	0.93±0.12 ^{a*}	0.86±0.15 ^{b*}
Serum Uric acid (mg/dl)	1.46±0.11	1.49±0.09	1.50±0.13	1.56±0.26
Serum Aspartate transaminase (U/L)	149±7.62	150±9.52	171±9.21 ^{a*}	159±6.08 ^{b*}
Serum Alanine transaminase (U/L)	47±3.22	47±4.19	66±9.40 ^{a#}	52±7.32 ^{b#}
Serum Alkaline phosphatase (U/L)	241.6±14.9	231.8±12.4	263.6±16.3 ^{a*}	238.3±11.9 ^{b#}

Data are expressed as mean±SD, * p value <0.001, # p value <0.05 was considered statistically significant.
 a= Group I compared with Group II and Group III
 b= Group III compared with Group IV

Table 3: Erythrocyte antioxidant and TBARS levels in control and experimental groups

Parameters	Group -I (n=8)	Group -II(n=8)	Group -III(n=8)	Group -IV(n=8)
Super oxide dismutase- (U/mg Hb)	4.17±0.81	3.95±0.68	2.49±0.65 ^{a*}	3.85±0.39 ^{b*}
Catalase (μ moles of H ₂ O ₂ utilized/min/mg Hb)	35.75±4.27	34.13±3.95	27.23±4.17 ^{a*}	33.63±4.47 ^{b#}
Glutathione peroxidase (μg of GSH consumed /min/mg Hb)	6.55±0.72	6.48±0.62	4.12±1.22 ^{a*}	6.20±0.60 ^{b*}
Glutathione (mg/dl RBC)	41.7±6.34	43.37±4.80	31.7±6.45 ^{a#}	36.6±5.38 ^{b#}
TBARS (n moles/mg Hb)	5.94±0.69	6.39±0.88	7.93±0.27 ^{a*}	5.95±0.75 ^{b*}

Data are expressed as mean±SD, * p value <0.001, # p value <0.05 was considered statistically significant.
 a= Group I compared with Group II and Group III
 b= Group III compared with Group IV

Table 4: Hepatic, Renal enzymes and TBARS levels in control and experimental groups

Parameters	Group -I (n=8)	Group-II (n=8)	Group -III (n=8)	Group-IV(n=8)
Hepatic Glycolic acid oxidase (n moles/mg protein/min)	3.13±0.59	2.98±0.45	5.79±0.86 ^{a*}	3.75±0.43 ^{b*}
Hepatic Lactate dehydrogenase (Units/mg protein)	1.38±0.34	1.49±0.35	4.29±0.59 ^{a#}	1.50±0.66 ^{b#}
Renal Lactate dehydrogenase (Units/mg protein)	2.88±0.50	2.74±0.49	2.05±0.37 ^{a#}	2.98±0.38 ^{b#}
Hepatic TBARS (n moles/mg protein)	1.92±0.41	2.03±0.38	3.81±0.91 ^{a*}	2.98±0.44 ^{b#}
Renal TBARS (n moles/mg protein)	2.89±0.61	2.71±0.82	4.26±0.53 ^{a*}	2.70±0.72 ^{b*}

Data are expressed as mean±SD, * p value <0.001, # p value <0.05 was considered statistically significant.
 a= Group I compared with Group II and Group III
 b= Group III compared with Group IV

IV. Discussion

Crystalluria was induced in male albino rats by oral administration of ethylene glycol at the dose of 0.2 gm/day for 15 days [20]. Ethylene glycol administration approximately 2 fold increased in urinary oxalate excretion and marked crystalluria. The increased urinary oxalate excretion could be due to dietary source, increased intestinal absorption, increased synthesis from ethylene glycol, altered renal tubular transport. Oxalate

is an end product of several metabolic pathways. The amount of oxalate in the urine reflects the sum total of intestinal absorption plus denovo synthesis. Oxalate is transported by a passive simple diffusion process [21]. In the present study all the animals were on normal chow diet, so hyperoxaluria could not be due to dietary origin. Oxalic acid formed was largely excreted in the urine as calcium oxalate and deposited in the renal tubules [22]. Oxalate seems to be freely ultra-filterable at the glomerulus and there is a insignificant amount of secretion by the tubules [19]. Thus, the increased oxalate excretion could not be due to altered renal tubular transport.

Ethylene glycol metabolized through glycol aldehyde, glycolic acid and glyoxylic acid too oxalic acid and carbon dioxide by glycolic acid oxidase (GAO) and lactate dehydrogenase (LDH) [23]. So the hyperoxaluria is due to increased synthesis from ethylene glycol mainly, which is also evident from the enhanced activities of oxalate synthesizing enzymes of the liver such as GAO and LDH. The specific activities of liver GAO and LDH have been found to be significantly elevated in ethylene glycol fed animals which may be due to substrate mediated induction of enzymes [24]. Tribulus terrestris administration result in a decline in oxalate excretion which could be due to inhibition of hepatic GAO and LDH activity as reported earlier [25].

In ethylene glycol treated rats there was a significant increase in urinary calcium and phosphorous. The serum calcium was normal whereas serum phosphorus was lowered. The concentration of calcium in urine depends mainly on the concentration of inhibitor, a sufficient level of urinary oxalate results in the inhibitor factors being overcome and oxalate then complexes with scanty amount of urinary calcium available. The formed complexes are insoluble and they precipitate in the tubular lumen. The filtered calcium bound to oxalate cannot be reabsorbed and apparent calcium excretion increases. In Tribulus terrestris treated rats urinary calcium excretion was significantly lesser than ethylene glycol treated rats. This could be due to diminished oxalate excretion as a result of diminished activity of GAO and LDH.

Citrate seems to have significant inhibitory effect on calcium oxalate crystals growth even at very low concentration [26]. In the present study there was a significant reduction of citrate excretion in ethylene glycol treated rats. It could be due to ethylene glycol metabolites such as lactate, glycolic acid and oxalic acid accumulating in the renal tissue resulting in acidosis which would lead to increased reabsorption of citrate in exchange for hydrogen ion in the renal tubules causing reduced urinary citrate. In Tribulus terrestris treated rats urinary citrate excretion was significantly higher than ethylene glycol treated rats. This could be due to protective effect of the against toxic metabolites of ethylene glycol, interference with EG metabolism resulting in reduced formation of toxic metabolites.

In ethylene glycol treated group there was hypermagnesemia. There is a high risk of calcium oxalate stone formation in magnesium deficient rats due to hyper absorption and retention of calcium and oxalate by the renal tubules [27]. In ethylene glycol treated rats AST, ALT, ALP activities were raised which could be due to the toxic effect of the metabolites of ethylene glycol. It is reported that glycoaldehyde and glyoxylate are the principle metabolites responsible for EG toxicity causing ATP depletion and enzyme destruction [28]. In Tribulus terrestris treated rats the hepatic enzymes activity was significantly lesser than ethylene glycol treated rats.

In ethylene glycol treated rats serum urea and creatinine were significantly elevated which is suggestive of renal dysfunction. In Tribulus terrestris treated rats serum urea and creatinine became normal because toxic intermediate formation was reduced. Renal cell damage is also associated with lipid peroxide production indicating cell injury due to the production of free radicals and damage appears primarily due to hyperoxaluria and is augmented crystal deposition in renal tubules [29]. In Tribulus terrestris treated rats TBARS levels were significantly decreased than in ethylene glycol treated rats. It could be due to decrease urinary calcium oxalate excretion, decreased GAO and LDH activity and antioxidant effect of Tribulus terrestris.

In Tribulus terrestris treated rats the antioxidant status became normal which could be antioxidant protection rendered by possible flavonoids present in Tribulus terrestris extract. In conclusion toxic metabolites and oxalate formed from ethylene glycol has been proposed to induce free radical damage in the kidney and promoting calculogenesis. It is evident from increased TBARS and diminished antioxidant enzyme status. Administration of Tribulus terrestris extract improve the antioxidant status which may be due to their flavonoids such as Kaempferol, Quercetin and Isorhamnetin.

References

- [1]. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC.: Time trends in reported prevalence of kidney stones in the United States: 1976-1994. *Kidney Int* 63: 1817-1823, 2003.
- [2]. Worcester EM, Coe FL.: Clinical practice. Calcium kidney stones. *N Engl J Med*.2010; 363: 954-963.
- [3]. Ravindra VK, Navneet BG, Alagawadi KR, Rudrabrabhu VS. Effect of Moringa oleifera Lam. root - wood on ethylene glycol induced urolithiasis in rats. *J Ethnopharmacol* 2006;105:306-314.
- [4]. Vyas BA, Vyas RB, Joshi SV, Santani DD. Antiuro lithiatic activity of whole-plant hydroalcoholic extract of Pergularia daemia in rats. *J Young Pharm* 2011;3:36-40.
- [5]. Tania AV, Cristina DD, Ana PS, Maria TR, Antonio JL, Caden S. Evaluation of the antiuro lithiatic activity of the extract of *Costus spiralis* Roscoe in rats. *J Ethnopharmacol* 1999;66:193-8.
- [6]. Robertson WG, Peacock M. Pathogenesis of urolithiasis. In SchneiderHJ ed. HS-V &. Urolithiasis, Etiology, Diagnosis. Chapter 3. New York, Springer 1985: 185-301.

- [7]. Hodgkinson A. Oxalic acid in Biology and Medicine. New York: Academic Press 1984.
- [8]. Robertson WG. Urinary Tract Calculi. In NordinBEC, AGNa , HAM, Hong Kong: Churchill Livingstone, 1993: 249.
- [9]. Laker MF. The clinical chemistry of oxalate metabolism. *Adv Clin Chem* 1983; 23: 259-97.
- [10]. Thamilselvan S, Khan SR, Menon M. Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. *Urol Res* 2003; 31: 3-9.
- [11]. Thamilselvan S, Byer KJ, Hackett RL, Khan SR. Free radical scavengers, catalase and superoxide dismutase provide protection from oxalate-associated injury to LLC-PK1 and MDCK cells. *J Urol* 2000; 164: 224-9.
- [12]. Thamilselvan S, Selvam R. Effect of vitamin E and mannitol on renal calcium oxalate retention in experimental nephrolithiasis. *Indian J Biochem Biophys* 1997; 34: 319-23.
- [13]. Donnan SK. The Thiobarbituric acid test applied to tissues from rats treated in various ways. *J Biol Chem* 1950, 182: 415-419.
- [14]. Lui NS, Roels OA. An improved method for determining glyoxylic acid. *Anal Biochem.* 1970 ;38(1):202-9.
- [15]. Kakkar P, Das B, Viswanathan PN .A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys.*1984; 21:130-132.
- [16]. Asru K. Sinha. Colorimetric assay of catalase. *Analytical Biochemistry.*1972; 47(2):389-394.
- [17]. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science.* 1973; 179(4073):588-590.
- [18]. Beutler, E. and Kelley, B.M. The effect of sodium nitrate on RBC glutathione. *Experientia.*1963; 19: 96-97.
- [19]. Hodgkinson A, Williams A. An improved colorimetric procedure for urine oxalate. *Clin Chim Acta.* 1972; 36(1):127-32.
- [20]. DePass LR, Garman RH, Woodside MD, Giddens WE, Maronpot RR, Weil CS. Chronic toxicity and oncogenicity studies of ethylene glycol in rats and mice. *Fundam Appl Toxicol.* 1986; 7(4):547-65.
- [21]. Binder HJ. Intestinal oxalate absorption. *Gastroenterology.* 1974; 67(3):441-6.
- [22]. Richardson KE. Endogenous oxalate synthesis in male and female rats. *Toxicol Appl Pharmacol.* 1965; 7(4):507-15.
- [23]. Gessner Pk, Parke Dv, Williams R. Studies in detoxication. 86. The metabolism of 14C-labelled ethylene glycol. *Biochem J.* 1961; 79:482-9.
- [24]. Murthy MS, Talwar HS, Thind SK, Nath R. Vitamin B6 deficiency as related to oxalate-synthesizing enzymes in growing rats. *Ann Nutr Metab.* 1982;26(3):201-8.
- [25]. D. Sangeeta, H. Sidhu, urinder kumar Thind, S. Vaidyanathan. Therapeutic response of Tribulus terrestris (Gokhru) aqueous extract on hyperoxaluria in male adult rats. *Phytotherapy Research.* 1993;7(2):116 - 119 .
- [26]. Tiselius HG1, Berg C, Fornander AM, Nilsson MA. Effects of citrate on the different phases of calcium oxalate crystallization. *Scanning Microsc.* 1993;7(1):381-90.
- [27]. Rattan V, Sidhu H, Jethi RK, Thind SK, Nath R. Calcium and oxalate uptake by the renal brush border membrane vesicles in magnesium-deficient rats. *Biochem Mol Biol Int.* 1994; 34(5):1017-26.
- [28]. Poldelski V, Johnson A, Wright S, Rosa VD, Zager RA. Ethylene glycol-mediated tubular injury: identification of critical metabolites and injury pathways. *Am J Kidney Dis.* 2001;38(2):339-48.
- [29]. Thamilselvan S, Hackett RL, Khan SR. Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. *J Urol.* 1997 ;157(3):1059-63.