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Investigation of Effect of Ethylene Glycol-Induced Nephrolithiasis on Calcium Oxalate Crystals and Oxidative Damage of Renal Cells

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Abstract
Nephrolithiasis is one of the common renal diseases that is attributed to multiple factors. On the other hand, calcium oxalate is the most common urine lithiasis, and urinary calcium and oxalate oversaturation is vital to calcium oxalate stone formation. The percent study examined the possible role of Ethylene Glycol-Induced Nephrolithiasis on Calcium Oxalate Crystals and Oxidative Damage of Renal Cells in stone formation. The ethylene glycol was administered at doses of 0.4% (LD), 0.75% (MD), and 1.0% (HD) in drinking water for 28 days in Wistar rats to induce Nephrolithiasis. The blood and urine samples were collected on 14 and 28 days of treatment and analyzed for various biochemical parameters. Ethylene glycol causes a significant dose-dependent reduction in body weight and dose and time-dependent increases in urinary calcium, oxalate, phosphate, and total protein excretion levels and decreased urinary magnesium excretion in urine samples. Ethylene glycol causes dose and time-dependent increases in calcium, phosphate, and total protein content and significantly reduces magnesium.

Keywords: Nephrolithiasis. Ethylene glycol, Calcium Oxalate Crystals, Oxidative Damage, Renal Cells

1. INTRODUCTION

1.1. Ethylene glycol

Ethylene glycol (EG)(ethane-1,2-diol) is an organic compound (a vicinal diol) with the formula $(CH_2OH)_2$. It is an odorless, colorless, sweet taste, flammable viscous liquid, density-1.1132 g/cm^3, boiling point-19703 ^{0}C, and melting point-12.9^{0}C, but it is toxic in high concentrations. French chemist Charles-Adolphe Wurtz (1817–1884) first prepared ethylene glycol in 1856.[1] He first treated "ethylene iodide" ($C_2H_4I_2$) with silver acetate and then hydrolyzed the resultant "ethylene diacetate" with potassium hydroxide. Wurtz named his new compound "glycol" because it shared qualities with both ethyl alcohol (with one hydroxyl group) and glycerin (with three hydroxyl groups).

The caterpillar of the Greater wax moth, *Galleria mellonella*, has gut bacteria that can degrade polyethylene (PE) into ethylene glycol(EG).[2][3][4]

Ethylene glycol is produced from ethylene (ethene) via the intermediate ethylene oxide. Ethylene oxide reacts with water to produce ethylene glycol according to the chemical equation:

$$C_2H_4O + H_2O \rightarrow HO{-}CH_2CH_2{-}OH$$

Ethylene glycol has relatively high mammalian toxicity when ingested with an oral LD_{Lo}=786 mg/kg for humans. Administration of EG in drinking water has been shown to result in consistent induction of hyperoxaluria, crystalluria, and calcium oxalate nephrolithiasis.[5]

Severe metabolic acidosis, cardiopulmonary complications, acute renal failure, coma, and death characterize ethylene glycol administration in humans and animals.[6] The critical and sub-chronic toxicity of ethylene glycol results from its metabolism to two toxic metabolites, initially glycolic acid, which is responsible for acidosis, and eventually oxalic acid, which precipitates as calcium oxalate monohydrate (COM) in numerous tissues, especially the kidney. The renal failure of ethylene glycol poisoning is associated with proximal tubular (PT) cell necrosis and with the appearance of COM crystals in the urine and kidney tissue[7]. In normal human proximal tubule (HPT) cells in primary culture, oxalate (≥2 mM) cause cell death[8]. Subsequent studies in HPT cells have indicated that COM, but not the oxalate ion, is responsible for the cytotoxicity. Studies in renal cell lines have confirmed that oxalate or COM produces kidney cell death in the 0.5–2 mM concentration range [9]. The toxic effects have been linked with increased free radical production [10] and lipid peroxidation[11]. The critical role of calcium oxalate in the etiology of renal failure has been recently confirmed in vivo. In rats fed diets containing ethylene glycol for 16 weeks [12], the development of nephropathy correlated highly with kidney oxalate crystal accumulation.

1.2. Nephrolithiasis

Nephrolithiasis is a significant health problem with a worldwide prevalence of between 2% and 20%.[13,14,15] According to epidemiological data, the incidence of nephrolithiasis has risen over the last decades in the United States and other parts of the world.[16,17,18,19,20] Kidney stones are linked with chronic kidney disease. Preventing reappearance is precise to the type of stone like calcium oxalate, calcium phosphate, cystine, magnesium ammonium phosphate, and uric acid stones.[21,.22]

Renal stone formation and the biggest chemical composition depend on age and gender. The majority of stones are formed in older age people. However, clinical interpretations have mentioned an altering frequency and composition of urinary calculi and a swing in gender and age-related incidences. Contributing risk factors for kidney stones are obesity, insulin resistance, gastrointestinal pathology, living in warmer climates, and specific dietary patterns and medications.[23,24,25]

The escalating frequency of nephrolithiasis is associated with rising imaging utilization for diagnosis, treatment planning, and post-treatment follow-up. Imaging in nephrolithiasis has risen over the years due to technological advancement and an improved understanding of the disease process. Since its beginning in the 1990s, unenhanced computed tomography has become the gold standard for the characterization of urinary stone disease.[26,27,28,29]

1.2.1. Basic pathophysiological mechanisms

Renal stone formation progresses in successive steps.[30]

(a) Nucleation: this regards the phase change of dissolved salts into a solid. It is dependent on the degree of saturation of urine in one solvent. One salt can remain dissolved in urine even though its concentration exceeds its solubility. In such cases, the urine is characterized as meta-stable. The limit of supersaturation above which precipitation of the dissolved salts occurs is the upper limit of metastability.[31] Nucleation can be either homogenous when crystal precipitation happens spontaneously in supersaturated urine or heterogeneous when it occurs at lower degrees of saturation in the presence of nucleating agents (*i.e.*, cells, crystals, urinary proteins, or components of the epithelial cells).
    (a) Retention of the initial nucleus in sites of the urothelium
    (b) Crystal's growth
    (c) Crystal's aggregation

There are three proposed pathogenetic mechanisms of Nephrolithiasis.[32,33]
    (a) The free particle theory
    (b) The fixed particle theory
    (c) Interstitial apatite plaque (Randall's plaque hypothesis)

2. MATERIALS AND METHODS

2.1. CHEMICALS

Ethylene glycol of Analytical Research grade was obtained from Merck Ltd., Mumbai, India. All the other chemicals were used in the study. Were of analytical grade and procured from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

2.2. EXPERIMENTAL ANIMALS

The experimental procedures were approved by the "Committee for the Purpose of Control and Supervision of Experiment on Animals" and the "Institutional Animal Ethics Committee" (CPCSEA IAEC Approval No. CPCSEA/IAEC/DIPS/DL/05/20/124), New Delhi, India. Healthy Wistar strain rats weighing 200-220 gm. of equivalent age group were obtained. They were acclimatized for 15 days in polypropylene cages under controlled conditions (temperature 25 ± 2^0C; relative humidity 50–55%; 12 h light/ dark cycle). Animals were maintained on certified pelleted rodent feed and potable water *ad libitum* throughout the study.

2.3. ETHYLENE GLYCOL-INDUCED NEPHROLITHIASIS MODEL

The initial study focused on determining the best possible dosage of ethylene glycol treatment to induce nephrolithiasis. Three different doses of ethylene glycol in drinking water (0.4%, 0.75%, and 1.0%, v/v) were selected to induce nephrolithiasis. For this, twenty animals were randomly divided into four groups (five per group) and caged separately. Group 1 (untreated control) animals were maintained without treatment and given free food and drinking water access. Animals of groups 2, 3, and 4 were administered ethylene glycol at doses of 0.4% (LD), 0.75% (MD), and 1.0% (HD) in drinking water for 28 days. Mortality rate, behavioral and clinical changes were noted in animals of all experimental groups. On completion of treatment, the body weight of rats was recorded individually, and meaningful consequences were calculated. The blood samples were collected on 14 and 28 days of treatment from the retro-orbital sinus under mild ether anesthesia and allowed to clot. Serum was separated by centrifuging the blood at 1000 rpm. for 10 min and was used to estimate calcium, phosphate, magnesium, and total protein. Also, the early morning urine samples collected on 14 and 28 days of calculi induction treatment were analyzed for calcium, oxalate, phosphate, Magnesium, and total protein.

2.3.1. BIOCHEMICAL ANALYSIS

2.3.1.1. Calcium content

The calcium content in the urine and serum was estimated[34]. In an alkaline medium, calcium reacts with ocresolphthalein to form an intense red-violet color chromophore that absorbs light at 570 nm. Magnesium and iron are excluded by complexing with 8-hydroxyquinoline. The calcium content was expressed as mg/dL for urine and serum.

2.3.1.2. Oxalate content

The oxalate content was estimated in urine[35]. Oxalate is oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. The hydrogen peroxide reacts with 3-methyl-2- benzothiazolinone hydrazone (MBTH) and 3-(dimethylamino) benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye which has an absorbance maximum at 590 nm. The intensity of the color produced is directly proportional to the concentration of oxalate in the sample. The oxalate content was expressed as mg/dL for urine.

2.1.2.3. Phosphate content

The phosphate concentration was estimated in the urine and serum[36]. Inorganic phosphorus combines with ammonium molybdate in the presence of strong acids to form phosphomolybdate. The formation of phosphomolybdate is measured at 340 nm and is directly proportional to the concentration of inorganic phosphorus. The phosphate content was expressed as mg/dL in the case of urine and serum.

2.3.2.4. Magnesium content

The magnesium content was estimated in urine and serum[37]. Magnesium ions react with calmagite in an alkaline medium to produce a red-colored complex measured spectrophotometrically at 532 nm. The intensity of color produced is directly proportional to magnesium concentration.

2.3.2.5. Total protein content

Total protein content was estimated using bovine serum albumin as a standard in urine and serum[38]. The method is based on the formation of a protein-copper complex and reduction of phosphomolybdate-phosphotungstate reagent (Folin-Ciocalteu phenol reagent) by tyrosine and tryptophan residues of protein to form a colored product, which was measured at 540 nm. The protein content was expressed as mg/dL in the case of urine and serum.

2.4. STATISTICAL ANALYSIS

Results are expressed as mean ± SEM; n = 5, $^{\alpha}$n=3. Values shown in parenthesis indicate percent change ^{b} as compared to untreated control. Level of significance *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3. RESULTS

3.1. ETHYLENE GLYCOL-INDUCED NEPHROLITHIASIS

3.1.1. Clinical observations

No treatment-related clinical signs were observed in control and ethylene glycol-treated animals. However, there was 40% mortality in group 4 rats, administered with 1.0% of ethylene glycol in drinking water.

3.1.2. Body weight

Table 1. shows the changes in body weight of untreated control and ethylene glycol-treated rats. No significant difference in body weight was noted in the control group (Group 1). However, ethylene glycol treatment (Groups 2, 3, and 4) caused a significant dose-dependent reduction in body weight (LD: 1.74%; MD: 7.80%; HD: 15.92%; r2 =0.9326; Fig.1) of rats in 14 days as well as on 28 days (LD: 5.83%; MD: 13.58%; HD: 22.94%; r2 =0.9894; Fig.1) as compared to untreated control.

3.1.3. URINARY BIOCHEMICAL ANALYSIS

3.1.3.1. Effect on calcium and oxalate content

The effect of ethylene glycol treatment on calcium and oxalate excretion in urine is presented in Table 2. A significant ($p<0.05$) dose and time-dependent increase in urinary calcium and oxalate excretion levels were noted in all ethylene glycol-treated animals (Groups 2, 3, and 4) on 14 days (LD: 40.98%; MD: 104.70%; HD: 162.00%, r2 = 0.9928, Fig. 2) as well as on 28 days (LD: 73.67%; MD: 160.20%; HD: 252.23%, r2 = 0.99756, Fig. 2) as compared to untreated control (Group 1). Similarly, there was a significant ($p<0.05$ increase, as compared to untreated control (Group 1), in urinary oxalate excretion level on 14 days (LD: 32.63%; MD: 82.27%; HD: 111.42%, r2 = 0.9901, Fig. 2) as well as on 28 days (LD: 134.59%; MD: 259.89%; HD: 341.95%, r2 = 0.98885, Fig. 2) in ethylene glycol-treated rats in a time-dependent manner.

3.1.3.2. Effect on phosphate and magnesium content

The effect of ethylene glycol treatment on the excretion of phosphate and magnesium in the urine of rats is shown in Table 3. The ethylene glycol administration caused, as compared to untreated control (Group 2), a significant dose and time-dependent ($p<0.05$) increase in urinary phosphate excretion over 14 days (LD: 174.91%; MD: 347.84%; HD: 655.50%, r2 = 0.9772, Fig. 3) as well as on 28 days (LD: 493.73%; MD: 685.93%; HD: 965.64%, r2=0.9616, Fig. 3) of treatment. Ethylene glycol administration significantly ($p<0.01$) decreased urinary magnesium excretion on 14 days (LD: 23.93%; MD: 45.84%; HD: 53.58%, r2 = 0.9581, Fig. 3) and 28 days (LD: 22.27%; MD: 60.81%; HD: 89.47%, r2 = 0.9906; Fig. 3) of treatment, as compared to untreated control (Group 2) in a dose and time-dependent manner.

3.1.3.3. Effect on total protein

The total protein content in the urine of ethylene glycol–treated animals was found to be significantly ($p<0.05$) increased when compared to animals of control groups (Table 4) in a dose and time-dependent manner. Fig.4 shows that the increase in protein content by ethylene glycol treatment was 17.07%, 99.2%, and 265.85% on 14 days (r2=0.8730) and by 48.88%, 195.29%, and 359.56% on 28 days (r2=0.9536).

3.1.3.4. Correlation analysis

Pearson correlation analysis between different parameters in urine was calculated and presented in Table 5 and Fig.5 . A strong positive correlation was observed among urinary excretion of calcium and oxalate($r>0.916$), calcium and phosphate($r>0.970$), calcium and total protein($r>0.973$), oxalate and phosphate($r>0.946$), oxalate and total protein ($r>0.850$), phosphate and total protein ($r>0.924$). However, magnesium excretion level negatively correlated with calcium($r >0.987$), oxalate ($r>0.905$), phosphate($r>0.924$), and total protein ($r>0.941$).

3.1.4. SERUM BIOCHEMICAL ANALYSIS

3.1.4.1. Effect on calcium and phosphate contents

Table 6 shows the effect of ethylene glycol treatment on calcium and phosphate contents in the serum of rats. Results revealed that administration of ethylene glycol caused a significant ($p<0.01$) dose and time-dependent increase in calcium content; LD, MD, and HD of the ethylene glycol decreased the calcium content by 20.82%, 34.25%, and 51.06% on 14 days (r2=0.9929). By 26.51%, 60.97%, and 80.66% on 28 days, respectively (r2=0.9903, Fig.6). Ethylene glycol administration also caused a significant ($p<0.05$) elevation in phosphate content in the serum of rats, as shown in Table 6. All three doses of ethylene glycol had dose - as well as time-dependent (LD: 30.42%; MD: 89.16%; HD: 118.98% on 14 days (r2=0.98139) and LD: 49.57%; MD: 135.04%; HD: 174.44% on 28 days; r2=0.98085) increase on phosphate content as compared to untreated control.

3.1.4.2. Effect on magnesium content

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Table 7 shows the effect of ethylene glycol on magnesium content in the serum of rats. As compared with the untreated control rats, ethylene glycol administration caused a significant reduction ($p<0.05$) in magnesium content over 14 days (LD: 26.30%; MD: 43.06%; HD: 73.12%; r2 = 0.9895) as well as on 28 days (LD: 34.67%; MD: 68.88%; HD: 83.66%; r2=0.9720). The effect was dose and time-dependent, as shown in Fig. 7.

3.1.4.3. Total protein content

The effect of ethylene glycol on total protein content in serum is presented in Table 7. A significant ($p<0.01$) increase in total protein was noted in all ethylene glycol-treated animals in a time and dose-dependent manner over 14 days (LD: 56.67%; MD: 143.04%; HD: 223.70%, r2=0.9928; Fig. 7) as well as on 28 days (LD: 86.83%; MD: 187.80%; HD: 260.60%; r2 = 0.99680). The effect was dose and time-dependent, as shown in Fig. 7.

3.1.4.4. Correlation analysis

The Pearson correlation analysis was done between different parameters in serum and was presented in Table 8. A strong negative correlation was observed among serum calcium and phosphate($r>0.992$), calcium and total protein ($r>0.972$), phosphate and magnesium($r>0.970$), and magnesium and total protein($r>0.990$). However, serum calcium with magnesium($r > 0.979$) and phosphate with total protein in serum ($r > 0.972$) levels was positively correlated.

Table 1. Ethylene glycol induced changes in the body weight (gm) of rats.

S. No.	Experimental Group	Day of Treatment		
		0	14	28
1	Untreated Control	215.370±10.576	215.660±9.153	214.333±6.583
2	Ethylene Glycol(Low dose; 0.40% in drinking water)	216.920±7.164	211.916±8.605 [b](1.74)	201.833±6.390 [b](5.83)
3	Ethylene Glycol(Mild dose; 0.75% in drinking water)	216.130±10.317	198.840±1.559 [b](7.80)	185.233±5.388 [b](13.58)
4	[a]Ethylene Glycol(High dose; 1.0% in drinking water)	216.140±7.822	181.320±2.583 [b](15.92)	165.167±3.291 [b](22.94)

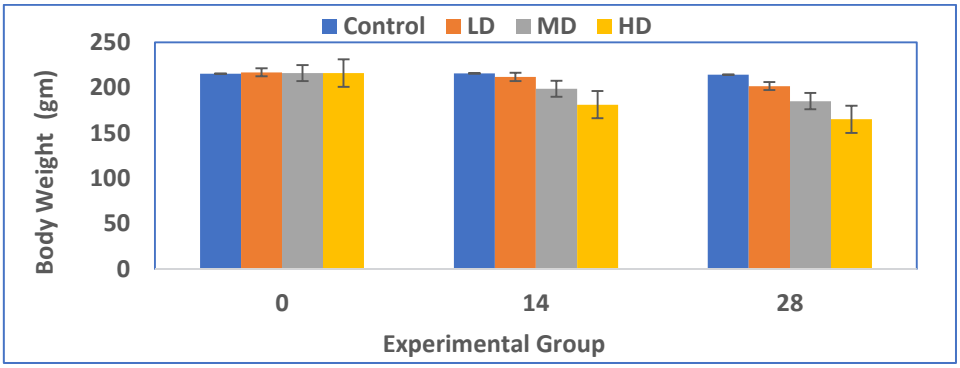

Figure 1. Ethylene glycol induced changes in the body weight (gm) of rats.

Table 2. Effect of ethylene glycol on urinary excretion of calcium and oxalate od rats.

S. No.	Experimental Group	Calcium (mg/dL)		Oxalate (mg/dL)	
		14 days	28 days	14 days	28 days
1	Untreated Control	5.452±0.793	5.470±0.849	24.700±1.245	24.980±1.170
2	Ethylene Glycol(Low dose;	7.686±0.393*	9.500±0.316*	32.760±1.086	58.600±1.265

	0.40% in drinking water)	[b](40.98)	[b](73.67)	[b](32.63)	[b](134.59)
3	Ethylene Glycol(Mild dose; 0.75% in drinking water)	11.160±0.36* [b](104.70)	14.233±0.183* [b](160.20)	45.020±0.566 [b](82.27)	89.900±1.14 [b](259.89)
4	[a]Ethylene Glycol(High dose; 1.0% in drinking water)	14.284±0.43* [b](162.00)	19.267±0.658* [b](252.23)	52.220±0.686 [b](111.42)	110.400±1.673 [b](341.95)

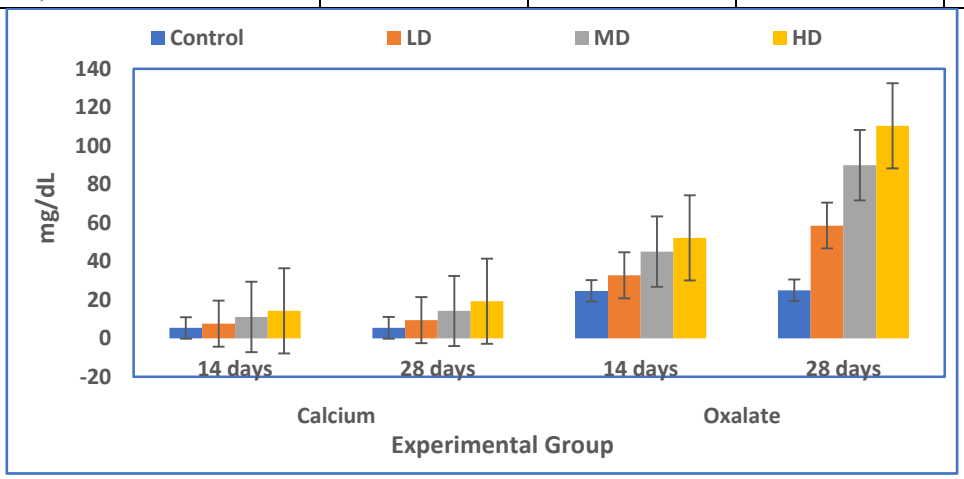

Figure 2. Effect of ethylene glycol on urinary excretion of calcium and oxalate od rats.

Table 3. Effect of ethylene glycol on urinary excretion of phosphate and magnesium of rats.

S. No.	Experimental Group	Phosphate (mg/dL)		Magnesium(mg/dL)	
		14 days	28 days	14 days	28 days
1	Untreated Control	16.180±0.686	16.910±0.745	5.170±0.381	5.060±0.283
2	Ethylene Glycol(Low dose; 0.40% in drinking water)	44.480±0.985 [b](174.91)	100.400±1.378 [b](493.73)	3.933±0.552** [b](23.93)	3.933±0.183** [b](22.27)
3	Ethylene Glycol(Mild dose; 0.75% in drinking water)	72.460±1.153 [b](347.84)	132.900±2.07 [b](685.93)	2.800±0.387* [b](45.84)	1.983±0.183* [b](60.81)
4	[a]Ethylene Glycol(High dose; 1.0% in drinking water)	122.240±2.373* [b](655.50)	180.200±0.949* [b](965.64)	2.400±0.316* [b](53.58)	0.533±0.183* [b](89.47)

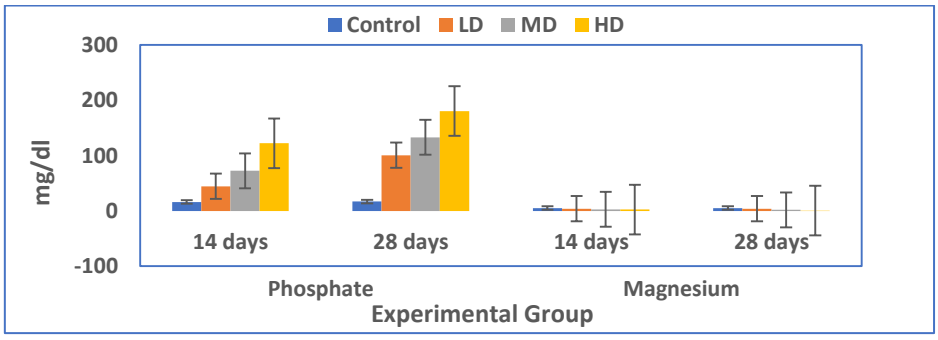

Figure 3. Effect of ethylene glycol on urinary excretion of phosphate and magnesium of rats.

Table 4. Effect of ethylene glycol on urinary excretion of Total Protein of rats.

S. No.	Experimental Group	Total Protein (mg/dL)	
		14 days	28 days
1	Untreated Control	2.050±0.158	2.060±0.173
2	Ethylene Glycol(Low dose; 0.40% in drinking water)	2.400±0.224 [b](17.07)	3.067±0.183 [b](48.88)
3	Ethylene Glycol(Mild dose; 0.75% in drinking water)	4.080±0.087* [b](99.02)	6.083±0.091* [b](195.29)
4	"Ethylene Glycol(High dose; 1.0% in drinking water)	7.500±0.224* [b](265.85)	9.467±0.183* [b](359.56)

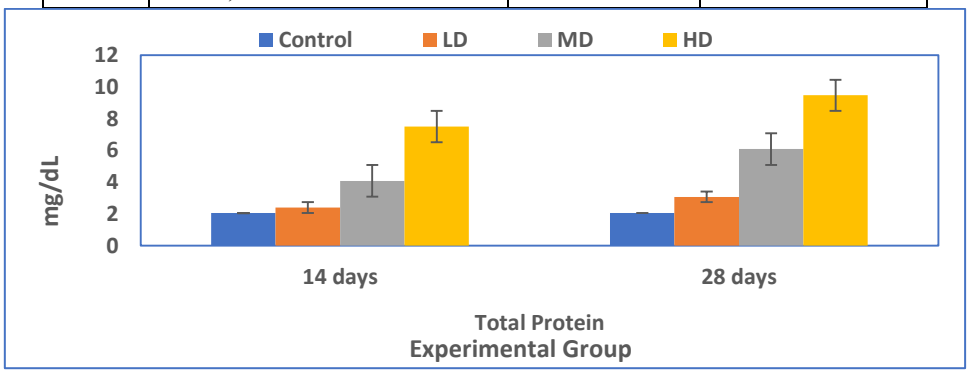

Figure 4. Effect of ethylene glycol on urinary excretion of Total Protein of rats.

Table 5. Pearson correlation analysis of the relationship between experimental parameters in the urine of rats

	Calcium	Oxalate	Phosphate	Magnesium	Total Protein
Calcium	-	0.9164**	0.9708***	-0.9879***	0.9736***
Oxalate	-	-	0.9462***	-0.9053**	0.8500**
Phosphate	-	-	-	-0.9421***	0.9247**
Magnesium	-	-	-	-	-0.9413***

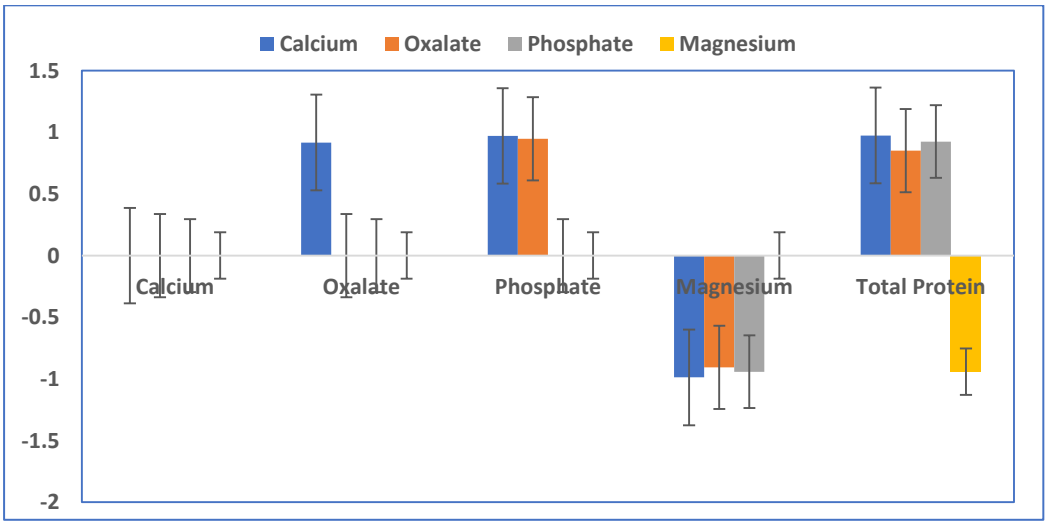

Figure 5. Pearson correlation analysis of the relationship between experimental parameters in the urine of rats

Table 6. Effect of ethylene glycol on calcium and phosphate content in the serum of rats.

S. No.	Experimental Group	Calcium (mg/dL)		Phosphate (mg/dL)	
		14 days	28 days	14 days	28 days
1	Untreated Control	9.460±0.132	9.480±0.087	3.320±0.308	3.510±0.235
2	Ethylene Glycol(Low dose; 0.40% in drinking water)	7.490±0.235** [b](20.82)	6.967±0.365** [b](26.51)	4.330±0.212* [b](30.42)	5.250±0.158* [b](49.57)
3	Ethylene Glycol(Mild dose; 0.75% in drinking water)	6.220±0.520* [b](34.25)	3.700±0.548* [b](60.97)	6.280±0.087* [b](89.16)	8.250±0.158* [b](135.04)
4	[α]Ethylene Glycol(High dose; 1.0% in drinking water)	4.630±0.803* [b](51.06)	1.833±0.183* [b](80.66)	7.270±0.087* [b](118.98)	9.633±0.483* [b](174.44)

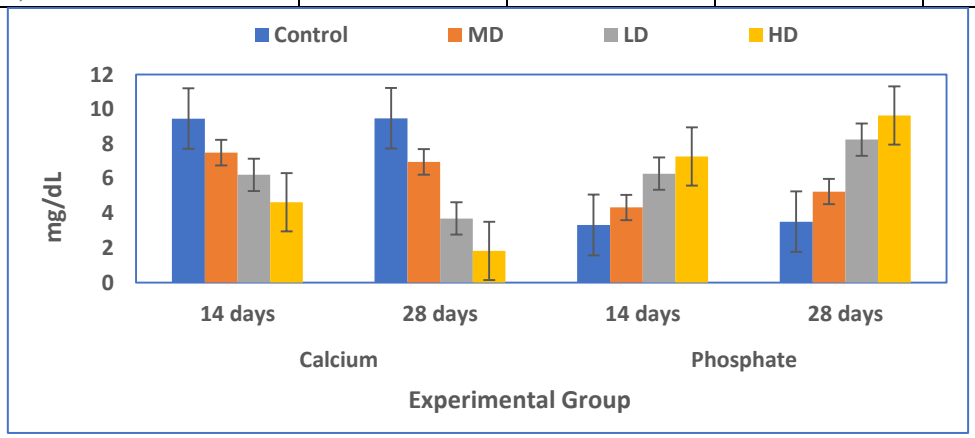

Figure 6. Effect of ethylene glycol on calcium and phosphate content in the serum of rats.

Table 7. Effect of ethylene glycol on magnesium and protein content in the serum of rats.

S. No.	Experimental Group	Magnesium(mg/dL)		Total Protein (mg/dL)	
		14 days	28 days	14 days	28 days
1	Untreated Control	3.460±0.132	3.470±0.087	2.802±0.113	2.810±0.071
2	Ethylene Glycol(Low dose; 0.40% in drinking water)	2.550±0.158** [b](26.30)	2.267±0.091** [b](34.67)	4.390±0.693* [b](56.67)	5.250±0.158* [b](86.83)
3	Ethylene Glycol(Mild dose; 0.75% in drinking water)	1.970±0.141* [b](43.06)	1.080±0.063* [b](68.88)	6.810±0.071** [b](143.04)	8.067±0.091** [b](187.80)
4	[α]Ethylene Glycol(High dose; 1.0% in drinking water)	0.930±0.087* [b](73.12)	0.567±0.091* [b](83.66)	9.070±0.087** [b](223.70)	10.133±0.365** [b](260.60)

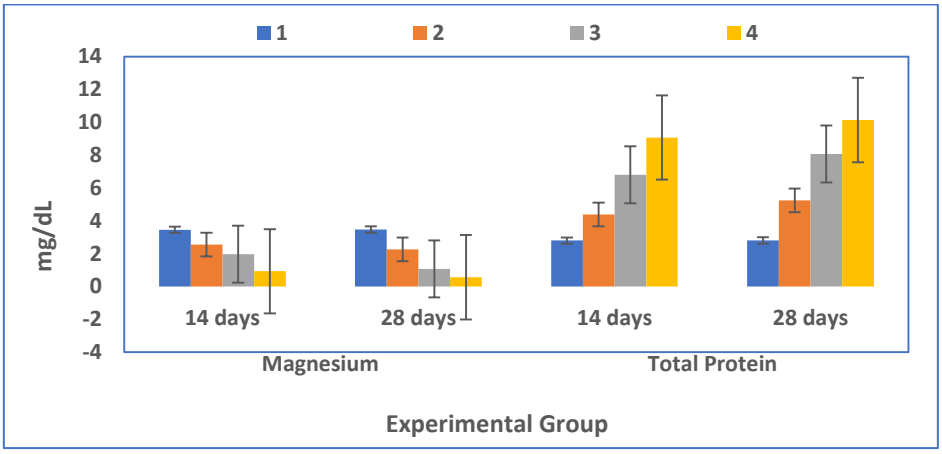

Figure 7. Effect of ethylene glycol on magnesium and protein content in the serum of rats.

Table 8. Pearson correlation analysis of the relationship between experimental parameters in the urine of rats

	Calcium	Phosphate	Magnesium	Total Protein
Calcium	-	-0.9926***	0.9799***	-0.9721***
Phosphate	-	-	-0.9700***	0.9723***
Magnesium	-	-	-	-0.9907***

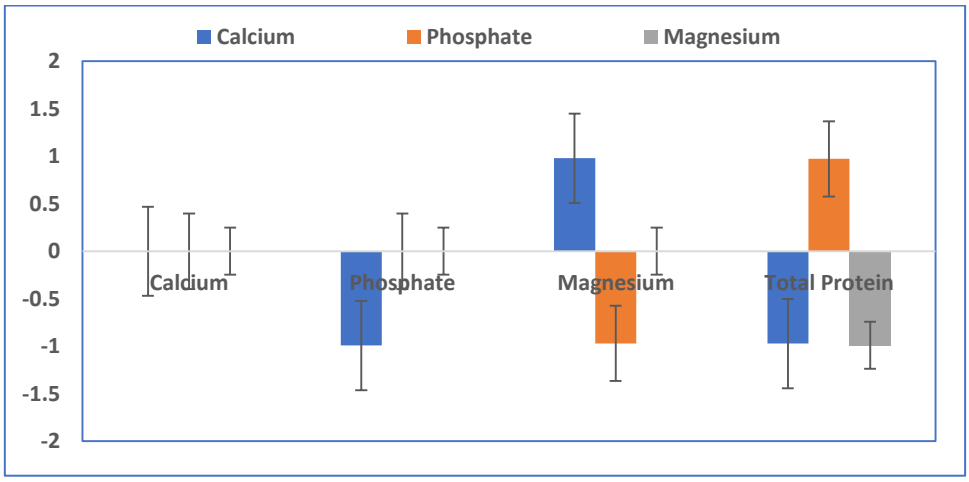

Figure 8. Pearson correlation analysis of the relationship between experimental parameters in the urine of rats

DISCUSSION

No treatment-related clinical signs were observed in control and ethylene glycol-treated animals. However, there was 40% mortality in group 4 rats, administered with 1.0% of ethylene glycol in drinking water. Khan (1997) also reported mortality in Sprague Dawley rats treated with 1.0% ethylene glycol in drinking water.[39] The administration of ethylene glycol in drinking water caused a dose-dependent significant reduction in the body weight of rats (Table 1; Fig. 1). A significant loss in body weight correlates with a decrease in feed consumption. A similar decrease in body weight due to the decrease in food consumption has also been reported in an earlier study.[40] A considerable

reduction in body weight and increased kidney weight in rats was also observed when treated with ethylene glycol in drinking water.[41]

Urinary chemistry is one of the crucial factors in determining the type of crystals formed and the nature of macromolecules included on the surface of the crystals. Hence, indicates related to calculi-forming minerals will provide a good indication of the extent of stone formation. The urinary supersaturation for stone-forming constituents is generally considered one of the causative factors in calculogenesis. The biochemical mechanism for this process is related to an increase in the urinary concentration of calcium and oxalate. In the present study, the administration of ethylene glycol in rats caused a significant increase in calcium and oxalate excretion levels compared to untreated control in a time and dose-dependent manner (Table 2, Figs. 2). The high urinary calcium concentrations led to increased urinary saturation of calcium salts and reduced urinary inhibitory activity by way of complexation with negatively charged inhibitors such as citrate.[42] Hyperoxaluria is also a major significant risk factor in the pathogenesis of renal stones. It has been reported that oxalate plays a vital role in stone formation and has about 15-fold more significant effect than urinary calcium.[43,44,45] In the present study, urinary oxalate was increased in ethylene glycol-induced urolithic rats (Table 2, Fig. 2). Similar rapid increases in calcium and oxalate excretion due to ethylene glycol treatment were also reported.[46]

The administration of ethylene glycol caused a significant elevation in phosphate excretion, compared to untreated control, in a dose and time-dependent manner (Table 3 Fig. 3). The increased phosphate excretion has been reported in stone formers.[47] The increased urinary phosphate excretion and oxalate stress provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces CaOx deposition.[48]. The treatment of ethylene glycol caused a significant reduction in magnesium excretion compared to untreated control in a dose and time-dependent manner (Table 3, Fig. 3). Magnesium is a well-known inhibitor of calcium stone formation, preventing crystal growth and aggregation. In a supersaturated CaOx solution, magnesium reduced the CaOx particle number by 50%.[49] Magnesium can form complexes with oxalate and thus decreases urinary supersaturation.

An increased urinary total protein excretion was observed in ethylene glycol-induced urolithiasis rats in a dose-dependent manner (Table 4, Fig. 4). The proteinuria reflects proximal tubular dysfunction. The supersaturation of urinary colloids results in precipitation as crystal initiation particles which, when trapped, act as a nidus leading to subsequent crystal growth. This is associated with proteinuria.

In calculi-induced rats, there is a significant reduction in serum calcium level due to ethylene glycol treatment in a dose and time-dependent manner (Table 6, Fig. 6). It has been reported that the oxalic acid, a metabolite of ethylene glycol, chelates serum calcium and precipitates as crystals in renal tubules, thereby causing depletion of serum calcium level.[50] However, there is a significant increase in serum phosphate levels in urolithiasis rats (Table 6, Fig. 6). These results are per the previous studies with ethylene glycol in various animal species.[51,52]

Results also indicated a dose-dependent decrease in magnesium content in serum after ethylene glycol treatment (Table 7, Fig. 7). Magnesium supplementation in subjects with magnesium deficiency had been reported to increase the excretion of citrate in the urine, which in turn, binds with calcium thereby reducing the concentration of CaOx aggregation.[53] Table 7 indicates the results of serum levels of total protein after ethylene glycol treatment (Fig. 7). There was a considerable increase in

serum total protein due to the ethylene glycol treatment in a dose-dependent manner. The main protein most likely to appear in the urine is albumin.[54] However, it has been reported that in CaOx urolithiasis, there has been a rise in oxalate-binding protein in serum, thereby promoting crystallization.[55]

SUMMARY AND CONCLUSIONS

This study aimed to establish the appropriate dose of ethylene glycol, a commonly used agent to induce urolithiasis in rat models. The ethylene glycol was administered at doses of 0.4% (LD), 0.75% (MD), and 1.0% (HD) in drinking water for 28 days in female Wistar rats to induce Nephrolithiasis. The blood and urine samples were collected on 14 and 28 days of treatment and analyzed for various biochemical parameters. The ethylene glycol significantly decreased body weight with a concurrent increase in urinary excretion of calcium, oxalate, phosphate, and total protein. Moreover, there was a significant reduction in serum calcium and magnesium, while a significant increase was observed in levels of phosphate and total protein in serum.

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