International Journal of Natural and Engineering Sciences 5 (2): 01-06, 2011
ISSN: 1307-1149, E-ISSN: 2146-0086, www.nobel.gen.tr

The Effects of Ethylene Glycol on Factors Influencing Urolithiasis in Rat

P. MARDANI[1] J. MOSHTAGHIAN[2] P. MAHZOONI[3] M. MORADI[4*]

[1] Biology Department, Payame Noor University, 19395-4697 Tehran, I. R. of IRAN

[2] Department of Biology, College of Science, Isfahan University, IRAN

[3] Department of Pathology, College of Medicine, Isfahan University, IRAN

[4] Department of Biology, Faculty of Science, Zanjan University, Zanjan, IRAN

*Corresponding Author

e-mail: moradi_g@yahoo.com

Received: January 03, 2011
Accepted: January 15, 2011

Abstract

The aim of this study was to investigate in vitro the relative toxic effects of ethylene glycol (EG), a major industrial chemical, on biomarkers of urolithiasis in rats. 60 adult male rats weighting 200-250 grams were randomly distributed into 10 groups of six each in two experiments. In experiment 1, Four of the animal groups were each treated either with 0.2, 0.4, 0.8 or 1.6 percent EG in drinking water, respectively and one group as control for a period of 4 weeks. Experiment 2, was exactly the same it, but for a period of 8 weeks. At the end of each of the treatment periods, 24-hour urine samples were collected from all respected animals using single metabolic cages. Urine samples were used to measure calcium, oxalate, phosphorus, uric acid, protein, creatinine, citrate, magnesium and pH. Blood samples were used to measure calcium, phosphorus and creatinine. Kidneys Sections were stained and pathologically investigated for stone formation. The results indicated EG or its metabolites caused dose and duration of treatment dependent significant changes in urolithiasis factors. Thus considering health principles, the wide spread industrial use of EG may not be as safe as it is thought to be.

Keyword: Ethylene glycol, Hyperoxaluria, Biomarkers and Rat

INTRODUCTION

Calcium oxalate calculi of the kidneys are a common clinical problem [1]. Hyperoxaluria is a major risk factor of human idiopathic calcium oxalate calculi disease [2]. In the early stage of urolithiasis, crystallization in supersaturated urine is an important process in the formation of mature renal stones. The retention of microcrystals by renal epithelial cells has been specifically proposed as a critical event in this process [3, 4]. Thamilselvan et al found that lipid peroxidation occurred in kidney tissue and urine samples of male rats treated with 75% ethylene glycol during all periods [5]. Ethylene glycol (EG) is a clear, odorless, slightly viscous liquid and a bitter-sweet-tasting dihydric alcohol (HO-CH2-CH2-OH) [6, 7] EG is a very useful industrial compound because of its low freezing point and high boiling point. EG is an intermediate in the synthesis of a number of commercial chemical products, including polyethylene terephthalate (PET) resins, unsaturated polyester resins, polyester fibers and films. It is also a constituent in antifreezes, deicing fluids, surface coatings, heat transfer fluids, industrial coolants, hydraulic fluids, surfactants and emulsifiers. General population or consumer exposure occurs primarily from the use of ethylene glycol in automotive antifreeze. Dermal or inhalation exposure to workers may occur during manufacturing or using of the chemical materials. It may also enter the environment from its uses in deicing airplane runways and solvents containing EG [8, 9]. EG is readily absorbed from the GI-tract, and the maximal blood concentration is reached within 1-4h, and the half-life is 3-8h. It has a low toxicity in itself, but is broken down In vivo by the enzymes to four organic acids: glycoladehyde, glycolic acid, glyoxylic acid and oxalic acid [10, 11]. The metabolites are cell toxins that suppress the oxidative metabolism, causing central nervous system depression, cardio-pulmonary and renal failure. This results in an accumulation of glycolic acid in the blood. Glycolic acid causes severe acidosis, and oxalate is precipitated as calcium oxalate in the kidneys and other tissues [12, 13, and 14]. In this experiment model, formation of calcium oxalate deposits in the kidney can be demonstrated in an EG different doses and periods of treatment time with the presence of renal stones that can be observed macroscopically. This would indicate an abnormal physiological condition using the existing experimental model in rats.

MATERIAL AND METHODS

In Juan 2007, 60 adult male Wister rats weighting about 200-250 grams were obtained from Isfahan medical university, Iran. Then, they were maintained at animal house in the biology department of Isfahan University, Iran. The animals randomly distributed into 10 groups of six each and were given commercial food pellets and tap water ad libitum. Ethylene glycol purchased from Polyacryil Company and dissolved in tap water. After 2 weeks, Rate habituated the climate of controlled conditions. In experiment 1, four of the animal groups were each treated either with 0.2, 0.4, 0.8 or 1.6 percent EG in drinking water, respectively for a period of 4 weeks. In experiment 2, each of another set of four animal groups was treated with exactly the same percentage of EG in drinking water respectively, but for a period of 8 weeks. For each of the 4-week or 8-week treatment periods, a separate control group of animals were considered that received regular drinking water. At the end of each of the treatment periods, the rats were transferred to individual metabolic cages before sacrificing and 24-hour urine samples were collected in a 50 ml centrifuge tube. Then, the animals were weighted and sacrificed for gross anatomy examinations and their blood and renal tissue samples were

collected. Urine samples were centrifuged for 10 min at 800g for subsequent analysis and were used to measure calcium, oxalate, phosphorus, uric acid, protein, creatinine, citrate and magnesium using diagnostic kits (Darman kave Diagnostics, Iran) and urine volume. Deposits were used for microscopic examination. Blood samples were taken to measure calcium, phosphorus and creatinine using diagnostic kits (Darman kave Diagnostics, Iran). Kidneys were excised, weighted and fixed with 10% buffered formalin. Renal sections were stained with Pizzolato methods and pathologically investigated for stone formation. Statistical significance was calculated using Microsoft SPSS 13.0 for Windows. Duncan test was used for multiple comparisons only if a one-way factorial ANOVA showed a significant difference [15]. Statistical significance was assumed when the P-value was less than 0.05. All results were expressed as the mean ± SD.

RESULTS

Urinary pH values ranged from 8.28±0.41 to 6.8±0.45 and 8.10±0.56 to 6.38±0.52 in experiments1 and 2. This reduction was significant in the most of groups (Fig.2).

There was a gradual increase in urinary oxalate in all treatment groups. This increase was dependent on EG concentration and duration of treatment of animals with special drinking water significantly. The urine oxalate values in experiments1 and 2 ranged from 1.81±0.36 to 15.06±0.64 and 3.96±0.28 to 13.84±0.27 respectively. In contrast, urinary oxalate excretion for control groups in experiments1 and 2 showed 0.47±0.12 and 0.5±0.11 mg/dl respectively (Fig.1). Similar increase in urinary calcium excretion was observed in all groups. Calcium excretion in all groups was significantly greater than the control groups with the exception of group 0.2% in experiment 1. However serum calcium concentrations in the experimental groups reduced rather than control groups significantly (Fig.3). In both of the experiments, phosphorus excretion reduced a little but with higher concentration of EG, a gradual increase was observed in all groups. Maximum phosphorus excretion was 18.01±0.85 and 28.5±0.84 in experiments 1 and 2 respectively. These changes were significant rather than control in groups 0.4, 0.8 and 1.6%. Instead, serum phosphorus concentrations indicated an increase depending on doses and duration of treatment. (Fig.4). Urinary uric acid values ranged from 1.03±0.02 to 4.97±0.38 and 1.026±0.01 to 4.92±0.19 in experiments 1 and 2 respectively. These values were significant with the exception of group 0.2% (Fig.5). A multiple-fold increase in protein excretion was observed in EG-treated groups significantly (Fig.7). Additive doses of EG caused major reduce in citrate and magnesium excretion, but in highest concentration of EG, an increase was observed rather than other groups. Citrate excretion greatest was related to control. Groups 1.6% and 0.8% indicated magnesium excretion greatest in experiments 1 and 2 respectively (Fig8, 9). When renal function was evaluated using the creatinine clearance (Ccr) method, it was observed that the values in all groups were already lower than control significantly. These values increased in highest concentration of EG (Fig.6). Crystal's deposits were confirmed to be calcium oxalate crystals using Pizzolato staining. Calcium oxalate crystals developed rapidly in broad areas of the kidneys, especially in the rats high concentrations of EG in their drinking water. There was no crystal deposit formation in group 0.2% of experiment 1 (Fig.10).

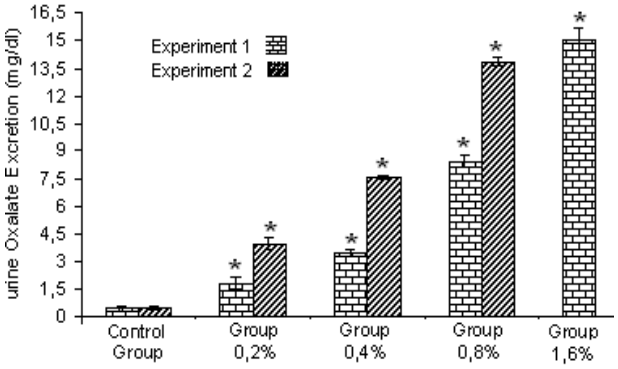

Figure 1. Urinary oxalate excretion in each group. Urinary oxalate in all groups was significantly increased with increase in dose of EG and was time dependent compared to the control group (*P<0.05).

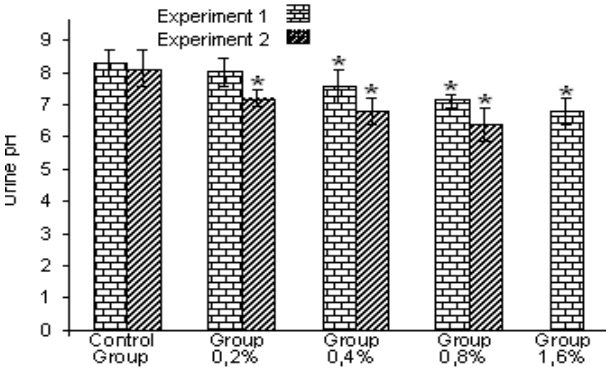

Figure 2. pH values were significantly lower than the control group (*P<0.05) with the exception of group 0.2% in experiment 1 (*P>0.05).

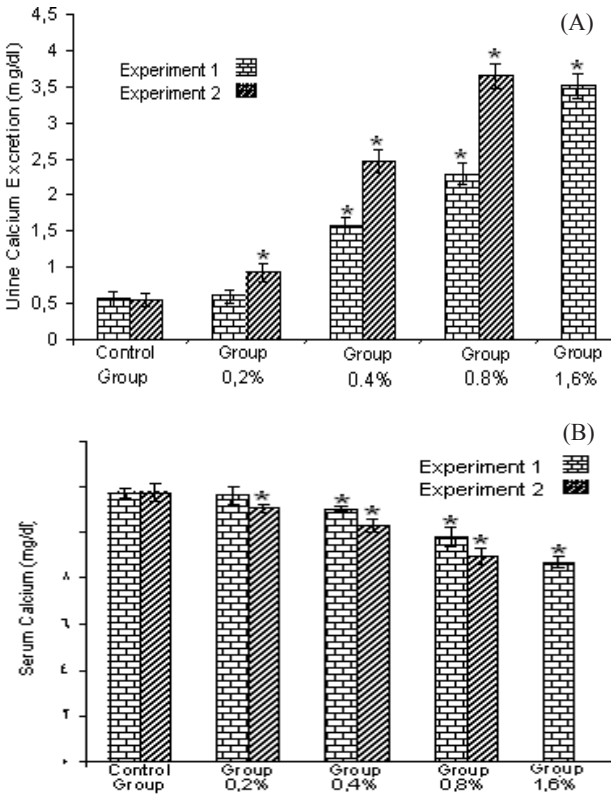

Figure 3. Calcium changes in each group. (A) Urine calcium excretion. (B) Serum calcium changes. In both of them, changes were significant (*P<0.05) with the exception of 0.2% group in experiment 1 (*P>0.05).

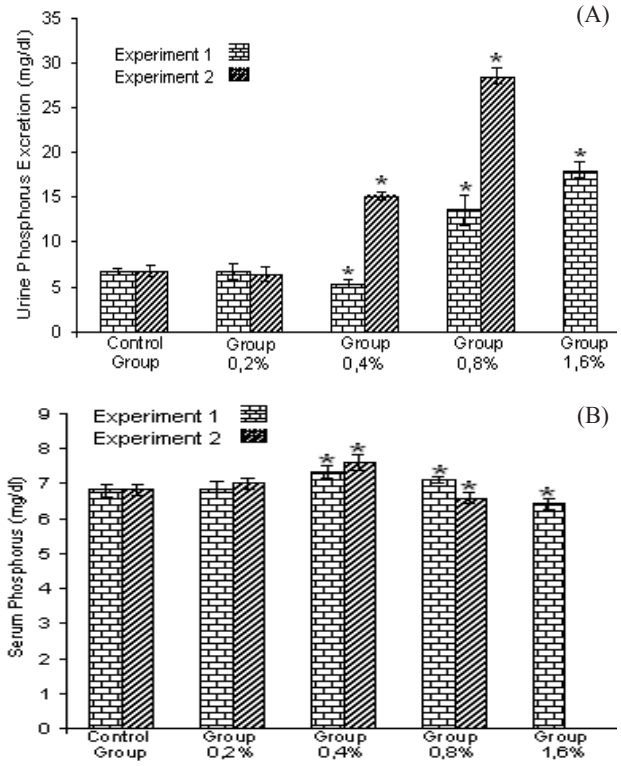

Figure 4. Phosphoros changes in each group. (A) Urine Phosphoros excretion. (B) Serum Phosphoros changes. In both of them, changes were significant (*P<0.05) with the exception of 0.2% group (*P>0.05).

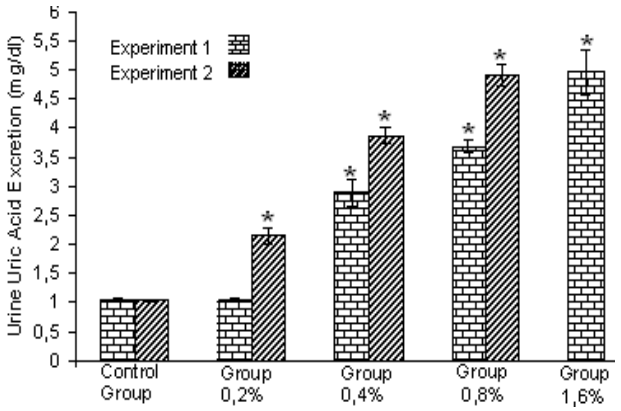

Figure 5. Urine uric acid excretion in each experiment

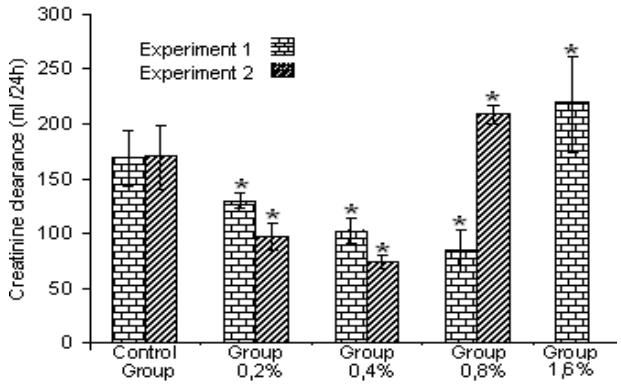

Figure 6. Creatinine clearance significant changes in each experiment (*P<0.05).

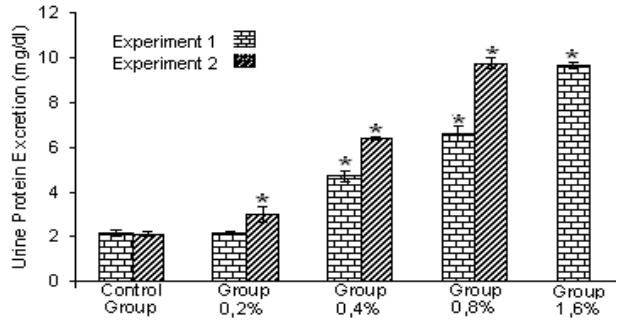

Figure 7. Urine protein excretion in each group. These values were significant in the most of them (*P<0.05).

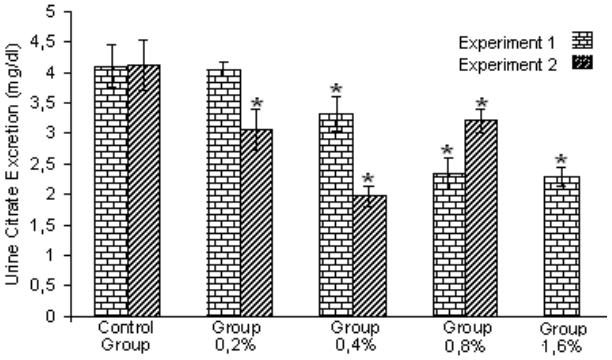

Figure 8. Urine citrate excretion in each experiment. Citrate change was not significant in group 0.2% experiment 1 than control group (*P>0.05).

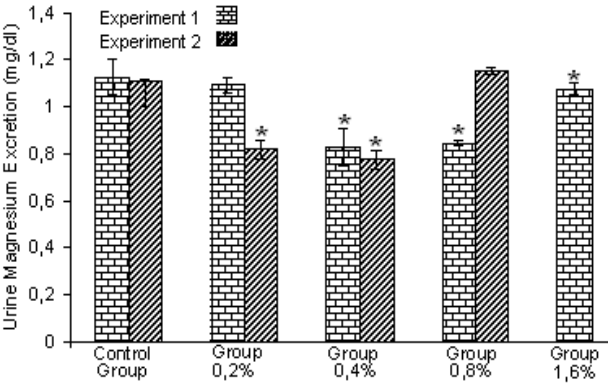

Figure 9. Urine magnesium excretion in each experiment

DISCUSSION

Urinary chemistry is one of the important factors in determining the type of crystals formed and the nature of macromolecules included on the surface of the crystals. Hence the study of urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation [16]. Oxalate and calcium excretion is progressively increased in EG-treated groups. Similar results have been obtained when rats were treated with ethylene glycol and BOS or ammonium oxalate [17]. It is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria [18, 19]. Increased excretion of calcium has been reported in humans as well as rats [20, 21, and 22]. An increased urinary calcium concentration is a factor favoring

the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine subsequent crystal growth [23]. It can be as the cause of the tissue failure and its role in forming stone in treated group with EG. Oxalate interferes with the binding of polysaccharides to crystals. This can be envisioned to occur through changes in the crystals surface properties or by induction of functional and secondary structural changes of urinary macromolecular inhibitors such as glycosaminoglycans, resulting in a decrease of their inhibitory activity against Calcium oxalate monohydrate (COM) crystallization. Thus in urine, a high oxalate may increase the rate of crystallization both by increasing the super saturation and by decreasing the inhibitory potential of the urine [24].The results indicate that both oxalate and calcium oxalate crystals are injurious to renal epithelial cells in the kidneys as well as culture [25]. A gradual increase in urinary phosphorus and uric acid excretion is observed in EG-treated animals. Increase in excretion of phosphorus and uric acid has been reported in stone formers [26, 27]. And hyperoxaluric rats [28, 29]. Increased urinary phosphorus excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition [30]. Uric acid interferes with calcium oxalate solubility [31]. And it binds and reduces the inhibitory activity of glycosaminoglycans [32]. 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 [33]. Lower doses of EG are caused decreased glomerular filtration and chronic renal failure that as the result of these two factors phosphorus increased in serum. It has been suggested that lactic acid reduces the renal excretion of uric acid by competitively inhibitory its secretion by the proximal tubule [34]. In the other hands, it is presumed that the cellular damage and tissue failure results from a membrane irritation effect of oxalate calcium crystals or an intracellular effect of oxalate ions. That induces phosphorus and uric acid excretion in higher concentration of EG. Protein excretion is increased in hyperoxaluric rats. Increased excretion of protein in hyperoxaluric rats as well as stone formers has been reported [35]. Super saturation of urinary colloids results in precipitation as a crystal initiation particle, which when trapped, acts as a niduce, leading to subsequent crystal growth [36]. It is suggested that EG is hyper osmotic factor and induces dehydration followed by proteinury [37]. Normal urine contains many inorganic and organic inhibitor of crystallization, magnesium is one such well-known inhibitor. The level of magnesium is slightly lowered in hyperoxaluric rat urine. Low levels of magnesium are also encountered in stone formers as well as stone forming rats [38]. Magnesium can reduce the super saturation of calcium oxalate by reducing the saturation of calcium oxalate. Magnesium has been found to decrease the growth and nucleation rates of calcium oxalate crystals. Previous studies have described the inhibitory effects of citrate on calcium oxalate crystallization in place of crystal growth, but the effects of citrate on matrix proteins of stones has not been studied in vivo [39] . Calcium stone disease is attributable to super saturation of the urine with calcium and other salts, the presence of substances that promote crystallization and a deficiency of inhibitors of crystallization. Citrate is a potent inhibitor of calcium oxalate and calcium phosphate stone formation whose excretion is diminished in some patients with stone disease owing to idiopathic causes or secondary factors such as bowel disease and use of thiazides. The pH within the proximal tubule cells is an important determinant of citrate excretion. Previous studies have shown that magnesium deficiency accelerates renal tubular calcium oxalate monohydrate deposition in rats on chronic hyperoxaluric, lithogenic protocols. The present study was conducted to investigate the effect of magnesium deficiency on intratubular calcium oxalate formation in rats from the 1st day of administration of a hyper oxaluric agent. The objectives were to delineate early ultra structural features of the formation, mechanisms of retention, and development of renal tubular crystal deposits and to characterize the crystalluria in rats on the hyperoxaluric/hypomagnesuric protocol.

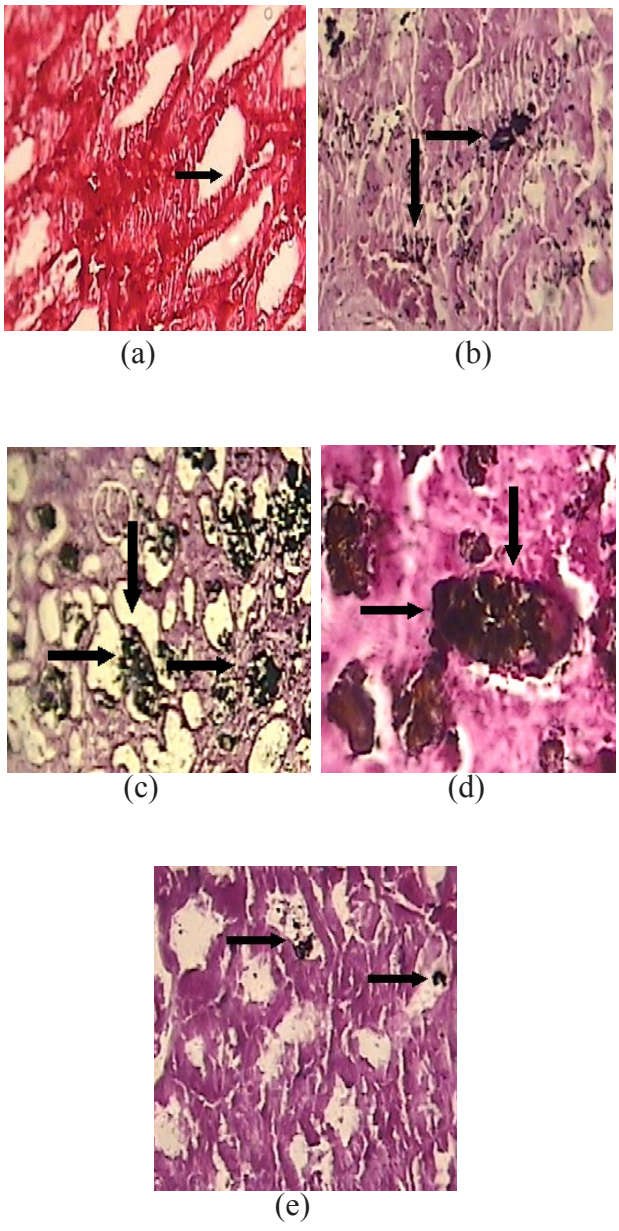

Figure 10. Pathological findings in the rats treated with EG. (a) Control group. (b, c and d) groups 0.4% , 0.8% and 1.6%; (e) group 0.2% in experiment 1. Crystals showed with black arrows. It is observed increase dependent on EG doses and duration of treatment in size and number of crystals.

REFERENCES

[1] Yasui, T., K. Fujita, M. Sato, M. Sugimoto, M. Iguchi, Sh. Nomura and K. Kohri, 1999. The effect of takusha, a kampo medicine, on renal stone formation and osteopontin expretion in a rat urolithiasis model. Urol. Res., 27: 194-199

[2] Huang, H.S. and J. Chen, 2002. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. The J. of Urol., 167: 2584-2593.

[3] Finlayson, B. and F. Reid, 1978. The expection of free and fixed particles in urinary stone disease. Invert. Urol., 15: 44, 39,402-8.

[4] Khan S.R, 1995. Experimental calcium oxalate nephrolithiasis and the formation if human urinary stones. Scanning Microsc., 9(1): 89-100.

[5] Thamilselvan, S., R.L. Hackett and S.R. Khan, 1997. Lipid peroxidation in ethylene glycol induced hyperoxaluric and calcium oxalate nephrolithiasis. J. Urol., 157: 1059 - 1063.

[6] Leth, P. M. and M.M. Gregerson, 2005. Ethylene glycol poising. Forensic Sci. . Int 155(2): 179–184

[7] Staples, C. A., J.B. Williams, G.R. Craig and K.M. Roberts, 2001. Fate, effect and potential environmental risks of ethylene glycol: a review. Chemosphore., 43(3): 377-383.

[8] Cruzan, G., R.A. Corley, G.C. Hard and J.W. Mertens, 2004. Subchronic toxicity of ethylene glycol in wistar and F-344 rats related to metabolism and clearance of metabolites. Toxicol. Sci., 81(2): 502-511.

[9] Henderson, W. R. and J. Brubacher, 2002. Methanol and ethylene glycol poisoning: a case study and review of current literature. Can. J. of Emerg. Med., 4(1).

[10] Klug, S., H.J. Merker and R. Jackh, 2001. Effects of ethylene glycol and metabolites on in vitro development of rat embryos during organogenesis. Toxicol. in Vitro., 15(6): 635-642.

[11] Scalley, R.D., D.R. Ferguson, J.C. Piccaro, M.L. Smart and T.E. Archie, 2002. Treatment of ethylene glycol poisoning. Am. Fam. Physician., 66(5): 807-12.

[12] Spiegel, M. R, 1991. Statistics, Schaum's outline Series. 2ed. ed. McGrow Hill Book Co. New York.

[13] Selvam, R. and M. Adhirai, 1997. Vitamin E pretreatment prevents cyclosporine A-induced crystal deposition in hyperoxaluric rats. Nephron., 75(1): 77–81.

[14] Selvam, R., P. Kalaiaiselve, A. Govindaraj, V.B. Bala Murugan and A.S. Sathish Komar, 2001. Effect of Lanata leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate. Urolithiasis during experimental hyperoxaluria. Pharmacol. Res., 43(1): 89-93.

[15] Tisselius, H.G, 1996. Solution chemistry of supersaturation. In: Kidney stones: medical and surgical management. Edited by F.L. Coe, M.J. Favus, C.Y. Pak, J.H. Parks and G.M. Preminger. Lippincott Raven, Philadelphia, pp: 33.

[16] Bek-Jensen, H. and H.G. Tiselius, 1997. Evaluation of urine composition and calcium salt crystallization properties in standardized volumeadjusted 12-h night urine from normal subjects and calcium oxalate stone formers. Urol Res., 25(5): 365–372.

[17] Bek-Jensen, H. and H.G. Tiselius, 1998. Repeated urine analysis in patients with calcium stone disease. Eur. Urol., 33(3): 323–332.

[18] Lemann, J.Jr., E.M. Worcestor and R.W. Gray, 1991. Hypercalciuria and stones. Am. J. Kidney Dis., 17(4): 386–91.

[19] Cao, L.C., G. Deng, E.R. Boeve, J.C. Romijn, W.C. Bruijn, C.F. Verkoelen and F.H. Schroder, 1997. Does urinary oxalate interfere with the inhibitory role of glycosaminoglycans and semisynthetic sulfated polysaccharides in calcium oxalate crystallization? Eur. Urol., 31(4): 485-92.

[20] Thamilselvan, S. and S.R. Khan, 1998. Oxalate and calcium oxalate crystals are injurious to renal epithelial cells: results of in vivo and in vitro studies. J. Nephrol., 1: 66-9.

[21] Ettinger, B., A. Tang, J.T. Citron, B. Livermore and T. Williams, 1989. Randomized trial of allopurinol in the prevention of calcium oxalate in vitro. Proc. Soc. Exp. Biol. Med., 315(22): 1386–9.

[22] Yamaguchi, S., J. H. Wiessner and A.T. Hasegawa, 2005. Study of a rat model for calcium oxalate crystal formation without severe renal damage in selected conditions. Int. J. of Urol., 12: 303-311.

[23] Rengaraju, M. and R. Selvam, 1987. Role of citrate as an inhibitor of calcium oxalate stone formation in experimental urolithiasis. Arogya. J. Health Sci., XIII: 49–54.

[24] Roger, K., M.D. Low and M.L. Stoller, 1997. Uric acid nephrolithiasis. Urol. Clin. N. Am., 24: 135–48.

[25] Grover, P.K. and M.I. Resnick, 1995. Evidence for the presence of abnormal proteins in the urine of recurrent stone formers. J. Urol., 153: 1716–21.

[26] Grover, P.K. and R.L. Ryall, 1994. Urate and calcium oxalate stones: From repute to rheotoric to reality. Miner. Electrolyte. Metab., 20(6): 361–70.

[27] Kalaiselvi, P., K.L. Udayapriya and R. Selvam, 1999. Uric acid binding proteins in calcium oxalate crystallization. Br. J. Uro., 83: 919–23.

[28] Yamamoto, T., Y. Moriwaki and S. Takahashi,. 2005. Effect of ethanol on metabolism of purine bases (hypoxanthine, xanthine, and uric acid). Clin. Chim. Acta., 356(1-2): 35-57.

[29] Grover, P.K., P.L. Ryall and V.R. Marshall, 1993. Dissolved urate promotes calcium oxalate crystallisation: Epitaxy is not the cause. Clin. Sci., 85: 303–7.

[30] Subha, K. and P. Varalakshmi, 1993. Alterations in some risk factors and urinary enzymes in urolithiatic rats treated with sodium pentosan polysulfate. Biochem. Mo. Biol. Int., 29: 271–80.

[31] Finch, A.M., G.P. Kasidass and G.A. Rose, 1981. Urine composition in normal subjects after oral ingestion of oxalate rich foods. Clin. Sci., 60(4): 411–8.

[32] Xu, X. and R. K. Wang, 2003. The role of water desorption on optical clearing of biotissue: studied with near infrared reflectance spectroscopy. Med. Phys., 30(6): 1246-53.

[33] Khan, S.R, 1997. Animal models of kidney stone formation: an analysis. World J. Urol., 15(4): 236–43.

[34] Rushton, H.G. and M. Spector, 1982. Effects of magnesium deficiency on intratubular calcium oxalate formation and crystalluria in hyperoxaluric rats. J. Urol., 127(3): 598-604.

[35] Grases, F., C. Genestar, A. Conte, P. March and A. Costa-Bauza, 1989. Inhibitory effect of pyrophosphate, citrate, magnesium and chondriotin sulfate in calcium oxalate urolithiasis. Br. J. Urol., 64(3): 235–7.

[36] Ryall, R.L., R.M. Harnet and V.R. Marshall, 1991. The effect of urine pyrophosphate, citrate, magnesium and glycosaminoglycans on the growth and aggregation of calcium oxalate crystals in vitro. Clin. Chim. Acta., 112: 349–56.

[37] Ashby, R. A. and R. J. Sleet, 1992. The role of citrate complexes in preventing urolithiasis. Clin-Chim-Acta., 210(3): 157-65.

[38] Yasui, T., M. Sato, K. Fujita, K. Tozawa, S. Nomura and K. Kohri, 2001. Effects of citrate on renal stone formation and osteopontin expression in a rat urolithiasis model. Urol. Res., 29(1): 50–56.

[39] Goldberg, H., L. Grass, R. Vogl, A. Rapoport and D.G. Oreopoulos, 1989. Urine citrate and renal stone disease. Can. Med. Assoc. J., 141(3): 217-221.