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Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats

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Abstract

Renal injury is considered as one of the prerequisites for calcium oxalate retention. In order to determine the role of lipid peroxidation related effects for hyperoxaluria, we evaluated the alterations in lipid peroxidation, antioxidants and oxalate synthesizing enzymes in lithogenic rats with response to vitamin E + selenium treatment. In kidney of lithogenic rats, the level of lipid peroxidation and the activities of oxalate synthesizing enzymes were found to be increased whereas the levels/activities of non-enzymatic and enzymatic antioxidants were found to be decreased. The urinary excretion of both oxalate and calcium were significantly elevated. Supplementation of lithogenic rats with vitamin E + selenium decreased the levels of lipid peroxides and the activities of oxalate synthesizing enzymes like glycolic acid oxidase (GAO), lactate dehydrogenase (LDH), xanthine oxidase (XO) with a concomitant increase in the activities of enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glucose-6-phosphate dehydrogenase (G6PDH) and increased levels of non-enzymatic antioxidants like ascorbic acid, α-tocopherol and reduced glutathione (GSH). The urinary excretion of oxalate and calcium were normalized. The antioxidants vitamin E + selenium thereby protected from hyperoxaluria. © 2003 Elsevier Inc. All rights reserved.

Keywords: Vitamin E; Selenium; Urolithiasis; Antioxidants; Lipid peroxidation

1. Introduction

Urolithiasis is a very painful disease that has afflicted a wide sector of human population since ancient times [1]. The mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty of treatment. Hyperoxaluria is one of the main risk factors of human idiopathic calcium oxalate disease. Oxalate, the major stone-forming constituent is known to induce lipid peroxidation which causes disruption of the cellular membrane integrity [2,3]. Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. This alters the membrane fluidity, permeability and thereby affects the ion transport across the cellular organelle [4,5].

Free radical reaction can injure renal tubular cells and promote calcium oxalate crystallization [6,7]. Antioxidants are necessary for preventing the formation of free radicals and the deleterious actions of reactive oxygen species [8]. Our earlier investigations have demonstrated that the involvement of enhanced lipid peroxidation reactions in cellular and sub-cellular levels, as an important factor for the etiology of stone formation [9-11]. Decreased functional efficiency in the antioxidant defense system and increased lipid peroxidation has been suggested to be one of the primary factors that contribute to lithogenesis [12,13]. They affect the equilibrium between pro-oxidant and antioxidant balance in biological systems and thereby lead to modifications in vital biomolecules [14].

Vitamin E is the major lipid-soluble antioxidant present in blood and cell membranes. It acts synergistically with other antioxidants in cells, to protect them from oxidative damage [15]. Our earlier studies show that vitamin E reduces the risk of stone formation in rats [16,17]. Selenium in normal metabolism is incorporated in glutathione peroxidase, which is the main well-characterized functional selenoprotein [18]. Selenium compounds have also been found to inhibit tumorigenesis in a variety of animal models, and

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Table 1
Lipid peroxidation and antioxidant status in the liver of control and experimental rats

Parameters	Group I (Control)	Group II (CPD)	Group III (CPD + vit-E)	Group IV (CPD + Se)	Group V (CPD + vit-E + Se)
LPO	2.86 ± 0.22	4.01 ± 0.35*	2.99 ± 0.23[b]	3.16 ± 0.26[b]	2.83 ± 0.26[b]
SOD	7.92 ± 0.63	5.21 ± 0.41*	7.11 ± 0.61[a,b]	6.81 ± 0.52[a,b]	7.87 ± 0.66[b,d]
CAT	64.39 ± 5.43	40.62 ± 4.08*	55.32 ± 4.86[a,b]	49.37 ± 4.12[a,b]	59.13 ± 5.85[b,d]
GPx	9.97 ± 0.81	6.92 ± 0.61*	9.01 ± 0.87[b]	9.26 ± 0.92[b]	10.09 ± 0.93[b,c]
G6PDH	2.11 ± 0.17	1.26 ± 0.13*	1.72 ± 0.16[a,b]	1.66 ± 0.14[a,b]	1.94 ± 0.17[b,c,d]
GSH	12.75 ± 0.95	9.14 ± 0.73*	10.96 ± 0.99[a,b]	11.86 ± 0.96[b]	12.59 ± 0.97[b,c]
Vitamin C	3.11 ± 0.27	1.80 ± 0.22*	2.96 ± 0.26[b]	2.47 ± 0.28[a,b]	3.08 ± 0.29[b,d]
Vitamin E	1.82 ± 0.20	1.16 ± 0.12*	1.84 ± 0.17[b]	1.52 ± 0.16[a,b]	1.89 ± 0.20[b,d]

Each values are expressed as mean ± SD for six rats in each group

[LPO - nmoles of MDA formed/mg protein; SOD - Units/min/mg protein; CAT - μmoles of H_2O_2 consumed/min/mg protein; Gpx - μmoles of GSH oxidized/min/mg protein; G6PDH - Units/min/mg protein; GSH - μg/mg protein; vitamin C - μg/mg protein; vitamin E - μg/mg protein]

On comparing Group I with Group II -* $p<0.001$

[a] Group I compared with Group III, Group IV and Group V

[b] Group II compared with Group III, Group IV and Group V

[c] Group III compared with Group V

[d] Group IV compared with Group V

[a, b, c, d] represent $p<0.05$

recent studies indicate that supplemental selenium in human diets may reduce cancer risk [19]. These defense systems protect the lipophilic membrane and soluble fractions of the cell from attack by reactive free radicals [20]. Studies on the combined effect of vitamin E and selenium in lithogenic rats are scarce. Hence, the present study was designed to evaluate their valuable role under this condition.

2. Materials and methods

Adult male Wistar rats weighing 170-200g were purchased from Veterinary College, Tamilnadu Veterinary University, Chennai, India. All chemicals and reagents used in this study were of reagent grade. Hyperoxaluria was induced by feeding calculi producing diet (CPD) [21]. The animals were divided in five groups. Group I: served as control and received normal diet and physiological saline, Group II: received CPD through diet for 30 days, Group III: CPD along with vitamin E (400mg/kg body weight/30 days); Group IV: CPD along with sodium selenite 0.2 mg/kg body weight/day and Group V: CPD along with vitamin E (400 mg/kg) + sodium selenite (0.2 mg/kg) for 30 days.

At the end of experimental period, 24-hr urine was collected in ice-jacketed beaker. Acidified urine was used for estimation of oxalate [22] and calcium [23] was estimated by Atomic absorption spectrophotometer. The rats were sacrificed by cervical decapitation. Liver and kidney were excised immediately, immersed in ice-cold physiological saline, and blotted with filter paper. A known amount of tissue was weighed and homogenized in 0.01M Tris-HCl buffer, pH 7.4 to get a 10% homogenate. The following estimations were carried as detailed: protein, lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PDH), reduced glutathione (GSH), vitamin C, vitamin E, glycolic acid oxidase (GAO), lactate dehydrogenase (LDH) and xanthine oxidase (XO) [12,13].

2.1. Statistical analysis

Values are mean ± SD for six rats in each group and significance of the differences between mean values were determined by one-way analysis of variance coupled with the Student-Newman-Kuel multiple comparison test. *P*-values of less than 0.05 were considered to be significant.

Statistical significance of differences between the control (Group I) and CPD-fed rats (Group II) were determined by Student's t-test. The levels of significance were evaluated with *p*-values. Correlation coefficient *r* was calculated for the parameters analyzed in urine, liver and kidney. Statistical Package for Social Sciences (SPSS) was used for statistical analysis.

3. Results

Table 1 represents the liver lipid peroxidation and antioxidant status in control, CPD-fed rats and vitamin E + selenium treated rats. A significant increase in lipid peroxidation ($p < 0.001$) and decrease in antioxidants ($p < 0.001$) were observed in CPD-fed rats (Group II) when compared to control rats (Group I). Supplementation of vitamin E + selenium to CPD-fed rats brought down the level of lipid peroxidation while elevated the levels of non-enzymatic antioxidants like GSH, vitamins C and E, and the activities of enzymatic antioxidants such as SOD, CAT, GPx, and G6PDH.

Comparison of kidney lipid peroxidation, enzymatic and

Table 2
Lipid peroxidation and antioxidant status in the kidney of control and experimental rats

Parameters	Group I (Control)	Group II (CPD)	Group III (CPD + vit-E)	Group IV (CPD + Se)	Group V (CPD + vit-E + Se)
LPO	2.35 ± 0.21	3.83 ± 0.33*	2.59 ± 0.25^{b}	2.71 ± 0.32^{a,b}	2.34 ± 0.22^{b}
SOD	5.62 ± 0.46	3.97 ± 0.41*	5.06 ± 0.40^{a,b}	4.91 ± 0.48^{a,b}	5.57 ± 0.50^{b,d}
CAT	47.12 ± 4.30	22.16 ± 2.07*	42.27 ± 3.95^{b}	31.16 ± 3.97^{a,b}	45.07 ± 4.23^{b,d}
GPx	9.23 ± 0.82	5.78 ± 0.53*	8.13 ± 0.71^{a,b}	8.91 ± 0.65^{b}	9.18 ± 0.85^{b,c}
G6PDH	1.67 ± 0.13	1.12 ± 0.12*	1.47 ± 0.16^{a,b}	1.36 ± 0.12^{a,b}	1.62 ± 0.15^{b,c}
GSH	8.65 ± 0.72	6.16 ± 0.56*	7.93 ± 0.69^{b}	8.04 ± 0.67^{b}	8.74 ± 0.65^{b}
Vitamin C	1.87 ± 0.17	1.30 ± 0.14*	1.68 ± 0.16^{b}	1.53 ± 0.12^{a,b}	1.81 ± 0.15^{b,d}
Vitamin E	1.25 ± 0.16	0.75 ± 0.08*	1.22 ± 0.11^{b}	1.09 ± 0.13^{b}	1.26 ± 0.14^{b,d}

Each values are expressed as mean ± SD for six rats in each group
[LPO - nmoles of MDA formed/mg protein; SOD - Units/min/mg protein; CAT - μmoles of H_2O_2 consumed/min/mg protein; Gpx - μmoles of GSH oxidized/min/mg protein; G6PDH - Units/min/mg protein; GSH - μg/mg protein; vitamin C - μg/mg protein; vitamin E - μg/mg protein]
On comparing Group I with Group II -* $p<0.001$
[a] Group I compared with Group III, Group IV and Group V
[b] Group II compared with Group III, Group IV and Group V
[c] Group III compared with Group V
[d] Group IV compared with Group V
[a, b, c, d] represent $p<0.05$

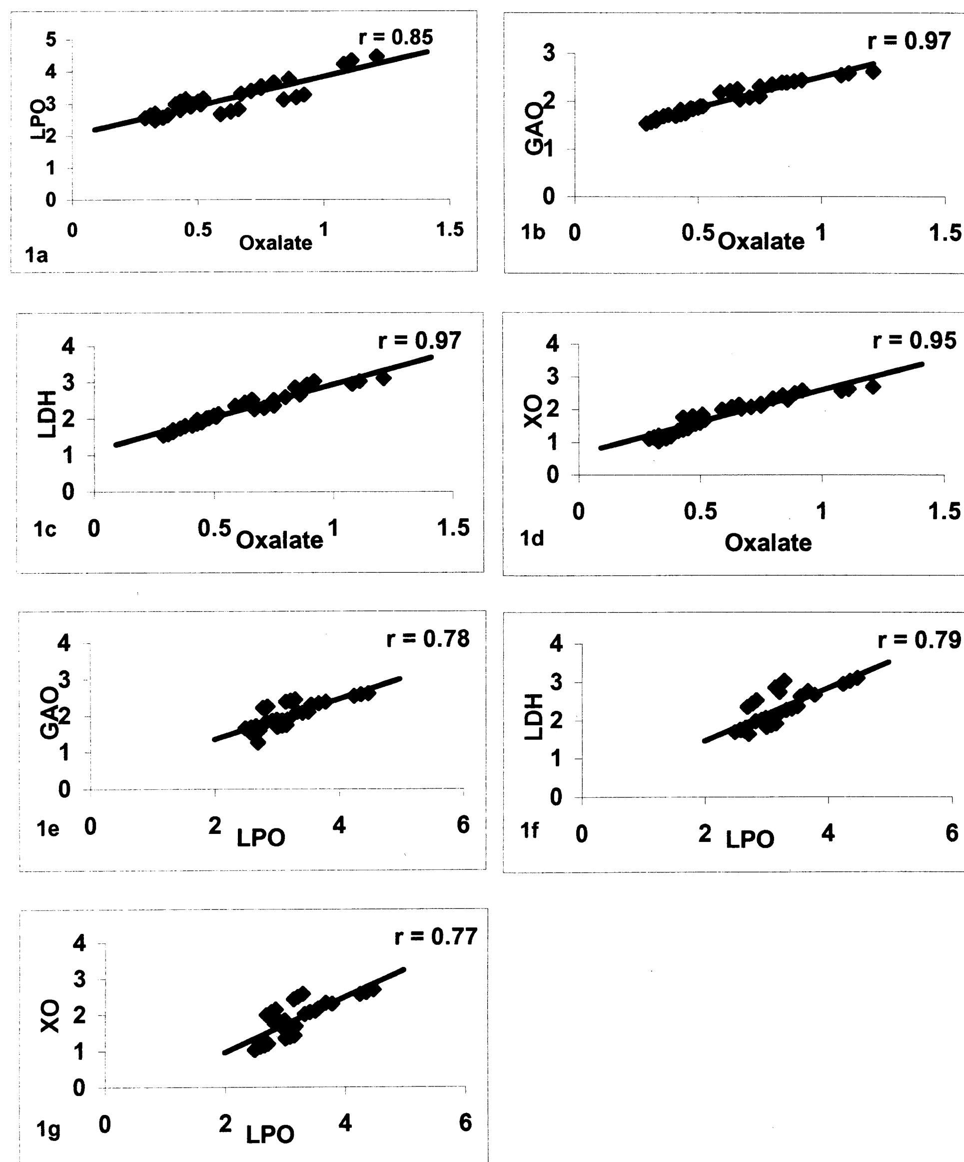
non-enzymatic antioxidants in control, lithogenic and lithogenic rats supplemented with vitamin E + selenium is given in Table 2. Lipid peroxidation was significantly high ($p < 0.001$) whereas antioxidants were markedly lowered ($p < 0.001$) in CPD-fed rats (Group II) as compared to control rats (Group I). After 30 days of vitamin E + selenium treatment to CPD-fed rats lipid peroxide level was significantly reduced while the antioxidants GSH, vitamins C and E, and the activities of SOD, CAT, GPx and G6PDH were significantly increased ($p < 0.05$) in Group III, Group IV and Group V rats when compared to Group II rats. Selenium supplementation alone protected against alteration in lipid peroxidation or glutathione peroxidase activity while vitamin E protected both superoxide dismutase and catalase under CPD-fed condition. Supplementation with both vitamin E and selenium restored the level of LPO and the activities of the antioxidant enzymes to normalcy as that of control animals.

Table 3 represents the activities of oxalate synthesizing enzymes GAO, LDH and XO in liver and kidney and urinary risk factors of control, lithogenic rats and vitamin E + selenium treated lithogenic rats. Significant increase ($p < 0.001$) in the activities/levels of oxalate synthesizing enzymes and urinary risk factors were observed in lithogenic

Table 3
Oxalate synthesizing enzymes of liver and kidney and urinary risk factors of control and experimental rats

Parameters	Group I (Control)	Group II (CPD)	Group III (CPD + vit-E)	Group IV (CPD + Se)	Group V (CPD + vit-E + Se)
Liver					
GAO	1.65 ± 0.08	2.47 ± 0.12*	2.32 ± 0.10^{a,b}	1.97 ± 0.11^{a,b}	1.78 ± 0.09^{b,c,d}
LDH	1.73 ± 0.14	2.81 ± 0.23*	2.69 ± 0.26^{a}	2.16 ± 0.15^{a,b}	1.91 ± 0.17^{b,c,d}
XO	1.27 ± 0.12	2.43 ± 0.20*	2.28 ± 0.22^{a}	1.93 ± 0.14^{a,b}	1.36 ± 0.25^{b,c,d}
Kidney					
LDH	2.01 ± 0.23	3.51 ± 0.28*	3.13 ± 0.24^{a,b}	2.43 ± 0.21^{a,b}	2.23 ± 0.20^{b,c}
XO	0.73 ± 0.05	1.25 ± 0.08*	1.19 ± 0.07^{a}	1.07 ± 0.05^{a,b}	0.87 ± 0.06^{b,c,d}
Urinary risk factors					
Calcium	0.31 ± 0.05	0.61 ± 0.12*	0.54 ± 0.08^{a,b}	0.41 ± 0.06^{a,b}	0.35 ± 0.06^{b,c}
Oxalate	0.37 ± 0.06	0.98 ± 0.18*	0.76 ± 0.13^{a,b}	0.59 ± 0.12^{a,b}	0.43 ± 0.07^{b,c,d}

Each values are expressed as mean ± SD for six rats in each group
[GAO - nmoles of glyoxalate formed/mg protein; LDH - Units/min/mg protein; XO - Units/min/mg protein; Calcium - mg/24h urine and Oxalate mg/24hr urine]
On comparing Group I with Group II -* $p<0.001$
[a] Group I compared with Group III, Group IV and Group V
[b] Group II compared with Group III, Group IV and Group V
[c] Group III compared with Group V
[d] Group IV compared with Group V
[a, b, c, d] represent $p<0.05$



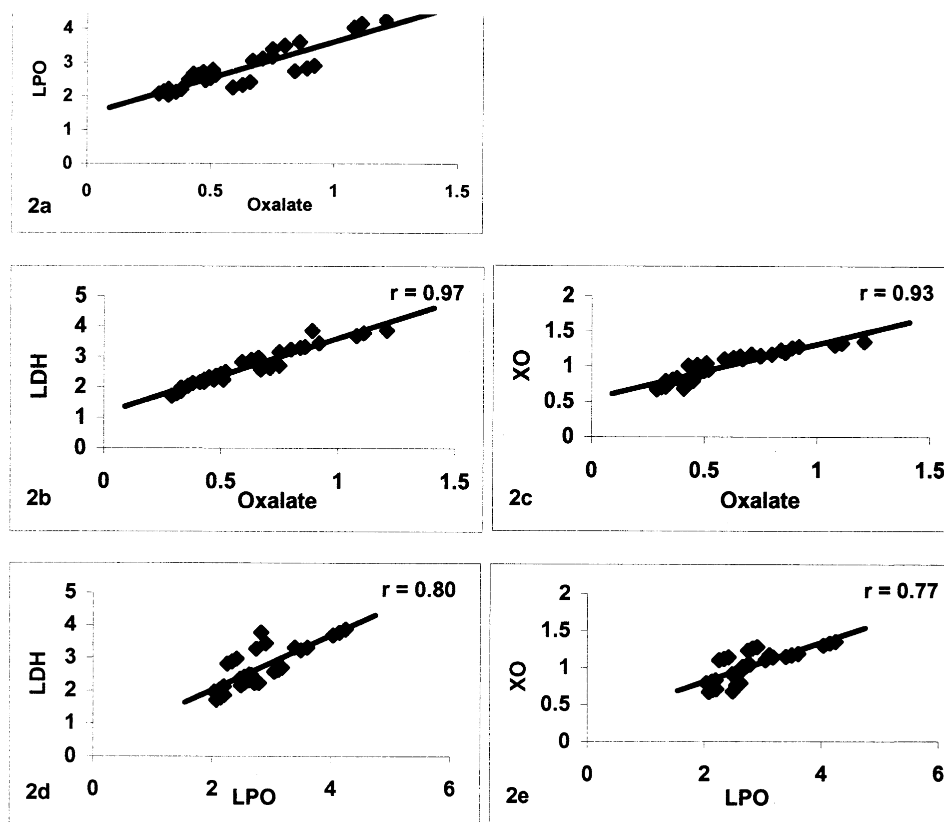
[LPO - nmoles of MDA formed/mg protein
GAO -nmoles of glyoxalate formed/mg protein
LDH - Units/min/mg protein
XO - Units/min/mg protein
Oxalate - mg/24h urine]

Fig. 1. Correlation between oxalate and lipid peroxidation and oxalate synthesizing enzymes in liver.

rats, whereas a significant decrease ($p < 0.05$) was observed in Group III and Group IV rats. However, 30 days of vitamin E + selenium supplementation normalized the activities/levels of GAO, LDH, XO, calcium and oxalate in hyperoxaluric condition.

A positive correlation between oxalate and lipid peroxidation ($r = 0.85$) [Fig. 1a] oxalate and oxalate synthesizing enzymes such as GAO ($r = 0.97$), [LDH ($r = 0.97$) and XO ($r = 0.95$) [Fig. 1b,c,d]and LPO and oxalate synthesizing enzymes GAO ($r = 0.78$), LDH ($r = 0.79$) and XO ($r = 0.77$) [Fig. 1e,f,g] were obtained in liver. Similarly a positive correlation between oxalate and lipid peroxidation ($r = 0.90$) [Fig. 2a] and oxalate synthesizing enzymes such as LDH ($r = 0.97$) and XO ($r = 0.93$) [Fig. 2b,c] were obtained in kidney. Further, a positive correlation between kidney lipid peroxidation and oxalate synthesizing enzymes like LDH ($r = 0.80$) and XO ($r = 0.77$) [Fig. 2d,e] was obtained.

Histopathological examination by Von Kossa's staining revealed prominent deposition of calcium oxalate crystals in



[LPO - nmoles of MDA formed/mg protein
GAO -nmoles of glyoxalate formed/mg protein
LDH - Units/min/mg protein
XO - Units/min/mg protein
Oxalate - mg/24h urine]

Fig. 2. Correlation between oxalate and lipid peroxidation and oxalate synthesizing enzymes in kidney.

the kidney of CPD fed rats (Fig. 3b). A few small sized crystals were seen in vitamin E or selenium alone treated rat kidney (Fig. 3c,d). However it is very interesting to that no deposition of crystals was observed in vitamin E+ selenium treated rats (Fig. 3e). Denudation and shedding of epithelial lining of the tubules, dilation of tubules and swelling of epithelials were seen in CPD fed rats, whereas in the vitamin E or selenium treated rat kidney very mild congestion was observed. Normal architecture was maintained in vitamin E+ selenium treated rats.

4. Discussion

The process of lipid peroxidation generates hydroperoxides, aldehydes and other free radical intermediates, which can react with essential proteins, enzymes and nucleic acids and can render them inactive [24]. In the present study, the enhanced lipid peroxidation in CPD-fed rats may be due to either increased promoters of lipid peroxidation or decreased antioxidant potency. Administration of vitamin E + selenium decreased the level of lipid peroxidation in CPD-fed rats. Oxalate has been shown to be a potent stimulator of lipid peroxidation in rats feeding sodium glycolate or administered sodium oxalate [9,10]. Calcium oxalate crystals cause oxidation of membrane and might lead to tissue injury [25]. A correlation between oxalate binding and lipid peroxidation has been reported earlier by us [26].

Administration of methionine [27], vitamin E[26] and GSH monoester [13] to hyperoxaluric rats has been found to effectively prevent deposition of calcium oxalate crystals. The mechanism for this action is attributed to its action of prevention of cell injury, which is a prerequisite factor for the retention of calcium oxalate crystals.

α-Tocopherol, a known biological antioxidant protects membranes from oxidative stress because it is hydrophobic and quenches free radicals [28]. Further, vitamin E is a potent antioxidant in the membrane, preventing damage from hydroxyl and alkoxyl radicals [29]. Chen et al. [30] have observed inhibition of lipid peroxidation in tissue

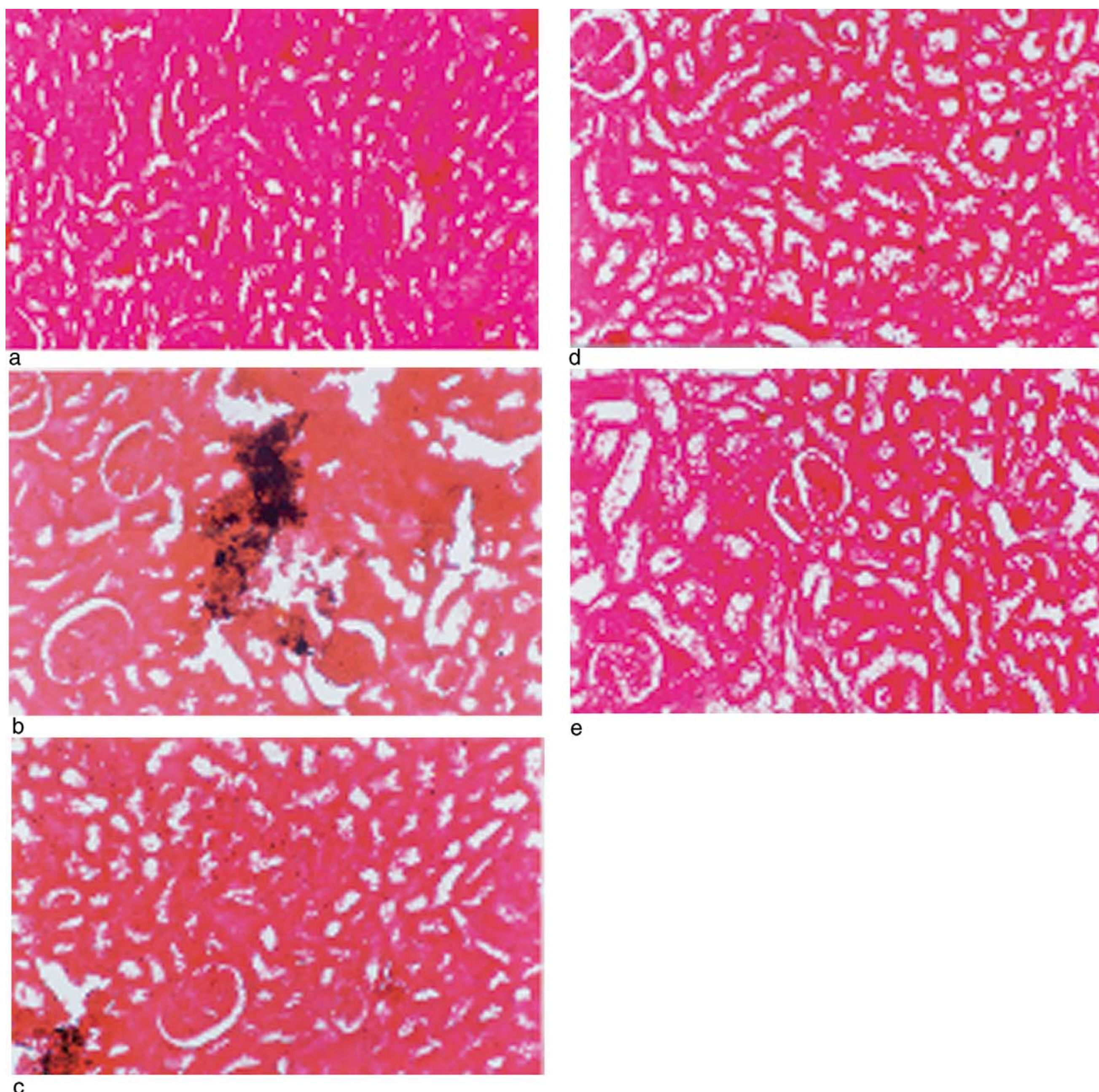


Fig. 3. Histopathology (Von Kossa's stain) of rat renal cells under drug treatment during hyperoxaluria (40X) a: Control b: CPD fed (stone induced) c: Vitamin E alone supplemented. d: Selenium alone supplemented e: Vitamin E + selenium supplemented.

homogenate after co-administration of vitamin E and selenium.

The activities of superoxide dismutase, catalase and glutathione peroxidase are found to be decreased in the tissue of lithogenic rats. On administration of vitamin E + selenium in CPD-fed rats the activity of SOD is restored to near normal possibly due to its free radical scavenging action. Catalase requires NADPH for its regeneration from inactive form and hence the decreased activity in CPD-fed rats may be associated with decreased availability of NADPH or enhanced oxidative stress. Further catalase has been shown to be inhibited by oxalate [10] and the observed decrease in the activity of CPD-fed rats may also be correlated with the enhanced level of oxalate. The decline in catalase activity can be attributed to ineffective scavenging of H_2O_2 resulting in increased H_2O_2 level which can react with $O_2^{\cdot-}$ to $^{\cdot}OH$ radical and thus increased lipid peroxidation and macromolecular damage. The observed decrease in the activity of glutathione peroxidase in CPD-fed rats may be correlated to decreased availability of its substrate, reduced glutathione as well as increased lipid peroxidation. Selenium is an essential nutrient and exerts its action as an antioxidant via its incorporation into glutathione peroxidase. The activity of glutathione peroxidase in most tissues is highly sensitive to

dietary levels of selenium [31], which is bound to the active site as a selenocysteine and serves as the redox center in catalysis. Deficiency of selenium is accompanied by a decrease in the activity of glutathione peroxidase [32]. Glutathione peroxidase requires reduced glutathione for detoxification of hydroperoxides. The fall in the activity of G6PDH observed in CPD-fed rat may be responsible for a decrease in the generation of NADPH and thereby the reduction of oxidized glutathione to reduced glutathione. Administration of vitamin E + selenium in CPD-fed rats restores the activity of glutathione peroxidase. Vitamin E and selenium serve as antioxidants and stabilize the membrane and regulate its function. Increase in glutathione level has been reported following vitamin E + selenium supplementation [33] as seen in this study.

In the present study, a significant reduction in the levels of reduced glutathione, ascorbic acid and α-tocopherol is observed in CPD-fed rats. Supplementation of vitamin E + selenium normalizes them suggesting a protective role against oxalate mediated free radical reactions.

Reduced glutathione thwarts peroxidative damage [34]. A decrease in the GSH level in CPD-fed rats may be due to the decrease in -SH group through the oxidation of free radicals. Oxidized glutathione has been shown to stimulate oxalate binding [11] whereas GSH inhibits oxalate binding. There seems to be a critical threshold level for GSH that is needed to regulate oxalate binding and beyond which cells undergo damage [35] mediated by protein thiol oxidation [36]. Reduced glutathione also keeps up the cellular levels of the active forms of vitamin C and E. These vitamins participate in neutralizing the free radicals and are closely interlinked to each other [37]. Combination of vitamin E + selenium is effective in maintaining intracellular GSH level and thereby reduce injury to tubules caused in calculogenic rats.

Oxalate is synthesized by GAO, XO and LDH in liver and GAO absent in kidney. A significant increase in the activities of GAO, LDH and XO and the urinary excretion of calcium and oxalate is observed in CPD-fed rats. This may be due to the increase in the synthesis of these enzymes during lithogenesis due to the availability of its substrate. Glycolic acid oxidase and xanthine oxidase are known to synthesize oxalate and to produce hydrogen peroxide and superoxide anions [38]. The increase in the activities of these enzymes in hyperoxaluric rats might result in an increase in free radicals and thereby lipid peroxidation. Dietary supplementation of vitamin E + selenium enhances the level of eicosapentanoic acid (EPA) and decosahexanoic acid (DCHA) [39]. EPA and DCHA are also known to significantly reduce urinary calcium and oxalate excretion in recurrent hypercalcuric stone formers [40-42]. Administration of vitamin E + selenium normalizes the activities of oxalate synthesizing enzymes and the alter urinary calcium and oxalate levels and thereby prevent stone formation.

These results suggest that dietary supplementation of vitamin E and selenium can reduce urinary risk factors, prevent the tissue of lipid peroxidation, inhibit oxalate synthesis and enhance enzymatic and non-enzymatic antioxidant status in liver and kidney under lithogenic environment. In conclusion, combination of these antioxidants may be therapeutically advocated for the treatment of urolithiasis.

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