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Effect of depletion of reduced glutathione and its supplementation by glutathione monoester on renal oxalate retention in hyperoxaluria

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The effect of glutathione (GSH) depletion followed by administration of glutathione monoester (GME) on the metabolism of oxalate in hyperoxaluric condition was investigated. Renal GSH was depleted by intraperitoneal administration of buthionine sulfoximine (BSO, 4 mmol/kg b.w) twice a day for 20 days to rats with or without hyperoxaluria induced by adding 0.75% ethylene glycol (EG) in drinking water. GME was administered intraperitoneally (5 m mol in water/kg body weight) simultaneously. Tissue GSH was depleted by 47% and 58% by treatment with BSO and BSO + EG, respectively. Oxalate content was enhanced maximally (125% of control) only in BSO + EG treated group. A polarized light microscopic examination showed prominent deposition of calcium oxalate crystals only in the kidney of BSO- + EG-treated rats. GME treatment brought down kidney oxalate and calcium content dramatically and reduced calcium oxalate retention. However, GME did not have any effect on urinary oxalate level. The observed decreased creatinine clearance, elevated urinary excretion of lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γ-GT), and decreased tissue nonenzymatic and enzymatic antioxidants, and thiol status in BSO + EG treated rats were all restored to normal values on GME supplementation. GSH depletion increases the retention of calcium oxalate in renal cells and normalization of GSH by administration of glutathione monoester prevents it. (J. Nutr. Biochem. 8:445–450, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Renal injury is considered as one of the prerequisite for calcium oxalate retention.[1] But the biochemical mechanism behind renal tubular injury and crystal retention is still not understood. Our earlier studies have shown the involvement of enhanced lipid peroxidation reactions in stone formation.[2,3] Further, depletion of antioxidants and decreased activities of antioxidant enzymes have been noted in the calculus kidneys of experimental urolithic rats.[3] Supplementation of vitamin E or methionine has been found to normalise antioxidant status and prevent stone formation.[3] These studies suggest a role for antioxidants in the pathology of calcium oxalate deposition.

GSH is one of the major antioxidants of the cell. Besides being an important source of thiol, it plays a unique role in cellular defense against reactive oxygen species and reactive intermediates.[4] It is also involved in the regeneration of other reduced antioxidants like vitamin E and C through redox cycle.[5] Depletion of GSH has been implicated in lipid peroxidation mediated cellular injury of many disease and toxic conditions[6,7] and administration of GME has been shown to reverse it by replenishing the tissue GSH.[8] However the effect of depletion of cellular GSH on oxalate metabolism is unknown. This study focused on oxalate metabolism in cellular GSH-depleted condition brought out by the administration of buthionine sulfoximine (BSO), a known inhibitor of GSH synthesis.[8]

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Table 1 Effect of BSO and GME treatment on body weight, organ weight, and protein content of liver and kidney in control and EG-treated rats

Particulars	Body weight[1]		Organ weight[1]		Protein content[2]	
	Initial	Final	Liver	Kidney	Liver	Kidney
Control	107.3 ± 8.51	150.9 ± 7.07	5.12 ± 0.46	1.08 ± 0.07	151.7 ± 8.49	123.5 ± 8.47
BSO	109.7 ± 5.52	135.7 ± 6.83**	4.73 ± 0.35	0.99 ± 0.06*	148.3 ± 8.08	110.0 ± 9.38*
EG	106.1 ± 7.97	152.5 ± 9.20	5.28 ± 0.59	1.12 ± 0.17	152.4 ± 13.38	117.6 ± 5.22
BSO + EG	107.3 ± 5.72	133.8 ± 6.46**	5.25 ± 0.49	1.35 ± 0.17*	140.5 ± 7.58*	103.9 ± 7.30**
Control + GME	104.7 ± 6.83	173.3 ± 10.05**	6.00 ± 0.59*	1.29 ± 0.07	155.4 ± 7.61	130.8 ± 8.00
BSO + GME	108.8 ± 6.04	156.3 ± 6.52	5.33 ± 0.57	1.10 ± 0.11	151.5 ± 8.54	122.4 ± 7.92
EG + GME	106.1 ± 8.05	161.6 ± 5.23	4.98 ± 0.85	1.18 ± 0.12	154.2 ± 10.61	122.9 ± 6.8
BSO + EG + GME	107.3 ± 7.80	151.8 ± 8.07	5.18 ± 0.46	1.11 ± 0.08	149.6 ± 9.87	121.0 ± 7.48

Values are mean ± SD of six animals in each group; values are expressed as [1]g; [2]mg/g tissue;
Values are statistically significant when $^*P < 0.05$; $^{**}P < 0.01$.

Methods and materials

Male albino wistar rats weighing 100 to 120 gm were purchased from FIPPET, Madras. Hyperoxaluria was induced by feeding 0.75% ethylene glycol (EG) in water orally.[9] At this concentration it did not produce crystal deposition in kidney in our study. Cellular GSH was depleted by administration of BSO intraperitoneally.[8] GME was synthesised by the method of Anderson et al.[10]

Animals were divided into eight groups. Group I served as control and received saline intraperitoneally for 20 days; Group II received BSO (4 mmol/kg bw) twice a day for 20 days; Group III received 0.75% EG in drinking water for 20 days; Group IV received BSO + EG as in Group II and Group III for 20 days. Group V to VIII animals were treated in the same order as per Group I to IV with simultaneous administration of GME (5 mmol in water/kg bw) once a day for 20 days. All animals were fed with rat chow and water ad libitum. At the end of experimental period, 24-hr urine was collected in ice-jacketed beaker for assay of γ-glutamyl transpeptidase[11] and lactate dehydrogenase.[12] Acidified urine was used for estimation of oxalate[13] and calcium.[14] The animals were killed by decapitation. Liver and the kidneys were excised immediately and kept in ice-cold saline. The tissue was homogenized in 0.01M Tris-HCl buffer pH 7.2 to give 10% homogenate. Tissue GSH was estimated by the method of Moron et al.[15] and LPO by the method of Devasagayam.[16] Antioxidants-ascorbic acid[17] and vitamin E,[18] and antioxidant enzymes, superoxide dismutase (SOD),[19] catalase (CAT),[20] and glutathione peroxidase (GPX)[21] activities were also determined. Total, nonprotein and protein thiols were measured by the method of Sedlak and Lindsay.[22] Oxalate synthesizing enzymes, glycolate oxidase (GAO),[23] lactate dehydrogenase (LDH),[12] xanthine oxidase (XO)[24] tissue calcium, and oxalate were also estimated.

Comparison of mean values between groups was made using Students *t* test.

Results

Treatment of rats with BSO alone or in presence of hyperoxaluria caused a moderate decrease ($P < 0.01$) in body weight gain when compared with that of controls. Kidney weight was decreased ($P < 0.05$) on BSO treatment and it was more prominently increased ($P < 0.001$) in BSO + EG treatment (*Table 1*). EG-treated rats and rats supplemented with GME did not show any change in body weight gain or organ weights compared with control.

Polarized light microscopic examination revealed prominent deposition of calcium oxalate crystals in the kidney of BSO + EG-treated rats (*Figure 1D*) a few small sized crystals in EG-treated rats (*Figure 1C*) and no deposition at all in BSO-administered rats (*Figure 1B*). Denudation and shedding of epithelial lining of the tubules and dilation of tubules were observed in BSO + EG-treated group. In the BSO-treated group, there was edema and swelling of epithelials. It was significant to note that on supplementation with GME, there were relatively very few crystals and very mild congestion in BSO + EG-treated group. EG and BSO-treated groups showed normal architecture on GME administration.

Intraperitoneal injection of BSO resulted in inhibition of γ-GCS activity significantly ($P < 0.001$). The decrease in γ-GCS activity caused marked depletion (47%) ($P < 0.001$) of GSH level. In EG-fed animals, a decrease (27%) ($P < 0.05$) in GSH level was observed without any change in γ-GCS activity. However, when BSO was administered along with EG, there was a significant depletion of GSH content (58%) ($P < 0.001$) when compared with that of control. Kidney total, nonprotein, and protein thiol levels were decreased by 38, 36, and 29% ($P < 0.001$), respectively, in BSO-treated rats; by 24, 18 and 14% ($P < 0.01$), respectively, in EG-treated rats and by 46, 51, and 45% ($P < 0.001$) in BSO + EG-treated rats (*Table 2*). The decreased thiol status observed in BSO and BSO + EG treated groups was significant ($P < 0.001$) even when compared with EG alone-treated animals (*Figure 2*). The observed decrease in the thiol status in experimental groups were brought to normal levels on supplementation with GME.

LPO potential (TBARS release) showed concomitant elevation after GSH depletion and it reached a maximum (185%) in BSO + EG-treated group. On administration of GME simultaneously along with BSO, EG, or BSO + EG, LPO potential was nearly normalized to that of the control. The observed decreased levels of ascorbic acid and vitamin E in the BSO-treated rats with or without hyperoxaluria, were also remarkably prevented by GME administration. Activities of antioxidant enzymes, SOD and GPX in BSO and BSO + EG-treated groups were notably decreased ($P < 0.001$) compared to controls and EG treated groups. However, catalase activity decreased to the same extent in both EG ($P < 0.001$) and BSO ($P < 0.001$) treated group. On supplementation with GME, there was normalization of antioxidant enzymes activity in all the experimental groups.

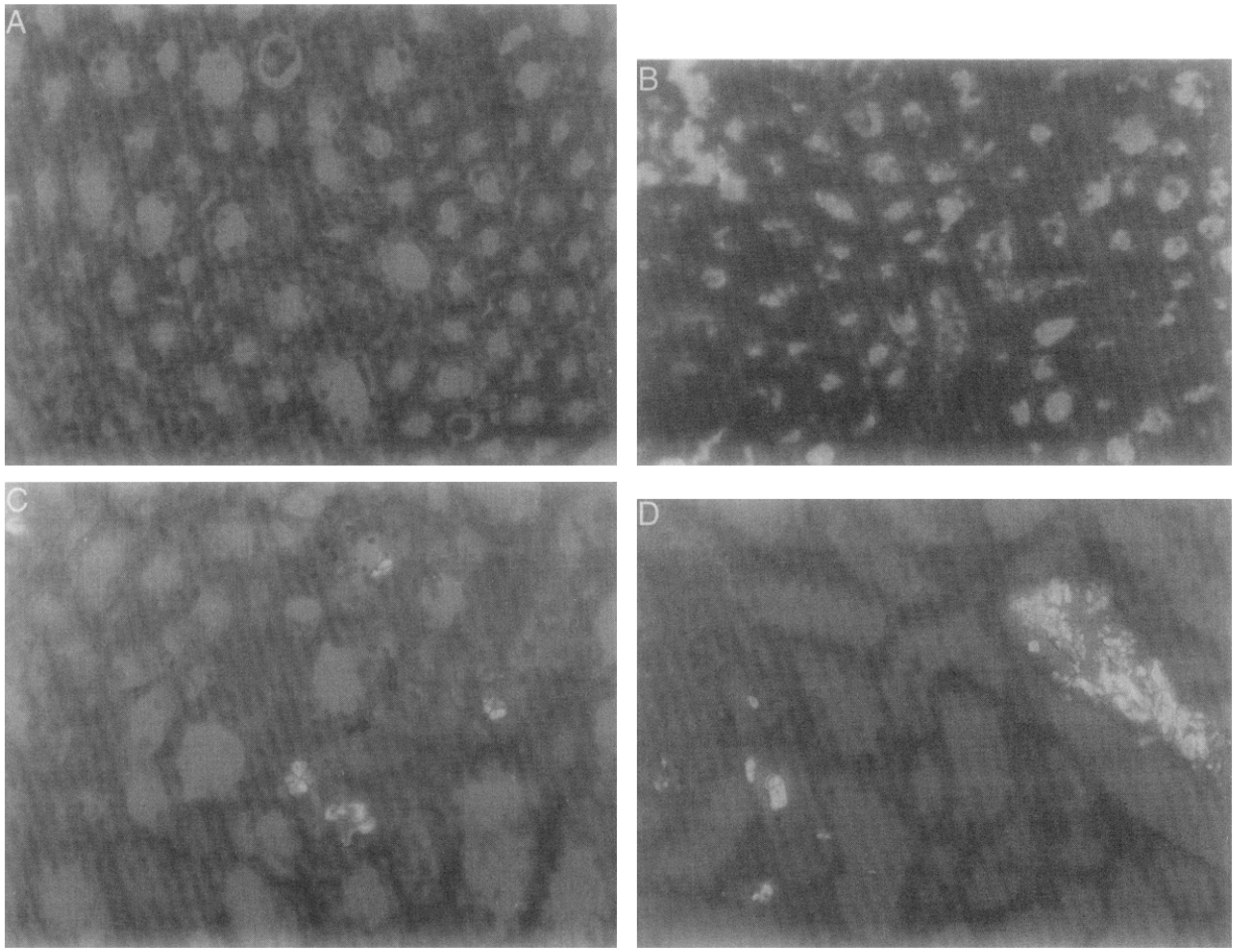

Figure 1 Cross section of the kidney of control and experimental rats as viewed under polarised microscope. (Hematoxylin and Eosin 100×) *A*, Control; *B*, BSO; *C*, EG; *D*, BSO + EG.

The effect of BSO treatment on the activity of oxalate synthesising enzymes is given in *Table 3*. BSO had no effect on GAO activity. However, hyperoxaluria increased GAO activity remarkably ($P < 0.001$). LDH and XO activities were elevated in BSO group ($P < 0.01$) and the elevation more marked ($P < 0.001$) in BSO + EG-treated group. Administration of GME normalized the activities of LDH and XO but not GAO under hyperoxaluric condition. Tissue calcium and oxalate were remarkably high ($P < 0.001$) in BSO + EG-treated group but absolutely normal when supplemented with GME (*Table 3*). There was a negative correlation of tissue oxalate with GSH depletion (*Figure 3*).

Data on plasma creatinine, creatinine clearance, and renal marker enzymes activity are shown in *Table 4*. No

Table 2 Effect of BSO administration on renal γ-GCS, antioxidants, and LPO in control and EG-treated animals with or without GME treatment

Particulars	Control	BSO	EG	BSO + EG	Control + GME	BSO + GME	EG + GME	BSO + EG + GME
γ-GCS[1]	65.64 ± 4.59	36.15 ± 4.70***	63.02 ± 4.65	31.70 ± 5.94***	60.45 ± 6.77	36.94 ± 4.20	63.42 ± 7.54	38.01 ± 4.98
GSH[2]	4.72 ± 0.61	2.51 ± 0.30***	3.45 ± 0.42**	1.98 ± 0.26***	5.10 ± 0.56	4.82 ± 0.50	4.23 ± 0.85	3.99 ± 0.66
Ascorbic acid[3]	1.85 ± 0.08	1.18 ± 0.06***	1.51 ± 0.17**	1.06 ± 0.11***	1.90 ± 0.28	1.83 ± 0.11	1.81 ± 0.90	1.76 ± 0.10
Vitamin E[4]	1.04 ± 0.04	0.79 ± 0.15**	0.88 ± 0.09**	0.64 ± 0.12***	1.05 ± 0.18	1.06 ± 0.11	1.00 ± 0.08	0.99 ± 0.13
SOD[5]	5.10 ± 0.37	3.88 ± 0.30***	4.56 ± 0.22*	3.37 ± 0.41***	5.28 ± 0.65	4.97 ± 0.28	5.15 ± 0.44	4.82 ± 0.46
CAT[6]	163.20 ± 7.70	121.13 ± 8.79***	120.41 ± 9.41***	107.56 ± 8.13***	164.20 ± 14.57	158.79 ± 5.62	153.66 ± 9.80	151.57 ± 10.13
GPX[7]	8.29 ± 0.66	5.14 ± 0.67***	6.89 ± 0.50**	3.79 ± 0.31***	8.34 ± 0.56	8.24 ± 0.53	8.55 ± 0.41	7.96 ± 0.81
LPO[8]	2.25 ± 0.35	3.18 ± 0.28***	2.83 ± 0.06**	4.17 ± 0.29***	1.98 ± 0.07	2.28 ± 0.18	2.13 ± 0.32	2.43 ± 0.25

Values are mean ± S.D. of six rats; μg of P_i released/min/mg protein[1]; μg/mg protein[2,3&4]; units/mg protein[5] (one unit is equal to the amount of enzyme that inhibits autoxidation of pyrogallol by 50%); μmol of H_2O_2 consumed/min/mg protein[6]; μg of GSH consumed/min/mg protein[7]; nmoles of MDA released/mg protein.[8] *P* values: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs control.

Table 3 Effect of BSO on oxalate synthesising enzymes and tissue calcium and oxalate in control and EG treated rats with or without GME treatment

Particulars	Control	BSO	EG	BSO + EG	Control + GME	BSO + GME	EG + GME	BSO + EG + GME
Liver								
GAO[1]	1.67 ± 0.08	1.55 ± 0.12	2.46 ± 0.13***	2.42 ± 0.16***	1.60 ± 0.05	1.63 ± 0.14	2.59 ± 0.18	2.40 ± 0.11
LDH[2]	1.12 ± 0.11	1.28 ± 0.13*	1.37 ± 0.16*	1.41 ± 0.12**	1.14 ± 0.15	1.17 ± 0.13	1.13 ± 0.08	1.14 ± 0.12
XO[3]	2.24 ± 0.32	2.90 ± 0.25**	2.77 ± 0.14**	2.96 ± 0.35**	2.15 ± 0.33	2.22 ± 0.23	2.25 ± 0.19	2.36 ± 0.27
Kidney								
LDH[2]	0.87 ± 0.04	0.94 ± 0.03**	0.94 ± 0.06*	1.03 ± 0.06***	0.86 ± 0.03	0.88 ± 0.04	0.89 ± 0.03	0.88 ± 0.05
XO[3]	2.15 ± 0.19	2.84 ± 0.16***	2.50 ± 0.11**	3.07 ± 0.28***	2.14 ± 0.10	2.13 ± 0.04	2.22 ± 0.17	2.30 ± 0.26
Calcium[4]	36.02 ± 3.89	43.05 ± 4.70*	44.67 ± 5.93*	94.31 ± 7.41***	38.24 ± 4.55	38.69 ± 2.10	42.04 ± 6.38	45.10 ± 6.41
Oxalate[5]	0.44 ± 0.13	0.49 ± 0.08	0.66 ± 0.15*	1.02 ± 0.24***	0.46 ± 0.04	0.40 ± 0.12	0.52 ± 0.06	0.61 ± 0.21

Values are mean ± S.D. of six rats; nmoles of glyoxylate formed/mg protein[1]; nmol of pyruvate formed/mg protein[2]; units/mg protein (one unit is equal to the amount of the enzyme that brings about a change in O.D by 0.01/min), *n* mol/mg protein[4]; mg/gm tissue[5]. *P* values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs control.

significant difference in plasma creatinine was observed among control, EG- and GME-supplemented groups. In BSO-administered groups, there was an increase ($P < 0.01$) in plasma creatinine and it reached very high ($P < 0.001$) in BSO + EG-treated group. Complementing this, creatinine clearance showed a prominent decrease ($P < 0.001$) in BSO + EG-treated group. In GME-supplemented groups, plasma creatinine and creatinine clearance were near normal to that of controls. The increased urinary excretion of γ-GT and LDH observed in BSO and BSO + EG groups was also normalized by GME supplementation.

Discussion

Oedema and swelling of epithelial lining of the kidney tubules are observed in rats treated with BSO. When BSO treatment was combined with EG, there is denudation and shedding of epithelial lining of the tubules. This is accompanied by significant accumulation of calcium oxalate crystals. In EG alone fed rats, except for mild congestion, no morphological change, when calcium oxalate deposition has been observed.

Administration of BSO, a specific inhibitor of γ-GCS has brought about a significant decrease in the level of cellular GSH.[8] EG feeding also has depleted GSH but without inhibiting γ-GCS. BSO when combined with EG, has severely depleted GSH. EG is a precursor of oxalate. It contributes to oxalate production through glycolaldehyde and glycolate.[25] This is reflected in the elevated activity of GAO. The BSO + EG-treated group also showed a similar increase in GAO activity but not in the BSO-alone-treated group, suggesting that GSH may not be involved directly in oxalate synthesis. Though increase in other two oxalate synthesizing enzymes, LDH and XO was noted in BSO treatment, it was not accompanied with enhanced oxalate production. This also proves an earlier finding that these two enzymes have only a minor role to play in oxalate synthesis.[26] Nonetheless, XO is an important source of

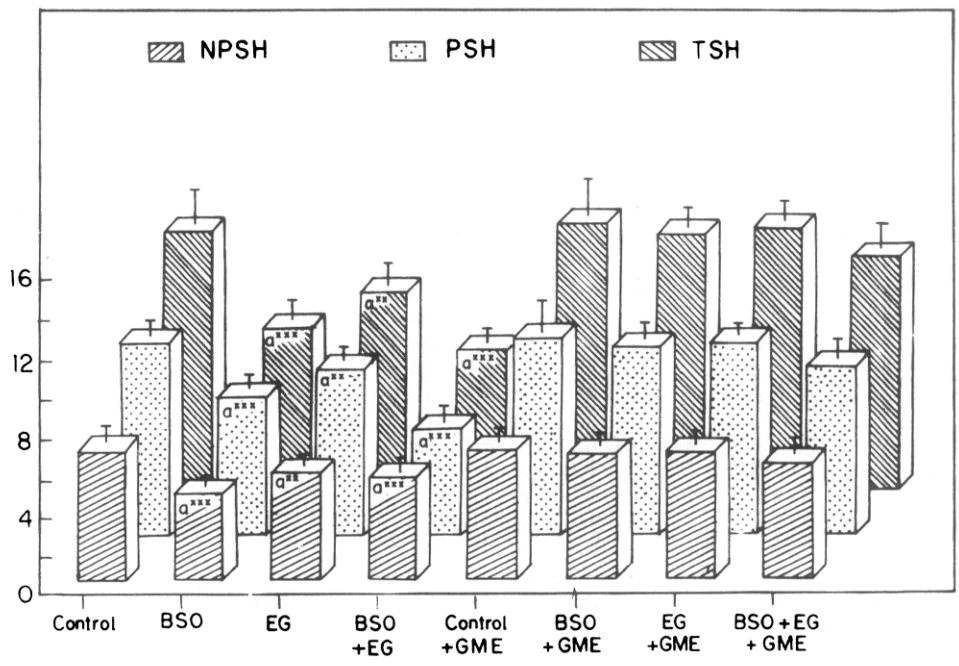

Figure 2 Effect of BSO and GME administration on the thiol status of kidney in control and EG-treated rats.

Table 4 Effect of BSO on urinary parameters in control and EG treated rats with and without treatment of GME

Particulars	Control	BSO	EG	BSO + EG	Control + GME	BSO + GME	EG + GME	BSO + EG + GME
Plasma creatinine[1]	0.68 ± 0.04	0.80 ± 0.06**	0.74 ± 0.06	1.47 ± 0.08	0.66 ± 0.02	0.69 ± 0.05	0.70 ± 0.03	0.75 ± 0.09
Creatinine clearance[2]	0.99 ± 0.12	0.82 ± 0.01*	0.91 ± 0.10	0.53 ± 0.04***	0.97 ± 0.15	1.01 ± 0.07	0.94 ± 0.06	0.94 ± 0.11
γ-GT[3]	1.36 ± 0.19	2.21 ± 0.28***	1.51 ± 0.14	3.76 ± 0.89***	1.38 ± 0.10	1.44 ± 0.35	1.36 ± 0.21	1.60 ± 0.33
LDH[4]	0.23 ± 0.02	0.31 ± 0.06*	0.29 ± 0.13	0.45 ± 0.13**	0.23 ± 0.14	0.25 ± 0.07	0.23 ± 0.09	0.26 ± 0.15
Oxalate[5]	0.62 ± 0.06	0.59 ± 0.11***	1.52 ± 0.17***	1.14 ± 0.25***	0.63 ± 0.14	0.65 ± 0.04	1.49 ± 0.32***	1.37 ± 0.20***
Calcium[6]	0.51 ± 0.06	0.61 ± 0.06*	0.74 ± 0.10***	0.82 ± 0.13***	0.52 ± 0.09	0.51 ± 0.04	0.65 ± 0.06*	0.68 ± 0.05**

Values are mean ± S.D. of six rats; mg/dL[1]; mL/min[2]; μmoles of p-nitroaniline formed/mg creatinine/hr[3]; μmoles of pyruvate formed/mg creatinine/hr[4]; mg/24-hr urine[5,6]; *P* values: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs control.

reactive oxygen species (ROS) and has been implicated as critical mediator of ischemic-reperfusion injury.[27]

Studies have shown oxalate to be an inducer of LPO, mediated through inhibition of catalase.[2] Catalase takes part in the removal of H_2O_2. GSH too participates in scavenging free radicals directly and through GSH-dependent reactions.[4,8] Accumulation of reactive oxygen species caused by catalase inhibition in EG treatment may exert an extra burden on GSH-dependent reactions, thereby contributing to GSH depletion. EG treatment, thus combined with BSO has led to severe GSH depletion.

GSH is essential for maintaining XO in its reduced form, and depletion of GSH as seen in the present study may enhance conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XO) form.[28] XO is a source of ROS via formation of superoxide radicals. ROS are very toxic to antioxidant enzymes in the absence of GSH.[27] GSH is required for regeneration of vitamin E and ascorbic acid from their radical form.[5] The overall depletion of the antioxidants as a consequence of cellular GSH depletion leads to tilt from the usually well balanced proantioxidant state to the pro-oxidant state. This results in an oxidative stress condition as demonstrated by marked enhancement in LPO potential (TBARS release). Elevated LPO has been observed in isolated hepatocytes treated with a number of GSH depleting agents[7] as well as in experimental urolithic conditions.[2,3] In the present study, LPO potential is more remarkable in BSO- and BSO + EG-treated groups. Elevated LPO potential accompanying GSH depletion has been proposed for cell killing in formaldehyde toxicity.[29]

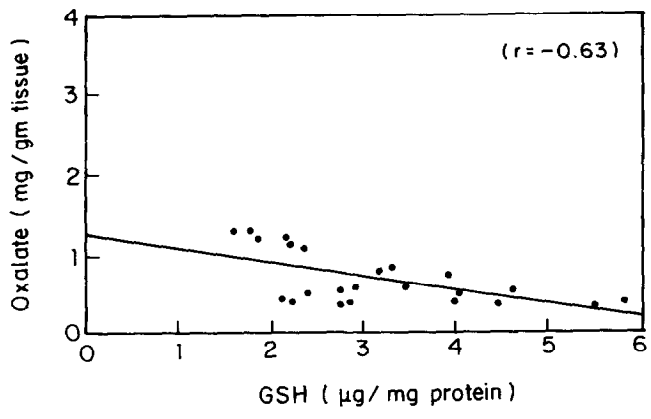

Figure 3 Correlation between GSH and oxalate in rat kidney.

Renal injury is confirmed by measuring the urinary excretion of renal marker enzymes LDH and γ-GT.[9] They are significantly excreted in BSO- and BSO + EG-treated groups. Most of the urinary enzymes originate in kidneys and have been demonstrated to be more sensitive than other biochemical and morphological indicators of renal function.[30] Decreased renal function is observed as demonstrated by increased plasma creatinine and decreased creatinine clearance levels in BSO and BSO + EG treated groups suggesting renal injury.

EG at 0.75% concentration does not induce the deposition of calcium oxalate. However, significant deposition of calcium oxalate in the kidney is observed only in rats administered with BSO + EG simultaneously. Under this condition, kidney weight is increased as evidenced by oedema and swelling of epithelial lining of the kidney tubules (*Figure 1D*). Existence of oxalate binding protein has been demonstrated in renal mitochondria[31] and nuclei[32] and enhanced oxalate binding activity has been reported on peroxidation of mitochondria[33] and nuclei.[34] Further oxidized glutathione has been shown to stimulate mitochondrial oxalate binding,[35] whereas GSH inhibits oxalate binding. There seems to be a critical threshold level of GSH that is needed to regulate oxalate binding and beyond which cell undergo damage,[7] mediated through protein thiol oxidation,[29] which favors increased oxalate binding. This is supported by a significant correlation between oxalate retention and GSH depletion as observed in this study, confirming a significant role of GSH on oxalate metabolism.

When GME has been supplemented to these experimental groups, there is remarkable restoration of GSH level. GME is a source for reduced glutathione. It is readily transported into cells and hydrolysed by esterases to form GSH and ethanol, thereby producing a dramatic elevation in tissue GSH content.[8] Restoration of GSH has normalized the level of free radical scavengers, vitamin E and ascorbic acid and also antioxidant enzymes. This is evident from the prevention of LPO potential and deposition of calcium oxalate in GME supplemented groups. Addition of reduced GSH has been shown to inhibit microsomal peroxidation of lipids.[36] As a result, oxidation of protein thiols was prevented. GSH has been suggested to act as a buffer against

the oxidation of protein thiols.[37] Net effect was prevention of renal injury evidenced from restoration of normal creatinine and renal marker enzyme level. GSH is shown to have a special role in maintaining renal function and structure[4] and intracellular calcium homeostasis[37] and to completely suppress renal injury.[38] It can be concluded that GSH depletion facilitates calcium oxalate retention and prevents deposition of calcium oxalate crystals on normalization.

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