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Oxalate-mediated oxidant–antioxidant imbalance in erythrocytes: role of N-acetylcysteine

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The present in-vivo study was to observe the effect of N-acetylcysteine (NAC) on oxalate-induced oxidative stress on rat erythrocytes. A total of 15 Wistar rats were divided into three groups. The control group received normal saline by single intraperitoneal injection. Hyperoxaluria was induced by single intraperitoneal (i.p.) dose of sodium oxalate (70 mg/kg body weight in 0.5 mL saline) to a second group. The third group was administered single i.p. dose of NAC according to 200 mg/kg body weight dissolved in 0.5 mL saline, half an hour after oxalate dose. NAC administration normalized antioxidant enzyme activities (superoxide dismutase and catalase) and reduced malondialdehyde content (indicator of lipid peroxidation) in hyperoxaluric rat's red blood cell (RBC) lysate. NAC administration also resulted in a significant improvement of thiol content in RBC lysate via increasing reduced glutathione content and maintaining its redox status. Oxalate-caused alteration of cholesterol/phospholipid ratio (determining membrane fluidity) was also rebalanced by NAC administration. Further, after NAC administration, electron microscopy showed improved cell morphology presenting its prophylactic properties. Above results indicate that NAC treatment is associated with an increase in plasma antioxidant capacity and a reduction in the susceptibility of erythrocyte membranes to oxidation. Thus, the study presents positive pharmacological implications of NAC against oxalate-mediated impairment of erythrocytes.

Key words: catalase; oxalate; oxidative stress; red blood cell; superoxide dismutase

Introduction

Oxalate is the main constituent of the kidney stones, produced by many metabolic pathways in the body. This byproduct cannot be processed further and is excreted by kidneys.[1] Removal of oxalate in the kidney is facilitated by a variety of transport systems at the apical and basolateral surfaces of both proximal[2–4] and distal[5] tubular cells. A defect in oxalate transport system causes increase in oxalate level in serum that result in hyperoxaluria. In a report by Baggio,[6] nephrolithiatic patients showed an abnormal transmembrane oxalate flux in their red blood cells (RBCs) due to a common defect in chloride/bicarbonate exchange protein of erythrocyte membrane, that is, anion exchanger1(AE1).[7] This functional variability in transmembrane oxalate flux has also depicted high oxalate self-exchange in erythrocytes.[7]

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High oxalate exposure has been shown to cause oxidative stress in various tissues.[8,9] The membrane phospholipid polyunsaturated fatty acid composition is a crucial mediator of oxalate transport in erythrocytes of calcium renal stone former.[10] In addition, erythrocytes are vulnerable to lipid peroxidation due to their high content of polyunsaturated lipids.[11] From the observation that oxalate can increase production of free radicals, it follows that polyunsaturated fatty lipids of erythrocytes involved in oxalate transport are vulnerable to lipid peroxidation.

In our previous report, we have shown that a well-known antioxidant N-acetylcysteine (NAC) has showed curative properties toward hyperoxaluric manifestations in liver.[8] So, the present study was to investigate the prophylactic properties of NAC on oxalate-induced oxidative stress in RBCs of Wistar rats where the levels of malondialdehyde (MDA) and glutathione were considered as main factors for determining the lipid peroxidation and cellular thiol status, respectively. Erythrocyte

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antioxidant status and electron microscopy have also been performed to assess pharmacological implications of NAC on oxalate caused alterations.

Materials and methods

Healthy male rats of the Wistar strain weighing between 150 and 200 g of equivalent age groups were obtained from central animal house of Panjab University, Chandigarh. The rats were acclimatized for one month in polypropylene cages under hygienic conditions and were provided standard animal feed and water *ad libitum*. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals and were approved by the local care of Experimental Animals Committee.

A total of 15 rats were taken and divided into three groups (having five rats each). The control group (group 1) rats were given 0.5 mL of normal saline (0.9% NaCl). Group 2 rats were administered intraperitoneally with single dose of sodium oxalate (Merck) dissolved in 0.5 mL saline according to 70 mg/kg body weight. Group 3 rats were administered NAC (Merck, Darmstadt, Germany) at a dose of 200 mg/kg body weight dissolved in 0.5 mL saline, half an hour after sodium oxalate challenge. After 24 h, 6.0–7.0 mL blood was taken from orbital sinus with the help of capillaries, out of which 4.0–5.0 mL was taken to centrifuge tube without anticoagulant for extracting serum and the remaining 2.0 mL was immediately fixed in 2.5% glutaraldehyde made in phosphate buffer (pH 7.2) for scanning electron microscopy. After dissection, the blood was taken from the heart through intracardiac puncture with the anticoagulant heparin.

Preparation of serum
Freshly drawn blood taken without anticoagulant was allowed to clot at room temperature for 10 min and then centrifuged for 15 min at 3000 rpm. Supernatant was collected as serum for the estimation of oxalate.

Preparation of RBCs
Blood samples collected via intracardiac puncture with heparin (0.06 mg/mL) anticoagulant were centrifuged. Plasma and buffy coat were removed by centrifugation at 3000 rpm for 10 min. The RBCs in pellet were washed three times with an equal volume of cold saline and the resulting washed RBC lysate was obtained.

Oxalate
Serum oxalate concentrations were measured by the method as described by Hodgkinson and Williams.[12] Oxalic acid is coprecipitated with $CaSO_4$, reduced to glyoxalic acid by boiling with dilute H_2SO_4 in the presence of zinc, which was measured at 540 nm.

Lipid peroxidation
The quantitative measurement of lipid peroxidation was performed according to method of Buege and Aust.[13] The MDA formed after a series of peroxidation reaction as end product was measured by the reaction with thiobarbituric acid at 532 nm. The results were expressed as nanomoles (nmoles) of MDA per milligram of protein using the molar extinction coefficient of malondialdehyde–thiobarbituric chromophore ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

Superoxide dismutase
The assay was performed according to the method of Kono.[14] The extent of inhibition of reduction of nitro blue tetrazolium to blue formaxon by the addition of the enzyme was measured at 560 nm. The activity of enzyme was expressed as units per milligram of protein, where one unit of enzyme is defined as the amount inhibiting the rate of reaction by 50%.

Catalase
Catalase (CAT) was measured by UV spectrophotometer method described by Luck.[15] The amount of H_2O_2 hydrolyzed was calculated based on molar extinction coefficient of H_2O_2 at 240 nm ($0.071\ mM^{-1}\ cm^{-1}$). The result is expressed as nanomoles (nmoles) of H_2O_2 decomposed per minute per milligram of protein.

Total lipid extraction and estimation
Lipids were extracted by the method of Folch.[16] The content of total lipids was analyzed by the method of Frings.[17]

Phospholipid content
The content of phospholipids was determined by the method of Bartlett.[18] The organic phosphorous converted to inorganic phosphorous by digestion with perchloric acid. When this acid hydrolysate is treated with molybdate, it forms phosphomolybdic acid with inorganic phosphorus. The phosphomolybdic acid is reduced by the addition of 1,2,4-amino-naphthol sulfonic acid reagent to give a blue color, the intensity of which is read at 740 nm.

Cholesterol content

Cholesterol was analyzed in the lipid fraction by the method of Zlatkis.[19] Cholesterol in the presence of sulfuric acid and glacial acetic acid forms a violet colored complex with ferric chloride, which is measured colorimetrically at 540 nm. The reaction involves initial dehydration of cholesterol to 3,5-cholestadiene or 2,4-cholestadiene, which polymerizes to dimmer or trimer. The polymers react with $FeCl_3$–H_2SO_4 to form a colored complex, the absorbance of which can be read at 540 nm

Proteins

Proteins were measured in the entire samples by the method of Lowry.[20]

Scanning electron microscopy

The rat blood was collected from the orbital sinus by a capillary tube and immediately fixed in 2.5% glutaraldehyde made in phosphate buffer (pH 7.2). After 1½–2 h of fixation, the cells were centrifuged 2–3 times in phosphate buffer. Then the pellet so obtained is suspended in triple distil water. The sample is loaded on conductive silver plate and coated with gold to a thickness of 100 Å. The specimens were observed under electron microscope, JSM-6100, Japan.

Statistical analysis

Data are expressed as mean ± SD for five animals in each group and analyzed by Student's *t*-test.

Results

Figure 1 shows the significant ($P < 0.001$) increase in serum oxalate level, 24 h after the administration of single dose of sodium oxalate as compared to control rats. Moreover, the post treatment with NAC (group 3) also showed significantly elevated level of serum oxalate content.

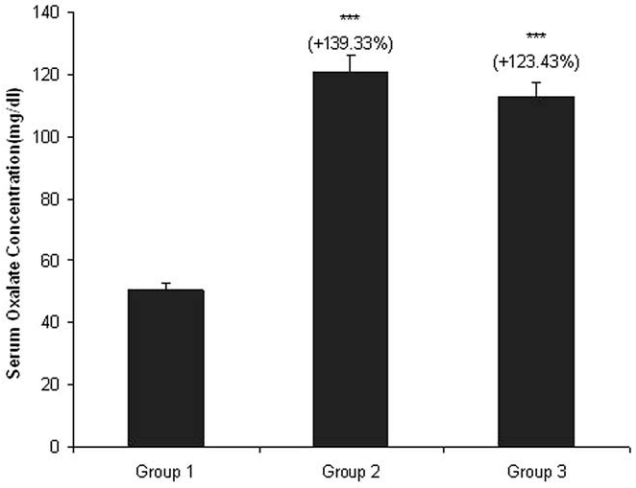

Figure 1 Concentration of oxalate in serum of oxalate exposed rats (group 2) and N-acetylcysteine-treated oxalate exposed rats (group 3). [Values in brackets are % increase (+) or % decrease (–) as compared to control (group 1), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$: indicates significant change in comparison to control group 1].

Table 1 shows that with the increase in oxalate content in the serum, there was an increase in MDA (80% as compared to control) level of erythrocytes. Treatment with NAC caused significant decrease ($P < 0.01$) in MDA status as compared to oxalate-exposed rats. The oxalate induction causes significantly increased ($P < 0.001$) in superoxide dismutase (SOD) activity in RBC lysate. SOD activity was found to be increased by 59.63% as compared to control group rats (Table 1). NAC treatment normalized SOD activity in oxalate-exposed NAC-treated rats and the decrease in its activity as compared to oxalate-exposed rats is highly significant. Similarly, in case of CAT activity, oxalate exposure caused its significant rise ($P < 0.001$) in RBC lysate and here again NAC treatment of oxalate-exposed rats reverted activity of CAT enzyme to near control levels.

Further on assessing the effect of NAC on glutathione antioxidant defense system, oxalate

Table 1 Effect of NAC treatment on MDA content and activities of SOD and CAT in erythrocytes from oxalate-exposed rats.

	MDA nmoles of MDA/mg of protein	*SOD U/mg protein*	*CAT nmoles of H_2O_2 decomposed/min/mg protein*
Group 1 (control)	1.5 ± 0.32	47.72 ± 3.14	189.17 ± 15.73
Group 2 (oxalate exposed)	2.7 ± 0.41 (+80.00%)***	76.18 ± 5.12 (+59.63%)***	226.09 ± 22.14 (+19.51%)***
Group 3 (NAC treated)	1.9 ± 0.37 (+26.66%)***###	46.23 ± 2.16 (–3.12%)###	194.16 ± 19.15 (+2.63%)***###

Values are mean and SD of five determinations.
Values in brackets are % increase (+) or % decrease (–) as compared to control (group 1).
$*P < 0.05$, $**P < 0.01$, $***P < 0.001$: indicates significant change in comparison to control group 1.
$^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$: indicates significant change between groups 2 and 3.
NAC, N-acetylcysteine; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

Table 2 Effect of NAC treatment on the glutathione (reduced and oxidized) level and redox ratio (GSH/GSSG) in erythrocytes from oxalate-exposed rats

	GSH (nmoles/mg protein)	*GSSG (nmoles/mg protein)*	*Redox Ratio (GSH/GSSG)*
Group 1 (control)	16.9 ± 1.35	1.33 ± 0.112	12.7 ± 0.98
Group 2 (oxalate exposed)	10.1 ± 0.79 (−40.23%)***	1.55 ± 0.151 (+16.54%)***	6.5 ± 0.34 (−48.81%)***
Group 3 (NAC treated)	14.7 ± 1.12 (−13.01%)*##	1.41 ± 0.107 (+6.01%)**###	10.4 ± 0.81 (−18.11%)###

Values are mean and SD of five determinations.
Values in brackets are % increase (+) or % decrease (−) as compared to control (group 1).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: indicates significant change in comparison to control group 1.
#$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$: indicates significant change between group 2 and group 3.
NAC, N-acetylcysteine; GSH, reduced glutathione; GSSG, oxidized glutathione.

induction significantly ($P < 0.001$) lowered the level of reduced glutathione (GSH) and the reduction is 40.23% as compared to control rats (Table 2). Although NAC-treated rats showed a marginal reduction in GSH level, still the fall in GSH content of these rats is much less (13.01%) as compared to oxalate-exposed rats. On the other hand, oxidized glutathione (GSSG) content increased by 16.54% following oxalate exposure and NAC treatment reduced this rise to approximately 6.01%. In addition, NAC-treated oxalate-exposed rats normalized redox ratio to near control levels as shown in Table 2.

Oxalate induction causes significantly diminished ($P < 0.001$) total lipids in group 2 animals and NAC treatment to oxalate-induced rats had tried to normalize the total lipid content in group 3 rats (as shown in Table 3). A similar trend was observed in case of phospholipid (PLP) content after oxalate induction, where its level was decreased by 23.46% as compared to control rats. But after NAC treatment, the PLP level was quite close to levels of control (group 1) rats. The change in cholesterol (CHL) level has shown some reversed order, in which its level was increased as compared to control following oxalate exposure. No significant change (as compared to control rats) was observed in the CHL content of RBCs in NAC-treated rats. However, the ratio of CHL/PLP was found to be increased by 40.74% after oxalate exposure and NAC treatment has reduced it by 9.44% as compared to group 1 rats.

Scanning electron microscopy of RBCs in all the three groups showed prominent change in their appearance. They looked distorted with irregular margins, peripheral protuberance, and clumped together (Figure 2B). However, a semi-quantitative interpretation is that treatment with NAC apparently decreased distorted cells in the blood as shown in Figure 2C.

Discussion

A single intraperitoneal dose (70 mg/kg body wt) of sodium oxalate in rats presented an increase in serum oxalate level after 24 h. Post treatment of NAC to sodium oxalate pre-treated group did not cause any significant decrease in serum oxalate level, which clearly shows that the RBCs were exposed to higher oxalate level even after NAC treatment. Similar results were also found in our previous reports indicating sufficiency of both doses.[8,21] The RBC and WBC cell count after the end of treatment period was not altered in any of the treated group.

The precise sequence of events by which oxalate increases free radical production are yet to be elucidated. But its consequences are determined through estimation of great variety of stable and intermediate or end product, such as MDA,[21] one of the byproduct of lipid peroxidation which is a marker of oxidative stress. A report by Singh and Barjatiya[22] showed that in addition to a rise in oxalic acid in the serum

Table 3 Effect of NAC treatment on TL, PLP, CHL contents and CHL/PLP ratio in the erythrocytes of oxalate exposed rats.

	TL (mg/dL)	*PLP (mg/dL)*	*CHL (mg/dL)*	*CHL/PLP (mg/dL)*
Group 1 (control)	357 ± 4.30	179 ± 3.20	91 ± 2.70	0.508 ± 0.84
Group 2 (oxalate exposed)	247 ± 3.14 (−30.81%)***	137 ± 3.41 (−23.46%)***	98 ± 1.14 (+7.69%)*	0.715 ± 0.33 (+40.74%)***
Group 3 (NAC treated)	289 ± 4.10 (−19.04%)**###	167 ± 2.71 (−6.70%)*###	93 ± 1.15 (+ 2.19%)	0.556 ± 0.424 (+9.44%)###

Values are mean and SD of 5 determinations.
Values in brackets are % increase (+) or % decrease (−) as compared to control (group 1).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: indicates significant change in comparison to control group 1.
#$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$: indicates significant change between groups 2 and 3.
NAC, N-acetylcysteine; TL, total lipid; PLP, phospholipid; CHL, cholesterol.

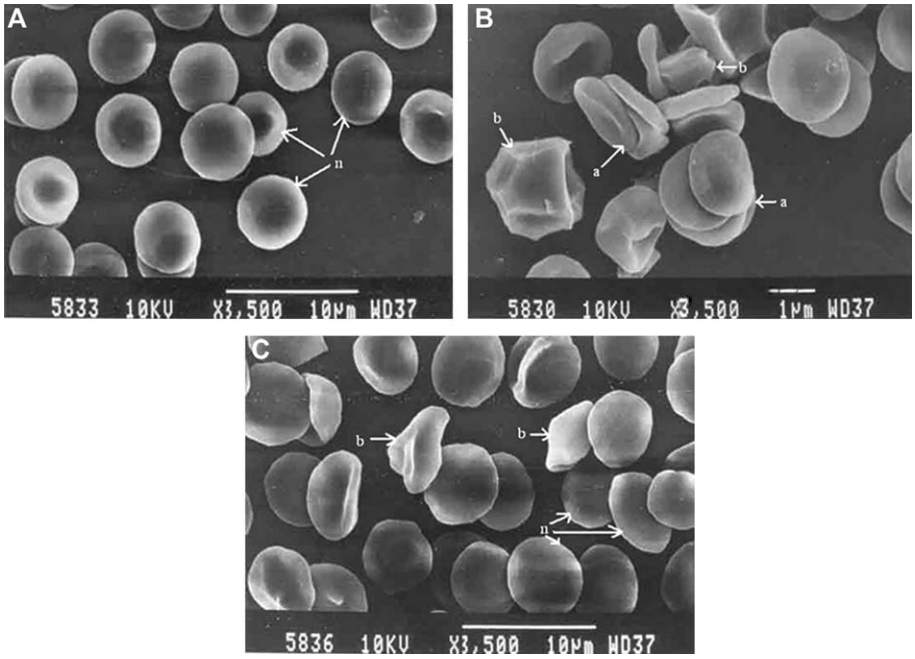

Figure 2 Electron micrographs of erythrocytes; (A) Control group 1 rats showing normal concave (n) configuration; (B) Oxalate-exposed group 2 rats showing clumped erythrocytes (a) and distorted erythrocytes (b); (C) NAC-treated group 3 rats showing fewer distorted (b) erythrocytes and many normal (n) concave configuration of erythrocytes (magnification 3500×).

of stone formers, an increase in oxidative stress in their blood is also observed. Similarly, erythrocyte membrane lipid peroxidation was also observed in hyperoxaluric rats given ethylene glycol for 28 days.[23] In our study, the increased MDA level was observed in oxalate-treated group, whereas MDA content was found to be reduced in NAC-treated group animals. Free thiol group of NAC might have quenched free radicals causing lipid peroxidation and MDA content diminished spontaneously.

The decline in phospholipid content of RBCs may be due to its degradation by phospholipase A2 (PLA2). It has been shown that PLA2 activity is associated with increase in peroxidase activity.[24] It can be postulated that oxalate exposure causes lipid peroxidation and finally results in RBC membrane disintegration (as shown in Figure 2B). NAC post treatment elevated phospholipid level possibly by decreasing PLA2 activity or synthesis.

The erythrocyte has several membrane systems to protect itself against oxidative damage; these includes antioxidant enzymes like SOD, glutathione peroxidase, and CAT. Antioxidant enzyme SOD was observed more active than control after oxalate exposure. This change is possibly an adaptive response of this enzyme to increased production of superoxide ions that might have been produced by the activation of of NAD(P)H oxidase via cytokine transcriptional growth factor β1 (TGF-β1) induction.[25] The increased SOD activity further elevates the level of H_2O_2. To contend increased H_2O_2 production, CAT enzyme activity (that causes decomposition of hydrogen peroxide) increased as observed in our experimentation. NAC treatment to oxalate pre-treated group normalized RBC's antioxidant enzymes activity. Study shows that Transcriptional growth factor β (TGFβ) activates NAD(P)H oxidase that causes production of superoxide ion. A study by Shan[26] on mouse mesangial cells shows decrease in TGFβ level after NAC treatment followed by decreased superoxide radical formation. Similarly in our study, NAC might have decreased superoxide radicals' production and resulted in decreased requirement for SOD enzyme. Due to presence of thiol group on NAC, it has also been observed to reduce H_2O_2-induced damage to epithelial cells *in vitro*.[27] Increased CAT activity also normalized after NAC administration due to decrease in H_2O_2 production.

Glutathione plays an important role in maintaining the erythrocyte membrane integrity by quenching free radicals formed by various detoxification processes. The cells with lower level of GSH are more susceptible to hemolysis.[28] Decreased GSH/GSSG ratio is another indicator of oxalate-induced oxidative stress. NAC treatment has maintained the ratio close to control group rats. NAC being a

precursor of GSH[29] raised GSH levels even after oxalate exposure and maintained the redox ratio of oxalate-exposed rat erythrocytes.

In addition to lipid peroxidation, Weismann[30] showed the damage of membrane protein after oxidative stress, resulting in alteration and impairment of membrane dynamics or fluidity. Highly reactive MDA (marker of lipid peroxidation) leads to cross-linking reactions of the lipid-lipid and lipid-protein that results in rigidity of membrane and finally decrease fluidity.[31] Thus, suggesting that oxidative stress leads to decreased membrane fluidity. Major determinants that contribute to decreased membrane fluidity are high cholesterol to phospholipid molar ratio.[32] The elevated cholesterol to phospholipid ratio in oxalate-induced group clearly indicates decreased membrane fluidity and pertains as another oxidative stress marker in erythrocytes. NAC post treated oxalate-exposed rats restored this ratio and provides an evidence of its efficacy to relieve oxalate-induced oxidative stress.

The morphological changes in RBCs (Figure 2B) might be due to destruction of phospholipids bilayer membrane. Many evidences have shown that a change in membrane lipid composition was the key reason for deformities in the shape of RBCs in response to any chemical treatment.[33] Therefore, an increase in oxalate level in serum resulted in induction of free radicals that further activated PLA2 causing phospholipid metabolism.[34] However, treatment of these hyperoxaluric rats with NAC showed improved membrane integrity of RBCs. The number of deformed cells in the plasma of these rats was significantly reduced as seen in Figure 2C. This reduction in the number of deformed cells can be attributed to the reduction in phospholipid metabolism. A study by Guo and McMartin showed that calcium oxalate crystals are responsible for hemolysis of RBCs in a concentration-dependant manner.[35] In our previous report, NAC have shown to reduce the number of calcium oxalate crystals in the urine of hyperoxaluric rats.[36] From this, we can infer that a 24-hour dose of NAC increases its level sufficiently in the serum and provide its prophylactic properties to erythrocytes by reduction in calcium oxalate crystals.

We have found the effect of NAC in restoring morphology of liver and kidney tissue following hyperoxaluric manifestations.[8,36] In the present study, the results confirmed oxalate-induced free radical production followed by oxidative stress, also inflict impairment of normal morphology of erythrocytes, and that is responded by increased antioxidant enzymes activity. NAC has shown marked ameliorative effects corroborated by decreased oxidative stress. An additional group of animals given NAC dose alone could perhaps throw more to the present findings. NAC has shown its potential ability to reduce the greater impact of free radicals produced by oxalate exposure and reverted the impact to control level.

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References

1 Senekjian, OH, Weinman, EJ. Oxalate transport by proximal tubule of the rabbit kidney. *Am J Physiol* 1982; **243**: F271–F275.
2 Fritzsch, G, Rumrich, G, Ullrich, KJ. Anion transport through the contraluminal cell membrane of renal proximal tubule. The influence of hydrophobicity and molecular charge distribution on the inhibitory activity of organic anions. *Biochim Biophys Acta* 1989; **978**: 249–256.
3 Karniski, LP, Aronson, PS. Anion exchange pathways for CV transport in rabbit renal microvillus membranes. *Am J Physiol* 1987; **253**: F513–F521.
4 Kvo, SM, Aronson, PS. Oxalate transport via the sulfate/HCO3exchanger in rabbit renal basolateral membrane vesicles. *J Biol Chem* 1988; **263**: 9710–9717.
5 Wang, TS, Agulian, K, Giebisch, G, Aronson, PS. Effects of formate and oxalate on chloride absorption in rat distal tubule. *Am J Physiol* 1993; **264**: F730–F736.
6 Baggio, B, Gambaro, G, Marchini, F, Cicerello, E, Borsatti, A. Raised transmembrane oxalate flux in red blood cells in idiopathic calcium oxalate nephrolithiasis. *Lancet* 1984; **2**: 12–13.
7 Gambaro, G, Patrarulo, M, Nardelotto, A, Marangella, M, Baggio, B. Erythrocyte transmembrane flux and renal clearance of oxalate in idiopathic calcium nephrolithiasis. *Kidney Int* 1995; **48**: 1549–1552.
8 Bijarnia, RK, Kaur, T, Singla, SK, Tandon, C. Reversal of hyperoxaluria-induced alteration in rat liver by administration of N-acetylcysteine. *Drug Chem Toxicol* 2007; **30**: 229–240.
9 Khan, SR. Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. *Urol Res* 2005; **33**: 349–357.
10 Baggio, B, Gambaro, G. Mechanisms of oxalate cellular transport in idiopathic calcium nephrolithiasis. *J Nephrol* 1998; **11**: 63–65.
11 Puppo, A, Halliwell, B. Formation of hydroxyl radicals from hyderogen peroxide in the presence of iron. *Biochem J* 1998; **249**: 185–190.
12 Hodgkinson, A, Williams, A. An improved colorimetric procedure for urine oxalate. *Clin Chim Acta* 1972; **36**: 127–132.

13 Buege, JA, Aust, SD. Microsomal lipid peroxidation method. *Methods Enzymol* 1978; **52**: 302–310.
14 Kono, Y. Generation of superoxide radical during auto oxidation of hydroxylamine and assay of SOD. *Arch Biochem Biophys* 1978; **186**: 189–215.
15 Luck, H. Catalase. In: Bergmeyer, H, (ed), Methods of enzymatic analysis. New york: Acadmic press; 1971. p. 885–893.
16 Folch, J, Lees, M, Sloane-Stanley, GH. A simple method for isolation and purification of total lipids from animal tissue. *J Biol Chem* 1957; **226**: 497–509.
17 Frings, CS, Fendley, TW, Dunn, RT, Queen, CA. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. *Clin Chem* 1972; **18**: 673–674.
18 Bartlett, GR. Phosphorous assay in column chromatography. *J Biol Chem* 1959; **234**: 466–468.
19 Zlatkis, A, Zak, B, Boyle, AJ. A new method for the direct determination of serum cholesterol. *J Lab Clin Med* 1953; **41**: 486–492.
20 Lowry, OH, Rosebrough, NJ. Farr, Al, Randall, RJ. Protein measurement with follin-phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
21 Loidl-Stahlbofen, A, Spiteller, G. Alpha-Hydroxyaldehydes, products of lipid peroxidation. *Biochim Biophys Acta* 1994; **1211**: 156–160.
22 Singh, PP, Barjatiya, MK. Peroxidative stress and antioxidant status in relation to age in normal population and renal stone formers. *Indian J Nephrol* 2002; **12**: 10–15.
23 Veena, CK, Josephine, A, Preetha, SP, Varalakshmi, P. Effect of sulphated polysaccharides on erythrocyte changes due to oxidative and nitrosative stress in experimental hyperoxaluria. *Hum Exp Toxicol* 2007; **26**: 923–932.
24 Frei, B, Stocker, R, Ames, BN. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci USA* 1988; **85**: 9748–9752.
25 Rashed, T, Menon, M, Thamilselvan, S. Molecular mechanism of oxalate-induced free radical production and glutathione redox imbalance in renal epithelial cells: effect of antioxidants. *Am J Nephrol* 2004; **24**: 557–568.
26 Shan, *et al*. Intracellular glutathione influences collagen generation by mesangial cells. *Kidney Int* 1994; **46**: 388–395.
27 Cotgreave, IA, Moldeus, P. Lung protection by thiol-containing antioxidants. *Bull Eur Physiopathol Respir* 1987; **23**: 275–277.
28 Smith, JE, Osburn, BI. Glutathione deficiency in sheep erythrocytes. *Science* 1967; **158**: 374–375.
29 De Rosa, SC, *et al*. N-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest* 2000; **30**: 915–929.
30 Wiseman, H. Dietary influences on membrane function: importance in protection against oxidative damage and disease. *J Nutr Biochem* 1996; **7**: 2–15.
31 Levin, G, Cogan, U, Levy, Y, Mokady, S. Riboflavin deficiency and the function and fluidity of rat erythrocyte membranes. *J Nutr* 1990; **120**: 857–861.
32 Shinitzky, M. Membrane fluidity and cellular function. In: Shinitzky, M, (ed), Physiology of membrane fluidity. Bocca Raton: CRC Press; 1984. p. 1–51.
33 Fee, J, Teitelbaum, H. Evidence that Superoxide dismutase plays a role in protecting red blood cells against peroxidative hemolysis. *Biochem Biophys Res Commun* 1972; **49**: 150–158.
34 Frei, B, Stocker, R, Ames, BN. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci USA* 1988; **85**: 9748–9752.
35 Guo, C, McMartin, KE. The cytotoxicity of oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation. *Toxicol* 2005; **208**: 347–355.
36 Bijarnia, RK, *et al*. Modulatory effects of N-acetylcysteine on hyperoxaluric manifestations in rat kidney. *Food Chem Toxicol* 2008; **46**: 2274–2278.