

NADPH Oxidase as a Mediator of Calcium Oxalate Nephrolithiasis

Oxalate and calcium oxalate crystals induce activation of NADPH oxidase in renal tubular epithelium; this in turn promotes calcium oxalate deposition and inflammation within tubulointerstitial tissue, and renal stone formation. Phycocyanobilin, by inhibiting NADPH oxidase, hence has the potential to prevention oxalate nephrolithiasis, as demonstrated by the utility of oral phycocyanin in a rat model of this disorder. However, whole spirulina may not be ideal to use for prevention of renal stones, owing to its high content of nucleic acids and protein; it had a net negative impact on nephrolithiasis in ethyleneglycol-fed rats.

- 1 Joshi S, Peck AB, Khan SR. NADPH oxidase as a therapeutic target for oxalate induced injury in kidneys. *Oxid Med Cell Longev* 2013;2013:462361.

Ref ID: 41643

Abstract: A major role of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family of enzymes is to catalyze the production of superoxides and other reactive oxygen species (ROS). These ROS, in turn, play a key role as messengers in cell signal transduction and cell cycling, but when they are produced in excess they can lead to oxidative stress (OS). Oxidative stress in the kidneys is now considered a major cause of renal injury and inflammation, giving rise to a variety of pathological disorders. In this review, we discuss the putative role of oxalate in producing oxidative stress via the production of reactive oxygen species by isoforms of NADPH oxidases expressed in different cellular locations of the kidneys. Most renal cells produce ROS, and recent data indicate a direct correlation between upregulated gene expressions of NADPH oxidase, ROS, and inflammation. Renal tissue expression of multiple NADPH oxidase isoforms most likely will impact the future use of different antioxidants and NADPH oxidase inhibitors to minimize OS and renal tissue injury in hyperoxaluria-induced kidney stone disease

- 2 Joshi S, Saylor BT, Wang W, Peck AB, Khan SR. Apocynin-treatment reverses hyperoxaluria induced changes in NADPH oxidase system expression in rat kidneys: a transcriptional study. *PLoS ONE* 2012;7(10):e47738.

Ref ID: 41644

Abstract: PURPOSE: We have previously shown that production of reactive oxygen species (ROS) is an important contributor to renal injury and inflammation following exposure to oxalate (Ox) or calcium-oxalate (CaOx) crystals. The present study was conducted, utilizing global transcriptome analyses, to determine the effect of Apocynin on changes in the NADPH oxidase system activated in kidneys of rats fed a diet leading to hyperoxaluria and CaOx crystal deposition. APPROACH: Age-, sex- and weight-matched rats were either fed regular rat chow or regular rat chow supplemented with 5% w/w hydroxy-L-proline (HLP). Half of the rats on the HLP diet were also placed on Apocynin-supplemented H₂O. After 28 days, each rat was euthanized, their kidneys freshly explanted and dissected to obtain both cortex and medulla tissues. Total RNA was extracted from each tissue and subjected to genomic microarrays to obtain global transcriptome data. KEGG was used to identify gene clusters with differentially expressed genes. Immunohistochemistry was used to confirm protein expressions of selected genes. RESULTS: Genes encoding both membrane- and cytosolic-NADPH oxidase complex-associated proteins, together with p21rac and Rap1a, were coordinately up-regulated significantly in both renal medulla and cortex tissues in the HLP-fed rats compared to normal healthy untreated controls. Activation of NADPH oxidase appears to occur via the angiotensin-II/angiotensin-II receptor-2 pathway, although the

DAG-PKC pathway of neutrophils may also contribute. Immuno histochemical staining confirmed up-regulated gene expressions. Simultaneously, genes encoding ROS scavenger proteins were down-regulated. HLP-fed rats receiving Apocynin had a complete reversal in the differential-expression of the NADPH oxidase system genes, despite showing similar levels of hyperoxaluria. CONCLUSIONS: A strong up-regulation of an oxidative/respiratory burst involving the NADPH oxidase system, activated via the angiotensin-II and most likely the DAG-PKC pathways, occurs in kidneys of hyperoxaluric rats. Apocynin treatment reversed this activation without affecting the levels of hyperoxaluria

- 3 Khan SR. Reactive oxygen species as the molecular modulators of calcium oxalate kidney stone formation: evidence from clinical and experimental investigations. *J Urol* 2013;189(3):803-11.

Ref ID: 41645

Abstract: PURPOSE: Idiopathic calcium oxalate kidney stones form while attached to Randall plaques, the subepithelial deposits on renal papillary surfaces. Plaque formation and growth mechanisms are poorly understood. Plaque formation elsewhere in the body is triggered by reactive oxygen species and oxidative stress. This review explores possible reactive oxygen species involvement in plaque formation and calcium oxalate nephrolithiasis. MATERIALS AND METHODS: A search of various databases for the last 8 years identified literature on reactive oxygen species involvement in calcium oxalate nephrolithiasis. The literature was reviewed and results are discussed. RESULTS: Under normal conditions reactive oxygen species production is controlled, increasing as needed and regulating crystallization modulator production. Reactive oxygen species overproduction or decreased antioxidants lead to oxidative stress, inflammation and injury, and are involved in stone comorbidity. All major chronic inflammation markers are detectable in stone patient urine. Patients also have increased urinary excretion of the IalphaI and the thrombin protein families. Results of a recent study of 17,695 participants in NHANES III (National Health and Nutrition Examination Survey) showed significantly lower antioxidants, carotene and beta-cryptoxanthin in those with a kidney stone history. Animal model and tissue culture studies revealed that high oxalate, calcium oxalate and calcium phosphate crystals provoked renal cell reactive oxygen species mediated inflammatory responses. Calcium oxalate crystals induce renin up-regulation and angiotensin II generation. Nonphagocytic NADPH oxidase leads to reactive oxygen species production mediated by protein kinase C. The P-38 MAPK/JNK transduction pathway is turned on. Transcriptional and growth factors, and generated secondary mediators become involved. Chemoattractant and osteopontin production is increased and macrophages infiltrate the renal interstitium around the crystal. Phagocytic NADPH oxidase is probably activated, producing additional reactive oxygen species. Localized inflammation, extracellular matrix and fibrosis develop. Crystallization modulators have a significant role in inflammation and tissue repair. CONCLUSIONS: Based on available data, Randall plaque formation is similar to extracellular matrix mineralization at many body sites. Renal interstitial collagen becomes mineralized, assisting plaque growth through the interstitium until the mineralizing front reaches papillary surface epithelium. Plaque exposure to pelvic urine may also be a result of reactive oxygen species triggered epithelial sloughing

- 4 Thamilselvan V, Menon M, Thamilselvan S. Selective Rac1 inhibition protects renal tubular epithelial cells from oxalate-induced NADPH oxidase-mediated oxidative cell injury. *Urol Res* 2012;40(4):415-23.

Ref ID: 41646

Abstract: Oxalate-induced oxidative cell injury is one of the major mechanisms implicated in calcium oxalate nucleation, aggregation and growth of kidney stones. We previously demonstrated that oxalate-induced NADPH oxidase-derived free radicals play a significant role in renal injury. Since NADPH oxidase activation requires several regulatory proteins, the

primary goal of this study was to characterize the role of Rac GTPase in oxalate-induced NADPH oxidase-mediated oxidative injury in renal epithelial cells. Our results show that oxalate significantly increased membrane translocation of Rac1 and NADPH oxidase activity of renal epithelial cells in a time-dependent manner. We found that NSC23766, a selective inhibitor of Rac1, blocked oxalate-induced membrane translocation of Rac1 and NADPH oxidase activity. In the absence of Rac1 inhibitor, oxalate exposure significantly increased hydrogen peroxide formation and LDH release in renal epithelial cells. In contrast, Rac1 inhibitor pretreatment, significantly decreased oxalate-induced hydrogen peroxide production and LDH release. Furthermore, PKC alpha and delta inhibitor, oxalate exposure did not increase Rac1 protein translocation, suggesting that PKC resides upstream from Rac1 in the pathway that regulates NADPH oxidase. In conclusion, our data demonstrate for the first time that Rac1-dependent activation of NADPH oxidase might be a crucial mechanism responsible for oxalate-induced oxidative renal cell injury. These findings suggest that Rac1 signaling plays a key role in oxalate-induced renal injury, and may serve as a potential therapeutic target to prevent calcium oxalate crystal deposition in stone formers and reduce recurrence

- 5 Zuo J, Khan A, Glenton PA, Khan SR. Effect of NADPH oxidase inhibition on the expression of kidney injury molecule and calcium oxalate crystal deposition in hydroxy-L-proline-induced hyperoxaluria in the male Sprague-Dawley rats. *Nephrol Dial Transplant* 2011;26(6):1785-96.

Ref ID: 41647

Abstract: **BACKGROUND:** Renal calcium oxalate (CaOx) crystal deposition is associated with epithelial injury and movement of inflammatory cells into the interstitium. We have proposed that oxalate (Ox)- and CaOx crystal-induced injury is most likely caused by reactive oxygen species (ROS) produced by activation of membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. **METHODS:** Present study was undertaken to determine the effect of NADPH oxidase inhibitor apocynin on the expression of kidney injury molecule-1 (KIM-1) and renal CaOx crystal deposition in rats with hyperoxaluria. We also investigated the urinary excretion of KIM-1, osteopontin (OPN) and monocyte chemoattractant protein-1 (MCP-1) and renal expression of OPN and ED-1. Male Sprague-Dawley rats were fed a diet containing 5% hydroxyl-L-proline (HLP) and 4 mmol apocynin to drink for 28 days. Urine was collected on Days 7, 14, 21 and 28. After that, rats were sacrificed and their kidneys processed for various microscopic and molecular investigations. **RESULTS:** HLP consumption produced heavy deposits of CaOx crystals. Renal expression of KIM-1 and OPN and urinary excretion of KIM-1, OPN, H(2)O(2) and MCP-1 was significantly increased. ED-1-positive cells migrated into renal interstitium. Apocynin treatment caused significant reduction of crystal deposits, injured and dilated tubules; renal expression of KIM-1, OPN and ED-1 and urinary excretion of KIM-1, OPN, MCP-1 and H(2)O(2). Apocynin had no effect on the urinary excretion of Ox. **CONCLUSIONS:** This is the first study of urinary excretion and renal expression of KIM-1 in association with renal CaOx crystal deposition, experimental or clinical. The results indicate that NADPH oxidase inhibition leads to reduction in KIM-1 expression and urinary excretion as well as renal CaOx crystal deposition. KIM-1 is an important marker of renal epithelial injury. The results provide further support to our proposal that renal epithelial injury is critical for crystal retention and that injury is in part caused by the production of ROS with the involvement of NADPH oxidase

- 6 Yoshioka I, Tsujihata M, Akanae W, Nonomura N, Okuyama A. Angiotensin type-1 receptor blocker candesartan inhibits calcium oxalate crystal deposition in ethylene glycol-treated rat kidneys. *Urology* 2011;77(4):1007.

Ref ID: 41648

Abstract: **OBJECTIVES:** To investigate whether an angiotensin type-1 receptor blocker could inhibit calcium oxalate crystal deposition using ethylene glycol-treated rats. The

renoprotective effect has been reported to be another role of angiotensin type-1 receptor blockers in addition to their role in lowering blood pressure. Recent research has suggested that inhibiting reactive oxidative species generation and tubulointerstitial inflammation is the major role of angiotensin type-1 receptor blockers. These 2 factors are also important in the mechanism of calcium oxalate stone formation. **METHODS:** We divided 28 rats, aged 7 weeks, into 4 groups: group 1, control rats; group 2, candesartan-treated rats; group 3, stone-forming rats; and group 4, candesartan-treated stone-forming rats. The kidney crystal deposits were examined, and the oxidative stress biomarker, nicotinamide adenine dinucleotide phosphate oxidase activity, general and urinary variables, and the transforming growth factor-beta level in kidney tissue were compared among the 4 groups. **RESULTS:** The candesartan-treated rats were healthy and had weight gain similar to that of the control rats, although a significant reduction in blood pressure was observed. The urinary components associated with calcium oxalate stone formation were not influenced by candesartan treatment; however, significantly fewer crystal deposits were observed in group 4. The oxidative biomarker and nicotinamide adenine dinucleotide phosphate oxidase activity decreased, and the level of transforming growth factor-beta was suppressed in group 4. **CONCLUSIONS:** Candesartan had substantial effects on crystal formation in the rat kidney by suppressing nicotinamide adenine dinucleotide phosphate oxidase and the transforming growth factor-beta levels

- 7 Khan SR, Khan A, Byer KJ. Temporal changes in the expression of mRNA of NADPH oxidase subunits in renal epithelial cells exposed to oxalate or calcium oxalate crystals. *Nephrol Dial Transplant* 2011;26(6):1778-85.

Ref ID: 41649

Abstract: **BACKGROUND:** Exposure of renal epithelial cells to oxalate (Ox) or calcium oxalate (CaOx) crystals leads to the production of reactive oxygen species and cell injury. We have hypothesized that Ox and CaOx crystals activate NADPH oxidase through upregulation of its various subunits. **METHODS:** Human renal epithelial-derived cell line, HK-2, was exposed to 100 μmol Ox or 66.7 $\mu\text{g}/\text{cm}^2$ CaOx monohydrate crystals for 6, 12, 24 or 48 h. After exposure, the cells and media were processed to determine activation of NADPH oxidase, production of superoxide and 8-isoprostane (8IP), and release of lactate dehydrogenase (LDH). RT-PCR was performed to determine mRNA expression of NADPH subunits p22(phox), p40(phox), p47(phox), p67(phox) and gp91(phox) as well as Rac-GTPase. **RESULTS:** Exposure to Ox and CaOx crystals resulted in increase in LDH release, production of 8-IP, NADPH oxidase activity and production of superoxide. Exposure to CaOx crystals resulted in significantly higher NADPH oxidase activity, production of superoxide and LDH release than Ox exposure. Exposure to Ox and CaOx crystals altered the expression of various subunits of NADPH oxidase. More consistent were increases in the expression of membrane-bound p22(phox) and cytosolic p47(phox). Significant and strong correlations were seen between NADPH oxidase activity, the expression of p22(phox) and p47(phox), production of superoxide and release of LDH when cells were exposed to CaOx crystals. The expressions of neither p22(phox) nor p47(phox) were significantly correlated with increased NADPH oxidase activity after the Ox exposure. **CONCLUSIONS:** As hypothesized, exposure to Ox or CaOx crystals leads to significant increases in the expression of p22(phox) and p47(phox), leading to activation of NADPH oxidase. Increased NADPH oxidase activity is associated with increased superoxide production and lipid peroxidation. Different pathways appear to be involved in the stimulation of renal epithelial cells by exposure to Ox and CaOx crystals

- 8 Thamilselvan V, Menon M, Thamilselvan S. Oxalate-induced activation of PKC-alpha and -delta regulates NADPH oxidase-mediated oxidative injury in renal tubular epithelial cells. *Am J Physiol Renal Physiol* 2009;297(5):F1399-F1410.

Ref ID: 41650

Abstract: Oxalate-induced oxidative stress contributes to cell injury and promotes renal deposition of calcium oxalate crystals. However, we do not know how oxalate stimulates reactive oxygen species (ROS) in renal tubular epithelial cells. We investigated the signaling mechanism of oxalate-induced ROS formation in these cells and found that oxalate significantly increased membrane-associated protein kinase C (PKC) activity while at the same time lowering cytosolic PKC activity. Oxalate markedly translocated PKC- α and - δ from the cytosol to the cell membrane. Pretreatment of LLC-PK1 cells with specific inhibitors of PKC- α or - δ significantly blocked oxalate-induced generation of superoxide and hydrogen peroxide along with NADPH oxidase activity, LDH release, lipid hydroperoxide formation, and apoptosis. The PKC activator PMA mimicked oxalate's effect on oxidative stress in LLC-PK1 cells as well as cytosol-to-membrane translocation of PKC- α and - δ . Silencing of PKC- α expression by PKC- α -specific small interfering RNA significantly attenuated oxalate-induced cell injury by decreasing hydrogen peroxide generation and LDH release. We believe this is the first demonstration that PKC- α - and - δ -dependent activation of NADPH oxidase is one of the mechanisms responsible for oxalate-induced oxidative injury in renal tubular epithelial cells. The study suggests that the therapeutic approach might be considered toward attenuating oxalate-induced PKC signaling-mediated oxidative injury in recurrent stone formers

- 9 Li CY, Deng YL, Sun BH. Effects of apocynin and losartan treatment on renal oxidative stress in a rat model of calcium oxalate nephrolithiasis. *Int Urol Nephrol* 2009;41(4):823-33. Ref ID: 41651

Abstract: BACKGROUND: Tissue culture studies found that renal epithelial cells suffer oxidative injury on exposure to high levels of oxalate (Ox) and calcium oxalate (CaOx) crystals; nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a major source of reactive oxygen species (ROS) production in kidney, has been shown to be involved in this event. The present study aimed to investigate whether this in vitro feature of NADPH oxidase could be confirmed in vivo. METHODS: Animal model of nephrolithiasis was established in adult male Sprague-Dawley rats by administration of 0.8% ethylene glycol (EG) in drinking water for 4 weeks. Simultaneous treatment with apocynin (0.2 g kg⁻¹ day⁻¹) or losartan (30 mg kg⁻¹ day⁻¹) by intragastric administration was performed in rats. At the end of the study, urinary 8-IP, a product of lipid peroxidation, and enzymatic activity of superoxide dismutase (SOD) in kidney homogenates were assessed as markers for state of renal oxidative stress (OS). Expression of NADPH oxidase subunit p47phox in kidney was localized and evaluated by immunohistochemistry, real-time polymerase chain reaction (PCR), and Western blotting. The concentration of angiotensin II in kidney homogenates was determined using radioimmunoassay method. RESULTS: Compared with control, OS developed significantly in rats received EG, with increased expression of p47phox messenger RNA (mRNA) and protein in kidneys. Renal angiotensin II also increased significantly. Treatment with apocynin or losartan significantly reduced excretion of urinary 8-IP, restored SOD activity, with decrease in expression of p47phox in kidney, but levels of those OS markers in apocynin- or losartan-treated rats were still higher than in normal controls. CONCLUSIONS: These results suggest that renal Ang II and its stimulation of NADPH oxidase may partially account for the development of OS in kidney in this rat model of CaOx nephrolithiasis

- 10 Umekawa T, Tsuji H, Uemura H, Khan SR. Superoxide from NADPH oxidase as second messenger for the expression of osteopontin and monocyte chemoattractant protein-1 in renal epithelial cells exposed to calcium oxalate crystals. *BJU Int* 2009;104(1):115-20. Ref ID: 41652

Abstract: OBJECTIVE To test the hypothesis that exposure of a renal epithelial cell line, NRK52E, to calcium oxalate monohydrate crystals (COM) would up-regulate NADPH oxidase subunit p47(phox), enhance superoxide production and increase monocyte chemoattractant protein-1 (MCP-1) and osteopontin mRNA levels. MATERIALS AND

METHODS Confluent cultures of NRK52E cells were exposed to COM (66.7 microg/cm²) with or with no pretreatment with diphenyleneiodium chloride (DPI, 10 x 10⁻⁶m) an inhibitor for NADPH oxidase, under serum-free conditions. The conditioned medium was collected and total cellular RNA isolated from the cells, and subjected to enzyme-linked immunosorbent assay and real-time polymerase chain reaction (PCR). Production of reactive oxygen species (ROS) was estimated by dihydroethidium (DHE) staining using a fluorescence microscope. Immunohistochemistry and real-time PCR were used to analyse p47(phox) in NRK52E cells. **RESULTS** In COM treated NRK52E cells there was enhanced expression of p47(phox) and production of superoxide. COM-induced production of MCP-1 and osteopontin was significantly reduced after treatment with DPI. **CONCLUSIONS** While the generation of a lot of ROS might play a major role in tissue injury or death, the regulated generation of low concentration of ROS, possibly by NADPH oxidase, may represent a second messenger system for generation of COM-induced MCP-1 and osteopontin production in the renal tubules

- 11 Huang HS, Ma MC, Chen J. Low-vitamin E diet exacerbates calcium oxalate crystal formation via enhanced oxidative stress in rat hyperoxaluric kidney. *Am J Physiol Renal Physiol* 2009;296(1):F34-F45.

Ref ID: 41653

Abstract: Vitamin E was previously reported to reduce calcium oxalate (CaOx) crystal formation. This study explored whether vitamin E deficiency affects intrarenal oxidative stress and accelerates crystal deposition in hyperoxaluria. The control (C) group of rats received a standard diet and drinking water, while the experimental groups received 0.75% ethylene glycol (EG) in drinking water for 42 days. Of the latter, one group received a standard diet (EG group), one received a low-vitamin E (LE) diet (EG+LE group), and the last received an LE diet with vitamin E supplement (4 mg) (EG+LE+E group). The C+LE and C+LE+E groups were the specific controls for the last two experimental groups, respectively. In a separate experiment, EG and EG+LE rats were studied on days 3-42 to examine the temporal relationship between oxidative change and crystal formation. Urinary biochemistry and activity/levels of antioxidative and oxidative enzymes in glomeruli and tubulointerstitial specimens (TIS) were examined. In EG rats, CaOx crystal accumulation was associated with low antioxidative enzyme activity in TIS and with increased oxidative enzyme expression in glomeruli. In the EG+LE group, marked changes in antioxidative and oxidative enzyme levels were seen and correlated with massive CaOx deposition and tubular damage. The increased oxidative stress seen with EG+LE treatment was largely reversed by vitamin E supplementation. A temporal study showed that decrease in antioxidative defense and increased free radical formation in the EG+LE group occurred before crystal deposition. This study shows that low vitamin E disrupts the redox balance and causes cell death, thereby favoring crystal formation

- 12 Umekawa T, Byer K, Uemura H, Khan SR. Diphenyleneiodium (DPI) reduces oxalate ion- and calcium oxalate monohydrate and brushite crystal-induced upregulation of MCP-1 in NRK 52E cells. *Nephrol Dial Transplant* 2005;20(5):870-8.

Ref ID: 41654

Abstract: BACKGROUND: Our earlier studies have demonstrated upregulation of monocyte chemoattractant protein-1 (MCP-1) in NRK52E rat renal epithelial cells by exposure to oxalate (Ox) ions and crystals of calcium oxalate monohydrate (COM) or the brushite (Br) form of calcium phosphate. The upregulation was mediated by reactive oxygen species (ROS). This study was performed to investigate whether NADPH oxidase is involved in ROS production. **METHODS:** Confluent cultures of NRK52E cells were exposed to Ox ions or COM and Br crystals. They were exposed for 1, 3, 6, 12, 24 and 48 h for isolation of MCP-1 mRNA and 24 h for enzyme-linked immunosorbent assay (ELISA) to determine the secretion of protein into the culture medium. We also investigated the effect of free radical scavenger,

catalase, and the NADPH oxidase inhibitor diphenyleneiodium (DPI) chloride, on the Ox- and crystal-induced expression of MCP-1 mRNA and protein. The transcription of MCP-1 mRNA in the cells was determined using real-time polymerase chain reaction. Hydrogen peroxide and 8-isoprostane were measured to investigate the involvement of ROS.

RESULTS: Exposure of NRK52E cells to Ox ions as well as the crystals resulted in increased expression of MCP-1 mRNA and production of the chemoattractant. Treatment with catalase reduced the Ox- and crystal-induced expression of both MCP-1 mRNA and protein. DPI reduced the crystal-induced gene expression and protein production but not Ox-induced gene expression and protein production. **CONCLUSIONS:** Exposure to Ox ions, and COM and Br crystals stimulates a ROS-mediated increase in MCP-1 mRNA expression and protein production. Reduction in ROS production, lipid peroxidation, low-density lipoprotein release, and inducible MCP-1 gene and protein in the presence of DPI indicates an involvement of NADPH oxidase in the production of ROS

- 13 Huang HS, Ma MC, Chen J, Chen CF. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. *J Urol* 2002;167(6):2584-93. Ref ID: 41655

Abstract: **PURPOSE:** We investigated the possible mechanism of increased free radicals, the role of antioxidant enzymes and their correlation with renal tubular damage in the kidney after feeding 0.75% ethylene glycol to male Wistar rats. **MATERIALS AND METHODS:** Rats were divided into 7 experimental groups according to the duration of ethylene glycol feeding (1, 3, 5, 7, 9, 21 or 42 days) and into age matched control groups. Chemiluminescence levels were examined in blood samples (renal artery and vein) and in the kidney. The activities of oxidase and antioxidant enzymes were measured in kidney homogenates. The nitroblue tetrazolium perfusion method and immunohistochemical stains with ED1 and CD45 were performed. Urinary levels of alpha and mu-glutathione S-transferase (GST) were also measured and expressed in gm. urinary creatinine. **RESULTS:** Chemiluminescence levels of renal venous blood samples were elevated on days 1, 3 and 7 ($p < 0.05$), and those of the kidney were elevated only on days 3 and 42 ($p < 0.05$) compared with controls. The infiltration of CD45 positive cells in the kidney increased on day 7 and a further increase in these positive cells was noted on day 21. Fused ED1 positive cells surrounding the calcium oxalate crystals and adjacent to the nitroblue tetrazolium positive area were found on day 42. Xanthine oxidase activity showed no significant change, whereas nicotinamide adenine dinucleotide dependent oxidase activity was higher on day 5 and nicotinamide adenine dinucleotide phosphate dependent activity was elevated in all experimental groups ($p < 0.05$). The activities of catalase and manganese superoxide dismutase were elevated in the early stage. On day 42 almost all antioxidant enzyme activities were attenuated ($p < 0.05$) except that of catalase. The urinary levels of alpha-GST were elevated from day 7 until day 42, whereas levels of mu-GST were elevated from day 3 until day 42 except day 5. **CONCLUSIONS:** The possible mechanism that causes free radical elevation in the kidney may be different in the course of nephrolithiasis after ethylene glycol treatment. Initially the systemic circulation may bring the toxic substance into the kidney and cause it to produce free radicals. In the late stage gradually infiltrating leukocytes and decreased antioxidant enzyme activities may cause the kidney to remain under excessive oxidative stress

- 14 Farooq SM, Ebrahim AS, Subramhanya KH, Sakthivel R, Rajesh NG, Varalakshmi P. Oxalate mediated nephronal impairment and its inhibition by c-phycocyanin: a study on urolithic rats. *Mol Cell Biochem* 2006;284(1-2):95-101. Ref ID: 41656

Abstract: The assumption of oxidative stress as a mechanism in oxalate induced renal damage suggests that antioxidants might play a beneficial role against oxalate toxicity. An in vivo model was used to investigate the effect of C-phycocyanin (from aquatic micro algae; *Spirulina* spp.), a known antioxidant, against calcium oxalate urolithiasis. Hyperoxaluria was

induced in two of the 4 groups of Wistar albino rats (n = 6 in each) by intraperitoneally injecting sodium oxalate (70 mg/kg body weight). A pretreatment of phycocyanin (100 mg/kg body weight) as a single oral dosage was given, one hour prior to oxalate challenge. An untreated control and drug control (phycocyanin alone) were employed. Phycocyanin administration resulted in a significant improvement ($p < 0.001$) in the thiol content of renal tissue and RBC lysate via increasing glutathione and reducing malondialdehyde levels in the plasma of oxalate induced rats ($p < 0.001$), indicating phycocyanin's antioxidant effect on oxalate mediated oxidative stress. Administering phycocyanin after oxalate treatment significantly increased catalase and glucose-6-phosphate dehydrogenase activity ($p < 0.001$) in RBC lysate suggesting phycocyanin as a free radical quencher. Assessing calcium oxalate crystal retention in renal tissue using polarization microscopy and renal ultrastructure by electron microscopy reveals normal features in phycocyanin-- pretreated groups. Thus the study presents positive pharmacological implications of phycocyanin against oxalate mediated nephronal impairment and warrants further work to tap this potential aquatic resource for its medicinal application

- 15 Farooq SM, Ebrahim AS, Asokan D, Sakthivel R, Savitha S, Rajesh N Get al. Credentials of Spirulina diet on stability and flux related properties on the biomineralization process during oxalate mediated renal calcification in rats. *Clin Nutr* 2005;24(6):932-42.

Ref ID: 41657

Abstract: **BACKGROUND:** High Spirulina diet is a potential risk factor for nephrolithiasis since it has the capacity to increase urinary oxalate and uric acid level, facilitating lithogenesis. Our aim was to identify the effect of Spirulina diet during hyperoxaluric condition in Wistar albino rats. **METHODS:** The animals were divided into four groups: control (G1, n=6); ethylene glycol (EG) induced (G2, n=6); EG+Spirulina (G3, n=6); Spirulina alone (G4, n=6). EG at 0.75% was administered to G2 and G3 through drinking water for 4 weeks and Spirulina 1500 mg/kg feed was administered to G3 and G4. **RESULTS:** Urinary parameters like oxalate, uric acid, calcium, urea, and creatinine ($P < 0.001$) were found increased after Spirulina diet under hyperoxaluric conditions compared to the same without Spirulina diet. Similarly the BUN, plasma contents of uric acid, urea, creatinine ($P < 0.001$) were found to be raised in G3. The renal and RBC GSH levels, as estimated by HPLC, seemed decreased when compared to G2. **CONCLUSIONS:** The present study shows that free radicals aid in the progression of nephrolithiasis. The crystal deposition was found to be high in the renal cells of G3 than G2 and TEM revealed damage in renal cell of G3 implying that the disease deteriorates by free radical injury. In contrast the Spirulina diet alone (G4) did not induce any features relating to stone forming condition suggesting that free radical release might have been suppressed due to enrichment of dietary antioxidants and vitamins. Thus the present investigation demonstrates that during hyperoxaluric conditions the Spirulina diet must possibly be avoided and can be considered in normal subjects checked for family history of renal stone deposition

- 16 Farooq SM, Asokan D, Sakthivel R, Kalaiselvi P, Varalakshmi P. Salubrious effect of C-phycocyanin against oxalate-mediated renal cell injury. *Clin Chim Acta* 2004;348(1-2):199-205.

Ref ID: 41658

Abstract: **BACKGROUND:** C-phycocyanin, a biliprotein pigment found in some blue green algae (*Spirulina platensis*) with nutritional and medicinal properties, was investigated for its efficacy on sodium oxalate-induced nephrotoxicity in experimentally induced urolithic rats. **METHODS:** Male Wistar rats were divided into four groups. Hyperoxaluria was induced in two of these groups by intraperitoneal infusion of sodium oxalate (70 mg/kg), and a pretreatment of phycocyanin (100 mg/kg) as a single oral dosage was given to one of these groups by 1 h prior to sodium oxalate infusion challenges. The study also encompasses an untreated control group and a phycocyanin-alone treated drug control group. The extent of

lipid peroxidation (LPO) was evaluated in terms of renal concentrations of MDA, conjugated diene and hydroperoxides. The following assay was performed in the renal tissue (a) antioxidant enzymes such as superoxide dismutase (SOD) and catalase, (b) glutathione metabolizing enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and glucose 6-phosphate dehydrogenase (G6PD), (c) the low molecular weight antioxidants (GSH, vitamins E and C) and protein carbonyl content. RESULTS: The increased concentrations of MDA, conjugated diene and hydroperoxide (index of the lipid peroxidation) were controlled ($P < 0.001$) in the phycocyanin-pretreated group. At the outset, the low molecular weight antioxidants were appreciably increased ($P < 0.001$), whereas the tissue protein carbonyl concentration was decreased ($P < 0.001$), suggesting that phycocyanin provides protection to renal cell antioxidants. It was noticed that the activities of antioxidant enzymes and glutathione metabolizing enzymes were considerably stabilized in rats pretreated with phycocyanin. CONCLUSION: We suggest that phycocyanin protects the integrity of the renal cell by stabilizing the free radical mediated LPO and protein carbonyl, as well as low molecular weight antioxidants and antioxidant enzymes in renal cells. Thus, the present analysis reveals that the antioxidant nature of C-phycocyanin protects the renal cell against oxalate-induced injury and may be a nephroprotective agent

- 17 Farooq SM, Asokan D, Kalaiselvi P, Sakthivel R, Varalakshmi P. Prophylactic role of phycocyanin: a study of oxalate mediated renal cell injury. *Chem Biol Interact* 2004;149(1):1-7.

Ref ID: 41659

Abstract: Oxalate induced renal calculi formation and the associated renal injury is thought to be caused by free radical mediated mechanisms. An in vivo model was used to investigate the effect of phycocyanin (from *Spirulina platensis*), a known antioxidant, against calcium oxalate urolithiasis. Male Wistar rats were divided into four groups. Hyperoxaluria was induced in two of these groups by intraperitoneal infusion of sodium oxalate (70 mg/kg) and a pretreatment of phycocyanin (100 mg/kg) as a single oral dosage was given, 1h prior to sodium oxalate infusion. An untreated control and drug control (phycocyanin alone) were also included in the study. We observed that phycocyanin significantly controlled the early biochemical changes in calcium oxalate stone formation. The antiurolithic nature of the drug was evaluated by the assessment of urinary risk factors and light microscopic observation of urinary crystals. Renal tubular damage as divulged by urinary marker enzymes (alkaline phosphatase, acid phosphatase and gamma-glutamyl transferase) and histopathological observations such as decreased tubulointerstitial, tubular dilatation and mononuclear inflammatory cells, indicated that renal damage was minimised in drug-pretreated group. Oxalate levels ($P < 0.001$) and lipid peroxidation ($P < 0.001$) in kidney tissue were significantly controlled by drug pretreatment, suggesting the ability of phycocyanin to quench the free radicals, thereby preventing the lipid peroxidation mediated tissue damage and oxalate entry. This accounts for the prevention of CaOx stones. Thus, the present analysis revealed the antioxidant and antiurolithic potential of phycocyanin thereby projecting it as a promising therapeutic agent against renal cell injury associated kidney stone formation