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Physico-chemical alterations of urine in experimental hyperoxaluria: a biochemical approach with fucoidan

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Abstract

Urinary supersaturation-induced crystal formation has been attributed as one of the key factor for the pathogenesis/progression of lithogenesis. This study was aimed at investigating whether fucoidan, a naturally occurring sulfated glycosaminoglycan, could ameliorate the biochemical changes in urine induced by stone formation. Two groups of male albino Wistar rats (120 ± 20 g) received 0.75% ethylene glycol (EG) for 28 days to induce hyperoxaluria, and one of them received sulfated polysaccharides (fucoidan from *Fucus vesiculosus*, 5 mg kg^{-1}, s.c.), commencing from the 8^{th} day of the experimental period. One group was maintained as normal control group and another group served as drug control, which received sulfated polysaccharides. The urine collected from all the groups was analysed for changes in pH, volume, oxalate, calcium, phosphorus, uric acid, magnesium, citric acid and glycosaminoglycans. Urinary crystals were analysed with a light microscope. Renal tissues were studied under polarized light for deposition of crystals and also analysed for their oxalate and calcium content. The changes in extracellular matrix on crystal deposition were also evaluated. The urinary pH and volume were altered in rats treated with EG along with an increase in weight of the kidney. Further, administration of EG to rats increased the supersaturation of urine by escalating the levels of the stone-forming constituents, such as oxalate, calcium, phosphorus and uric acid, which was completely restored by fucoidan treatment. The decrease in the inhibitors, like citrate, magnesium and glycosaminoglycans, in urine was prevented by the co-treatment with fucoidan. In hyperoxaluric rats, there was an increased excretion of calcium oxalate monohydrate crystals in urine along with crystal deposition in renal tissues; this was prevented by fucoidan treatment. Fucoidan administration reversed even the tissue levels of calcium and oxalate. The increased accumulation of collagen and expression of transforming growth factor-β_1 in hyperoxaluria was normalized on fucoidan administration. These results suggest that the physico-chemical alterations in urine produced during hyperoxaluria can be reversed by fucoidan administration.

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Introduction

Nephrolithiasis is a real problem for public health and its increased prevalence and peak of frequency among men between the third and sixth decade explains its high socio-economic cost (Sowers et al 1998). The advent of minimally invasive surgical options, such as extracorporeal shockwave lithotripsy and laser lithotripsy, has significantly changed the treatment strategies for urolithiasis. However, a preventive prophylactic programme is essential because besides the high recurrence rate of kidney stones, exposure to shock waves even in therapeutic doses may cause acute renal injury and decrease in renal function (Willis et al 2006). In general, kidney stone formation is a multifactorial process involving a cascade of events, including supersaturation, nucleation, aggregation, growth and retention of crystals in the renal tubules (Jonassen et al 2005). Disturbance in the balance between supersaturating and inhibiting factors in urine is considered to be a primary aspect in altering the supersaturation of urine and initiating stone formation. The production of concentrated urine frequently initiates crystal formation in the kidney and persistent inadequate elimination of crystal material with the urine can eventually lead to formation of a stone in the urinary tract (Selvam 2002). Retention of microliths in the urinary tract facilitates further development

of crystals into stones and reports suggest that alteration in extracellular matrix (ECM) plays a crucial role in stone retention (Khan 2004). Hence, drugs that can alter the excretion of stone-forming constituents/modulators in urine and prevent the disturbance in ECM would be beneficial in the treatment of urolithiasis.

Glycosaminoglycans (GAGs) are recognized as potent inhibitors of urolithiasis through numerous in-vitro and in-vivo experiments (Cao et al 1997a). GAGs are polysaccharide chains formed from alternating hexosamine and uranyl residues. Except in the urinary tract, these polyanions are not found as free chains but as constituents of proteoglycans that usually contain one or more GAG chains. As a consequence of proteoglycans degradation, GAGs appear in the urine after being filtered through the glomerulus. Although numerous in-vitro studies have described the inhibitory effect of GAGs on stone formation, controversy exists in published reports that have compared GAG levels in patients and controls (Michelacci et al 1989; Nikkila 1989). Recent research on these molecules has shown their biphasic nature. GAGs from marine algae, especially fucoidan, a sulfated polysaccharide extracted from brown algae, are endowed with important properties, including anti-oxidant, anti-coagulant, anti-thrombotic, anti-angiogenic and anti-inflammatory activity, which are of potential value (Berteau & Mulloy 2003). Zhang et al (2003) showed that fucoidan could also alleviate chronic renal failure in rats, suggesting a possible nephro-protective role for fucoidan. Sodium pentosan polysulfate, a heparin analogue, was able to alter the stone forming constituents in urolithic rats (Subha & Varalakshmi 1993). Our previous studies have also shown that fucoidan was able to modulate the oxidative stress associated with hyperoxaluria (Veena et al 2007). Therapeutic agents that can modulate the damage in renal tissue as well as alter the urinary biochemical composition would be beneficial in the prognosis of urolithiasis. Hence, this study was initiated to explore the effect of fucoidan on urinary stone-forming constituents/modulators.

Materials and Methods

Chemicals

Fucoidan from *Fucus vesiculosus* and BSA were procured from Sigma Chemical Co. (St Louis, MO). All other chemicals and reagents used were of analytical grade.

Animal model

Male Wistar albino rats, 120 ± 20 g, were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The rats were maintained under standard conditions of humidity, temperature (25 ± 2°C) and light (12-h light–dark cycle) and were fed with standard rat pelleted diet (Amrut rat/mice feed; M/s Pranav Agro Industries Ltd, India) and allowed free access to water. Experimental rats were handled with humane care according to the guidelines of the institutional animal ethics committee (IAEC).

Experimental setup

The rats were randomly divided into four groups consisting of six rats each as shown in Table 1. In the ethylene glycol (EG) model of hyperoxaluria induction, different concentrations are established (0.5% EG + 25-hydroxy cholesterol, 1% EG, 2% EG, EG + ammonium chloride, etc). The dose used in this study was 0.75% v/v EG in drinking water for 28 days. The provision of this dose of EG has generated hyperoxaluria in as little as 3 days (Huang et al 2002) and as long as 60 days (Thamilselvan et al 1997), with no discernible effect on renal function as judged by creatinine clearance (Huang et al 2003). At the end of 28 days, the rats were housed in metabolic cages for 24-h urine collection. Urine collected was used for analysis of urinary components and to measure urinary volume, pH and crystalluria. The rats were sacrificed and the kidneys were rinsed in ice-cold physiological saline. A portion of the tissue was fixed in 10% formalin for polarized microscopic analysis of crystals and the remainder was homogenized in Tris-HCl buffer (0.01 M, pH 7.4) to give a 10% homogenate and was suitably processed.

Biochemical analysis of urine and renal tissue

Urinary oxalate and tissue oxalate were estimated after acid extraction as described by Hodgkinson & Williams (1972). Urinary uric acid, phosphorus and citrate were estimated by the methods of Caraway (1963), Fiske & Subbarow (1925) and Rajagopal (1984), respectively. Calcium and magnesium measurements were performed by atomic absorption spectrophotometry. For the measurement of GAG it was precipitated with cetylpyridinium chloride and then reacted with dimethylmethylene blue to produce a complex with the polyanionic molecule of sulfated GAGs (Panin et al 1986). Protein was estimated by the method of Lowry et al (1951).

Urinary crystal study

Twenty-four hour urine was collected and a drop of it was allowed to spread over a clean glass slide and visualized under a light microscope (ECLIPSE E400; Nikon, Japan).

Table 1 The study design

Group	Treatment
I	Vehicle-treated control rats
II	Rats were administered ethylene glycol (0.75% in drinking water) for 28 days
III	Rats were administered sulfated polysaccharides (*Fucus vesiculosus*; Sigma Chemicals, St Louis, MO), 5 mg kg^{-1} dissolved in saline and passed through a 0.2-μm filter before subcutaneous administration
IV	Rats were administered ethylene glycol for 28 days and sulfated polysaccharide commencing on day 8 of the experimental period

Polarized microscopic studies

Kidneys harvested from rats were examined for crystals after they were fixed in formalin and embedded in paraffin. Sections (5 μm thick) were stained by eosin solution and examined by polarized light microscopy (Euromex Stereo microscope, Holland).

Evaluation of extracellular matrix (ECM) alterations

Collagen content of renal tissues was estimated by the method of Esteban et al (2005) using Sirius red. Transforming growth factor-β_1 (TGF-β_1) expression in renal tissues was assessed using reverse transcriptase–polymerase chain reaction (RT-PCR). To determine the expression of TGF-β_1 mRNA in each group, total RNA was isolated from kidney using a total RNA extraction kit (Trizol; Medox Biotech Pvt Ltd, India). The following primer pairs were used: sense: 5′-ACT GAT ACG CCT GAG TGG CTG T-3′; anti-sense: 5′-CTC TGT GGA GCT GAA GCA GTA G-3′ (Shi et al 2004). The expected product size of TGF-β_1 mRNA was 303 bp. PCR amplification was carried out with a thermal cycler using a one-step RT-PCR kit (Quagen one step RT-PCR kit, Germany) according to a protocol: initial denaturing at 94°C for 15 min; then 39 cycles at 94°C for 15 s (denaturing), at 60°C for 1 min (annealing) and 72°C for 30 s (extension); and a further extension at 72°C for 5 min. The PCR products were resolved on a 2% agarose gel in Tris-borate-EDTA buffer. Ribosomal protein L19 (RPL-19) was used as an internal standard.

Data analysis

The results are expressed as mean ± standard deviation (s.d.) for six rats in each group. Differences between groups were assessed by one-way analysis of variance using the SPSS software package for Windows. Post-hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test; significance at P values < 0.001, < 0.01 and < 0.05 have been given respective symbols in the tables and figures.

Results

Table 2 delineates the changes in the body weight, kidney weight and urinary pH. There was no significant change in the body weight between the experimental groups. However, the weight of the kidney in group II rats was found to be increased from that of controls ($P < 0.001$). The pH of the ethylene glycol (EG)-treated rats was decreased ($P < 0.001$) when compared with the control, indicative of an acidic scenario favouring crystal retention. Treatment with fucoidan accentuated the abnormal decrease in the pH of the urine ($P < 0.001$) and also reversed the increase in kidney weight to near normalcy ($P < 0.01$).

Figure 1 shows the protein content and urinary volume of the different experimental groups. An increase in urinary volume was observed in EG-treated rats ($P < 0.01$). Treatment with fucoidan profoundly increased the urinary volume of group IV rats when compared with group II, illustrating the diuretic activity of fucoidan ($P < 0.001$). The excretion of protein in urine was increased in hyperoxaluric rats, indicating damage to the kidney ($P < 0.001$). Fucoidan administration decreased the protein excretion in urine ($P < 0.001$).

Table 3 shows the urinary content of the major stone-forming constituents, such as calcium, oxalate, uric acid and phosphorus. Oxalate, calcium and phosphorus were significantly increased ($P < 0.001$), more than uric acid ($P < 0.05$), in group II rats. In EG + fucoidan-treated rats, the stone-forming constituents were decreased when compared with the group II rats ($P < 0.001$). In EG-treated rats, the drastic decrease in the inhibitor constituents, such as citrate ($P < 0.05$), magnesium ($P < 0.001$) and GAGs ($P < 0.001$), favours a milieu for crystal nucleation and retention. Administration of fucoidan was able to alleviate these abnormal changes ($P < 0.001$).

Light microscopic observation of urinary crystals revealed the presence of aggregated calcium oxalate monohydrate crystals in EG-treated rats (Figure 2B) whereas calcium oxalate dihydrate crystals were present in EG + fucoidan-treated rats (Figure 2D). Control and rats treated with fucoidan showed the presence of calcium phosphate crystals (Figure 2A, C).

Table 4 shows the oxalate and calcium content of the kidney. There was a significant increase in the level of the stone-forming constituents, such as calcium and oxalate, in the kidney ($P < 0.001$). The increased accumulation of the stone-forming constituents in renal tissue was successfully reversed with fucoidan treatment ($P < 0.001$).

The examination of the renal sections under polarized microscope revealed that the EG-treated nephrolithic rats had large aggregated crystals in all the major areas of the kidney, especially the tubules (Figure 3B). In contrast, the kidneys treated with EG + fucoidan showed limited calcium oxalate deposition (Figure 3D). Sections from control and drug control rats showed no crystal deposition (Figure 3A,C).

Table 2 Effect of fucoidan on rat body weight, kidney weight and urinary pH in experimental hyperoxaluria

	Group I, control	Group II, EG	Group III, fucoidan	Group IV, EG + fucoidan
Body weight (initial) (g)	125.83 ± 13.23	122.17 ± 10.65	123.33 ± 14.33	127.67 ± 11.64
Body weight (final) (g)	142.67 ± 13.25	136.67 ± 15.32	144 ± 13.76	145.17 ± 12.19
Kidney weight (g)	1.03 ± 0.12	1.43 ± 0.14[a,***]	1.03 ± 0.12	1.19 ± 0.12[a,*,b,**]
Urinary pH	7.53 ± 0.96	5.10 ± 0.77[a,***]	7.71 ± 0.51	7.68 ± 0.61[b,***]

Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]Group I and Group II, III, IV; [b]Group II and Group IV (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

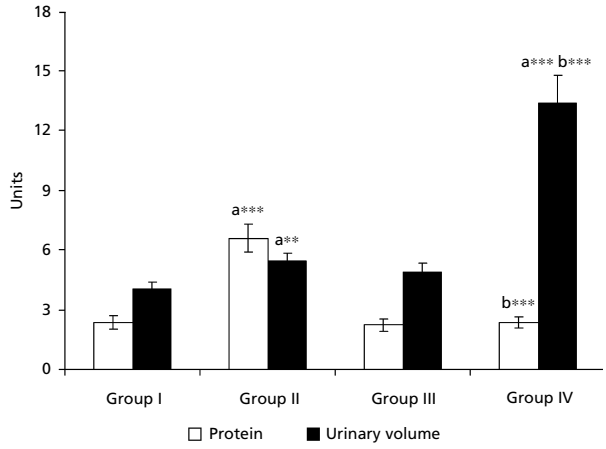

Figure 1 Effect of fucoidan on urinary protein content and urinary volume in experimental hyperoxaluria in rats. Units: protein, mg/24 h; urinary volume, mL/24 h. Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]Group I and Group II, III, IV; [b]Group II and Group IV (**$P < 0.01$, ***$P < 0.001$).

TGF-β_1 mRNA and collagen levels in renal tissue showed an increase in ECM synthesis during hyperoxaluria. Rats treated with EG showed a 1.68-fold increase in collagen when compared with control (Figure 4). Elevated renal collagen indicates an increase in fibrosis during hyperoxaluria. Fucoidan administration significantly decreased the collagen levels in kidney tissue ($P < 0.001$). Figure 5A shows the RT-PCR analysis of TGF-β_1 mRNA. A single transcript was observed at 303 bp in all the groups. Densitometric analysis showed a 1.32-fold increase in TGF-β_1 mRNA expression under hyperoxaluric condition (Figure 5B). On fucoidan administration the TGF-β_1 mRNA was significantly modulated in hyperoxaluric rats ($P < 0.001$).

Discussion

Two forces are known to control crystallization in human urine: urinary supersaturation with respect to stone salts and the presence of crystallization modulators. Urinary chemistry is a determining factor of supersaturation and subsequent formation of crystals. Evidence has shown that inhibition of crystallization, by decreasing the supersaturation, might be an important defence against stones (Kato et al 2004; Tungsanga et al 2005). Our preliminary studies indicated that fucoidan was able to improve the antioxidant status of hyperoxaluric rats (Veena et al 2007) and this interested us to further explore the changes in urinary chemistry after fucoidan administration to oxalate goaded rats.

Hyperoxaluric rats showed a mild reduction in body weight with a marked increase in kidney weight, which might be due to the increased synthesis of ECM. High oxalate concentrations in tubule fluid might have injured the renal epithelial cells resulting in disturbance of pH regulation (Coe et al 2005), hence a low urinary pH was observed in this study. The low urinary pH was also indicative of an environment conducive to stone formation. The urinary volume was increased in hyperoxaluric rats; interestingly fucoidan + EG-treated rats showed a profound increase in urine volume, attributing a diuretic property to fucoidan. Fucoidan-treated rats exhibited a 20% increase in urine volume when compared with control rats, indicative of a diuretic action. Besides these abnormal changes in urine, the protein excretion in urine was also increased, suggesting renal damage. Increased urinary protein could act as a nucleating platform, an architectural framework/cement, which was insufficient to resist the thermodynamic pressure of supersaturated urine and eventually results in stone formation (Ryall 1999). Further, oxidative damage to the kidney and increased protein excretion, along with the supersaturating environment, might initiate crystal nucleation (Jonassen et al 2005). The elevation of protein excretion positively correlated with increased excretion of urinary enzymes in our previous studies (Veena et al 2006).

The increased urinary excretion of oxalate, calcium and uric acid observed in this study corroborated positively with the previous report, which showed an upsurge in the excretion of these urinary stone-forming constituents in patients susceptible to stone formation (Coe et al 2005). Increased urinary excretion of oxalate might be due to the increase in oxalate as a consequence of ingestion of EG or secretion of oxalate by the tubules (Coe et al 2005). Data linking calcium excretion to stone risk is supportive of the idea that it is a graded risk factor (Curhan et al 2001). Oxalate alters intracellular calcium level through its potential to mobilize calcium from intracellular stores (Iida et al 2003). In addition, reactive oxygen species produced by oxalate can also damage the renal tubules, leading

Table 3 Alterations in rat urinary constituents on administration of fucoidan in experimental hyperoxaluria

Urinary constituent (mg/24 h)	Group I, control	Group II, EG	Group III, fucoidan	Group IV, EG + fucoidan
Oxalate	0.29 ± 0.02	1.86 ± 0.19[a,***]	0.22 ± 0.03	0.50 ± 0.05[a,**b,***]
Calcium	1.13 ± 0.19	2.16 ± 0.18[a,***]	1.1 ± 0.15	1.18 ± 0.16[b,***]
Phosphorus	3.13 ± 0.32	4.36 ± 0.52[a,***]	3.15 ± 0.33	3.25 ± 0.28[b,***]
Uric acid	2.48 ± 0.23	2.77 ± 0.24[a,*]	2.43 ± 0.19	2.49 ± 0.23[b,*]
Citrate	1.39 ± 0.19	1.10 ± 0.19[a,*]	1.43 ± 0.20	1.38 ± 0.20[b,*]
Magnesium	2.25 ± 0.29	1.17 ± 0.17[a,***]	2.31 ± 0.16	2.23 ± 0.20[b,***]
GAGs	1.87 ± 0.20	0.47 ± 0.05[a,***]	2.21 ± 0.16[a,**]	1.96 ± 0.17[b,***]

Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]Group I and Group II, III, IV; [b]Group II and Group IV (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

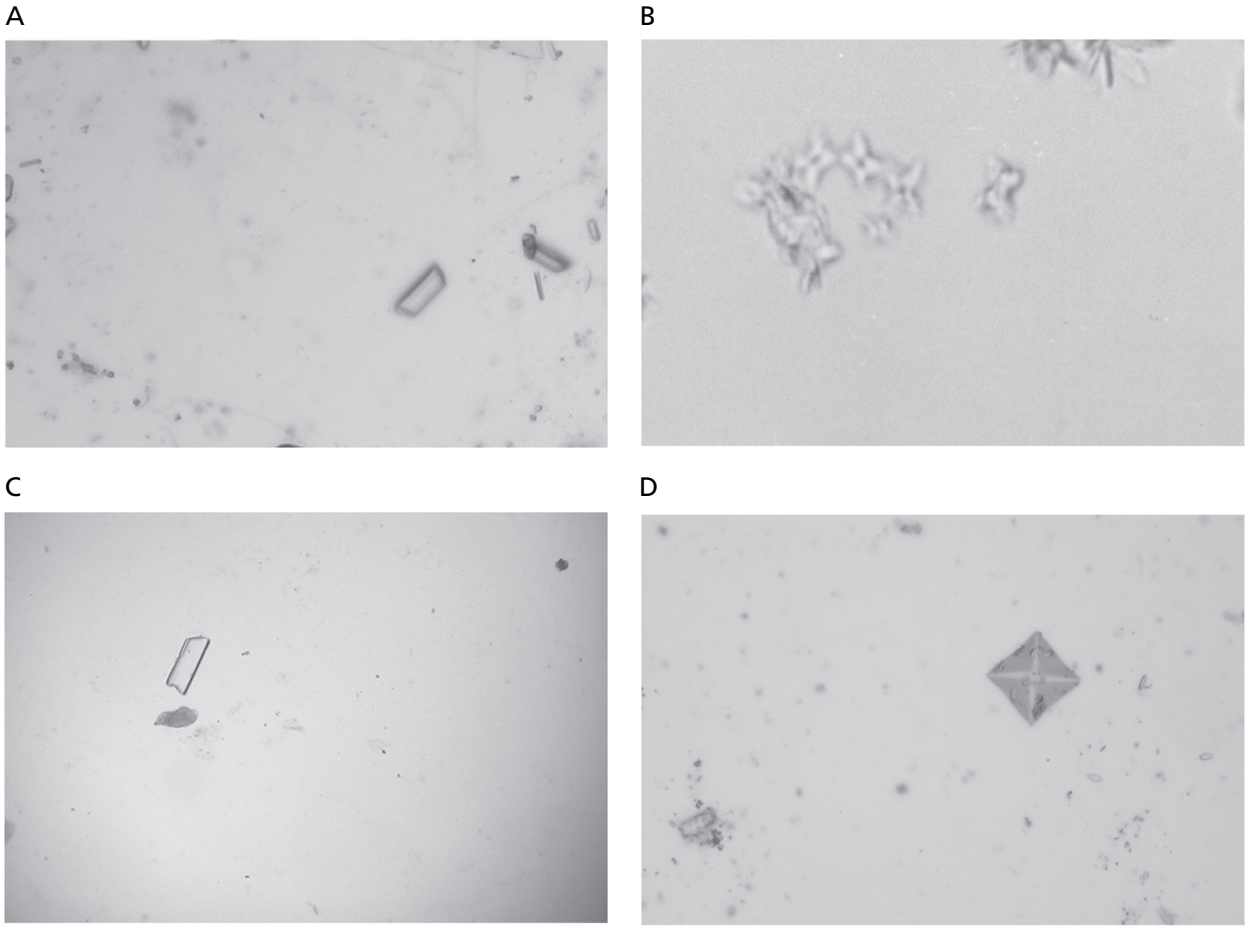

Figure 2 Light microscopic analysis of urinary crystals (400×). A. Control urine showing the presence of calcium phosphate crystals. B. Urine from hyperoxaluric rats showing the presence of calcium oxalate monohydrate crystals. C. Urine from fucoidan-treated rats showing the presence of calcium phosphate crystals. D. Urine from EG + fucoidan-treated rats showing the presence of small calcium oxalate dihydrate crystal.

Table 4 Oxalate and calcium content of rat kidney in experimental hyperoxaluria

	Group I, control	Group II, EG	Group III, fucoidan	Group IV, EG + fucoidan
Oxalate (mg g^{-1})	1.97 ± 0.14	3.22 ± 0.3[a,***]	1.94 ± 0.15	2.14 ± 0.26[b,***]
Calcium (mg g^{-1})	5.34 ± 0.52	10.27 ± 0.97[a,***]	5.22 ± 0.56	6.05 ± 0.66[b,***]

Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]Group I and Group II, III, IV; [b]Group II and Group IV (***$P < 0.001$).

to decreased calcium reabsorption with a consequent increase in calcium excretion in urine (Selvam 2002).

Further, reactive oxygen species can peroxidize the membrane and perturb calcium homoeostasis by decreasing the activity of calcium-ATPase, thereby increasing calcium concentration (Selvam 2002). Our previous report has already shown a decrease in calcium-ATPase activity in experimental hyperoxaluria and its normalization with fucoidan (Veena et al 2006). Any of these mechanisms can increase urinary calcium level and subsequent supersaturation. The acidic pH of urine, along with increased excretion of uric acid and phosphate, might favour the formation of uric acid and calcium phosphate crystals (Coe et al 2005). It has been suggested that calcium phosphate and uric acid crystals increase the propensity of calcium oxalate crystals by heterogenous nucleation (Khan 2004).

Furthermore, increase in uric acid decreases the solubility of calcium oxalate in addition to aborting the inhibitory activity of GAGs (Grases et al 1991). Increased degradation of protein in tubules might have contributed to the increase in uric acid. Studies have shown that hyperuricosuria, independent of the formation of uric acid crystals, could also potentiate calcium oxalate monohydrate crystal retention in the kidney (Farell et al 2004). Further, oxalate is known to induce apoptosis, which might also contribute to increase the uric acid

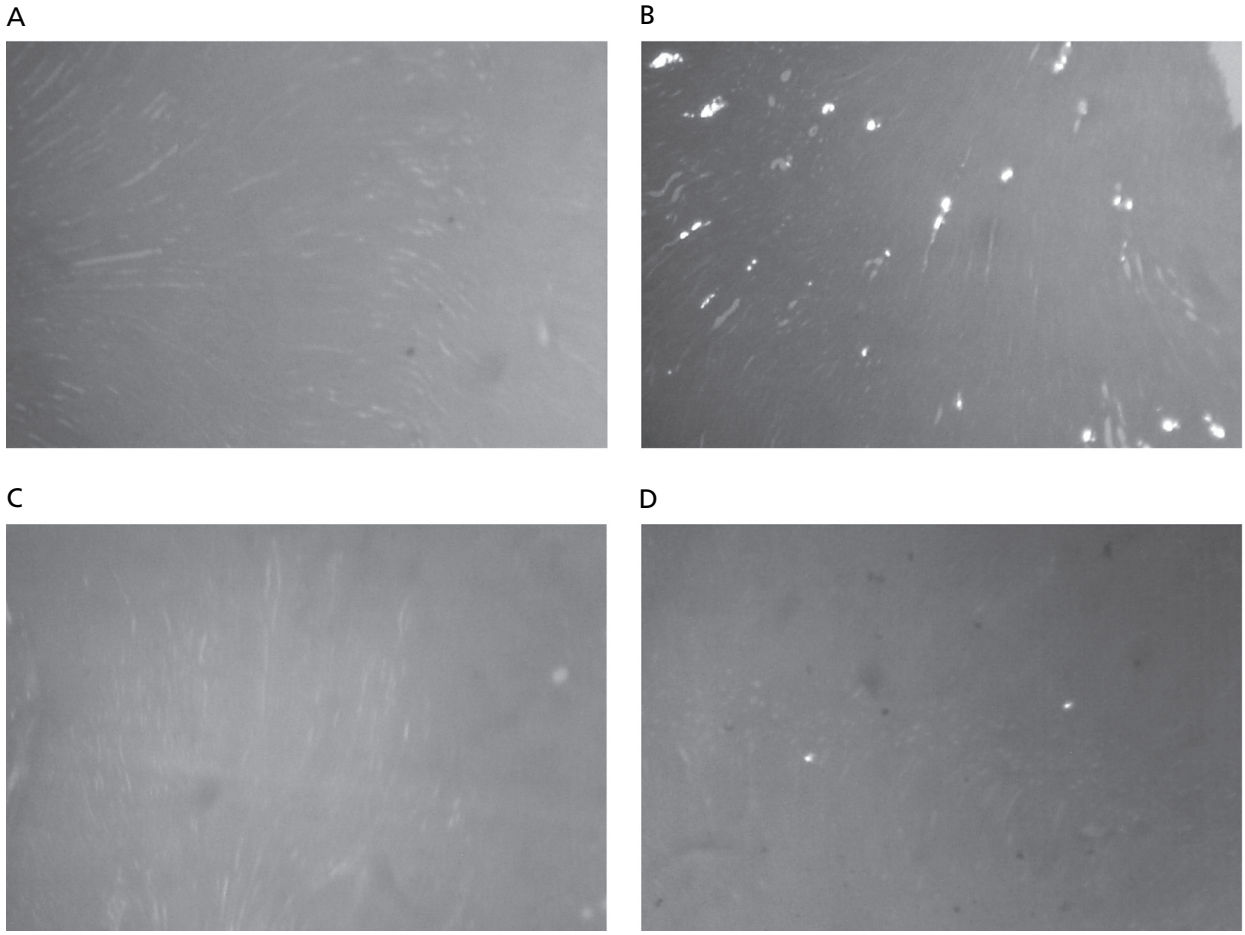

Figure 3 Polarized microscopic analysis of rat renal tissues (40×). A. Sections from control rats showing no crystals deposition. B. Sections from EG-treated rats showing the presence of aggregated crystals. C. Sections from fucoidan-treated rats showing no crystal deposition. D. Sections from EG + fucoidan-treated rats showing the presence of tiny crystals.

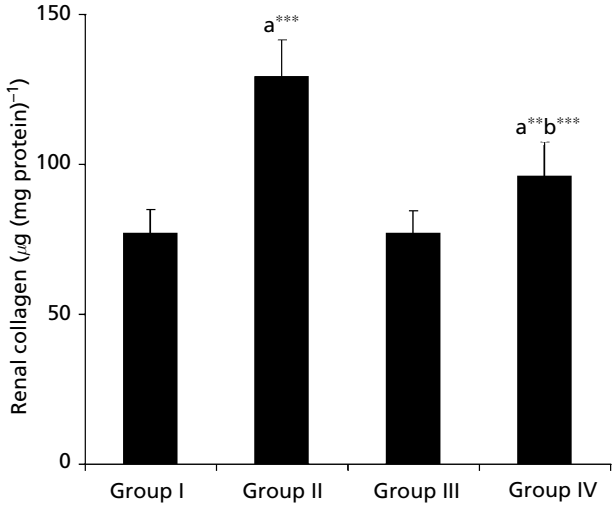

Figure 4 Effect of fucoidan on rat renal collagen level in experimental hyperoxaluria. Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]Group I and Group II, III, IV; [b]Group II and Group IV (**$P < 0.01$, ***$P < 0.001$).

and phosphate (Sarica et al 2001). Increase in the stone-forming constituents, as observed in this study, raises the supersaturation, thus increasing the chances of crystal nucleation and deposition in kidneys.

Increase in the concentration of stone-forming constituents was accompanied by a decrease in the concentration of inhibitors, like magnesium, citrate and GAGs. Magnesium exhibits its inhibitory activity by binding to oxalate and increasing its excretion by the formation of soluble magnesium oxalate (Kato et al 2004). Previous studies have already suggested the role of citrate in binding calcium and inhibiting calcium crystallization (Lieske et al 1996). Urine citrate concentration is determined mainly by tubule reabsorption, and diminution in the level of magnesium and citrate as observed in this study predicts the increased propensity to stone formation in hyperoxaluric rats. Decrease in the GAGs content during hyperoxaluria decreases the inhibitory potential of the urine and favours an environment for the precipitation of crystals. Reports have shown that increase in oxalate content, apart from increasing the supersaturation, also decreases the inhibitory potential of GAGs (Cao et al 1997b). Decrease in these potential inhibitors of stone formation in this study indicates that they might have been utilized in the process of

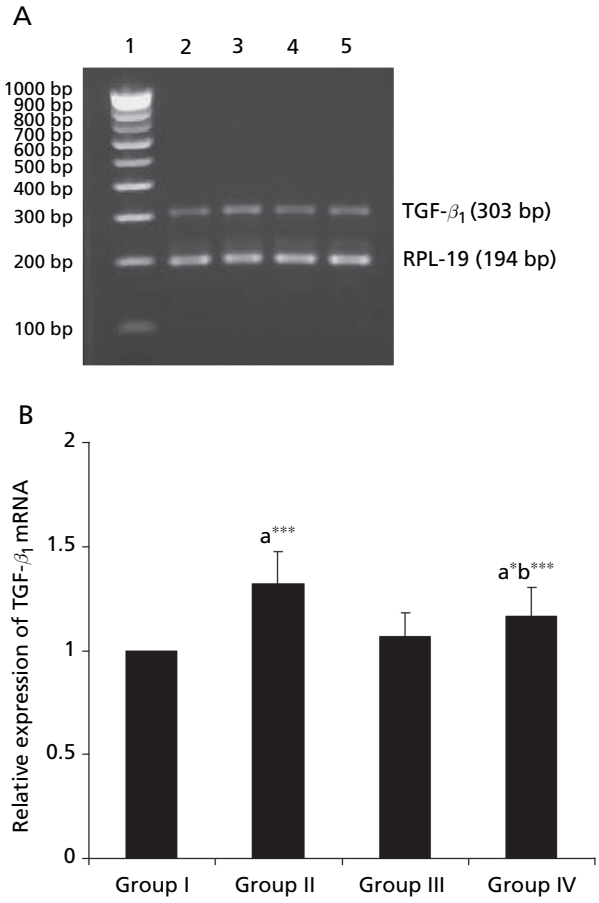

Figure 5 Effect of fucoidan on TGF-β_1 mRNA expression in kidney tissue of experimental rats in hyperoxaluria. A. The 303 and 194 bp fragments represent transcript TGF-β_1 and RPL-19 as internal standard, respectively. Lane 1, 100 bp DNA ladder; lane 2, control; lane 3, EG; lane 4, fucoidan; lane 5, EG + fucoidan. B. Ratio of TGF-β_1 mRNA expression relative to RPL-19 mRNA. Values are expressed as mean ± s.d. for 6 rats in each group. Comparisons are made between: [a]Group I and Group II, III, IV; [b]Group II and Group IV ($*P < 0.05$, $***P < 0.001$).

stone formation, which results in decrease in their concentration in this study. Analysis of urinary stones has shown that these constituents are present in the stone matrix; further, it has also been proved that these components competitively inhibit the crystallization process (Lieske et al 1995).

Fucoidan administration was able to decrease the supersaturation and crystal retention in EG-treated rats possibly due to its effect on glycolic acid oxidase and its anti-adherent property, as it is a naturally occurring GAG. The modulatory effect of fucoidan on glycolic acid oxidase and its diuretic property might be responsible for the decreased excretion of stone-forming constituents (Veena et al 2007). Our previous report showed that fucoidan decreases the activity of glycolic acid oxidase, a flavoprotein that catalyses the two-step oxidation of glycolate to oxalate with glyoxylate as an intermediate (Veena et al 2007). This enzyme is localized in the liver and its activity increases during hyperoxaluria. Fucoidan was also able to modulate the activity of xanthine oxidase, which also contributes to increase oxalate to a minor extent. Additionally, it is also found to increase the inhibitor concentration and this might be responsible for its protective activity against lithogenesis. Similar effects have been reported for sodium pentosan polysulfate, a heparin analogue, under oxalate stress (Subha & Varalakshmi 1993).

The urinary stone analysis showed that administration of fucoidan resulted in calcium oxalate dihydrate crystals whereas free and aggregated calcium oxalate monohydrate crystals were found in hyperoxaluric rats. Although both type of crystals are found in hyperoxaluria, calcium oxalate monohydrate crystals predominate under oxalate stress (Khan 1997; Lieske et al 1999). Moreover, it has been reported that the crystal morphology is mainly determined by the supersaturation of the urine. Carvalho & Vieira (2004) have shown that calcium oxalate dihydrate crystals are formed in urine at low supersaturation and, as the supersaturation increases, the number of crystals is increased as well as the morphology changes. Hence, the formation of calcium oxalate monohydrate crystals in EG-treated rats might be due to the increased supersaturation of urine. Increased excretion of calcium oxalate monohydrate is usually associated with stone formation as calcium oxalate monohydrate crystals have greater affinity for renal tubules than calcium oxalate dihydrate or calcium phosphate (Wesson et al 1998). The formation of calcium oxalate dihydrate in fucoidan-administered rats is another standing evidence of the protective action of fucoidan against urolithiasis.

The selective reabsorption capacity of segments of nephron poses different risks for crystallization at the various sites in the nephron. Renal tissues in this study showed an increase in accumulation of calcium and oxalate. The concentration of these substances in the renal tissue plays a key role in the pathogenesis of papillary calcification and eventual stone formation. Decreased glutathione and calcium-ATPase activity might account for the increased calcium content. Increase in oxidized glutathione leads to the formation of protein-S-glutathione disulfide, which favours an increase in cytosolic calcium during peroxidation (Bellomo et al 1983). Moreover, the loss of the critical –SH group of calcium-ATPase, which maintains the calcium pump, leads to the perturbation of cellular calcium homoeostasis (Mark et al 1995). Decreased calcium-ATPase activity and reduced glutathione have already been reported by us, nevertheless fucoidan was able to prevent the oxidation of –SH group of calcium-ATPase and increase the glutathione content of the renal tissues (Veena et al 2006). These properties of fucoidan might be responsible for the decreased retention of oxalate and calcium in the renal tissues in this study.

Fucoidan's effect in decreasing the retention of the crystals in the renal tissue was further demonstrated by the polarized microscopic studies, which showed decreased crystals in the renal tissue on treatment with fucoidan. Apart from decreasing the concentration of stone-forming constituents, like oxalate and calcium, which decrease the supersaturation of urine, fucoidan, being a GAG, can also bind to the potential growth sites of crystals and block crystal growth (Cao et al 1997a). Besides, it can also cover the crystals and prevent their retention in the renal tissues (Cao et al 1997b). These mechanisms might lie behind the decreased retention of crystals in the renal tissue of fucoidan + EG-treated rats.

Numerous reports support the fact that fucoidan is functionally similar to heparin and its mode of action can be related with heparin (Pereira et al 1999; Shanmugam & Mody 2000). It has been suggested that exogenous GAG administration in the form of low-molecular-weight heparin improves the anionic charges on the tubular epithelium, which is depleted during calculogenesis and thereby decreases tubular uptake of crystals (Rajeswari & Varalakshmi 2006). This coating phenomenon principally defies calcium oxalate crystals and oxalate from gaining easy access to renal tubular epithelium by way of charge repulsion action. Hence, it can be hypothesized that fucoidan exerts its protective effect similarly to heparin by decreasing the tubular uptake of crystals. In this study, polarized images showed numerous crystals accumulated in the tubular lumen on EG administration and only a few interstitial crystals on EG + fucoidan treatment. This further supports the hypothesis that fucoidan prevents tubular uptake of crystals. Oxidative stress and associated injury have been recognized as the key factors in crystal retention and subsequent formation of stones. Fucoidan was proved to decrease oxidative stress and renal injury through our previous studies (Veena et al 2007); hence, decrease in renal injury on fucoidan administration can also be attributed to the decreased crystal retention of the renal tissues.

Recent studies have shown that crystal deposition is associated with increased ECM synthesis resulting in renal fibrosis (Khan 2004). Light and electron microscopic studies of a renal papilla and associated calcium oxalate kidney stones showed distinct signs of injury and inflammation and the presence of a large amount of collagen, indicative of renal fibrosis. Increase in collagen content of the renal tissue in this study shows the increase in fibrotic nature as well as increase in the ECM favouring stone retention. Toblli et al (1999) have shown an increase in collagen expression in experimental hyperoxaluria and have suggested that decreasing collagen accumulation is favourable for circumventing hyperoxaluria. TGF-β_1 has a central role in regulating renal fibrosis and increasing ECM synthesis during lithogenesis. Numerous studies have reported that TGF-β_1 inhibits matrix degradation, regulates type I, type III and type VI collagen synthesis and also participates in apoptosis. Similar increase in collagen content and TGF-β_1 expression has already been reported in hyperoxaluria (Toblli et al 1999). An increased ECM synthesis results in tissue remodelling and exposure of crucial crystal-binding molecules, which favours crystal retention (Toblli et al 1999; Umekawa 2004). Various actions of TGF-β_1 are mediated by oxidative stress; fucoidan, due to its antioxidant potential, might have decreased the expression of TGF-β_1. Apart from this indirect effect, fucoidan may act on the intracellular signalling machinery, such as the process of transcription factor activation. Fucoidan is known to bear functional similarity with heparin and heparin is known to migrate into the nucleus and suppress AP-1-mediated transcription in smooth muscle cells and hepatoma cells (Dudas et al 2000). A similar effect of fucoidan is also possible in hyperoxaluria. Decrease in TGF-β_1 expression may account for the decrease in collagen as TGF-β_1 can modulate the expression of collagen.

Conclusion

In conclusion, we have demonstrated the impact of fucoidan on urinary supersaturation as well as crystal retention in the renal tissues. Our findings imply that by decreasing supersaturation, fucoidan could modulate the urinary crystallization process. Further, fucoidan was able to abort the stone retention. Alteration in ECM, which is also an important determinant favouring stone retention, was normalized with fucoidan administration. These findings highlight the protective role of fucoidan in experimental hyperoxaluria.

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