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Beneficial role of sulfated polysaccharides from edible seaweed *Fucus vesiculosus* in experimental hyperoxaluria

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Abstract

Sulfated polysaccharides from marine algae are known to possess numerous properties of pharmacological importance. The present study is an attempt to evaluate the efficacy of the sulfated polysaccharides from edible seaweed, *Fucus vesiculosus* in ameliorating the abnormal biochemical changes in experimental hyperoxaluria. Two groups of male albino rats of Wistar strain (140 ± 20 g) received 0.75% ethylene glycol for 28 days to induce hyperoxaluria, and one of them received sulfated polysaccharides (fucoidan from *F. vesiculosus*, 5 mg/kg b.wt., s.c.) treatment, commencing from the 8th day of the experimental period. One group was maintained as a control group and another group served as a drug control, which received only sulfated polysaccharides. Incongruity in the renal tissue enzymes (ALP, β-Glu and γ-GT) were observed during hyperoxaluria along with an increased activity of oxalate metabolizing enzymes like LDH, GAO and XO. These changes were reverted to near normalcy with sulfated polysaccharide administration. Alterations were observed in the activities/levels of tissue enzymic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase and glucose-6-phosphate dehydrogenase) and non-enzymic (reduced glutathione, ascorbate and α-tocopherol) antioxidants, along with high malondialdehyde levels in the hyperoxaluric group. However, normalized lipid peroxidation status and antioxidant defences were noticed with sulfated polysaccharide administration. Biochemical discrepancies observed in hyperoxaluria disrupt membrane integrity, favouring a milieu for crystal retention. Advocation of sulfated polysaccharides enhanced the antioxidant status, thereby preventing membrane injury and alleviating the microenvironment favourable for stone formation.
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Keywords: Hyperoxaluria; Sulfated polysaccharides; *Fucus vesiculosus*; Lipid peroxidation; Antioxidants

1. Introduction

Seaweeds have been used as a foodstuff in the Asian diet for centuries and are considered an under-exploited resource (Nisizawa, Noda, Kikuchi, & Watamaba, 1987). They have also proven to be rich sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential. Seaweeds have been used as thickening and gelling agents in foods. Seaweeds are low in fats but contain vitamins and bioactive compounds, like terpenoids and sulfated polysaccharides, the latter being a potential natural antioxidant not found in land plants (Lahaye & Kaffer, 1997). Sulfated polysaccharides, the naturally occurring glycosaminoglycans, are a class of compounds containing hemi-ester sulphate groups in their sugar residues (Shanmugam & Mody, 2000). Sulfated polysaccharides from brown algae are generally known as fucoidans, as they are rich in the sugar, fucose. They are reported to have blood anticoagulant, anti-tumour, anti-mutagenic, anti-complementary, immunomodulating, hypoglycaemic, antiviral, hypolipidemic and anti-inflammatory activities (Shanmugam & Mody, 2000). Exploring the biomedical potential of sea-

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weeds has opened a new era of research, resulting in the application of seaweeds, to remodel the treatment regime in various pathologies. Recently, the potential of seaweeds to ameliorate chronic renal failure in rats has also been reported (Zhang, Li, Xu, Niu, & Zhang, 2003).

Hyperoxaluria is considered as one of the major risk factors for idiopathic calcium oxalate stones. Supersaturation, the initial event in the sequence of stone formation is influenced by oxalate rather than calcium (Kok & Khan, 1994). Oxalate, an inert end product of carbon assimilation, is mainly excreted by the kidney. Abnormalities in oxalate metabolism have been suggested as a cause for the pathogenesis of stone disease, as an excessive excretion of oxalate leads to calcium oxalate crystalluria. Calcium oxalate stones have been known to haunt mankind for centuries, despite the development of novel means of treatment, as the basic mechanism of stone formation and the identity of predictors of recurrence are still largely shrouded in uncertainty. Application of exogenous glycosaminoglycans (GAGs) to prevent stone formation and recurrence is considered as a promising prophylactic approach (Cao et al., 1997). Reports have stated a significant difference in GAG excretion between the stone formers and non-stone formers (Cao et al., 1997). Exogenous supplementation of the GAGs, like sodium pentosan polysulfate (SPP), has been effective in the treatment of urolithiasis (Subha, Baskar, & Varalakshmi, 1992). In experimental diabetic nephropathy, Gambaro, Venturini, and Noonan (1994) demonstrated that the administration of glycosaminoglycans prevents morphological and functional alterations of the kidney and appeared to reverse established diabetic lesions. Synthetic polysaccharides have been studied in our and other laboratories as potential inhibitors of crystal agglomeration and nucleation (Senthil, Subha, Saravanan, & Varalakshmi, 1996; Verkoelen, Romijn, Boeve, De Bruijn, & Schroder, 1995). Synthetic polysaccharides, like low molecular weight heparin (LMWH), have been reported to have renoprotective effects (Deepa & Varalakshmi, 2003). The sulfated polysaccharides isolated from marine organisms bear similarity with heparin, possessing less anticoagulant activity and greater pharmacological approach (Shanmugam & Mody, 2000). Hence, the nephroprotective action of heparin derivatives like LMWH and SPP can also be extended to sulfated polysaccharides. Moreover, the potential of the sulfated polysaccharides to ameliorate nephrotic lesions and their accumulation in the kidneys has led us to investigate the beneficial role of sulfated polysaccharides, fucoidan, from the edible seaweed *Fucus vesiculosus*, in experimental hyperoxaluria.

2. Materials and methods

2.1. Animal model

Male Wistar albino rats of body weight 140 ± 20 g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were maintained under standard conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark). They were fed standard rat pelleted diet (M/s Pranav Agro Industries Ltd., India), under the trade name Amrut rat/mice feed, and had free access to water. The experiments were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC).

2.2. Experimental protocol

The rats were randomly divided into four groups consisting of six animals each. Group I rats served as vehicle treated control. Group II received ethylene glycol (EG, 0.75% in drinking water) for 28 days, to induce a chronic low grade hyperoxaluria and generate calcium oxalate deposition in kidneys. Group III rats served as drug controls and were given sulphated polysaccharides, fucoidan from *F. vesiculosus* (Sigma Chemicals, St. Louis, MO, USA), 5 mg/kg body weight dissolved in saline and passed through a 0.2 μm filter before subcutaneous administration. Group IV rats received ethylene glycol for 28 days and sulfated polysaccharide commencing on day 8 of the experimental period.

At the end of the 28 days, the animals were sacrificed and the liver and kidney were excised, rinsed in ice-cold physiological saline and homogenized in Tris–HCl buffer (0.1 M, pH 7.4) to give a 10% homogenate. Tissue homogenates were suitably processed for the assessment of enzymes, lipid peroxidation and antioxidant status.

2.3. Enzymic indices of cellular damage

Alkaline phosphatase (ALP) was assayed using disodium phenyl phosphate as substrate (King, 1965). γ-Glutamyl transferase (γ-GT) and β-glucuronidase (β-Glu) were measured by the methods of Orlowski and Meister (1965) and Kawai and Anno (1971) with L-γ-glutamyl *p*-nitroanilide and *p*-nitrophenyl-β-D-glucuronide as substrates, respectively.

2.4. Assessment of oxalate metabolizing enzymes

Lactate dehydrogenase (LDH) was assayed by the method of Liao and Richardson (1973), using glyoxylate as substrate. Glycolic acid oxidase (GAO) was assayed by the method of Lui and Roels (1970). Xanthine oxidase (XO) was assayed by the method of Fried and Fried (1966).

2.5. Assessment of lipid peroxidation

Lipid peroxidation (LPO) was determined by the procedure of Hogberg, Larson, Kristoferson, and Orrenius (1974). The formation of malondialdehyde (MDA), a thiobarbituric acid reactive end product served as the index of LPO. The coloured product formed gave an absorption maximum at 532 nm. The ferrous sulphate and ascorbate-induced LPO system contained 10 mM

ferrous sulphate and 0.2 mM ascorbate as inducers (Devasagayam, 1986).

2.6. Determination of the activities of enzymic antioxidants

Superoxide dismutase (SOD) was assayed by the method of Marklund and Marklund (1974). The unit of enzyme activity is defined as the enzyme required for 50% inhibition of pyrogallol auto-oxidation. The activity of catalase (CAT) was assayed by the method of Sinha (1972). It is based on the reduction of dichromate to chromic acetate when heated in the presence of hydrogen peroxide, with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus formed is measured colorimetrically at 610 nm. Glutathione peroxidase (GPX) was assayed by the method of Rotruck et al. (1973), which is based on the reaction between the glutathione remaining after the action of GPX and 5,5′-dithiobis-(2-nitrobenzoic acid), to give a compound that absorbs light at 412 nm. Glutathione S-transferase (GST) was assayed by the method of Habig, Pabst, and Jakoby (1974). Glutathione reductase (GR), which utilizes NADPH to convert oxidized glutathione (GSSG) to the reduced form (GSH), was assayed by the method of Staal, Visser, and Veeger (1969). The estimation of glucose-6-phosphate dehydrogenase (G6PD) was carried out according to the method of Beutler (1983). It is based on the increase in absorbance on addition of the substrate glucose-6-phosphate. Protein estimations were carried out according to the method of Lowry, Rosebrough, Farr, and Randall (1951).

2.7. Estimation of non-enzymic antioxidants

Total reduced glutathione (GSH) was estimated by the method of Moron, Depierre, and Mannervik (1979), where the colour developed was read at 412 nm. Ascorbic acid (Vitamin C) was assayed by the method of Omaye, Turnbull, and Sauberlich (1979). Ascorbic acid was oxidized by copper to form dehydroascorbic acid and diketoglutaric acid, and was treated with 2,4-dinitrophenylhydrazine to form the derivative of *bis*-2,4-dinitrophenylhydrazine which undergoes a rearrangement, to form a product which was measured at 520 nm. α-Tocopherol (Vitamin E) was estimated by the method of Desai (1984).

2.8. Data analysis

The results are expressed as mean ± standard deviation (SD). Differences between groups were assessed by one-way ANOVA 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.

3. Results

Table 1 delineates the activities of renal enzymes in experimental animals. The activity of ALP, β-Glu and γ-GT were markedly decreased in Group II animals when compared to that of the control animals ($P < 0.001$). This abnormal enzymic profile was reverted to near normalcy on administration of sulfated polysaccharides ($P < 0.001$) suggesting the membrane protective effects of the sulfated polysaccharides.

Table 2 shows the abnormal rise in the oxalate metabolizing enzymes of liver and kidney. The marked increase in the LDH and XO activity in the kidney ($P < 0.001$) and liver ($P < 0.01$, $P < 0.001$) was normalized with sulfated polysaccharide administration. The liver GAO activity which showed an upsurge in hyperoxaluria, was also normalized with sulfated polysaccharide administration.

Table 3 shows the lipid peroxidative damage in kidney, induced by increased concentration of oxalate/calcium oxalate and the protection rendered by the sulfated polysaccharides. LPO was increased by 1.37-, 1.34- and 1.45-fold in basal, ferrous sulfate and ascorbate-induced conditions, respectively, in hyperoxaluric rat kidneys, when compared with the control animals. Increase in LPO was culminated by the administration of sulfated polysaccharides.

Table 4 presents the altered activities of enzymic antioxidants in the kidney of control and experimental animals. Compared with the control group, SOD, CAT and GPX activities decreased by 23.35%, 31.80% and 34.02%, respectively, in-group II rat kidneys. The activities of GST, GR and G6PD were also significantly ($P < 0.001$) decreased in hyperoxaluric rats. The abnormal alterations associated with hyperoxaluria were effectively prevented ($P < 0.001$) with sulfated polysaccharides treatment.

Table 1
Effect of sulfated polysaccharides on the renal marker enzymes in experimental hyperoxaluria

Enzymes	Group I, control	Group II, EG	Group III, sulfated polysaccharide	Group IV, EG + sulfated polysaccharide
ALP	1.65 ± 0.17	1.20 ± 0.13[a,***]	1.58 ± 0.13	1.55 ± 0.11[b,***]
γ-GT	8.68 ± 0.77	6.18 ± 0.74[a,***]	8.33 ± 0.83	8.35 ± 0.78[b,***]
β-Glu	0.62 ± 0.08	0.36 ± 0.03[a,***]	0.59 ± 0.05	0.60 ± 0.06[b,***]

Values are expressed as mean ± SD for six animals in each group. Unit: ALP, μmol of phenol liberated/min/mg protein; γ-GT, nmol × 10 *p*-nitroaniline liberated/min/mg/protein; β-Glu, nmol of *p*-nitrophenol liberated/min/mg/protein. Comparisons are made between: [a] – group I and group II, III, IV; [b] – group II and group IV. The symbols represent statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 2
Effect of sulfated polysaccharides on oxalate metabolizing enzymes of kidney and liver

Enzymes	Group I, control	Group II, EG	Group III sulfated polysaccharide	Group IV, EG + sulfated polysaccharide
Kidney				
LDH	2.13 ± 0.19	$3.39 \pm 0.35^{a,***}$	1.99 ± 0.27	$2.53 \pm 0.23^{a,*,b,***}$
XO	0.91 ± 0.084	$1.43 \pm 0.15^{a,***}$	0.91 ± 0.089	$1.36 \pm 0.16^{a,*,b,***}$
Liver				
LDH	2.36 ± 0.16	$2.72 \pm 0.19^{a,**}$	2.41 ± 0.20	$2.44 \pm 0.20^{b,*}$
XO	1.34 ± 0.12	$2.49 \pm 0.20^{a,***}$	1.35 ± 0.15	$1.38 \pm 0.22^{b,***}$
GAO	2.80 ± 0.21	$5.41 \pm 0.46^{a,***}$	2.65 ± 0.23	$2.49 \pm 0.34^{b,***}$

Values are expressed as mean ± SD for six animals in each group. LDH, XO, units/mg protein (1U = the amount of enzyme that bring about a change in O.D. of 0.01 min); GAO, nmol of glyoxylate formed/mg protein. Comparisons are made between: [a] – group I and group II, III, IV; [b] – group II and group IV. The symbols represent statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 3
Lipid peroxidation levels in the kidney of treated and untreated animals in experimental hyperoxaluria

Lipid peroxidation	Group I, control	Group II, EG	Group III, sulfated polysaccharide	Group IV, EG + sulfated polysaccharide
Basal	2.07 ± 0.24	$2.84 \pm 0.32^{a,***}$	1.91 ± 0.24	$2.12 \pm 0.32^{b,***}$
Ascorbate-induced	4.23 ± 0.43	$6.15 \pm 0.62^{a,***}$	4.25 ± 0.49	$4.75 \pm 0.38^{b,***}$
Ferrous sulfate-induced	12.62 ± 1.26	$16.85 \pm 1.96^{a,***}$	12.96 ± 1.57	$13.06 \pm 1.62^{b,***}$

Values are expressed as mean ± SD for six animals in each group. Unit: LPO, nmol of MDA formed/mg protein. Comparisons are made between: [a] – group I and group II, III, IV; [b] – group II and group IV. The symbols represent statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 4
Activities of antioxidant enzymes in hyperoxaluric and sulfated polysaccharides treated rats

Antioxidants	Group I, control	Group II, EG	Group III, sulfated polysaccharide	Group IV, EG + sulfated polysaccharide
SOD	5.44 ± 0.48	$4.17 \pm 0.42^{a,***}$	5.49 ± 0.51	$5.43 \pm 0.45^{b,***}$
CAT	165.20 ± 16.48	$112.67 \pm 10.29^{a,***}$	167 ± 15.27	$150.83 \pm 16.32^{b,***}$
GPX	8.2 ± 0.80	$5.41 \pm 0.58^{a,***}$	8.03 ± 0.82	$7.63 \pm 0.68^{b,***}$
GR	1.53 ± 0.19	$0.95 \pm 0.092^{a,***}$	1.47 ± 0.16	$1.36 \pm 0.14^{b,***}$
GST	1.44 ± 0.17	$0.87 \pm 0.09^{a,***}$	1.51 ± 0.21	$1.30 \pm 0.24^{b,***}$
G6PD	1.88 ± 0.13	$1.06 \pm 0.092^{a,***}$	1.90 ± 0.15	$1.64 \pm 0.22^{a,*,b,***}$

Values are expressed as mean ± SD for six animals in each group. Units: SOD, units/mg protein (1U = amount of enzyme required to bring about 50% inhibition of auto-oxidation of pyrogallol); CAT, μmol of H_2O_2 utilized/min/mg protein; GPX, μg of GSH utilized/min/mg protein; GR, μmol of NADPH oxidized/min/mg protein; GST, nmol of 1-chloro-2,4-dinitro benzene-GSH conjugate formed/min/mg protein; G6PD, nmol of NADP reduced/min/mg protein. Comparisons are made between: [a] – group I and group II, III, IV; [b] – group II and group IV. The symbols represent statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 5
Effect of sulfated polysaccharides on the non-enzymic antioxidants in the kidney of experimental animals

Non-enzymic antioxidants	Group I, control	Group II, EG	Group III, sulfated polysaccharide	Group IV, EG + sulfated polysaccharide
GSH	4.60 ± 0.56	$2.88 \pm 0.34^{a,***}$	4.67 ± 0.49	$4.62 \pm 0.39^{b,***}$
Vitamin C	2.04 ± 0.24	$1.04 \pm 0.19^{a,***}$	2.01 ± 0.2	$1.97 \pm 0.24^{b,***}$
Vitamin E	1.05 ± 0.11	$0.30 \pm 0.024^{a,***}$	1.01 ± 0.091	$1.06 \pm 0.15^{b,***}$

Values are mean ± SD for six rats. Units: GSH, Vit C and Vit E: μg/mg protein. Comparisons are made between: [a] – group I and group II, III, IV; [b] – group II and group IV. The symbols represent statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

The values reported in Table 5 shows the effect of the sulfated polysaccharides on the levels of non-enzymatic antioxidants in rat kidney. Significant reduction in the level of non-enzymic antioxidants ($P < 0.001$) GSH, vitamin C and vitamin E in hyperoxaluric animals were normalized with sulfated polysaccharide administration.

4. Discussion

In recent years, a broad series of polysaccharides from edible seaweeds have emerged as an important class of bioactive natural products, possessing many important properties of pharmacological relevance (Shanmugam &

Mody, 2000). Sulfated polysaccharides are widespread in nature, occurring in a great variety of marine organisms. It is well established that the damaged epithelium of the kidney, due to an increased oxidative stress and with reduced anti-adherent glycosaminoglycan layer, might act as a nidus for stone formation (Selvam, 2002). Culminating the production of free radicals or regeneration of the kidney epithelial membrane might be an effective treatment in stone pathogenesis. It is known that sulfated polysaccharides from marine organisms are poorly taken up from the intestine because of their size and electrical charge but it is found that the sulfated polysaccharides, upon systemic administration, accumulated predominantly in the kidney (Guimaraes & Mourao, 1997), suggestive of the possible role of these naturally-occurring glycosaminoglycans in renoprotection.

Abnormal changes in renal enzyme levels can indicate tubular dysfunction and the specific tubular segments that are involved. Calcium oxalate crystals are known to damage proximal tubular epithelium and are generally associated with shedding of the brush border membrane and leakage of enzymes in urine (Thamilselvan & Menon, 2005). ALP present in the brush border of the kidney is implicated in the calcification process and the observed decrease in ALP activity in the present study might be due to the decreased translocation of the enzyme across the epithelial membrane or the leak of the enzyme in general circulation from the collateral circulation, due to the damage of the membranes by calcium oxalate crystals. γ-GT, an amphipathic dimeric protein with the ability to accumulate glutamyl peptides in kidney, was also found to be decreased in hyperoxaluric condition. Altered enzyme activity might implicate a defective transport mechanism, as the hydrophobic part of the enzyme spans the membrane and may be directly involved in the transport of cations. These enzymes are localized in the proximal tubule, the part of the nephron where the renal handling of oxalate is maximum (Hautman & Osswald, 1985). Decreased excretion of these enzymes has already been reported in hyperoxaluric state and can be directly correlated with the extent of membrane damage. In the present study, the abysmal decrease in renal enzymes was in line with the increased oxalate metabolizing enzymes. Decrease in β-Glu, another membrane-bound enzyme, is due to the damage to the membrane inflicted by the calcium oxalate crystals. This abnormal enzymic profile was corrected to near normalcy with sulfated polysaccharides administration. The sulfated polysaccharides maintained the integrity of the cell membrane and, consequently, prevented the adhesion of calcium oxalate crystals, as calcium oxalate crystals are known to adhere to damaged epithelium. These observations are in line with our previous findings, where administration of SPP, a heparin analogue, was found to have membrane protective effect in experimental hyperoxaluria (Subha et al., 1992).

Increase in oxalate synthesizing enzymes observed in the present study, might be due to the increased availability of their substrate. GAO, a flavoprotein, catalyses the two step oxidation of glycolate to oxalate, with glyoxylate as an intermediate. This enzyme is localized in the liver and its activity is found to be increased during hyperoxaluria (Pragasam, Kalaiselvi, Sumitra, Srinivasan, & Varalakshmi, 2005). LDH and XO of kidney and liver were also increased during hyperoxaluria. LDH, a cytosolic enzyme, catalyses the coupling of oxidation and reduction of glyoxylate, resulting in the formation of glycolate and oxalate (Liao & Richardson, 1973). Due to the breaks encountered in the electron transport pathway and the inhibition of oxidative phosphorylation, a high concentration of $FAD^{+}/FADH_2$ and $NAD^{+}/NADH$ ratios is easily provided in the hyperoxaluric rats, which favour the increase in GAO and LDH activity (Ichiyama et al., 2000). Apart from increasing the concentration of the oxalate, GAO and XO release hydrogen peroxide and/or superoxide anions as end products. In normal tissues XO exists as a dehydrogenase, utilizing NAD^{+} as coenzyme, instead of oxygen as electron-acceptor. Xanthine dehydrogenase can be converted to XO by oxidation of its critical sulfhydryl groups (Nishino, 1994). Depletion of antioxidants in hyperoxaluric condition favours the formation of XO from xanthine dehydrogenase, further increasing the oxalate load. Increased XO can react with free iron in urolithic conditions resulting in the generation of hydroxyl radicals (Biemond, Swaak, Beindorff, & Koster, 1986). Oxalate, and the free radicals generated during the metabolism of oxalate, impose an oxidative stress on hyperoxaluric animals, as evident from the increased LPO in the present study. The increase in oxalate metabolizing enzymes was circumvented with administration of sulfated polysaccharide, a potential antioxidant which corroborates well with the previous observation, where administration of antioxidants, vitamin E and selenium was effective in hyperoxaluric condition (Kumar & Selvam, 2003).

LPO, a degenerative pathway of the membrane components mediated through the free radicals produced in the cell, is a hallmark feature of oxidative stress. In the present study, there is an upsurge in LPO in rat kidney during hyperoxaluric condition and this was further increased in the presence of inducers like ascorbate and ferrous sulphate. LPO was found to increase when LLC-PK(1) cells were incubated with oxalate and this was further elevated on incubation with calcium oxalate crystals (Thamilselvan, Khan, & Menon, 2003). The kidney, being highly vascular in nature, is more susceptible to the toxic effects of lipid peroxides secondary to erythrocyte membrane LPO (Sumathi, Jayanthi, & Varalakshmi, 1993). The evidence of the involvement of oxalate in free radical-mediated LPO reaction is further strengthened by previous observations (Huang, Ma, Chen, & Chen, 2003; Selvam, 2002). Ascorbic acid, a precursor of oxalate biosynthesis, has been shown to promote LPO in vitro in tissue non-enzymatically. Decrease in antioxidant enzymes in the present study might also be partly attributed to the elevation in the LPO. Abnormal rise in LPO was reverted back to near normalcy

with sulfated polysaccharide administration, due to their antioxidant activity, emphasized through numerous in vitro experiments (Ruperez, 2001; Xue, Yu, Hirata, Terao, & Lin, 1991). This is in line with the previous observation where LMWH supplementation could circumvent the elevated LPO, associated with nephrotoxic condition (Deepa & Varalakshmi, 2003).

Complexes of free radical scavenging enzymes, including SOD, CAT and GPX have evolved to prevent excessive oxidant stress. The development of tissue injury probably depends on the balance of the generation of reactive oxygen species and the tissues antioxidant defense mechanism. SOD is the primary enzyme involved in the dismutation of the superoxide radical to hydrogen peroxide. CAT and GPX are involved in the splitting of hydrogen peroxide to water and hydrogen. Decrease in the activities of antioxidant enzymes observed in the present study might be due to the increase in free radicals during hyperoxaluria. The free radicals when present in high concentrations are capable of interacting with the enzymes and inactivating them (Pigeolet et al., 1990). Direct evidence of decrease in antioxidant enzymes in hyperoxaluria and restoration to normal values on antioxidant therapy has been reported (Thamilselvan & Menon, 2005). Decrease in the activity of CAT might be attributed to direct inhibition of CAT by oxalate and decreased regeneration of CAT from its inactive form, due to lesser availability of NADPH (Kirkman & Gaetani, 1984). Reduction in the activity of GPX might be due to the decreased availability of its substrate GSH and partly due to its inhibition by the superoxide radicals accumulated, due to the decreased activity of SOD. Supplementation with exogenous sulfated polysaccharides was found to increase the activity of the antioxidant enzymes and correlates positively with the observation made with LMWH supplementation on nephrotoxicity (Deepa & Varalakshmi, 2003). Zhang et al. (2003) have reported an increase in antioxidant status in aging mice on supplementation with polysaccharide fraction from *Porphyra haitanesis*.

Reduced glutathione, an important oxidant defence, functions in the reduction of oxidized tissue components. The observed decrease of GSH in hyperoxaluric animals might be due to its increased conversion to GSSG. Increased oxidative stress increases the formation and efflux of GSSG (Rashed, Menon, & Thamilselvan, 2004). Anundi, Hogberg, and Stead (1979) reported that GSH depletion induces LPO and ultimately cell lysis. Replenishing the GSH levels is, therefore, necessary for the maintenance of the overall thiol status in the cell. GSH reduction can additionally explain a decreased concentration of the non-enzymic antioxidant vitamin C, which enters the cell mainly in the oxidized form, where it is reduced by GSH (Packer, 1992). The diminution of this vitamin is very detrimental, because additionally to its antioxidant function, vitamin C plays a role in sparing other antioxidants like vitamin E (Packer, 1992). The decrease in the levels of these vitamins increases the risk of LPO. This correlates with previous observations where induction of hyperoxaluria causes a significant decrease in non-enzymic antioxidants (Farooq, Asokan, Sakthivel, Kalaiselvi, & Varalakshmi, 2004).

Decrease in the levels of GSH may be correlated with decreased activities of the glutathione synthesizing enzymes GR and G6PD, both of which are influenced by oxidative assault. G6PD, the first enzyme in the hexose monophosphate shunt, is responsible for the production of the reducing equivalent NADPH. GR, at the expense of NADPH, regenerates GSH from GSSG. The modification of thiol groups in G6PD during oxidative stress leads to loss of activity, consequently leading to decreased NADPH levels (Yoshida & Huang, 1986). The observed decrease in the activity of the GR might be due to decreased availability of the cofactor NADPH. Reports demonstrate that decrease in glutathione metabolizing enzymes during hyperoxaluria can be normalized with supplementation of an antioxidant (Rashed et al., 2004). GST forms the group of multifunctional proteins catalyzing the detoxification of electrophilic compounds, to protect cells against peroxidative damage (Liebau, Wildenburg, Walter, & Henkle-Duhrsen, 1994). Diminution in the level of GST may be due to lack of the substrate GSH. Administration of sulfated polysaccharides to hyperoxaluric rats is found to increase the reduced milieu of the cell, thereby preventing oxidative stress mediated renal injury.

To summarize, the exogenous supplementation of sulfated polysaccharides to hyperoxaluric rats is effective in decreasing the oxidative stress, by increasing the activities of antioxidant enzymes like SOD, CAT, GPX, and limiting lipid peroxidation. Furthermore, sulfated polysaccharides were able to prevent crystal retention, by averting the membrane damage induced by the calcium oxalate crystals. It also had an appreciable effect on the levels of non-enzymic antioxidants. Similarity of the sulfated polysaccharides with heparin derivatives can be attributed as the possible reason for the protective effect of the sulfated polysaccharide observed in the present study, as the heparin derivatives like SPP and LMWH have renoprotective effects. Further studies are warranted to explore the mechanisms underlying the protective action of this drug as its pharmacological properties could be of therapeutic use to mankind.

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