4. SUMMARY

4.1 The efficacy of supplementation of vitamin E in preventing stone formation was assessed in stone formers and experimental urolithic rats.

4.2 More than 1.5 fold increase in levels of lithogenic substances such as oxalate, calcium, phosphorus and uric acid were observed in stone formers. The total protein excretion was also elevated in stone formers. Upon supplementation of vitamin E the levels were found to be within normal limits.

4.3 Urinary citrate, an important inhibitor of stone formation was found to be lowered in stone formers. It was normalized upon supplementation of vitamin E.

4.4 The excretion of total COM binding proteins was elevated in stone formers (2.1 mg / D) when compared with that of healthy individuals (0.8 mg / D). The vitamin E treated patients showed near normal levels after 9 months of supplementation.

4.5 COM binding proteins when eluted in DEAE cellulose column with Tris – HCl buffer alone and with buffer containing increasing concentrations of NaCl (0.05 & 0.3 M), yielded three protein peaks and they were designated as fractions I, II and III according to their order of elution.
4.6 Among the three COM binding protein fractions, the proportion of F I and F III was increased and F II was reduced in stone formers. Identical distribution pattern of COM binding proteins with those of healthy individuals were observed for vitamin E treated patients after 9 months.

4.7 DEAE cellulose column eluted F I when electrophorosed on 10 % SDS – PAGE showed a molecular weight of 45 k Da.

4.8 The thiol content of all the three fractions was decreased in stone formers. Supplementation of vitamin E restored the -SH contents.

4.9 Fraction I and II had oxalate binding activities at pH 7.4, while F III had oxalate binding activity at pH 4.5 only. Non-stone formers showed higher oxalate binding activity for F II (285.6 pmoles / mg protein) whereas stone formers showed higher oxalate binding activity for F I (334.2 pmoles / mg protein). These changes in oxalate binding protein are reverted back to control levels in treated patients.

4.10 Ca²⁺ chelators like EDTA and EGTA abolished the oxalate binding activity of F II. Oxidized glutathione increased the oxalate binding activity of all the three fractions. -SH group reducing agent such as β - mercapto ethanol and DTT completely abolished the oxalate binding activities of F I and F II. When F III was treated with lysine ε - amino group modifiers such as pyridoxal phosphate, it lost its capacity to bind oxalate. These results suggest the involvement of -SH group in F I and F II and lysine in F III to bind oxalate.
4.11 F I behaved as promoter of calcium oxalate crystallization, whereas F II and F III was nearly 2.2 fold increased in stone formers and also there was significant loss in the inhibitory activity of F II and III. These altered kinetics were moderated upon supplementation of vitamin E.

4.12 Crystal growth studies were carried out in presence of all the three DEAE cellulose fractions. F I showed promoting effect on crystal growth, while F II and III showed inhibitory effect. These effects were more aggravated / pronounced in stone formers and sublimed in vitamin E treated patients.

4.13 Light microscopic pictures of calcium oxalate crystals formed in the presence of F I derived from stone formers showed aggregated COM whereas control F I formed individual COM crystals. The extent of aggregation of COM was reduced when vitamin E was supplemented to stone formers.

IN EXPERIMENTAL UROLITHIASIS

To assess the protein

4.14 Hyperoxaluria was induced in rat model using EG. In another group of rats three weeks prior to hyperoxaluria induction, vitamin E pre – treatment was given. The urinary risk factors like oxalate, calcium, uric acid and total protein were found to be elevated in EG treated rats from 14th day of induction and in vitamin E pre – treated rats it was found to be lowered than in the EG treated rats.
4.15 Citrate excretion was decreased in EG induced rats and thus decrease in excretion was prevented in vitamin E pre-treated rats.

4.16 In order to assess the tissue injury, urinary marker enzymes like ALP, γ - GT, LDH and ACP were found to be elevated from 14th day (p<0.01) and during 28th day of induction (p < 0.001). Vitamin E pre-treated rats exhibited values well within the normal limits, thus suggesting the protective effect of vitamin E on renal cells.

IN RAT KIDNEY

4.17 The level of oxalate in EG treated rats during 28th day was 1.07 mg / g against control rats (0.71 mg / g). The activity of the enzyme was also found to be 66 % increased during 28th day. On vitamin E pre-treatment the extent of increase was reduced and thereby shows that vitamin E plays a role in preventing the kidney from the damage caused by EG.

4.18 The levels of both enzymatic and non-enzymatic antioxidants were reduced in the case of hyperoxaluric rats, suggesting the role of oxidative stress in the etiology of stone formation. Significant reduction in the activities of enzymatic antioxidants like SOD (23 %), catalase (39 %) and significantly low levels (P < 0.001) of non-enzymatic antioxidants like vitamin E and reduced GSH were observed during 28th day of EG induction. In the case of vitamin E pre-treated rats, the levels of both the antioxidants were only partially affected.
4.19 EG treated rat kidneys showed significantly reduced levels of thiol content \((p < 0.001)\) in all the three protein fractions during 28th day. Vitamin E pre-treatment maintained the thiol status of these proteins to near normalcy.

4.20 The levels of TBARS were found to be increased both in basal as well as in induced condition in EG treated rats. It was found to be 1.4 fold increased in basal condition during 28th day and 1.5 fold increased in the presence of ascorbate system. There is no increase in vitamin E pre-treated rats and thereby reveals that vitamin E combats LPO by antioxidant restoration.

4.21 When COM adsorbing proteins of rat renal tissue were fractionated on DEAE cellulose column, three protein fractions were identified and they were designated as fractions I, II and III.

4.22 There was gradual increase in distribution of F I and III following different days of EG induction. In the case of vitamin E pre-treated rats, the distributions of COM binding proteins were identical to that of control rats.

4.23 DEAE cellulose column eluted rat kidney F I when electrophoresed on 10 % SDS – PAGE showed a molecular weight of 74 kDa.

4.24 The thiol content of COM binding protein F I was found to be depleted during EG induction and it was found to be maximum during 28th day (70 %) when compared with control rats. Vitamin E pre-treatment prevented the depletion.
4.25 On spectrophotometric crystallization assay, hyperoxaluric rat F I promoted nucleation (58.8 %) and aggregation (57.6 %) during 28th day. On vitamin E pre – treatment, the protein exhibited lesser effect.

4.26 Similarly, hyperoxaluric rat F I showed increase in oxalate binding activity (29.8 %) during 28th day of EG induction and vitamin E pre – treated rats showed no marked increase.

4.27 Immunoblotting of hyperoxaluric rat urinary protein (74 kD) with antibodies raised against human urine 45 kDa protein showed cross – reactivity, increased excretion was observed in hyperoxaluric rats. In vitamin E pre – treated rats, it was slightly lesser when compared to that of hyperoxaluria induced rats.

4.28 Urinary excretion of COM binding protein F I was 4.5 fold increased in hyperoxaluric rats on 28th day. In the case of vitamin E pre – treated rats, the excretion profile was similar to that of control rats.