

Effect of alpha-tocopherol supplementation on renal oxidative stress and Na⁺/K⁺-adenosine triphosphatase in ethanol treated Wistar rats

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Ethanol intoxication resulted in high extent of lipid peroxidation, and reduction in antioxidant defenses (decreased GSH, GSH/GSSG ratio, and catalase, SOD and GPx activities) and (Na⁺/K⁺)-ATPase activity in kidney. Alpha-tocopherol treatment effectively protected kidney from ethanol induced oxidative challenge and improved renal (Na⁺/K⁺)-ATPase activity. Ethanol induced oxidative stress in the kidney and decreased (Na⁺/K⁺)-ATPase activity could be reversed by treatment with ascorbic acid.

Key words: Alpha-tocopherol, Ethanol, Kidney, Oxidative stress, Na⁺ / K⁺-ATPase,

Alcohol consumption has deleterious effects on kidney. It is known to induce oxidative stress, a condition associated with an increased rate of cellular damage induced by reactive oxygen species. Normally antioxidants quench these reactive oxygen species and protect the cell. Ethanol inhibits (Na⁺/K⁺)-ATPase *in vitro*¹, P-type cation pump that regulates active transport of Na⁺ and K⁺ ions. Acute ethanol intoxication causes oxidative challenge and down-regulation of (Na⁺/K⁺)-ATPase in lung². However its effect on renal (Na⁺/K⁺)-ATPase is not clear. Oxidative modifications especially at -SH groups of (Na⁺/K⁺)-ATPase by oxidative stress has been suggested, a molecular mechanism that could operate *in vivo* during ethanol intoxication. In addition, the authors believe that ethanol induced lipid peroxidation might also be playing a major role in

enzyme inactivation. The study was undertaken to investigate the influence of alpha-tocopherol (AT) supplementation, a lipid soluble dietary antioxidant³ on renal oxidative stress and (Na⁺/K⁺)-ATPase activity.

Chemicals— Chemicals used were of analytical grade and purchased from Merck Ltd. India, Sisco Research Laboratories Ltd. India and Sigma Chemicals, USA.

Drug— Vitamin E 400mg/cap (Tocofer-400, Torrent Pharmaceuticals Ltd, India)

Animals— Male Albino rats of Wistar strain (10-12 weeks old weighing 100-120 g were housed in plastic cages of size 35×22.5×20cm (6 rats in each cage) in a well-ventilated room at 22° ±2°C with a 12-hr light/dark cycle. All rats had free access to a standard diet and tap water. The Animal Ethics Committee, Sikkim Manipal Institute of Medical Sciences, Gangtok approved the procedures used.

Experimental design— The animals were divided into following 5 groups of 6 rats each in a group:

Gr I (Control): 1g double distilled water/kg body wt/day for 4 weeks, orally; Gr II: Ethanol treated rats (1.6g ethanol/kg body wt /day for 4 weeks, orally); Gr III: Ethanol + AT treated rats (1.6g ethanol + 80mg AT /kg body wt/day for 4 weeks, orally); Group IV: Ethanol followed by AT treated rats (1.6g ethanol/kg body wt/day for 4 weeks, followed by 80mg AT/kg body wt/day for next 4 weeks, orally); Group V: Ethanol treatment (1.6g ethanol/kg body wt/day, orally) for 4 weeks and followed by 4 weeks abstinence.

The dose of ethanol was determined as described previously⁴. Ethanol and alpha-tocopherol (AT) were freshly dissolved in double distilled water to get desired concentration.

Rats were weighed and killed by cervical dislocation under light ether anesthesia. Kidneys were removed, cleaned of the adhering tissues and weighed. Tissues were immediately processed for biochemical analysis and all parameters were estimated on the same day.

Biochemical analysis— Total protein⁵, extent of lipid peroxidation⁶, reduced glutathione (GSH)⁷, glutathione disulfide (GSSG)⁸, superoxide dismutase (SOD) (EC 1.15.1.1)⁹, catalase (EC 1.11.1.6)¹⁰, glutathione peroxidase (GPx) (EC 1.11.1.9)¹¹ and (Na⁺/K⁺)-ATPase activity¹² were estimated.

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Statistical analysis- were performed by Student's *t* test and significance of difference was set at $P < 0.05$.

Chronic ethanol intoxication resulted in high extent of lipid peroxidation and reduction in tissue GSH and GSH/GSSG ratio (Table 1). In addition activities of enzymatic antioxidants like catalase, SOD and GPx, and $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ were reduced on ethanol treatment (Table 2). Exogenous AT, to some extent could protect kidney from ethanol induced oxidative challenge and improve renal $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ activity. Preventive option was better than curative. Tissue thiobarbituric acid reactive substances (TBARS) level was significantly low in drug treated groups in comparison to ethanol treated group, and renal GSH and GSH/GSSG, and catalase, SOD and GPx activities were improved on AT administration.

Need for AT during chronic alcohol consumption is enhanced, due to its participation in oxidoreducing processes connected with ethanol metabolism, which leads to its irreversible destruction¹³. Exogenous AT effectively protected kidney from reactive oxygen species (ROS) as it considerably reduced the extent of

lipid peroxidation. Chronic ethanol treatment decreased GSH and reversed GSH/GSSG ratio and it was improved significantly by AT supplementation. Increase in GSH content may be due to activation of synthesis or decreased utilization. The later possibility is supported by improved catalase and SOD activity in AT treated rats than ethanol treated animals. The tissue GSH content reflects its potential for (i) detoxification (ii) preserving the proper cellular redox balance and (iii) its role as a cellular protectant¹⁴. Increased GSH may be channeled as a substrate for GPx, the activity of which was improved on AT treatment. GPx plays a significant role in the peroxy scavenging mechanism and in maintaining functional integration of the cell membranes¹⁵.

Reduced $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ activity in ethanol intoxicated rats was brought back to normal by AT treatment. It could be either due to the direct stimulatory effect and/or antioxidant effect of the vitamin. The latter possibility is supported by the fact that AT reduced renal TBARS and restored scavenger system. Studies in erythrocytes from essential hypertensive have shown

Table 1— Effect of alpha-tocopherol (AT) supplementation on tissue levels of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG ratio. Values are mean \pm SD of 6 observations.

	TBARS [#]	GSH [§]	GSSG [§]	GSH/GSSG
Control	26.37 \pm 1.30	1.364 \pm 0.06	0.241 \pm 0.024	6.06 \pm 0.24
Ethanol	42.81 \pm 2.13 ^a	0.672 \pm 0.02 ^a	0.443 \pm 0.026 ^a	1.55 \pm 0.09 ^a
Ethanol + AT	31.0 \pm 1.13 ^{ab}	9.24 \pm 0.09 ^{abc}	0.305 \pm 0.02 ^{abc}	3.38 \pm 0.1 ^{abc}
Ethanol followed by AT	32.3 \pm 1.12 ^{ab}	9.01 \pm 0.02 ^{abc}	0.318 \pm 0.02 ^{abc}	3.16 \pm 0.2 ^{abc}
Ethanol + abstinence	40.27 \pm 1.56 ^a	0.691 \pm 0.06 ^a	0.413 \pm 0.027 ^a	1.67 \pm 0.17 ^a

P values: < 0.05 compared with ^a control, ^b ethanol treated, and ^c abstinent group

[#]nanomol H_2O_2 consumed/mg tissue/min

[§]micromol/g

Table 2— Effect of alpha-tocopherol (AT) supplementation on tissue activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and $(\text{Na} + \text{K})\text{-adenosine triphosphatase (ATPase)}$

Values are mean \pm SD of 6 observations

	SOD [#]	Catalase [§]	GPx*	$(\text{Na} + \text{K})\text{-ATPase}^{\textcircled{a}}$
Control	11.18 \pm 0.73	0.33 \pm 0.01	0.387 \pm 0.015	12.06 \pm 0.68
Ethanol	7.39 \pm 0.55 ^a	0.16 \pm 0.02 ^a	0.172 \pm 0.012 ^a	8.45 \pm 0.87 ^a
Ethanol + AT	9.81 \pm 0.72 ^{abc}	0.20 \pm 0.01 ^{abc}	0.214 \pm 0.01 ^{ab}	10.21 \pm 0.2 ^{ab}
Ethanol followed by AT	9.80 \pm 0.4 ^{abc}	0.20 \pm 0.01 ^{abc}	0.208 \pm 0.01 ^a	9.9 \pm 0.3 ^{ab}
Ethanol + abstinence	7.73 \pm 0.12 ^a	0.17 \pm 0.03 ^a	0.181 \pm 0.006 ^a	9.29 \pm 0.33 ^a

P values: < 0.05 compared with ^a control, ^b ethanol treated, and ^c abstinent group

[#]micromole/ pyrogallol auto- oxidized /mg protein/min

[§]nanomole/ 1-chloro-2,4-dinitrobenzene conjugate formed/mg protein/min

*micromole/ 1-chloro-2,4-dinitrobenzene conjugate formed/mg protein/min

[ⓐ]micromole/ Pi/mg protein/hr

on lipid peroxidation and changes in membrane fatty acid composition modulates $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ activity. In the present study, lipid peroxidation might be a contributing factor in ethanol induced inactivation of $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ ¹⁷. Optimal interaction of $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ with membrane phospholipids is essential, considering that its activity is modulated by microenvironment given by the physicochemical properties of the membranes into which it is inserted¹⁶. Restoration of renal $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ after AT supplementation in ethanol-treated rat may protect the kidney function.

From the results, it concluded that alpha-tocopherol supplementation had protective effect on ethanol induced renal $(\text{Na}^+/\text{K}^+)\text{-ATPase}$ inactivation. Moreover it also ameliorate the ethanol-induced oxidative stress. Alpha-tocopherol execute its role by modulating free radical production there by preventing lipid peroxidation and/or stimulating $(\text{Na}^+/\text{K}^+)\text{-ATPase}$. To answer which one is more important, further investigation is needed.

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